OVERVIEW

Glial Cell Lines: An Overview

George H. De Vries · Anne I. Boullerne

Abstract The importance and essential functions of glial cells in the nervous system are now beginning to be understood and appreciated. Glial cell lines have been instrumental in the elucidation of many of these properties. In this Overview, the origin and properties of most of the existing cell lines for the major glial types: oligodendroglia, astroglia, microglia and Schwann cells, are documented. Particular emphasis is given to the culture conditions for each cell line and the degree to which the line can differentiate in vitro and in vivo. The major molecular markers for each glial cell lines are indicated. Finally, methods by which the glial cell lines have been developed are noted and the future directions of glial cell line research are discussed.

Keywords Oligodendrocytes · Schwann cells · Astrocytes · Microglia · Spontaneous immortalization · Cell culture · SV 40 antigen · Glial cell markers · Glioma cell lines · Glioblastoma cell lines · Oligodendroglioma cell line

Introduction

Glial cells are now recognized as functional components of the nervous system rather than being regarded as structural

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"glue" holding the nervous system together [1] In vitro studies of primary cultures of glial cells have been an important tool to understand the metabolic capabilities of these cells. However primary cell cultures are often difficult to prepare and only offer limited numbers of cells for study in a limited timespan. Cell lines, as a tool to study glial cell biology, have both advantages and disadvantages in comparison with primary cultures. One major advantage is the unlimited number of cells available for studies, versus the limited number of primary culture cells obtained after the sacrifice of animals for each preparation. Further, the use of a cell line guarantees a single cell type, ensuring that the investigator starts with a pure culture of cells, generally all at the same stage of differentiation. With primary cultures, occasional contaminating cells may be impossible to avoid, and eliminating these contaminating cells often requires additional purification steps. Moreover, isolated primary cultures may show some degrees of heterogeneity with cells at different stages of maturation, whereas cells of a cell line are synchronized. Another major advantage of cell lines is their ease of maintenance in culture. The media on the cells has to be changed about twice per week, and the cells are split into additional flasks when confluence is reached. The culture medium is usually DMEM medium supplemented with 10% fetal bovine serum, which is readily available and easy to prepare.

However, there are also significant disadvantages of cell lines. Most cell lines differ from primary cell cultures with regard to their ability to differentiate and reach full maturity. Cell lines have a strong tendency to divide, even in culture conditions designed to slow down proliferation and induce differentiation. Also, cell lines retain some degree of plasticity and their properties can change with the number of passages over time. As a result, a cell line can lose the characteristic that is under study, sometime during

a course of experiments. Given that it may be difficult to obtain a large number of primary cells, cell lines are a viable option, and sometimes the only option available to study certain aspects of glial cell biology such as molecular biology and signaling pathways. When choosing a cell line, it is imperative for the investigator to continuously monitor the property of interest to be sure that this property is expressed in all the passages used for the study.

The purpose of this Overview is to not only give an updated listing on many cell lines currently available for all the glial cell types (see Table 1), but also to delineate the conditions under which these cell lines are cultured and the morphology of cell lines. The utility of a given glial cell line is inversely related to the ease of culturing the primary cells. For example primary glial cells such as rat Schwann cells and rat astrocytes are relatively easy to obtain in large numbers and to expand in culture. Therefore cell lines derived from primary cultures of these cells are less critical considering the primary cultures are much easier to maintain and primary cells proliferate easily. However the usefulness of glial cell lines is also species specific. For example although rat and mouse Schwann cells are fairly easy to obtain and expand, human Schwann cells are much more difficult to obtain, culture and expand. Therefore there are many more human Schwann cell lines which have been developed relative to Schwann cells derived from rodent nerves. The ability of a glial cell line to differentiate in a manner similar to the primary cell is critically important. The ultimate differentiation challenge for glial cells derived from primary cultures of oligodendrocytes is to myelinate in vitro or in vivo. Therefore the ability of glial cell lines derived from the myelinating glial cells (oligodendroglia and Schwann cells) to myelinate in vivo or in vitro is documented. Finally methods which have been successfully used to immortalize cells to create new cell lines are documented, and future directions regarding the use and need for new glial cell lines are discussed.

Glial Cell Lines Currently Available

A number of glial cell lines have been created from rat, mouse, and human sources for each of the major types of glial cells. An abbreviated listing of the most common glial cell lines used for current studies is shown in Table 1. Note that this list is not exhaustive; only cell lines which were referenced at least 10 times in the literature (PubMed Database) are included in Table 1.

It is very useful to know the origin of the most common glial cell lines and the original description of the cell line. The original description and characteristics can serve as a "touchstone" for the investigator today who will use the cell lines. Comparison of the properties of a given cell line, as described in the original paper, with the properties of the current cell line will reveal the degree to which the line faithfully replicates the original properties. As documented in Table 2, some of these cell lines were derived from tumors while others were created by transformation of primary cells.

Media Conditions for Culture of Glial Cell Lines

There are many nuances to the type of media required for the culture of glial cell lines. Therefore it is important to understand the exact media conditions for each cell line. Common for all the glial cell cultures are the culture chamber conditions: 37°C in a humidified atmosphere with 5% CO_2/95% air. The details of other culture conditions follow.

Two supplements (derived from additives to a medium originally published by Bottenstein and Sato [2], also known as B&S medium or Sato medium), are widely used for the culture of oligodendrocyte cell lines: N1 supplement sold by Sigma–Aldrich; and N2 supplement sold by Gibco-Invitrogen. The two supplements differ only in the

Table 1 Chart of the most common glial cell lines

Oligodendrocyte cell lines	Schwann cell lines	Astrocyte cell lines	Microglia cell lines
CG4 (rat)	33B (rat)[a]	C6 (rat)[a,b]	HAPI (rat)
OLN-93 (rat)	RT4-D6P2T (rat)[a,b]	DI TNC1 (rat)[a,b]	BV-2 (mouse)
Oli-neu (mouse)	TSC(rat)	BALB SFME (mouse)[b]	EOC 2 (mouse)[b]
N19 (mouse)	IMS32 (mouse)	A172 (human)[a,b]	EOC 20 (mouse)[b]
N20.1 (mouse)	ST88-14 (human)	U-87MG (human)[a,b]	CHME-5 (human)
G26-20 (mouse)	STS-26T (human)		
G26-24 (mouse)	NF1T (human)		
HOG (human)	T265 (human)		
MO3.13 (human)	GOTO (human)		

[a] Commercially available through ECACC (European Collection of Cell Cultures founded 1984, part of Health Protection Agency founded 1920)

[b] Commercially available through ATCC (Cell Biology Collection founded 1962, part of American Type Culture Collection founded 1925)

Table 2 Cell lines specifications

Cell line name	Cell type	Method used to generate cell lines	References
CG4 (CG-4)	Oligodendrocyte	Spontaneous immortalization of OPC from mixed glial culture of neonatal cortex (Sprague–Dawley rat)	[4]
OLN-93	Oligodendrocyte	Spontaneous immortalization of OPC from mixed glial culture of neonatal brain (Wistar rat)	[9]
Oli-neu	Oligodendrocyte	Transfection with a *t-neu* oncogene of primary culture enriched in oligodendrocytes from day 15 embryos (NMRI mouse)	[14]
N19 N20.1	Oligodendrocyte	Transfection with temperature-sensitive *SV40 large T antigen* of primary culture enriched in oligodendrocytes from neonatal brain (BALB/cByJ mouse)	[20]
G26-20 G26-24	Oligodendrocyte	Clones derived from the glioma G26 induced by methylcholanthrene treatment (C57BL/6 mouse)	[29, 30]
HOG	Oligodendrocyte	Clone derived from a surgically removed oligodendroglioma (human)	[35]
MO3.13 (M03.13)	Oligodendrocyte	Fusion of a human tumor rhabdomyosarcoma RD cell line with a human primary culture of oligodendrocytes from surgery (human)	[38]
TSC (tSc)	Schwann cell	Transfection with *SV40 large T antigen* under the control of metallothionein promoter of secondary Schwann cell cultures from neonatal sciatic nerve (Sprague–Dawley rat)	[54]
33B	Schwann cell	Clone derived from the TR33B tumor of lumbar spinal cord and roots induced by N-ethyl-N-nitrosourea treatment (Wistar-Furth rat)	[66, 67]
RT4-D6P2T	Schwann cell	Subclone derived from the RT4-D6 cell line generated from the RT4 tumor of sciatic nerve induced by N-ethyl-N-nitrosourea treatment (BDIX rat)	[69, 70]
IMS32	Schwann cell	Spontaneous immortalization of Schwann cell from long-term primary culture of adult dorsal root ganglia and peripheral nerves (CD1 mouse)	[72]
ST88-14	Schwann cell	Clone derived from a surgically removed solid tumor of the peripheral nerve sheath from a patient diagnosed for NF1 (human)	[57–59]
STS-26T	Schwann cell	Clone derived from a surgically removed grade III malignant Schwannoma from a patient not diagnosed for NF1 (human)	[60]
NF1T	Schwann cell	Clone derived from a surgically removed malignant soft tissue sarcoma from a patient diagnosed for NF1 (human)	[62]
T265	Schwann cell	Clone derived from a surgically removed malignant Schwannoma from a patient diagnosed for NF1 (human)	[49]
GOTO	Schwann cell	Clone treated by bromodeoxyuridine, derived from a neuroblastoma cell line originally established after 5 years culture of adrenal neuroblastoma from a patient (human)	[47, 48, 64]
C6	Astrocyte	Clone derived from a brain tumor induced by N-nitrosomethylurea treatment of adult animals (outbred Wistar rat)	[81]
DI TNC1	Astrocyte	Transfection with *SV40 large T antigen* under the control of GFAP promoter of type 1 astrocytes in primary culture isolated from the diencephalon of neonatal brain (Sprague–Dawley rat)	[84]
BALB SFME	Astrocyte	Clone derived from spontaneous immortalization of day 16 embryonic brains cultured in medium deprived of serum (BALB/c mouse)	[85]
A172 (A-172)	Astrocyte	Clone derived from a surgically removed solid glioblastoma (human)	[86]
U-87MG (U87-MG)	Astrocyte	Clone derived from a surgically removed malignant astrocytoma grade III (human)	[88]
HAPI	Microglia	Spontaneous immortalization in primary microglia culture from brains of neonatal animals (Sprague–Dawley rat)	[92]
BV-2 (BV2)	Microglia	Infection with a *v-raf/v-myc* oncogene carrying retrovirus J2 of primary microglia culture from neonatal brains (C57BL/6 mouse)	[94]
EOC 2 EOC 20	Microglia	Spontaneous immortalization in primary microglia culture from brains of 10 day-old animals (C3H H2^k mouse)	[95]
CHME-5 (CHME5)	Microglia	Transfection with *SV40 large T antigen* of primary culture enriched in microglia from cortex and spinal cord of 8–10 week-old embryos (human)	[96]

Table 3 Medium additives for oligodendrocyte cell lines

Bottenstein-Sato additives [2]	N1 supplement (1X) [Sigma–Aldrich]	N2 supplement (1X) [Gibco-Invitrogen]
5 µg/ml insulin	5 µg/ml insulin	5 µg/ml insulin
100 µg/ml transferrin (apo, iron-free)	5 µg/ml transferrin (holo, iron-saturated)	100 µg/ml transferrin (holo, iron-saturated)
100 µM putrescine	100 µM putrescine	100 µM putrescine
20 nM progesterone	20 nM progesterone	20 nM progesterone
30 nM sodium selenite	30 nM sodium selenite	30 nM sodium selenite

N1 and N2 supplements are inspired from the additives of a medium originally published by Bottenstein and Sato in 1979 [2], also known as B&S medium or SATO medium

concentration of transferrin. The exact composition of each supplement is shown in Table 3.

