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Human glial cell culture models of inflammation in the central nervous system

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Research into human central nervous system (CNS) disorders has traditionally focused on interconnecting neurons, thought to be the most important functional elements in the CNS. Consequently, animal models have developed as the central paradigm in CNS drug development. However, evidence is accumulating that suggests glial cells play a much more important role in health and disease in the CNS than has been previously acknowledged. Brain development, neurotransmission, inflammatory and neuroprotective pathways and blood–brain barrier functions rely on glial cells. It is also the case that human glial cell cultures adequately mimic *in vivo* glial cell behaviour, providing a novel and valuable tool for CNS drug discovery and development.

For a long time, glial cells in the central nervous system (CNS), microglia, astrocytes and oligodendrocytes, were considered to be largely uninteresting matrix, 'glue', elements that only contributed to feeding and supporting neurons. However, evidence is rapidly accumulating that suggests glial cells, in particular astrocytes and microglia, are in fact very important players in CNS development, repair [1] and neurotransmission [2]. They are also central functional elements in CNS vascularization, inflammation and neuroprotection [3–5]. It is increasingly recognized that the most important CNS disorders, for which therapy is urgently needed, are not merely defined by the enigmatic emergence of dysfunctional neurons but are in fact orchestrated largely by glial-cell-controlled inflammatory processes. In multiple sclerosis, the archetypical inflammatory CNS disease, as well as in Alzheimer's disease [6], stroke [7] and Parkinson's disease [8], inflammation has been implicated in the disease process and the involvement of glial cells has been clearly shown.

Like nearly all other cell types in the mammalian body, glial cells, including astrocytes and microglia, function in the context of cell–cell and cell–matrix interactions and they receive continuous cues from soluble and cell-bound signalling molecules, even during homeostasis. Glial cells are engaged much less intimately in established multicellular networks than neurons, sometimes even avoiding contact with nearby sister cells, and they are known to migrate frequently in response to chemokine signals within the CNS. The dynamic, plastic and interactive properties of glial cells are, thus, not essentially different from other cell types that are involved in inflammation, such as cells of the peripheral immune system (from which microglia are actually derived). As with glial cells, these immune cells also function in close association with other cells and matrices in specialized organs – the thymus, spleen and lymph nodes. This has not prevented remarkably successful efforts, made by immunologists in the past, to dissect pivotal inflammatory pathways after disrupting the organs of the immune system and examining purified immune cells in culture. There appears to be no *a priori* reason why a similar strategy could not be equally successful for glial cells, providing a basic understanding of neuroinflammatory and neuroprotective pathways on which research can build.

One very important advantage of using glial cell cultures to dissect inflammatory and neuroprotective pathways in the CNS is the fact that such cultures can be set up using adult human glial cells, the final target of many CNS drugs. Because several brain banks worldwide collect fresh brain tissue very shortly after death and because of the acquisition of human brain biopsies from temporal lobotomies

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(performed for the surgical management of epilepsy) sufficient amounts of human brain or spinal cord samples can be collected to set up glial cell cultures in a routine manner. Also, for confidential corporate projects, human brain tissue samples can, in principle, be made available while fully respecting the ethical constraints associated with the acquisition and use of the tissue.

Crucially, the use of human glial cell culture systems eliminates any problems linked to species specificity. These problems continue to hamper the reliability of animal-model data in respect to complex human diseases and this often occurs with CNS disorders. Although some neurobiological targets and pathways are very well conserved among mammalian species, this does not necessarily apply to all of them. In fact, recent analysis has revealed that interspecies variation in gene expression is particularly prominent in the brain, even among primates [9]. As an example, some key mediators of appetite regulation and energy metabolism, important to issues of obesity in humans, are completely absent from the brains of several other mammalian species [10]. Also, because development of species-specific therapeutic biologicals (e.g. antibodies, cytokines and growth factors) versus more-traditional small-molecule based intervention is rapidly increasing in CNS disorders, the requirements for research models based on primary human cells will probably increase.