Note that in the B&S medium additives, the transferrin is iron-free (apo form), whereas in the N1 and N2 supplements, the transferrin is iron-saturated (holo form). Choosing which transferrin form to use depends upon the culture conditions. In media such as DMEM/F12 containing high levels of iron salts, apo transferrin is preferred.

The culture media for oligodendrocyte cell lines should be supplemented with 4.5 g/l D-glucose and 2 mM Glutamax® (supplier Gibco-Invitrogen).

The most frequent culture medium suitable for most cell lines is the DMEM medium supplemented with 10% fetal bovine serum (FBS). Since the serum component of the media is critical for the successful culture of glial cell lines, a brief comment on nomenclature and use of serum is in order. Animal serum contains a number of undefined growth factors and hormones which greatly affect the health of glial cell lines. Although the term "fetal calf serum" is often used in the scientific literature, technically there is no such entity. The term calf refers to an animal which is already born and is no longer a fetus or fetal. Thus the term "fetal calf" is an oxymoron. The term "fetal calf serum" is ambiguous since one could not determine if the serum was derived from a newborn calf or a fetus. The real issue is not the nomenclature but the composition of the serum. The exact developmental stage at which the serum is obtained will greatly influence the hormone and growth factor profile of the serum. Obviously fetal bovine serum is more difficult to obtain and thus more expensive. However it may be richer in the hormones and growth factors required for good glial cell line growth. On the other hand newborn calf serum is less expensive and although less enriched in growth factors and hormones may still be suitable for glial cell growth. Regardless, age of the fetus (or calf) and the time of the year that the blood is collected will affect how well the serum performs in a particular culture experiment because of the diet of the mother of the fetus or nursing calf or if the calf has started grazing. Therefore, it is usual to buy a small sample of several serum lots, determine which sample is best for glial cell growth, then purchase enough of that lot of serum to complete the experiments. It is critically important to inactivate the complement in the serum prior to using it in culture to avoid complement mediated lysis of the cultured cells. The established protocols for heat inactivation of complement should be followed carefully [3] In particular the inactivation should be carried out at a temperature not greater than 56°C and not longer than 35 min. It is also important to sterile filter the inactivated serum to remove precipitated material and to freeze it in small aliquots to avoid repeated freeze thaw cycles.

Oligodendrocyte Cell Lines

Oligodendroglial Cell Lines and Myelination

The degree to which a cell line is able to mature in vitro does not predict its capacity to fulfill its normal function in vivo. For example, the capacity of an oligodendrocyte cell line to differentiate in vitro is not correlated to its potential to myelinate upon being grafted into the central nervous system. The method by which the oligodendroglial cell line is obtained largely dictates its ability to myelinate in vivo. For example the CG4 cell line spontaneously arose from a rat primary culture. This cell line in vivo stops proliferating, initiates differentiation and myelinates axons normally. In contrast to the spontaneously arising CG4 cell line, oligodendrocyte cell lines obtained by transformation fail to myelinate axons. The mouse cell line N20.1, immortalized with a SV40 large T antigen, ensheaths axons loosely but never reaches the degree of compaction found in normal myelin. Another mouse cell line Oli-neu, generated by transformation with an oncogene, does not even initiate axon ensheathment upon graft in the demyelinated spinal cord. If the primary use of a cell line is for in vitro studies, its inability to behave normally in vivo may be overlooked. The extent to which an oligodendroglial cell line differentiates may be influenced by the substratum on

which it is grown. In contrast to primary oligodendrocytes, oligodendroglial cell lines do not require a polylysine substratum for maintenance. In addition some oligodendroglial cell lines such as OLN-93 do not even need polylysine to reach the membrane-production stage. To date, no systematic study has been carried out to determine the degree to which a polylysine substratum may promote the differentiation of the existing oligodendrocyte cell lines.

CG4 Rat Oligodendrocyte Cell Line: Origin and Characteristics

The CG4 cell line (also denoted as CG-4) was generated from spontaneous immortalization of a bipotential oligodendrocyte-type 2 astrocyte (O2A or OPC) progenitor cell present in a mixed glial culture isolated from newborn rat cortex [4] The CG4 cells have a normal karyotype. CG4 cells display the properties of normal oligodendrocyte progenitor OPC cells with expression of the early marker A2B5 but not the mature markers galactocerebroside (GalC) and myelin basic protein (MBP). CG4 cells retain the capacity to differentiate into type 2-astrocytes. Replacement of 30% B104-conditioned medium in the culture medium with 20% fetal bovine serum induces the CG4 cells to differentiate into type 2-astrocytes expressing glial fibrillary acidic protein (GFAP) [4, 5].

CG4 Rat Oligodendrocyte Cell Line: Culture Conditions and Morphology

CG4 cells can be propagated for unrestricted periods of time as OPC cells in culture dishes coated with polyornithine/fibronectin. The propagation medium is serum-free, composed of 70% DMEM plus N2 supplement (modified Sato medium), and 30% conditioned medium from the B104 neuroblastoma cells maintained in the same medium. The B104-conditioned medium can be substituted by bFGF plus PDGF (50 ng/ml) as potent mitogens for CG4 cells [4].

The morphology of CG4 cells varies as a function of cell density in the B104-conditioned medium. At low density, CG4 cells are bipolar similarly to oligodendrocyte progenitors. At higher density, CG4 cells become multipolar but compared to primary oligodendrocytes the processes are shorter and less branched [4].

Further differentiation into oligodendrocytes can be obtained after withdrawal of the B104-conditioned medium or the bFGF and PDGF mitogens. After a few days in culture with the modified Sato medium, CG4 cells display a characteristic large arborization of mature oligodendrocytes with expression of GalC and MBP [4]. In the exact same culture conditions after withdrawing B104-conditioned medium for 2 days, CG4 cells expressed GalC on a large arborization of processes, and after 5 days cells
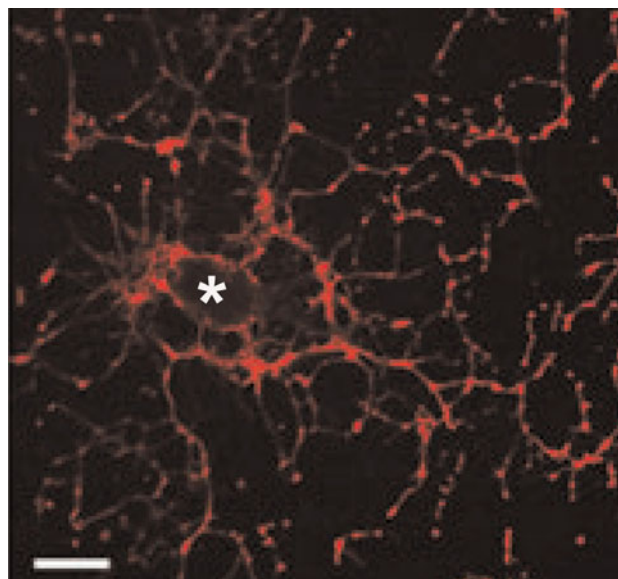

Fig. 1 Differentiated CG4 cell immunostained for Galc/O1. CG4 cells between passages 13 and 22 were cultured on glass coverslips coated with poly-L-lysine in modified SATO medium in absence of B104-conditioned medium. From the CG4 cell body (*) radiate an extensive network of processes with numerous branched extensions, scale bar 10 μm (5, with permission)

expressed the ultimate marker of differentiation, myelin oligodendrocyte glycoprotein (MOG), on numerous membrane extensions [6]. The CG4 cell line at early passages, between 13 and 22, differentiates quite well into oligodendrocytes on coverslips and in flasks coated with polylysine, in the absence of B104-conditioned medium [5]. CG4 cells extend an extensive network of processes visible by immunostaining, as illustrated in Fig. 1. However, the CG4 cell line is loses its ability to differentiate into oligodendrocytes in vitro after a certain number of passages, specifically not putting out processes, keeping a round shape and dividing all the time.

It is noteworthy that the CG4 cells can be used as a valid oligodendrocyte model for studying the cytotoxicity of the human neurotropic JC virus. The JC virus causes the fulminant disease Progressive Multifocal Leukoencephalopathy, which is posing a significant lethal risk upon treatment with strongly immunosuppressive medications recently put on the market. Transfecting CG4 cells with a single regulatory JC viral protein led to a delay of their differentiation with less complex outgrowth of processes and increased apoptosis [7].

CG4 Rat Oligodendrocyte Cell Line: Capacity to Myelinate In Vivo

CG4 cells possess the unique capacity to myelinate axons upon graft in vivo. CG4 cells of passages 31 to 48 were transplanted into the central nervous system of newborn

rats [8]. In the spinal cord of the myelin-deficient rat, the grafted CG4 cells migrated over several millimeters along the dorsal columns, where they divided and myelinated numerous axons over the course of two weeks. Similar migration of grafted CG4 cells occurred in the brain of normal newborn rats from the site of injection along axonal tracts. CG4 cells integrated the normal developing white matter along resident oligodendrocyte progenitors and generated myelin [8].

OLN-93 Rat Oligodendrocyte Cell Line: Origin and Characteristics

Similar to CG4cells, the OLN-93 cell line is derived from a spontaneously transformed oligodendrocyte progenitor cell which occurred in a mixed glial culture from neonatal rat brain [9]. When induced to differentiate, OLN-93 cells resemble primary oligodendrocytes in their antigenic properties, with absence of the early A2B5 marker, and expression of myelin-specific lipid GalC and myelin-specific proteins MBP, myelin-associated glycoprotein (MAG), and proteolipid (PLP). Expression of these mature markers, however, is restricted to the cell body and main cellular processes of OLN-93 cells. By contrast, primary oligodendrocytes express these markers on the totality of their network of processes and membrane extensions. OLN-93 cells do not express astrocytic markers such as GFAP or vimentin. OLN-93 cell extracts reveal the presence of a single MBP isoform of approximately 14 kDa, and two mRNAs specific for the proteolipids DM20 and PLP.

OLN-93 Rat Oligodendrocyte Cell Line: Culture Conditions and Morphology

OLN-93 cells can be expanded simply in non-coated culture dishes with the standard medium of DMEM-10% fetal bovine serum [9]. The morphological feature of OLN-93 cells at low cell density in these culture conditions is bipolar, similar to the OPC progenitor cells. At high cell density, OLN-93 cells form large clumps interconnected by long processes. The OLN-93 cell line can be maintained in DMEM-10% fetal bovine serum containing 4.5 g/l D-glucose, 25 mM HEPES and supplemented with 10 ng/ml PDGF-AA and bFGF [10].

When grown in low serum concentration (0.5% fetal bovine serum) or under total serum deprivation, OLN-93 cells are triggered to differentiate and grow a pronounced arborization of processes, akin to immature oligodendrocytes in primary culture. However, no membrane sheets are visible by staining for GalC or the myelin proteins MBP, PLP and MAG when grown in low serum medium [9]. When cultured in high 10% or low 0.5% fetal bovine

serum, the OLN-93 cell line was shown to express a broad range of markers at both molecular and protein levels: chondroitin sulfate proteoglycan NG2, cyclic nucleotide phosphodiesterase (CNP), MOG and MAG [11]. Whole-cell patch-clamp recordings showed that OLN-93 cells express mainly delayed-rectifying K + currents in high and low serum concentrations. Functional evidence for the voltage-gated potassium channel Kv1.3 obtained by using a selective blocker, confirmed a feature of immature oligodendrocytes. Based on these data, the OLN-93 cell line is at a developmental stage around late pre-oligodendrocyte (immature cell) regardless of serum concentration. However, it remains to be seen whether OLN-93 cells retain the delayed-rectifying K + currents in conditions triggering further differentiation. A more mature phenotype of OLN-93 cells is obtained by incubation with 10 ng/ml CNTF, 15 nM T3 and 5 μM forskolin which increases cyclic AMP level. This mix of chemicals promoted a fully mature stage characterized by multipolar arborization and an intense production of membrane sheets [10] as illustrated in Fig. 2.

When incubated in low serum (0.5%) with 1 mM cyclic AMP, OLN-93 cells grow a decent arborization of processes, not as extensive as in presence of CNTF and T3, but are enough differentiated to be refractory to nitric oxide production upon incubation with inflammatory cytokines. By contrast, OLN-96 cells grown in high serum medium
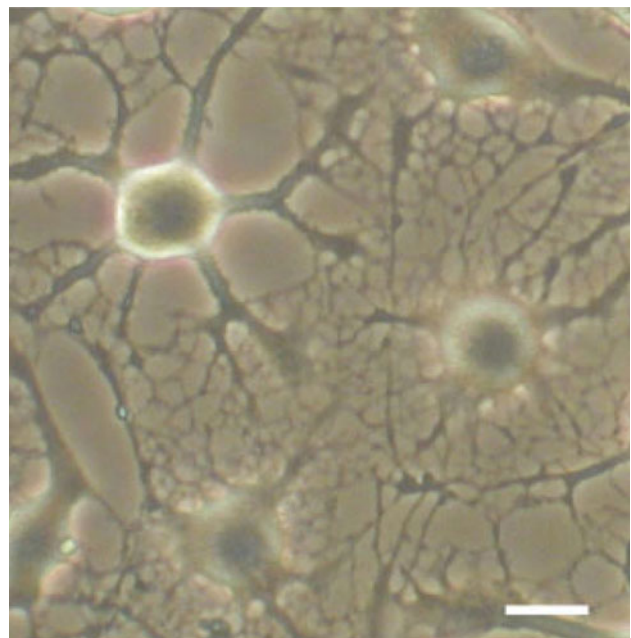

Fig. 2 Phase photograph of OLN-93 cells with a mature phenotype. OLN-93 cells cultured in low serum medium with CNTF, T3 and forskolin show a rich arborization of processes with membrane extensions visible as a lacy web between processes. Note that cell bodies are birefringent on the edges from their round shape, versus the flat thin darker membrane extensions, scale bar 10 μm (10, with permission)

showed a much less branched phenotype, expressed the inducible enzyme nitric oxide synthase, and produced nitric oxide [12]. Inducible nitric oxide synthase expression is a characteristic of immature oligodendrocytes which have little arborization [13]. These data support a functionally differentiated state of OLN-93 cells in low-serum medium in the presence of high level of cyclic AMP. It is interesting to note that this study demonstrated a differential effect of treating the OLN-93 cells with two drugs prescribed as therapy for multiple sclerosis: a glucocorticoid (inhibited nitric oxide production) and Glatiramer Acetate (no effect on nitric oxide production) [12].

OLN-93 Rat Oligodendrocyte Cell Line: Capacity to Myelinate In Vivo

OLN-93 cells have never been grafted in vivo to evaluate their capacity to myelinate.

Oli-neu Mouse Oligodendrocyte Cell Line: Origin and Characteristics

The murine cell line Oli-neu was established from primary cultures enriched in oligodendrocytes isolated from embryonic day 15 mouse embryos [14]. These cultures were infected with a retroviral vector carrying the *t-neu* oncogene coupled with the neomycin gene. The transformed cultures were maintained in Sato medium supplemented with 1% horse serum and selected on neomycin. The clone Oli-neu was derived from a transformed culture and shows a low expression of the *t-neu* oncogene at molecular and protein levels.

In basal conditions of Sato medium with 1% horse serum, the Oli-neu cell line does not express the astrocytic marker GFAP. Half the cells have a bipolar shape, the other half express O4 with a ramified arborization, while GalC is expressed on a minority of cells. Hence, the phenotype of Oli-neu in basal conditions is akin to immature oligodendrocytes, and was shown to be stable over many months [14]. When 10% fetal bovine serum was added to the culture medium, Oli-neu cells did not switch toward the astrocyte phenotype as characterized by a lack of GFAP expression [14]. We can therefore rule out a bipotential capacity of Oli-neu cells to differentiate in vitro into astrocytes. Oli-neu cells do not appear to share the characteristics of OPC cells.

Oli-neu Mouse Oligodendrocyte Cell Line: Culture Conditions and Morphology

Oli-neu cell line is expanded in Sato medium with 1% horse serum on non-coated culture dishes [14]. Further differentiation was induced by long-term incubation over 10 days
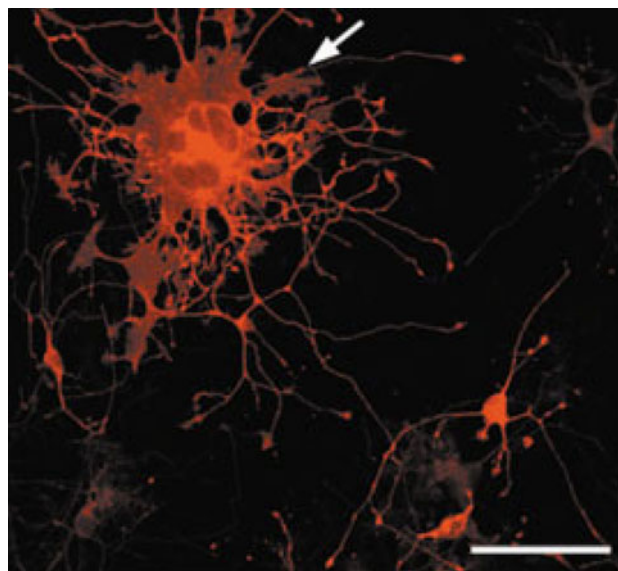

Fig. 3 Differentiated Oli-neu cells transfected with the gene for MAG and stimulated with cyclic AMP express MAG protein as stained in red. Oli-neu cell (*arrow*) shows membrane extensions around the cell body with radiating long branched processes, scale bar 97 μm (15, with permission)

with 1 mM dibutyryl cyclic AMP, which triggered a majority of Oli-neu cells to ramified a dense network of processes bearing occasional membrane sheets. Oli-neu cells expressed a myelin–specific lipid sulfatide recognized by the O4 antibody [14]. However O4 is a fairly immature marker, and only a third of cells expressed the more mature marker MAG while 10% cells expressed the mature marker GalC. The ultimate maturation stage was reached by Oli-neu cells upon transfection with the gene for MAG, on polylysine-coated culture dishes, and incubation with cyclic AMP. These conditions greatly enhanced membranes extension expressing MAG [15], as illustrated in Fig. 3.

Oli-neu Mouse Oligodendrocyte Cell Line: Capacity to Myelinate In Vivo

When Oli-neu cells were transplanted into experimentally demyelinated rat spinal cord, they multiplied in demyelinated lesions and migrated into the surrounding tissues [14]. There was more extensive differentiation of Oli-neu cells than seen in vitro in presence of cyclic AMP. Cells in close contact with demyelinated axons strikingly expressed the myelin-specific protein MAG. Despite increased MAG expression, no evidence was found of myelin ensheathment by grafted Oli-neu cells. After differentiation in serum-free medium with Sato additives and cyclic AMP, the Oli-neu cell line was shown to up-regulate expression of MAL2, an essential component for basolateral-to-apical transcytosis and indicator of oligodendrocyte differentiation [16].

Oli-neu Mouse Oligodendrocyte Cell Line: Recent Developments

Oli-neu cells have been used to screen libraries of pharmacologically active compounds inducing their differentiation. Several classes of inducers have been confirmed such as forskolin/cyclic AMP and new inducers were identified: steroids, ErbB2 inhibitors, and nucleoside analogs. These inducers of differentiation were subsequently confirmed on rat primary OPC and mixed cortical cultures [17]. Another study performed a microarray expression profiling of Oli-neu cells triggered to differentiate by inhibiting the Rho GTPase kinase ROCK with Y27632. This resulted in the identification of a novel transmembrane protein (Tmem10/Opalin), which was found enriched in primary cultures of maturing and mature oligodendrocytes. Opalin is not expressed in OPC, astrocytes, microglia or neurons [18]. Another study looked at the interaction between Oli-neu cells co-cultured with rat retinal explants with regard to neuritic growth. Oli-neu cells inhibited fiber outgrowth of retinal neurons. The inhibitory effect could not be neutralized after treatment of Oli-neu cells with the Rho-kinase inhibitor Y27632 inducing differentiation [19].

N19 and N20.1 Mouse Oligodendrocyte Cell Lines: Origin and Characteristics

N19 and N20.1 cell lines were immortalized from primary cultures enriched in oligodendrocyte prepared from neonatal mouse brain [20]. At the time around birth, a majority of cells are oligodendrocyte precursors undergoing transition into mature oligodendrocytes. Immortalization was performed by infection with a retroviral vector of simian virus 40 (SV40) large T antigen which carries a temperature-sensitive mutation. Transformed cells grow rapidly at the permissive temperature of 34°C (proliferative temperature). Under the restrictive temperature of 39°C (non-proliferative temperature), transformed cells significantly slow down and gear toward differentiation. The temperature-sensitive mutation allows in theory more control over cell differentiation.

The N19 and N20.1 cell lines express a number of glial cell markers at both molecular and protein levels. N19 and N20.1 cells both expressed the astrocyte marker vimentin, the oligodendrocyte progenitor marker A2B5, and the mature oligodendrocyte marker CNP. N19 cells have a more immature phenotype than N20.1 by expressing the GD3 ganglioside. In contrast to N19 cells, N20.1 cells do express GFAP but are seemingly more mature, because they express the myelin-specific lipid sulfatide recognized by the 007 antibody (same target as O4 antibody), and the myelin-specific lipid GalC recognized by the Ranscht antibody.