The use of adult human glial cell culture models to identify novel inflammatory targets and pathways in CNS disorders clearly holds promise but the possibilities and limitations of such models must be explored further. First and foremost, questions need to be addressed as to how human glial cells behave in culture and whether this situation accurately reflects their behaviour in an intact human brain or spinal cord. Also, the question arises as to what extent glial cell cultures from different donors, or different parts of the CNS, display functional diversity. Variations in cellular responses are an intrinsic quality of many primary adult human cell culture systems and glial cell cultures will probably be no exception to this fact. It will be crucial to the productive use of these cell culture systems that the extent of such variations and their biological consequences are properly understood. It should be noted, however, that biological variations (occurring in response to a molecular stimulus) observed in primary human cell cultures do not just represent a technological nuisance that must be avoided or, at least, minimized. Instead, this heterogeneity could be crucial for the therapeutic perspective of every candidate drug and this is an example of exactly the type of valuable information that primary human cell cultures can provide, whereas animal models cannot.

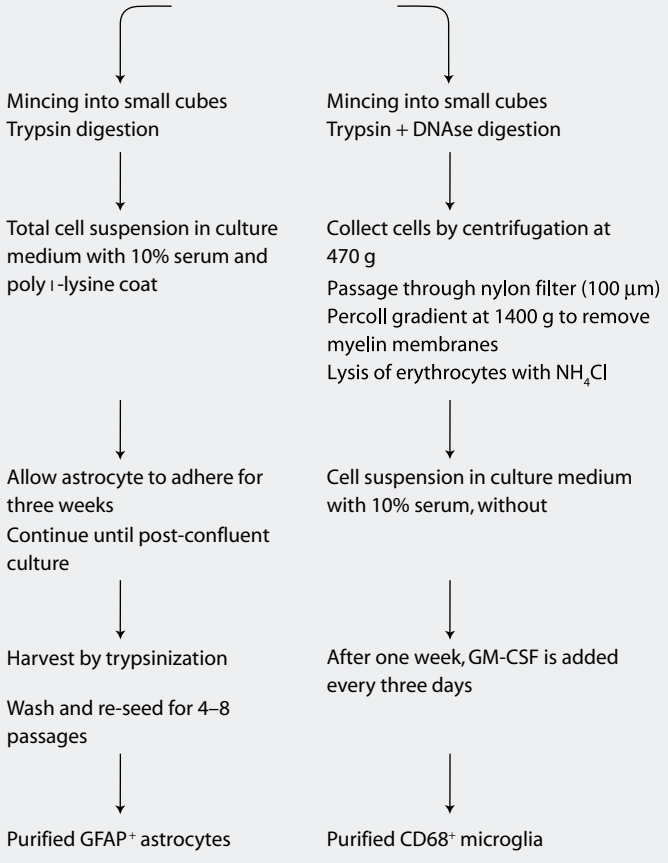
Are cultured human glial cells representative of glial cells *in vivo*?

Cell culture models of human glial cells centre on two major types of cell, namely astrocytes and microglia. Both cell types can be readily purified to homogeneity, can be kept in culture for several weeks and represent the two most important cell types in inflammatory control and neuroprotective pathways of the CNS (Box 1). Astrocytes can even be stored in a frozen state and re-cultured for several passages thereafter [11] (microglia cannot be stored frozen). In some systems granulocyte–macrophage colony-stimulating factor (GM-CSF) is used to promote microglial proliferation, whereas in other approaches no growth factor additions are included, as reviewed in detail by Lue and co-workers [6].

BOX 1

Methods to isolate and culture adult human glial cells

Fresh grey or white matter brain tissue (post-mortem delay less than six hours)



It is important to note that cultured astrocytes and microglia do not represent the highly ramified resident glial cells that are observed in the normal, healthy human CNS. Instead, cells, in particular microglia, usually adopt a much less ramified, bipolar (or even more-rounded) morphology depending on culture times and states of activation. Although post-confluent cultures can be obtained in which proliferation is minimized, both types of cultured glial cells represent cells that are activated to at least some extent [12]. This feature is an important consideration when comparing cultures with *in vivo* states but it does not render the cells irrelevant. After all, in most major CNS disorders microglia and astrocytes (in the affected tissue) will be rapidly activated to some extent (for example during gliosis, a common response to many different types of CNS insult). Glial cells obtained from cell cultures share a specific state of activation with disease-affected glial cells *in vivo*.

To validate the behaviour of cultured glial cells, histopathological data that assess cytokines, chemokines and growth factors (produced during CNS inflammation *in vivo*) can be compared with the results observed in cell culture systems after treatment with the same pro-inflammatory stimulus that is assumed to operate during neuroinflammatory disease. A large quantity of literature is available on histopathological studies of glial-derived inflammatory mediators in neuroinfection, neurotrauma and neurodegenerative diseases (e.g. multiple sclerosis and Alzheimer's disease) [13–15].