N19 and N20.1 Mouse Oligodendrocyte Cell Lines: Culture Conditions and Morphology

Culture medium for N19 and N20.1 cell lines is composed of DMEM/F12 supplemented with glucose, 15 mM HEPES, and 10% Nu-serum sold by the manufacturer BD Biosciences [20]. Cells are cultured on non-coated culture flasks for maintenance and expansion. For immunochemistry and differentiation, cells are cultured on glass coverslips and microchamber slides coated with polylysine.

N19 and N20.1 cell lines exhibit a certain number of differences with regard to their maturity stage, with N20.1 being more mature than N19 cell line. N20.1 cells express mRNAs for the myelin proteins MBP and PLP. When N20.1 cells are cultured at 39°C, the non-proliferative temperature, the proliferation rate decreases but no significant change of shape or marker expression occurs. N20.1 cells retain the expression of MBP and PLP mRNAs, but do not initiate the translation into MBP or PLP proteins [21]. When N20.1 cells are grown at 34°C or 39°C, a minority of cells expressed A2B5 with a bipolar shape, and a majority of cells expressed sulfatide (007 and O4 antibodies) and GalC with strong intensity on branched processes. When maintained in DMEM with 10% fetal bovine serum, instead of DMEM/F12 with 10% Nu-serum, N20.1 cells express MBP protein in a temperature-independent manner [22].

The conditions which favor differentiation of oligodendrocyte cell lines are specific to each cell line. Oligodendrocyte differentiation is usually defined as increased expression of the mature oligodendrocytic markers O4, GalC and MBP protein, as well as a more complex morphology with membrane production [23]. Using these criteria, transfection of N19 and N20.1 cells with the human transferrin gene triggers further differentiation. Transfection with the rat peripheral myelin protein P0 gene also triggered more differentiation in N20.1 cells, characterized by increased ratio of MBP/GFAP and PLP/GFAP expression [24]. However when N20.1 cells are grown under conditions which should favor differentiation (forskolin to raise cyclic AMP levels and 39°C which is non-permissive for proliferation), the N20.1 cells assumed an astroglial phenotype with a pronounced increase in GFAP expression [25]. This is in sharp contrast to other oligodendrocyte cell lines such as OLN-93 and Oli-neu, which change to a more differentiated phenotype when cyclic AMP levels are increased. Other differences have been reported between N20.1 cells and primary oligodendrocytes. For example, the sensitivity of either cell type to nitric oxide donors is much different with regard to concentration and mechanism [26, 27].

N19 and N20.1 Mouse Oligodendrocyte Cell Lines: Capacity to Myelinate In Vitro

In co-cultures of N20.1 cells with dorsal root ganglion neurons at the "differentiation" temperature of 39°C on collagen-coated Petri dishes, the N20.1 cells retained MBP and increased expression of GalC [22]. After 2 weeks co-culture at either temperatures of 34°C and 39°C, N20.1 cells markedly changed their morphology from rounded cells with few branching processes to highly reticulated cells in contact with neurites. After 8 weeks co-culture, N20.1 cells had wrapped membranous structures around the neurites of dorsal root ganglion neurons with areas of compaction similar to myelin, as visualized by electron microscopy [22]. Co-culture of N19 and N20.1 cells with cortical neurons in DMEM/F12 medium plus 1% fetal bovine serum at the "differentiation" temperature of 39°C for one week induced dramatic changes in morphology, especially in cells overexpressing transferrin [23], similarly to co-culture with dorsal root ganglion neurons. Changes included close contact with neurites and elaboration of membranous sheets, as illustrated in Fig. 4.

N20.1 Mouse Oligodendrocyte Cell Line: Capacity to Myelinate In Vivo

Upon transplantation into the neonatal brain of congenitally demyelinated shiverer mutant mice, the cell line with the most mature in vitro phenotype, N20.1, elaborated membranous processes around axons reminiscent of myelin ensheathment [28] Hence the mouse oligodendrocyte cell line N20.1 has a potential to myelinate in vivo.
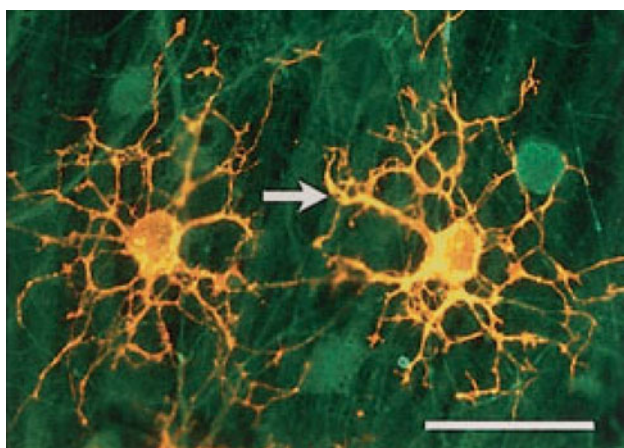

Fig. 4 N20.1 cells transfected with the human transferrin gene and stained for MBP in yellow are co-cultured with cortical neurons stained in green. N20.1 cells show an extensive arborization of branched processes and the *arrow* indicates that MBP has accumulated at the point of contact between N20.1 cell and the neurite, scale bar 12 micrometers (23, with permission)

G26-20 and G26-24: Mouse Oligodendrocyte Cell Lines: Origin and Characteristics

G26-20 and G26-24 cell lines are derived from a transplantable glioma named G26 induced by methylcholanthrene treatment of a mouse [29]. Glioma G26 has been classified as an immature glial cell with oligodendroglial and some astrocytic features. G26-20 and G26-24 cell lines have been shown to be oligodendroglioma with synthesis of large amounts of the oligodendrocyte markers sulfatide and galactosylceramide [30], and expression of proteolipid DM20 mRNA [31]. The G26-24 cells are currently used for cancer research, notably their membrane shed vesicles inducing neuron apoptosis [32].

G26-20 and G26-24: Mouse Oligodendrocyte Cell Lines: Culture Conditions

G26-20 cells are cultured in Ham's F12 medium with 10% fetal bovine serum [33]. G26-24 cells are cultured in DMEM with 10% fetal bovine serum [34]. Cells are maintained on non-coated culture dishes.

G26-20 and G26-24: Mouse Oligodendrocyte Cell Lines: Capacity to Myelinate In Vivo

G26 cells have never been grafted in vivo to evaluate their capacity to myelinate.

HOG: Human Oligodendrocyte Cell Line: Origin and Characteristics

HOG is a cell line derived from a surgically removed human oligodendroglioma [35]. HOG cells do not express the astrocyte marker GFAP, but express several oligodendrocyte markers such as the progenitor marker A2B5, low levels of the myelin-specific lipids sulfatide and GalC, and high level of the maturing marker CNP.

HOG: Human Oligodendrocyte Cell Line: Culture Conditions and Morphology

Cells do not need polylysine coating to grow or differentiate. HOG cells are maintained on non-coated culture dishes, chamber slides and glass coverslips in DMEM medium with 10% fetal bovine serum (high serum). When maintained in high-serum/DMEM, HOG cells have very few if any branches, and express the mRNA for CNP but do not express the mRNA for the mature markers MBP and MOG [36]. When HOG cells are maintained in low-serum DMEM medium supplemented with 0.5 μg/mL insulin, 50 μg/mL transferrin, 30nM Selenium, 30nM T3 and 0.05% fetal bovine serum, microarray analysis revealed
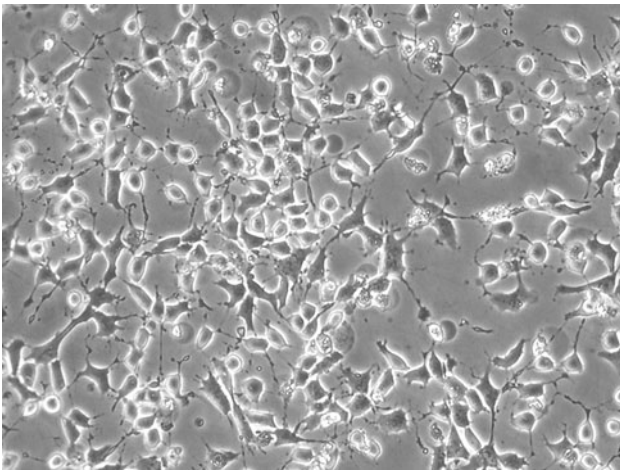
Fig. 5 Phase photograph of HOG cells after 24 h in low-serum N2 medium. Cells slow down division and start to branch short ramified processes. 50.4 × magnification (A.I.B. personal communication)

mRNA expression of CNP in addition to mRNA expression for all myelin proteins: MBP, MOG, MAG, myelin oligodendrocyte basic protein (MOBP), and oligodendrocyte myelin glycoprotein (OMgp) [36].

In a similar medium composed of high glucose (4.5 g/l) DMEM with N2 supplement, 30 nM T3 and 0.05% fetal bovine serum (low-serum N2 medium), HOG cells express at both mRNA and protein levels CNP, MAG, MBP and MOG, and the transcription factors Olig1, Olig2, Nkx2-2, SOX10 and PPAR delta, maturation markers which all specific to the oligodendrocyte lineage. Morphologically, HOG cells differentiate by extending branched processes and short membrane extensions (A.I.B. unpublished data). The phase contrast appearance of differentiated HOG cells is shown in Fig. 5.

Additional features of HOG cells are identical to primary cultures. For example, the receptor for advanced glycation end products RAGE involved in oxidative stress response, has been found at the mRNA and protein levels in HOG cells as well as primary rat oligodendrocytes. Addition of low hydrogen peroxide concentrations initiated a shedding of RAGE from membranes, followed by restoration of RAGE expression to normal levels in HOG and primary cells alike [37].

HOG: Human Oligodendrocyte Cell Line: Capacity to Myelinate In Vivo

Upon myelination, large amounts of myelin proteins and lipids need to be correctly targeted to generate a complex system of membranes. The intracellular trafficking is therefore highly segregated in maturing oligodendrocytes,

and parallels aspects of polarized epithelial cells. After differentiation in serum-free medium with Sato additives and cyclic AMP, the HOG cell line was shown to upregulate expression of MAL2, an essential component for basolateral-to-apical transcytosis. MAL2 was present at the enlarged tips of ramified processes, suggesting MAL2 might have an important role in the sorting of proteins and lipids for myelin assembly during differentiation [16]. However, HOG cells have never been grafted in vivo to evaluate their capacity to myelinate. In addition, they have not been co-cultured in vitro with myelin-competent neurites to observe the degree to which they may extending membrane sheets and attempt to myelinate neurites.

MO3.13: Human Oligodendrocyte Cell Line: Origin and Characteristics

The MO3.13 cell line was immortalized by fusing a human tumor rhabdomyosarcoma RD cell line with a human primary culture oligodendrocyte obtained from adult surgical specimens [38]. MO3.13 is a human–human hybrid cell line selected for its expression of the glial markers MBP and GFAP. MO3.13 was also found to express low level of GalC. In contrast to its parent tumor cell line rhabdomyosarcoma RD, the hybrid clone M03.13 expresses GalC, MBP and GFAP [38].