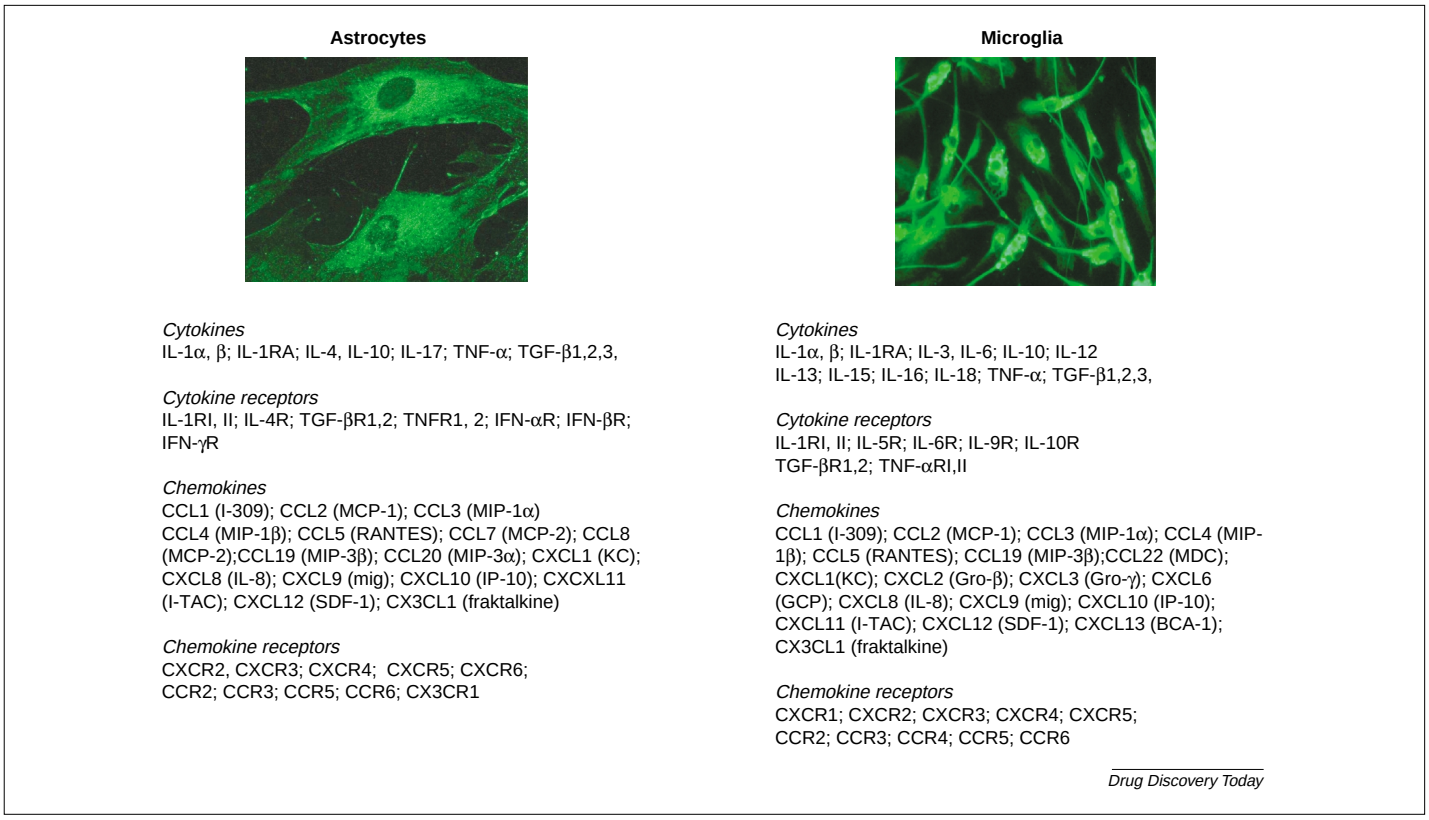


FIGURE 1

Cytokine and chemokine signalling by astrocytes and microglia. A compilation is presented of cytokines, chemokines and their receptors currently known to be expressed in cultured human astrocytes and microglia, illustrating their broad ability to participate in inflammatory pathways, based on data in [3–5,16–19].