MO3.13: Human Oligodendrocyte Cell Line: Culture Conditions and Morphology

MO3.13 cell line is cultured in DMEM with 10% fetal bovine serum on non-coated culture dishes [38, 39].

MO3.13 cells can be induced to differentiate in DMEM without fetal bovine serum after 70% confluence was reached, in presence of phorbol ester PMA that stops proliferation. Chronic treatment with PMA (but not treatment with cyclic AMP) induces a coordinated up-regulation of oligodendrocyte markers MBP and PLP messengers and down-regulates GFAP expression [38]. In differentiation medium, MO3.13 cells decrease proliferation and change morphology with the cell body becoming bipolar or triangular and growth of several elongated processes similar to filopodia. However, no formation of membrane structures was ever observed for MO3.13 cells in these culture conditions. At the molecular level, CNP expression was not affected, whereas MBP and MOG messengers were enhanced in medium promoting differentiation for MO3.13 cells [36].

LINGO-1 is a potent negative regulator of oligodendrocyte differentiation and hence may play a pivotal role preventing remyelination in diseases such as multiple

sclerosis. In the same culture condition with PMA, MO3.13 cells transfected with LINGO-1 were inhibited from process extension. LINGO-1 expression was also found to inhibit MBP transcription in the rat CG4 cell line. As in neurons, LINGO-1 physically associated with the endogenous p75 neurotrophin receptor in MO3.13 cells [40].

Another degenerative neurological condition, Krabbe disease is an inherited disorder characterized by accumulation of psychosine which leads to loss of oligodendrocytes, induction of gliosis and inflammation. For the first time a study reported the regulation of AMP-activated protein kinase by psychosine in oligodendrocytes using MO3.13 cells. Psychosine treatment significantly downregulated the AMP-activated protein kinase activity, resulting in increased biosynthesis of lipids including cholesterol and free fatty acid in MO3.13 cells [41].

MO3.13: Human Oligodendrocyte Cell Line: Capacity to myelinate in vivo

MO3.13 cells have never been grafted in vivo to evaluate their capacity to myelinate.

Schwann Cell Lines

Caveats Concerning Schwann Cell Lines

Primary Schwann cells isolated from neonatal rat sciatic nerves are very easy to expand in culture, and can be maintained and expanded for over 6 months without losing their properties. They can be frozen, thawed, and split with no apparent problems. Schwann cells in vitro require no specialized substratum and grow well on uncoated tissue culture grade plastic. Therefore there has never been a compelling need to create rodent Schwann cell lines.

However in contrast to rodent Schwann cells, human Schwann cells do not divide easily in culture. The Wood laboratory has developed and refined methods to reliably obtain and expand human Schwann cells [42]. The freshness and sterility of human tissue are two of the most pressing issues. To overcome these problems, the laboratory of Wood set up a program to obtain sterile peripheral nerves from organ donors and graciously provided primary human Schwann cells to a number of investigators for their own studies.

Given the difficulties of obtaining human Schwann cells, investigators have turned to Schwann cells derived from genetically-modified mice to gain new insights into human diseases such as neurofibromatosis. Neurofibromatosis Type 1 (NF-1) and Neurofibromatosis Type 2 (NF-2) are two diseases in which Schwann cells abnormally divide and form tumors. In NF-1, the absence of RAS-1

inactivating GTPase activating protein, termed neurofibromin, leads to hyperproliferation and tumor formation [43]. In NF-2, the loss of a protein termed merlin leads to Schwann cell tumor formation specifically on acoustic nerves. However, Schwann cells from transgenic mice do not always create the best model system in vitro to understand how Schwann cells are affected in a particular disease. For example, Schwann cells derived from the mouse model of NF-1, knock-out for neurofibromin, fail to proliferate in vitro [44], although Schwann cell proliferation is a hallmark of NF-1. Recent refinements in methodology [45] have allowed the preparation of neurofibromin deficient Schwann cells from single embryos at earlier stages of development. These Schwann cells appear to more faithfully replicate the properties of human NF-1 Schwann cells in that they grow rapidly without axonal contact and retain expression of Schwann cell markers. Given the difficulties of preparing either normal human Schwann cells or Schwann cells from transgenic mice which faithfully mimic human disease, there is a continuing need to create a human Schwann cell line which can be used to understand normal human Schwann cell biology.

Unfortunately, most of the reported human Schwann cell lines are derived from tumors. Therefore by virtue of their origin, they cannot be judged as "normal." For example, a large number of human Schwann cell lines are derived from malignant or benign tumors from NF-1 suffering patients such as the ST88-14; NF1T and T265 cell lines. In contrast to normal human Schwann cells, these cells divide intrinsically in culture and most Schwann cell lines lack neurofibromin, leading to signal transduction abnormalities. Restoration of the native domain of neurofibromin moved the cell lines toward normalcy (decreased proliferation and restored a more "spindle-shaped" morphology); however, the angiogenic potential of these cell lines is still greatly increased relative to normal human Schwann cells [46].

Neuroblastoma human cell lines are derived from malignant cells in the human adrenal which are neuroectodermal in origin. Most neuroblastoma cell lines can be induced to a neuronal phenotype such as neurite extension and catecholamine secretion [47]. The human Schwann cell line GOTO is derived from a parental neuroblastoma cell line by bromodeoxynucleotide treatment [47]. Although the GOTO cell line expresses some myelin specific proteins such as MBP and MAG to a limited extent [48], their tumor origin does not make them suitable candidates for the study of normal human Schwann cell biology.

In order to assess the extent to which the tumors derived Schwann cell lines are abnormal, a number of studies have analyzed gene expression in primary cultures of normal human Schwann cells compared with cell lines. Gene

expression analysis compared with the NF1-derived cell lines has revealed significant differences in basal gene expression between the two cell types. Over 900 genes were identified which were expressed differentially in the T265 cell line [49]. Miller and coll. [50] carried out a large-scale molecular comparison of normal human Schwann cells to human Schwann cell lines and found a 159-genome molecular signature which distinguished the cell lines from the normal cells. They also reported that Schwann cell differentiation markers were down-regulated in the cell lines whereas neural crest stem cell markers were up-regulated. A comparison of gene expression between primary normal human Schwann cells and Schwann cells from NF-2 patients revealed 41 genes whose expression levels differed by a factor greater than two-fold [51].

These studies underscore the unsuitability of the human Schwann cell lines to serve as surrogates for normal human Schwann cells. However, in some aspects, the human Schwann cell lines may still be useful if the property under investigation is evident in both normal human Schwann cells and Schwann cell lines. For example, Im and coll. [52] demonstrated that the molecule CD1d was expressed in both normal human Schwann cells and Schwann cell lines. This strategy may be applied to other Schwann cell molecular properties as well.

The best parameter by which to judge the appropriateness of a given cell line to faithfully replicate normal Schwann cell biology is the myelination response which occurs when the cell line is exposed to a cultured, myelin-competent naked neurite. Bunge and coworkers first reported that when normal Schwann cells in vitro are presented with a naked neurite in primary cultures of rat dorsal root ganglion, a rapid proliferation response occurred followed by Schwann cell ensheathment of neurites and subsequent myelination [53]. The ability of the existing human Schwann cell lines to myelinate in culture has not been applied since it appears unlikely that an intrinsically dividing cell line could stop dividing and ensheath a neurite in culture. However, when a rat Schwann cell line created by transfecting normal rat Schwann cells with a plasmid containing the SV40 large T antigen expressed under the control of a mouse metallothionen promoter, encountered either a neurite or basal lamina the cells ceased dividing, and extended processes to wrap around bundles of neurites initiating the first steps of myelination [54]. Although the creation of similar human Schwann cell lines has been postulated [55], these cell lines still do not exist.

Although the myelination potential of human Schwann cell lines has not been proven, a careful comparative analysis of human Schwann cell lines as model systems for myelin gene transcription studies has been reported [56]. In this study, the steady-state expression of Schwann cell markers (PMP22, P0, MBP, low-affinity NGF-R) were evaluated in six human Schwann cell lines at both the protein and mRNA level. The surprising conclusion of the study was that relative to primary rat Schwann cells undergoing myelination, only 2 cell lines expressed high levels of mRNA coding for myelin proteins and none of the cell lines expressed all of the myelin proteins typically expressed in myelinating Schwann cells. The authors concluded that the co-culture of Schwann cells with dorsal root ganglion may represent a more accurate model for future analysis of myelin gene promoters.

In conclusion, although a large number of Schwann cell lines are readily available, the relevance of any parameter to be studied relative to normal human Schwann cells should be carefully taken into consideration. If the given molecule under investigation can be shown to exist in both normal Schwann cells and a given cell line, some insights concerning its metabolism and function may be gained. The caveat in all cell line studies is that the factor(s) that made the cells immortal most likely alters the state of "normalcy" in the cell line.

ST88-14 Human Schwann Cell Line: Origin and Characteristics

The ST88-14 cell line was established from a peripheral nerve sheath tumor in the laboratory of Dr. Cynthia Morton by Dr. Jonathan Fletcher in the Department of Pathology at Brigham and Women's Hospital, Boston, MA [57]. This cell line was derived from a solid tumor in a patient diagnosed with NF1 [58, 59].

ST88-14 Human Schwann Cell Line: Culture conditions

The ST88-14 cell line is cultured in low-glucose DMEM supplemented with 10% fetal bovine serum.

STS-26T Human Schwann Cell Line: Origin and Characteristics

The STS-26T cell line was established in the laboratory of Dr. John Little by William Dalhberg at the Harvard School of Public Health, Boston, MA [60]. The line was derived from an isolated, grade III malignant schwannoma in an individual without Neurofibromatosis Type 1. This cell line was initially determined to be of Schwann cell origin based on electron microscopic studies. It has also been shown to contain clonal chromosomal aberrations and to form tumors in nude mice following transplantation (William Dahlberg, personal communication). The striking feature of this cell line is the presence of neurofibromin, in contrast to most other schwannoma cell lines derived from NF-1 patients which lack this protein [61].

STS-26T Human Schwann Cell Line: Culture
Conditions

The STS-26T cell line is cultured in low-glucose DMEM
supplemented with 10% fetal bovine serum.

NF1T Human Schwann Cell Line: Origin
and Characteristics

The NF1T cell line was derived from a malignant soft
tissue sarcoma in a patient diagnosed with NF1. This cell
line has been reported to be highly radio-resistant to
treatment with ionizing radiation during exponential
growth [62]. This increased level of radio-resistance may
reflect the ability of these cells to adequately repair
potentially lethal x-ray damage and thus be more incurable
in vivo with radiation therapy. Like the STS-26T cell line,
the NF1T cells contain neurofibromin [61].

NF1T Human Schwann Cell Line: Culture Conditions

The NF1T line is cultured in low-glucose DMEM supple-
mented with 10% fetal bovine serum.

T265 Human Schwann Cell Line: Origin
and Characteristics

The T265 cell line was established in the laboratory of
Dr. George H. DeVries by Karen Klein at the Medical
College of Virginia, Richmond, VA (now the Health Sci-
ences Division of Virginia Commonwealth University).
The tissue source for this cell line was a malignant
schwannoma derived from a patient with NF1 [49].