The qualitative aspects of such studies are usually the most informative because the quantitative aspects of the data are harder to assess. Based on current data, the use of different technologies in sample acquisition and sample treatment, the diversity in antibody-based staining and in *in situ* PCR techniques, and different image processing routines make it virtually impossible to quantify reliably the production of certain mediators by glial cells *in vivo*. In parallel, a lot of information has accumulated on cytokine and chemokine mediators produced by cultured human glial cells after stimulation with the prime mediators of CNS inflammation, namely interleukin (IL)-1β and tumour necrosis factor (TNF)-α [5,16–19] or interferon (IFN)-γ, IFN-γ is often used to mimic the frequent presence of activated T cells in brain infiltrates. The broad potential for glial cells to participate in neuroinflammatory pathways is clearly illustrated by the wide range of cytokines, chemokines and their receptors that are currently known to be expressed by cultured astrocytes and microglia derived from post-mortem brain tissue or biopsies. These data have been collected by different techniques that include PCR- and ELISA-based detection assays or by cDNA array-based gene profiling approaches (Figure 1) [3–5,16–19].

There is a remarkable correspondence between the cytokines and chemokines produced by glial cells in an inflamed human brain and those produced and/or induced by IL-1β, TNF-α or IFN-γ in glial cell cultures [18,19]. To date, no mediator has been documented during neuroinflammation *in vivo* that has not also been detected in cell culture. This comparison between (inducible) expression of mediators of neuroinflammation and neuroprotection in cultured glial cells and in glial cells in an intact human brain extends well beyond cytokines and chemokines. A wide range of functionally important molecules, including gap junction proteins [20], the Nogo and Nogo-receptor pair [21], receptors for binding amyloid β peptides [22], stress related enzymes, acute phase proteins and a variety of cell surface molecules for intercellular communication [6], are expressed by glial cells in culture and *in vivo*. Clearly, the picture is not yet complete and more-comprehensive technology, such as single-cell-specific genomic and proteomic profiling, will ultimately be required to answer this question in full, this is also the case regarding the quantitative similarities between glial cells in culture and *in vivo* [23]. Thus, evidence indicates that cultured glial cells express a wide range of functional molecules that are relevant to neuroinflammatory pathways and that respond to a variety of stimuli, very much like their *in vivo* counterparts.

What is the extent of donor-to-donor variation in human glial cell cultures?

Differences in the way seemingly identical cell types from different humans respond to stimuli is a fact of life, just as humans themselves respond differently to their environment (therapeutic intervention included). Unfortunately, interindividual variation in cell systems is frequently regarded as a technological nuisance and published reports either circumvent the issue, by the presentation of 'representative' data, or eliminate the issue, by pooling individual samples before analysis. As a consequence, very few studies explicitly address the issue of donor-to-donor variation in the behaviour or the response of human glial cell cultures. Based on our own evidence, as well as that reported by others, there appears

TABLE 1

Variability in the expression of relatively abundant gene products in cultured astrocytes

Gene product	Average signal [a]	Culture-to-culture variation (CV)[b]	Donor-to-donor variation (CV)[c]
Low affinity nerve growth factor receptor (NGFR)	1.232	3.9	2.1
Insulin-like growth factor binding protein 2 (IGFBP2)	1.159	4.1	5.1
Thymosin β-10 (TMSB10)	1.148	7.3	6.9
Pleiotrophin (PTN) + human nerve growth factor	1.075	5.3	5.3
CD70 (CD27 ligand)	1.029	4.7	6.0
CD147 (basigin, neurothelin)	1.005	7.6	4.5
Vascular endothelial growth factor receptor 1 (VEGFR1)	0.970	4.9	5.8
Insulin receptor (INSR)	0.864	6.8	7.3
Epidermal growth factor receptor (EGFR)	0.863	6.5	6.8
Interleukin-6 (IL-6)	0.806	39.4	15.6
BIGH3	0.792	10.0	13.1
CCL2 [monocyte chemotactic protein 1 (MCP-1)]	0.778	15.7	13.1
Interleukin-4 (IL-4)	0.770	29.8	9.8
Vascular endothelial growth factor (VEGF)	0.766	6.4	6.9
Fibroblast growth factor receptor 1 (FGFR1)	0.764	5.0	7.3
UFO/Axl receptor tyrosine kinase	0.762	8.7	5.4
TEK/TIE-2 receptor tyrosine kinase	0.717	6.4	3.7
Tumour necrosis factor α (TNF-α)	0.703	10.4	3.3
Connective tissue growth factor (CTGF)	0.702	6.3	3.0
Bone morphogenetic protein 2A (BMP-2A)	0.695	32.9	13.7

[a]Expression levels of mRNA relative to the mean of eight housekeeping genes.