T265 Human Schwann Cell Line: Culture conditions

The T265 cell line is cultured in low-glucose DMEM
supplemented with 10% fetal bovine serum.

GOTO Human Schwann Cell Line: Origin
and Characteristics

Neuroblastoma cell lines are derived from human adrenal
tumors and have been used extensively to examine neu-
ronal properties in culture since they can be easily differ-
entiated into cells with a neuronal phenotype. However,
Reynolds and Maples [63] discovered that treatment of
neuroblastoma cells promotes differentiation into glial or
Schwann cells. Following this observation, Tsunamoto and
colleagues [47] treated the GOTO cell line with bromo-
deoxyuridine. The GOTO cell line is derived from a neu-
roblastoma cell line originally established after 5 years
culture of adrenal neuroblastoma from one patient [64]. As

a result of bromodeoxyuridine treatment, the GOTO cells
become more Schwann-like in that cell growth was sup-
pressed, expression of the glial cell marker S-100 protein
was greatly increased, and morphology of the neuroblas-
toma cells changed to become smaller and more spindle-
shaped. The GOTO-derived cell line also expressed
myelin-specific proteins to a limited extent [48]. Recently,
it has been reported that when GOTO cells differentiate
into Schwann-like cells, they express CD44 and localize
this molecule to lipid rafts which is characteristic of
Schwann cells in culture [65].

GOTO Human Schwann Cell Line: Culture Conditions

The GOTO cell line is cultured in RPMI 1640 medium
supplemented with 10% fetal bovine serum. Cells are ini-
tially plated at a density of $5x10^5$ cells/25 cm^2 flask.

33B Rat Schwann Cell Line: Origin and Characteristics

The 33B cell line is derived from a tumor induced by
N-ethyl-N-nitrosourea injection into newborn rats [66].
Tumors arose in most animals 6-13 months after treatment.
A tumor named TR33B involving both lumbar spinal cord
and roots was transplanted several times, and the 33B cell
line was derived at the 17th transplantation passage. In
culture, the 33B cells extend many processes and resemble
another Schwann cell line. The 33B cell line is intrinsically
abnormal similarly to non-induced human tumor cell lines.

The 33B cell line is positive for the rat neural antigen-1
(Ran-1) and also reacts with a marker for normal Schwann
cells: the monoclonal antibody 217c [67]. It was later
found that the 217c antibody recognizes the Ran-1 antigen,
which is probably the NGF-R although this has not been
formally proven [68]. Despite the 33B cell line is listed as
oligodendroglioma by the European Collection of Cell
Cultures (ECACC), it was induced from spinal cord roots
and a large body of evidence indicates it is actually a
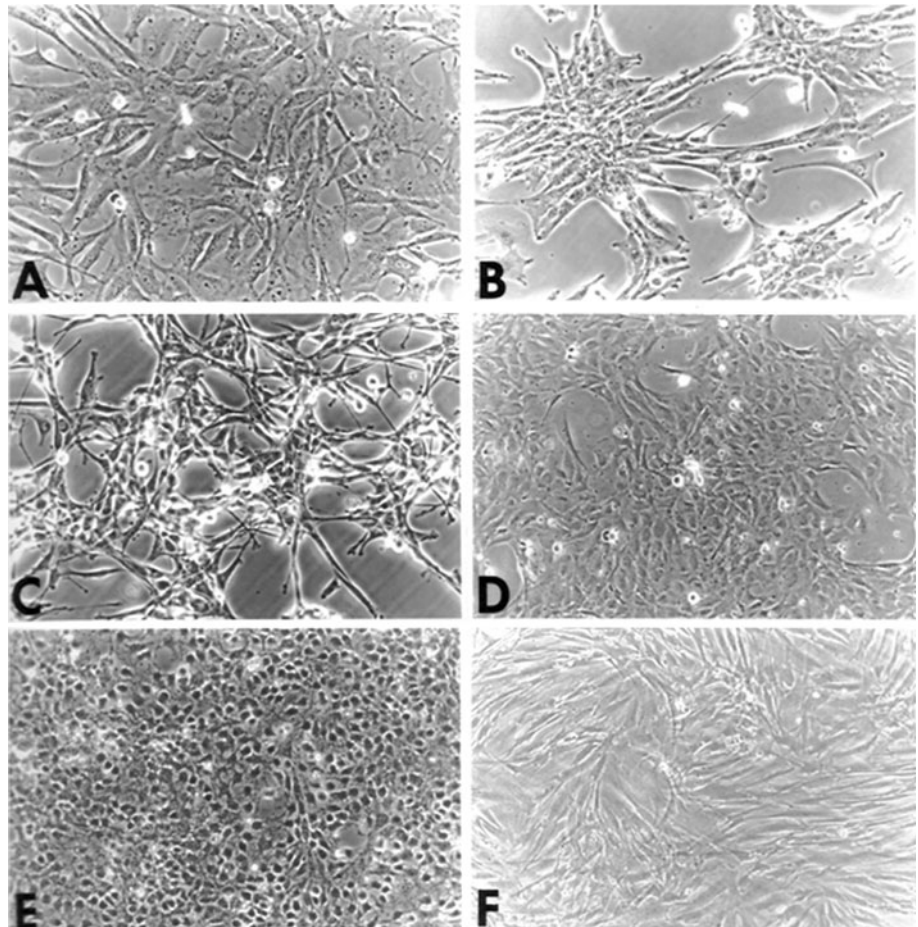schwannoma.

33B Rat Schwann Cell Line: Culture Conditions

The 33B cell line is cultured in DMEM with 10% fetal
bovine serum.

RT4-D6P2T Rat Schwann Cell Line: Origin
and Characteristics

The RT4-D6P2T is a schwannoma cell line derived
through several steps from a neurotumor of the sciatic
nerve, named RT4, isolated 6 months after subcutaneous
injection of N-ethyl-N-nitrosourea in a newborn rat [69].
The RT4-D6cell line was derived from the RT4

Fig. 6 Phase photographs of Schwann cell lines. **a** ST-88, **b** STS-26T, **c** NF1T, **d** T265, **e** TSC (SV40 Transformed rat Schwann cells), **f** Normal human fibroblasts. 50 × magnification (G.H.D.V. personal communication)

neurotumor using a technique to propagate a single stem cell. The RT4-D6 cell line was selected based on S-100 expression and a differentiated morphology of radial or bipolar processes depending presence of serum and cell density [69].

The subclone RT4-D6P2T, subsequently derived from the RT4-D6 cell line, has been shown to express GalC, sulfatide, and CNP [70], as well as the major myelin proteins of the peripheral nervous system P0 and P22 [56].

RT4-D6P2T Rat Schwann Cell Line: Culture Conditions

Culture medium for the RT4-D6P2T cell line is DMEM with 4.5 g/l glucose, 4 mM glutamine and 10% fetal bovine serum.

TSC Rat Schwann Cell Line: Origin and Characteristics

Secondary Schwann cells obtained from neonatal rat sciatic nerve were transfected with plasmids containing the SV40 large T antigen gene under the control of a metallothionein promoter, plus a gene carrying resistance to the aminoglycoside G418 [54]. Transfected Schwann cells were selected in the presence of G418 to derive a transformed Schwann cell line termed "TSC". The transfected cells had an antigenic profile very similar to the untransfected secondary Schwann cells. Morphologically, the transformed cells were much more pleotrophic and flattened (see Fig. 6 panel E) relative to the secondary Schwann cells which were spindle shaped and grew in fascicles. When the transformed cells are co-cultured with dorsal root ganglion neurites, the cells become more fusiform in appearance and send out processes that ensheath groups of neurites both close to the neuronal cell bodies and at some distance from it. The transformed cells were able to initiate some initial steps of the myelination process in that they segregated bundles of neurites. Although the TSC cells began ensheathing the neurites, no uncompacted or compacted myelin was evident.

TSC Rat Schwann Cell Line: Culture Conditions

The SV40 large T antigen transformed rat Schwann cell line TSC grows well in DMEM medium containing 10% fetal bovine serum.

Spontaneously Immortalized Schwann Cell Lines

Long term culture of Schwann cells sometime leads to spontaneous transformation of the mitotically quiescent primary cells to become an active proliferating cell line. Bolin and colleagues [71] derived a spontaneously immortal rat Schwann cell line by chronic culture of adult rat sciatic nerves. Subsequently, Watabe and colleagues [72] derived a spontaneously immortal mouse Schwann cell line (IMS32) via long term culture of adult mouse dorsal root ganglia or adult mouse sciatic nerves. The immortalized Schwann cell lines have proven useful in the study of the human sphingolipidoses, since mouse models for these diseases can be used to spontaneously derive mouse Schwann cell lines which can be useful in the understanding of the molecular basis for these diseases. For example, spontaneous mouse Schwann cell lines have been derived from appropriate mouse models for Niemann-Pick disease type c [73], globoid cell leukodystrophy [74], Sandhoff's disease [75] and metachromatic leukodystrophy [76]. A detailed description of the methods used to establish immortalized Schwann cells from murine disease models has been published [77].

Although Schwann cell lines which spontaneously arise from repeated passaging of primary cells are useful for some studies they fail the "myelin competent" test. The laboratory of Quarles [78] carried out a detailed study of three immortalized Schwann cell lines which expressed different levels of the myelin protein MAG. They reported that although all the cell lines expressed myelin-specific proteins at varying levels, none of them was able to myelinate dorsal root ganglion neurites in culture. They concluded that these cell lines may represent an immortalized stage of Schwann cell development prior to myelination, and that these cell lines could be useful for investigating the cell biology of MAG and other myelin-related components. The caveat with these cell lines is that by virtue of the fact that they have been transformed they have become less normal, even if they do represent an arrested stage of the development of Schwann cells. Therefore as much as possible it is important to confirm any findings with in vivo studies.

IMS32 Mouse Schwann Cell Line: Origin

The IMS32 is a mouse Schwann cell line established spontaneously from long-term cultures of dorsal root ganglia and peripheral nerves isolated from adult mice [72]. IMS32 cells express S-100, and proliferate under stimuli by platelet-derived growth factor (PDGF)-BB, acidic and basic fibroblast growth factors (aFGF, bFGF), and transforming growth factors (TGF)-beta 1 and -beta 2. The IMS32 cells themselves expressed TGF-beta 1, -beta 2, and -beta 3 mRNA transcripts. Co-culture of IMS32 cells also showed neurite growth of the neural cell line PC12. RNA transcripts of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), ciliary neurotrophic factor (CNTF), and glial cell line-derived neurotrophic factor (GDNF) were detected from IMS32 cells, which appear to have a certain degree of differentiation.

IMS32 Mouse Schwann Cell Line: Culture Conditions

The IMS32 cell line is cultured in DMEM with 10% fetal bovine serum [79].