[b]Comparing eight separate cultures derived from the same isolate.

[c]Comparing cultures from three different donors.

to be a difference between astrocytes and microglia at the level of donor-to-donor variations (explained in more detail below).

In a recent study, we performed a cDNA array-based gene profiling study on cultured astrocytes from different donors to examine their cytokine, chemokine and growth factor profile in a resting post-confluent culture, as well as after stimulation with IL-1β, TNF-α or IFN-γ (or a mixture of these pro-inflammatory mediators) [18]. Cells from one donor were examined on eight separate occasions using separately initiated cultures to examine the robustness of the culture system and the analytical tools themselves. This analysis revealed a remarkable degree of consistency in the products expressed by astrocytes in a standardized culture system, as well as in the gene products that appeared in response to a pro-inflammatory stimulus. In fact, results for different donors were usually no less consistent than results from separate astrocyte cultures derived from a single isolate. As shown in Table 1, the coefficients of variation for the most abundantly expressed cytokine, chemokine and growth factor genes (highlighting culture-to-culture variations only) did not, in most cases, exceed 15%. In fact, they were often well below 10%. Only in a few cases, *viz*. IL-4, IL-6 and bone morphogenetic protein 2A, did expression between different cultures vary more significantly, possibly reflecting the unusual sensitivity of these particular products for minor variations in environmental signals. In general, this analysis confirms that cultured adult human astrocytes represent a reproducible, reliable model system that examines inflammatory astrocyte pathways and donor-to-donor variation (at this level) is minimal. Marked parallels in the cytokine and chemokine responses of astrocytes to standardized stimuli, such as IL-1β and TNF-α, underpin this notion [3,5,18,19].

It should be noted, however, that the extension of the range of astrocyte genes under consideration (beyond inflammatory pathways) has, in fact, revealed some molecular diversity among astrocytes. Clues for such functional diversity in astrocytes from different parts of the CNS have been noted at the level of ion channel expression and the production of S100β (a neurite growth-promoting factor) [24,25]. Also, recent analyses of the transcriptional profile of astrocytes indicate that some diversity is a biological reality and might have consequences for their functional role in neurodegenerative diseases [26]. The extent of astrocyte heterogeneity in the human CNS will require further studies to clarify its origin, the range of functions affected by diversity and the neurobiological impact of the observed variations.

Although relevant to both glial cell types, cellular diversity is probably a more prominent feature of microglia than of astrocytes. Although microglial heterogeneity does not appear to be a popular subject on which to publish, some studies have documented variable gene-expression data, assessed by cDNA arrays in microglial cultures from rats [27] and mice [28]. Also *in vivo*, some microglial features appear to vary from one region of the CNS to another, as demonstrated by *in situ* analysis of phenotypic and response markers [29]. These studies suggest that microglial diversity is not merely a methodological artefact, but does indeed reflect the inherent diversity in microglial responsiveness in different parts of the CNS, possibly also as a function of age and disease. To address the issue of heterogeneity in adult human microglial cultures, we recently examined cytokine, chemokine and growth factor gene profiles from cultured microglia that were derived from nine different adult donors (this included analyzing their response to TNF-α and/or

TABLE 2

Variability in the expression of relatively abundant gene products in cultured microglia

Gene product	Average signal[a]	Donor-to-donor variation (CV)[b]
CCL2 (MCP-1)	1.415	20
Thymosin β-10 (TMSB10)	1.267	18
Neuromodulin (GAP43)	1.165	23
Migration inhibitory factor-related protein 14	1.141	22
Migration inhibitory factor-related protein 8 (MRP8)	1.042	30
Corticotrophin releasing factor receptor 1 (CRFR1)	1.010	21
Endothelin-2 (ET-2)	0.932	32
Bone morphogenetic protein 2A (BMP2A)	0.931	17
CXCL8 (IL-8)	0.929	49
CCL3 (MIP1-α)	0.921	24
Ribonuclease angiogenin inhibitor (RAI)	0.895	22
Interleukin-1 receptor antagonist protein (IL-1RA)	0.882	27
Bone morphogenetic protein 4 (BMP4) +BMP2B	0.862	42
BIGH3	0.853	21
Low-affinity nerve growth factor receptor (NGFR)	0.840	71
Pleiotrophin (PTN) + human nerve growth factor	0.830	62
Interleukin-1β (IL-1β)	0.714	31
Flt3 ligand (SL cytokine)	0.713	30
CD147 (basigin, neurothelin)	0.695	24
CD40	0.691	17

[a]Expression levels of mRNA relative to the mean of eight housekeeping genes.