Morphology of Human and Rat Schwann Cell Lines

Primary Schwann cells in culture have a typical bipolar spindle shaped appearance. This morphology is ideal for aligning with axonal extensions prior to myelination. The majority of the human cell lines have been derived from Schwann cell tumors which arise in Neurofibromatosis Type 1 disease. Compared to primary Schwann cells the morphology of human and rat Schwann cell lines are dramatically altered as shown in Fig. 6. It is evident that all the human Schwann cell lines and the rat Schwann cell lines are very pleotrophic and flattened more resembling fibroblasts (Panel F) than the typical bipolar primary Schwann cells. In spite of this appearance the Schwann cell lines have an antigenic profile consistent with a Schwann cell origin including reactivity for myelin proteins [54].

Astrocyte Cell Lines

C6 Rat Astrocyte Cell Line: Origin and Characteristics

The C6 cell line was derived from a tumor induced by repeated injections of N-nitrosomethylurea in an adult outbred Wistar rat over a period of 8 months [80]. When animals showed clinical signs, the tumors were removed and passaged several times by tissue culture. The C6 cell line was cloned from glioma "number 6", and shown to produce S-100 and GFAP proteins with a ten-fold increase of S-100 production upon reaching confluence [81]. Historically, the C6 cell line has been classified as astrocytoma based on histology. A microarray study to characterize similarities and differences with normal rat astrocytes confirmed that C6 cells overexpress numerous genes reported in human brain tumors, coding for growth factors and signaling pathways, including PDGFβ, IGF-1, EGFR, and TGFα, ErbB3/HER3 precursor proteins [82].

C6 Rat Astrocyte Cell Line: Culture Conditions

The original culture condition for C6 cells were Ham's F12 K medium with 2 mM glutamine adjusted to contain 1.5 g/l sodium bicarbonate, 15% horse serum and 2.5% fetal bovine serum. C6 cells can also grow well in the standard DMEM medium supplemented with 10% fetal bovine serum [83].

DI TNC1 Rat Astrocyte Cell Line: Origin
and Characteristics

The DI TNC1 cell line was established from primary cultures of type 1 astrocytes isolated from diencephalon of neonatal rat brains. The cultures were transfected with the SV40 large T antigen under the transcriptional control of the human GFAP promoter [84]. The cells retain characteristics consistent with the phenotype of type 1 astrocytes including GFAP immunoreactivity. The DI TNC1 cell line does not express any of the oligodendrocyte markers A2B5, O4 or GalC.

DI TNC1 Rat Astrocyte Cell Line: Culture Conditions

Growth medium for the DI TNC1 cell line is DMEM with 4 mM glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, and 10% fetal bovine serum.

BALB SFME: Mouse Astrocyte Cell Line: Origin
and Characteristics

BALB SFME is a neural stem cell line derived from embryonic day 16 embryos of BALB/c mice grown in serum-free medium [85]. Either serum or TGF-beta induces differentiation in astrocyte accompanied by GFAP expression. Furthermore, the presence of serum causes cell growth arrest which is reversible upon removal of the serum. When cultured in serum-free medium, BALB SFME cells can be propagated for extended periods without undergoing gross chromosomal aberration.

BALB SFME: Mouse Astrocyte Cell Line: Culture
Conditions

Growth of the BALB SFME cell line is density and temperature sensitive. Cell confluence above 80% will cause cells to form clumps and detach. Culture medium is DMEM/F12 with 2.5 mM glutamine, 1.2 g/l sodium bicarbonate, 15 mM HEPES and 0.5 mM sodium pyruvate supplemented with 10 μg/ml bovine insulin, 10 μg/ml human transferring, 1% chemically defined lipids (manufacturer LifeTechnologies), 50 ng/ml mouse epidermal

growth factor (EGF), and 10 nM sodium selenite. Omission of EGF causes the cells to undergo apoptosis.

A172 and U-87MG: Human Astrocyte Cell Lines:
Origin and Characteristics

The A172 cell line (also termed A-172) is an astrocytoma cell line derived from a human glioblastoma [86]. A172 cells express high level of GFAP, and induce the nitric oxide synthase type 2 enzyme upon incubation with inflammatory cytokines, which are both characteristic of astrocytes [87].

Similarly, U-87MG (also spelled U87-MG) is a cell line derived from a human tumor, a malignant astrocytoma grade III [88]. Grades III and IV are the most aggressive of malignant astrocytoma and indeed the cell line U-87MG is mostly used for cancer research. However, U-87MG cells do express GFAP protein, albeit at a very low level in basal culture conditions. GFAP expression can be enhanced several fold with an antisense construct for the proto-oncogene EGFR [89].

It is noteworthy that both A172 and U-87MG cell lines express high levels of peroxisome proliferator-activated receptor gamma (PPAR gamma) involved in control of cell proliferation and apoptosis [90]. Upon treatment with PPARgamma agonists of the thiazolidinedione class, the human cell lines U-87MG and A172, and the rat astrocytic line C6 undergo apoptosis, in contrast to primary murine astrocytes which are unaffected [83]. This suggests profound differences in energy metabolism at the mitochondria level between glioma cell lines and primary astrocytes [91].

A172 and U-87MG: Human Astrocyte Cell Lines:
Culture conditions

Growth medium for the A172 cell line is DMEM with 4 mM glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, and 10% fetal bovine serum. Growth medium for the U-87MG cell line is DMEM with 2 mM glutamine, 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 10% fetal bovine serum.

Microglia Cell Lines

HAPI Rat Microglia Cell Line: Origin
and Characteristics

HAPI is a microglia cell line spontaneously immortalized from a primary brain culture of neonatal rats [92]. HAPI was named after a "Highly Aggressively Proliferating Immortalized" clone appeared within one day in a shake-off culture enriched for microglia. HAPI cells are typically

round with few or no processes when cultured in 10% fetal bovine serum-DMEM. As the percentage of serum decreases, cells have more processes and exhibit phagocytosis in 5% fetal bovine serum-DMEM. HAPI cells express the microglia markers isolectin B4, OX-42 (CD11b) and GLUT5. HAPI cells do not express the oligodendrocyte progenitor marker A2B5 nor the astrocyte marker GFAP. HAPI cells exhibit cytokines and nitric oxide production upon lipopolysaccharide exposure.

HAPI Rat Microglia Cell Line: Culture Conditions

HAPI cells are maintained in 10% fetal bovine serum-DMEM [92] or in DMEM (low glucose), 5% fetal bovine serum and 4 mM glutamine [93].

BV-2 Mouse Microglia Cell Line: Origin and Characteristics

The BV-2 (also denoted as BV2) cell line was immortalized by infecting a primary microglia culture prepared from neonatal mouse brains with a *v-raf/v-myc* oncogene carrying retrovirus J2 [94]. BV-2 cells expressed Mac1 (CD11b) and Mac2 but were negative for the oligodendrocyte marker GalC and the astrocyte marker GFAP. BV-2 cells exhibited phagocytosis activity, and were able to secrete lysozyme and cytokines upon stimulation. In comparison with rat primary microglia cells, BV-2 and the rat HAPI microglia cell lines were shown to respond similarly with respect to migration, ionized calcium binding adaptor molecule 1 expression, and nitric oxide release. However, BV-2 and HAPI cells diverged from primary microglia following lipopolysaccharide treatment for expression of TNF-alpha, interleukin-1beta, interleukin-6 and monocyte chemoattractant protein-1. These results indicate that BV-2 and HAPI cells only partially model primary microglia [93].

BV-2 Mouse Microglia Cell Line: Culture Conditions

BV-2 cells are maintained in 10% fetal bovine serum-RPMI 1640 with 4 mM glutamine [94] or in DMEM supplemented with 10% fetal bovine serum, 1.1% Glutamax® (supplier Gibco-Invitrogen), at a density not exceeding 5×10^5 cells/ml [93].

EOC 2 and EOC 20 Mouse Microglia Cell Lines: Origin and Characteristics

EOC 2 and EOC 20 are two microglia cell lines spontaneously immortalized from primary brain cultures of

apparently normal 10 day-old mice [95]. Cells were cloned from 20 colonies after 3 weeks in soft agar culture, in the presence of colony stimulating factor 1. Clones were expanded on microcarrier beads, then transferred to culture dishes and subsequently passaged by scraping. The cell line is dependent on colony stimulating factor 1 to grow. Both cell lines exhibit phagocytic activity. Unlike EOC 20, EOC 2 cells do not constitutively express high levels of the major histocompatibility complex class II antigens and expression is not upregulated by murine interferon-gamma.

The EOC 2 and EOC 20 cell lines express the following antigens: CD11b/CD18 (Mac1), Mac2, Mac3, CD80 (B7-1), CD86 (B7.2), CD45, Ly-6C, F4/80, MHC Class I, MHC Class II, CD115 and FcR. Additionally, EOC 2 cells express CD86 (B7.2).

EOC 2 and EOC 20 Mouse Microglia Cell Lines: Culture Conditions

Growth medium for both EOC 2 and EOC 20 cell lines is DMEM with 4 mM glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10% fetal bovine serum and 20% conditioned media produced from the LADMAC cell line.

CHME-5 Human Microglia Cell Line: Origin and Characteristics

The CHME-5 (also termed CHME5) cell line was immortalized by infection of human primary microglia cultures with the SV40 large T antigen [96]. Primary culture cells were isolated from early embryonic stage of 8 to 10 week-old human embryos, when cells of the microglia lineage have just started colonizing the brain. CHME-5 cells do present a differentiated microglia phenotype. They express CD68 (EBM11), CD11b, FcR, and present a phagocytic activity. CHME-5 cells do not express CD11c, CD14, CD4, as well as the astrocyte marker GFAP nor the neuronal marker neurofilament. CHME-5 cells can be induced to express MHC class II antigens and IL-6 upon stimulation with cytokines. Infection of CHME-5 cells by the human coronavirus OC43 modulated the activity of matrix metalloproteinases 2, 9, and increased nitric oxide production [97], which are features similar to primary microglia. Similarities with primary macrophages were also observed upon infection with HIV-1 virus. The PI3 K/Akt pathway inhibitors alkylphospholipids promoted cell death in both HIV-1 infected macrophages and HIV-1 expressing CHME5 cells, ultimately restricting viral production [98] (Fig. 7).