[b]Comparing cultures from nine different donors.

IFN-γ) [19]. As illustrated in Table 2, the coefficients of variation in the expression levels of the most abundant gene products from untreated microglia (derived from different samples of subcortical white matter from post-mortem human brains) were generally 20–30% (some products showed a somewhat higher level of variability in their expression). Therefore, the consistency in the gene profile of microglial cultures appears to be lower than that of astrocytes, although not at levels that should raise immediate concerns for the applicability of microglial cultures. In contrast to the relatively stable behaviour of standardized and untreated microglia cultures, their response to IL-1β and/or TNF-α was occasionally found to display striking donor-to-donor variations [19]. In the majority of cases, cytokines and chemokines (induced in cultured microglia by IL-1β and/or TNF-α) mimic the products that are expressed by microglia in intact human brains during CNS inflammation. However, the levels of induction of these products sometimes varied markedly between different cultures, despite the standardized methodology. In some cases, a stimulus even induced expression of a certain gene in one culture while suppressing it in another.

Microglial heterogeneity, as observed in cell culture models and *in vivo*, is probably not merely a technological fault; it probably does reflect a biological reality. Interindividual variations (at the level of microglial responses) will probably be relevant to understanding the biology of every type of intervention in the CNS, or the effects of candidate CNS drugs. Thus, variability in microglial cell culture models should not be regarded as a shortcoming of the technology; it should be embraced as an opportunity to explore a highly relevant feature of the adult human CNS that would otherwise remain obscure.

Applicability of human glial cell culture models

The previous evaluation of adult human glial cell cultures indicates that astrocyte and microglial cultures respond to defined pro-inflammatory stimuli in ways that correspond closely with the responses documented in the intact human brain and spinal cord. It is also clear that, with respect to the inflammatory pathways, different astrocyte isolates yield consistent, reproducible cultures with few interindividual variations and, although variability is observed in untreated microglial cultures, this is completely acceptable. The difference between the two cell types is that there is a much higher level of variability in microglial isolates in response to a pro-inflammatory stimulus, a factor that probably also reflects the intrinsic heterogeneity of microglia *in vivo*.

A recent finding further substantiates the phenotypic parallel between cultured glial cells and glial cells *in vivo*. Following damage or inflammation in the CNS, glial cells are known to upregulate toll-like receptor (TLR) family members, key mediators of innate inflammatory reactions. In a detailed study of TLR expression in the brains of multiple sclerosis patients we noted that TLRs in inflamed areas were only expressed on the surface of astrocytes, whereas in microglia they were exclusively found in intracellular vesicles. In adult human glial cell cultures the expressed TLR family members and their subcellular distribution, as defined by immunocytochemical analysis, fully mimicked *in vivo* findings [30]. These data lend further support to the idea that cell culture models of adult human glial cells are particularly well-suited for the study of inflammatory CNS pathways. Essentially, all analytical techniques in cell biology can be applied to glial cell cultures and culturing conditions can be modified to examine the human glial cell response