Fig. 7 Phase photographs of microglia cell line. **a** CHME-5 cells in 10%FBS-DMEM for 5 days, **b** CHME-5 in N2 medium for 3 days, **c** human primary microglia in 10%FBS-DMEM for 2 weeks, **d** human primary microglia in N2 medium plus cytokines for 1 week. 51.2 × magnification (A.I.B. personal communication)
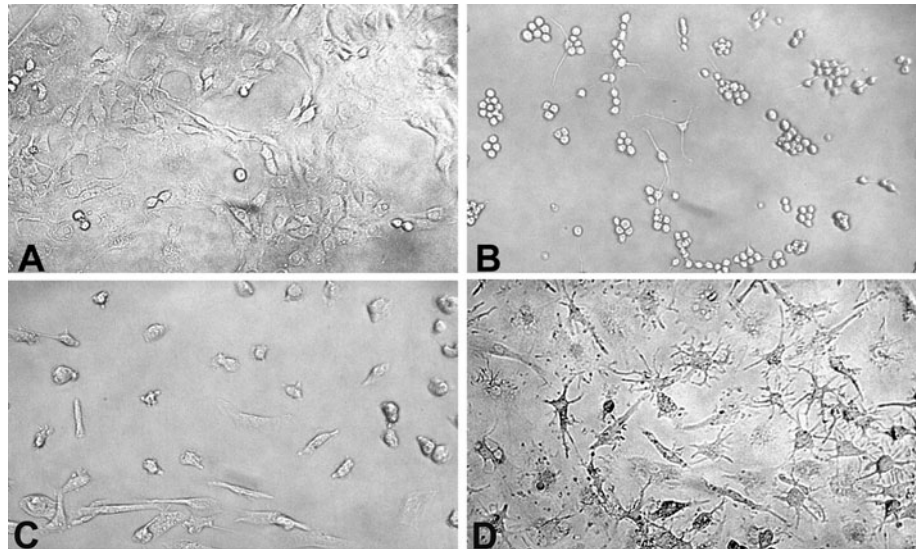
CHME-5 Human Microglia Cell Line: Culture Conditions

Culture medium for the CHME-5 cell line is DMEM with 10% fetal bovine serum.

Antigenic Markers for Glial Cell Lines

It is always desirable to characterize the antigenic properties of glial cell lines in culture with respect to the antigenic profile of the primary glial cell from which the glial cell line was derived. With this end in mind, Table 4 represents the most commonly used antigenic markers for the major glial cells.

As glial cell lines are passaged they may lose the expression of markers found in the primary cells, indicating a movement away from a true representation of the particular glial cell in vivo. Therefore it is desirable to characterize the antigenic profile of a cell line at the onset and end of a given study to be sure the cell line is representative of the native glial cell found in vivo.

Methods to Immortalize Primary Cells to Create a Cell Line

The first animal glial cell lines were derived from induced gliomas in the 1960's and 1970's [29, 30, 66, 69, 81]. Tumors were induced by direct treatment of a target tissue and by injecting a neonatal animal with a mutation-inducing chemical such as N-ethyl-N-nitrosourea, followed by several months for a tumor to appear. A cell line was then directly derived from the chemically-induced tumor, or indirectly subcloned from a cell line derived from the tumor. Chemicals other than N-ethyl-N-nitrosourea have been used to induce tumors. For example, G26-20 and G26-24 mouse oligodendrocyte cell lines were derived from a methylcholanthrene-induced tumor, and the C6 rat astrocytoma cell line was derived from animals treated with N-nitrosomethylurea. With time, the aggressive growth and poor differentiation in culture of such cell lines led to development of other strategies to immortalize cells with the aim of developing a cell line exhibiting a more differentiated phenotype closer to the one shown by primary cells.

Several methods are available to immortalize cells and create a permanent cell line. Two methods are favored. One is to infect the cells with a retroviral vector of simian virus 40 (SV40) large T antigen which carries a temperature-sensitive mutation. Transformed cells, such as the N19 and N20.1 mouse oligodendrocyte cell lines, grow rapidly at the permissive temperature of 34°C (proliferative), but significantly slow down proliferation and increase differentiation under the restrictive temperature of 39°C (non-proliferative), in theory allowing more control over differentiation. Alternatively, the SV40 large T antigen can be placed under the control of a gene promoter which upon activation will promote the differentiation of a cell line, such as the TSC rat Schwann cell line (metallothionein promoter) and the DI TNC1 rat astrocyte cell line (GFAP promoter).

The other method to immortalize cells is the fusion of a primary cell, such a human oligodendrocyte, with a cell line, such as the thioguanine-resistant mutant human rhabdomyosarcoma cell line, to generate a hybrid cell line of human oligodendrocyte such as the MO3.13 cell line. Cell fusion was accomplished via a lectin-enhanced

Table 4 Antigenic markers for glial cell lines

Marker	Full name and function	Localization	Cell type
A2B5 (Ab)	Ganglioside	Cell surface	Bipotential O2A progenitor/OPC
GD3	Ganglioside	Cell surface	Bipotential O2A progenitor/OPC
NG2	Chondroitin sulfate proteoglycan	Cell surface	Bipotential O2A progenitor/OPC
PDGFRα	Platelet-derived growth factor receptor alpha	Cell surface	Bipotential O2A progenitor/OPC
Transferrin	Iron storage glycoprotein	Cytoplasm	Oligodendrocyte
DM20	Proteolipid (short splicing)	Myelin	Oligodendrocyte
PLP	Proteolipid (long splicing)	Myelin	Oligodendrocyte
MOG	Myelin oligodendrocyte glycoprotein	Myelin	Oligodendrocyte
MOBP	Myelin oligodendrocyte basic protein	Myelin	Oligodendrocyte
OMgp	Oligodendrocyte myelin glycoprotein	Myelin	Oligodendrocyte
CNP	Cyclic nucleotide phosphodiesterase	Non-compacted myelin	Oligodendrocyte, Schwann cell
GalC/O1 (Ab)/Ranscht (Ab)	Galactocerebroside/galactosylceramide	Myelin	Oligodendrocyte, Schwann cell
MAG	Myelin-associated glycoprotein	Periaxonal myelin	Oligodendrocyte, Schwann cell
MBP	Myelin basic protein	Myelin	Oligodendrocyte, Schwann cell
O4/007 (Ab)	Sulfatide glycosphingolipid	Cell surface, myelin	Pre-oligodendrocyte, Schwann cell
L1	Neural adhesion molecule	Cell surface	Schwann cell
Merlin	NF2 tumor-suppressor protein	Cytoplasm	Schwann cell
NF1/Neurofibromin	Ras GTPase activating protein	Cytoplasm	Schwann cell
NGF-R/Ran-1/217c (Ab)	Nerve growth factor receptor	Cell surface	Schwann cell
P0	Protein zero	Myelin	Schwann cell
PMP22	Peripheral myelin protein 22	Myelin	Schwann cell
S-100	Small acidic calcium-binding protein	Cytoplasm	Schwann cell, astrocyte
GFAP	Glial fibrillary acidic protein	Cytoplasm	Astrocyte, Schwann cell
Vimentin	Intermediate filament of cytoskeleton	Cytoplasm	Astrocyte
CD11b/CD18/Mac1/OX-42(Ab)	Receptor of iC3b complement fragment	Cell surface	Microglia
CD68/EBM11(Ab)	Glycosylated mucin-like endosomal protein	Cell surface	Microglia
CD115	Colony stimulating factor 1 receptor	Cell surface	Microglia
FcR	Immunoglobulin receptor	Cell surface	Microglia
MHC class I and II	Major histocompatibility complex	Cell surface	Microglia
B4	Isolectin	Cell surface	Microglia
Mac2 (Ab)	Galactose-specific lectin binding IgE	Cell surface	Microglia

It can be confusing that an antigen and its corresponding antibody are often indiscriminately referenced in the literature

Ab antibody

polyethylene glycol procedure. Hybrids were selected in an aminopterin-containing media using the thioguanine resistance of the parent rhabdomyosarcoma RD mutant cell line.

Lastly, it is important to note that numerous cell lines have been obtained spontaneously. These cell lines have mostly two origins. The first origin is rodent primary cell cultures which contain progenitor cells, such as the rat oligodendrocyte cell lines CG4 and OLN-93, the mouse Schwann cell line IMS32, and the mouse microglia cell lines EOC 2 and EOC 20. The second source is a spontaneous tumors in patients such as oligodendroglioma, glioblastoma, malignant astrocytoma, peripheral nerve tumors in NF1 patients, malignant schwannoma, sarcoma in NF1 patients, and adrenal neuroblastoma from which were derived cell lines for all glial cell types except for microglia (see Table 2 for details and references).

Glial Cell Lines: Future Directions

One of the most active areas of current glial cell research is the study of central nervous system myelination. For these studies two human oligodendrocyte cell lines are available, HOG and MO3.13, which unfortunately show little potential to differentiate in vitro. HOG and MO3.13 cell

lines have never been grafted in vivo, since it does not seem likely that they would stop their intrinsic proliferation and begin myelination. Unfortunately both cell lines are more characteristic of the tumors from which they were derived: the HOG cell line from an oligodendroglioma, and the MO3.13 line obtained by fusion with a tumor rhabdomyosarcoma cell line. There is an urgent need for another human oligodendrocyte cell line that would be closer to the phenotype of a primary cell in its capacity to differentiate in vitro. Ideally, the cell line should have the capacity to differentiate to reach the stage of flat myelin membrane production, as shown by the rodent oligodendrocyte cell lines OLN-93 and Oli-neu when placed in the appropriate culture conditions. Ultimately, a human cell line should be able to myelinate axons, similar to the mouse N20.1 cell line co-cultured with neurons or upon graft in vivo like the rat CG4 cell line.

New oligodendrocyte cell lines were recently obtained by spontaneous immortalization and appear to have a phenotype capable of differentiation. Three human oligodendroglial cell lines were generated from a patient brain infected with the SV40 virus. These lines contain the SV40 large T antigen and were able to differentiate after silencing the viral mRNA [99]. A new cell line, BO-1, was established for the first time by spontaneous immortalization from a mouse source using a primary mixed glial culture derived from neonatal mouse pups [100]. The BO-1 line retains a bipotential phenotype: addition of serum causes it to differentiate into astrocytes while in other culture conditions the BO-1 cells express the markers O4, CNP and MBP on a network of processes. This phenotype is very similar to that of the rat CG4 cell line also obtained by spontaneous immortalization.

Another recent development is the availability of commercial vendors (e.g. Sigma–Aldrich) to modify a specific target gene in a particular cell line under the specification of the investigator in order to study a disease or to address a particular biological function. The commercial vendor uses a proprietary method to engineer (knockout/delete, mutate, add) a gene in a cell line supplied directly by the investigator or available commercially through ATCC or ECACC. This service named 'Custom Cell Engineering Service' holds great potential.

Lately, much emphasis has been put on differentiating embryonic stem cells in vitro to generate large number of myelinating glial cells for therapy in demyelinating diseases. Converting embryonic stem cells into a particular cell type has proven to be a tricky task. The in vitro manipulation of human embryonic stem cells to direct them to oligodendrocyte progenitor cells is delicate and involves numerous steps [101, 102]. The complexity of the procedure renders impractical a routine work for basic research on human oligodendrocytes. More accessible cells, such as human umbilical cord blood cells or human adult blood stem cells, have not yet been successfully differentiated into mature oligodendrocytes in vitro [103–105]. Nevertheless hard won success with human embryonic stem cells has been obtained to generate sufficient oligodendrocyte progenitor cells to begin Phase 1 trials for the use of these cells after spinal cord injury. This oligodendrocyte progenitor cell therapy proved successful in an animal model of spinal cord injury [106]. The success of this trial and subsequent studies should lead to the efficient generation of oligodendrocyte precursors in quantities similar to cell lines sufficient for future cell therapy. Although glial cell lines may not fill the niche as therapeutic agents, they will continue to be useful tools for further understanding the role that glial cells play in the functioning of the much neglected and increasingly important "Other Brain" [1].

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