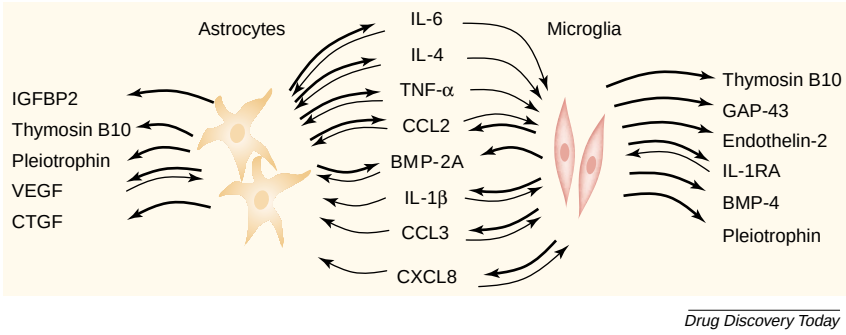


FIGURE 2

The most abundant cytokines, chemokines and growth factors that are produced by cultured astrocytes and microglia. Cytokines, chemokines and growth factors identified by gene profiling studies as the 20 most abundantly expressed genes in cultured (but otherwise untreated) adult human astrocytes and microglia [18,19]. Based on current information on receptor expression data, this information also indicates which of these abundant glial cell-derived mediators can signal to glial cells themselves (represented by thin arrows). Mediators positioned between astrocytes and microglia can, thus, be expected to play key roles in glia–glia communication although others might be primarily targeting other elements within the CNS. Abbreviations: IGFBP, insulin-like growth factor binding protein; VEGF, vascular endothelial growth factor; CTGF, connective tissue growth factor; BMP, bone morphogenetic protein.

to experimental conditions, stimuli, drug doses or combinations of drugs that could never be established in animal models or humans. For example, bacterial or viral infection with pathogens that specifically infect humans but that cannot infect other mammals. The awareness of the effects of neurotrophic infection in the CNS has recently been boosted by observations of unexpected side effects of Tysabri®, a novel drug for multiple sclerosis that provokes JC-virus-mediated pathology in some patients. The study of human-specific neurotrophic viruses, including JC virus, is almost impossible *in vivo* but it is readily amenable to experimental approaches in adult human glial cell culture models [31].

Therefore, glial cell culture models offer excellent opportunities for functional genomic and proteomic analyses, along with high-capacity screening possibilities. These options appear to be very useful for the discovery and validation of novel CNS targets, particularly because cell culture models allow much broader and more-detailed analyses than animal-model studies or *in situ* histopathological studies where the number of read-out parameters or markers examined is often necessarily limited. Gene profiling studies of cultured adult human astrocytes and microglia have recently confirmed the widely held notion that IL-1β and TNF-α are among the prime mediators of communication between activated glial cells in the human CNS, lending further credibility to these cell culture models [18,19]. At the same time, however, other cytokine and chemokine mediators have been revealed, which are expressed by cultured and partially activated glial cells at levels similar to the two 'traditional ones' (IL-1β and TNF-α), thus uncovering several other glial response mediators that deserve further attention. After all, many of the previous analyses on response mediators have been based on pre-selected candidates, ignoring a multitude of others. In Figure 2 we present a summary of the most abundant mediators in astrocyte–microglial communication in cell culture, derived from mRNA expression levels in cDNA array-based gene profiling studies [18,19]. Several of these mediators, notably IL-1β and TNF-α, have previously been documented and they are familiar key players in neuroinflammation, as well as in an intact human CNS. Other mediators, such as bone morphogenetic proteins 2A and 4, are less familiar. Similarly, comprehensive analyses of response markers have already uncovered mediators produced by cultured glial cells that have, until now, been considered to be specific for different cell types. One recent example of such an unexpected response mediator is IL-17. It has angiogenic activities and is readily produced by cultured astrocytes in response to IL-1β or TNF-α; it was previously considered to be a cytokine that was specifically produced by activated T cells only [18]. This emphasizes the fact that many of the mediators that are potentially involved in key pathways of neuroinflammation and neuroprotection have escaped our attention simply because they have not been looked for.

Conclusion

Cell culture models of adult human glial cells offer several important benefits for research on drug targets of CNS disorders and they can be considered to be valuable emerging tools that complement animal-model studies. In addition to cell culture systems that model CNS pathways, organotypic brain-slice cultures are gaining popularity and they can be prepared from human post-mortem brain [32]. Although offering a model system that is one step closer to the complexity of the intact CNS, brain-slice cultures pose considerable technical challenges and limitations, as recently reviewed for this journal by Sundstrom and colleagues [33]. Cell culture models of isolated human glial cell types lack the intercellular interactions that slice cultures partially offer but this review highlights the point that cultured glial cells mimic their *in vivo* behaviour closely, helping to uncover fundamental pathways in neuroinflammation and neuroprotection. Most importantly, glial cell cultures from human origin are now routinely available, circumventing species-specific issues. Therefore, these cultures provide an important tool that accelerates the development of urgently needed novel drugs for inflammatory diseases of the CNS.

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