Experimental Neurology 229 (2011) 181–194

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review

Clinical application of adult olfactory bulb ensheathing glia for nervous system repair

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ARTICLE INFO

Article history:
Received 22 July 2010
Revised 30 September 2010
Accepted 2 October 2010
Available online 12 October 2010

Keywords:
Spinal cord injury
Transplantation
Cell therapy
Paraplegia
Clinical trial
Regeneration
Legislation
Medicinal product
Advanced therapy

ABSTRACT

The ability of adult olfactory bulb ensheathing glia (OB-OEG) to promote histological and functional neural repair has been broadly documented. Pre-clinical studies show that beneficial effects of adult OB-OEG are repeatable in the same type of spinal cord injury initially tested, in other spinal cord and CNS injury models, in different species and after the administration of these cells in different forms (either alone or in combination with other cells, drugs, products or devices). These studies demonstrate the reproducibility, robustness, fundamental nature and relevance of the findings. Therefore, the use of adult OB-OEG for spinal cord injury repair meets the scientific criteria established by the International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) for the translation to human application. Because there is so much heterogeneity in the way adult OEG is administered, each of these different OEG-based therapies must be individually categorized to determine whether they fulfill the requisites dictated by the consolidated regulatory body to be considered or not as a medicine. In the case they do, in Europe, they shall be subjected to the Regulatory European Framework for Advanced Therapy Medicinal Products and the European Clinical Trials Directive (Directives 2001/20/EC and 2009/120/EC). After a deep analysis of the European Regulation we have concluded that grafts consisting of suspensions of purified adult OEG, to be used for the promotion of axonal regeneration in the CNS, do not comply with the definition of Medicinal Product provided by the European Medicines Agency. In contrast, experimental therapies using OEG in combination with other cell types, drugs, products or devices, or genetically-modified OEG fall under the definitions of Medicinal Product. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

Contents

Abbreviations: CATMP, Combined Advanced Therapy Medicinal Product; CBMP, Cell-Based Therapy Medicinal Product; CNS, Central Nervous System; EMP, Engineered Medicinal Product; GTMP, Gene-Therapy Medicinal Product; ICCP, International Campaign for Cures of Spinal Cord Injury Paralysis; SCMP, Somatic Cell Therapy Medicinal Product; OB-OEG, Olfactory bulb ensheathing glia.

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doi:10.1016/j.expneurol.2010.10.001

Introduction

Olfactory receptor neurons, located in the epithelium of the nasal cavity, undergo continuous turnover in adult mammals. The newly generated neurons grow their axons across the cribiform plate of the ethmoidal bone, traverse the piamater and enter the Central Nervous System (CNS) of the olfactory bulb (OB). Here they are able to navigate, reach and synapse with the dendrites of mitral, tufted and periglomerular cells in the olfactory glomeruli (Barber, 1982; Doucette et al., 1983; Graziadei and Graziadei, 1979; Graziadei and Monti Graziadei, 1980). Surprisingly, researchers discovered that olfactory axons possessed the ability to elongate and reconnect continuously throughout life, thus making the olfactory bulb a unique CNS structure capable of spontaneous axonal regeneration. The failure of axonal regeneration within the adult mammalian CNS has been attributed to the inhibitory environment created around the axons by the glial cells. As a result, scientists argued that this distinct capacity for axonal regeneration exhibited by the olfactory bulb (OB) and other CNS structures might be related to their unequal cellular composition. Going back to the first histological studies about the OB, it was intriguing to find that in 1875 Golgi (Golgi, 1875) and in 1898 Blanes (Blanes, 1898) described the presence of a fusiform glial type, exclusively located within the olfactory nerve and glomerular layers, which serve to isolate olfactory axons from other CNS cells. This was later confirmed at the ultrastructural level by other scientists (Barber and Lindsay, 1982; Doucette, 1984, 1990; Pixley, 1992; Raisman, 1985; Valverde and Lopez-Mascaraque, 1991; Valverde et al., 1992). They described a Schwann-like glial cell that surrounded and ensheathed olfactory axons through their entire path inside the bulb. This type of macroglial cells was exclusively encountered in a region of the CNS where axonal regeneration was possible during adulthood and, hence, suggested a key role in axonal growth for these cells. Blanes' macroglia was then named *olfactory ensheathing cells* (OEC) or *olfactory ensheathing glia* (OEG) to honor their main property, and because their ability to enfold and isolate olfactory axons from the hostile CNS environment was believed to be linked to their capacity to promote axonal growth.

Adult OEG play a critical role in the continuous regeneration of olfactory axons into the olfactory bulb, prompting scientists to consider their growth promoting properties in other regions of the CNS. If successful a new tool for nervous system repair could be available. To test this hypothesis, first OEG had to be isolated and purified from adult OBs and then these cultures had to demonstrate that their ability to enfold axons remained preserved. Cultures of adult OB-OEG were obtained and characterized (Ramon-Cueto and Nieto-Sampedro, 1992). Both, their *in vivo* and *in vitro* properties suggested that they were a unique glia cell type with axonal growth promoting properties (Ramon-Cueto and Valverde, 1995). Purified adult OB-OEG had to also maintain their ability to promote axonal elongation and ensheathment because these properties were considered essential to exert their positive and potentially therapeutic effect. Co-culture studies confirmed that after isolating OEG from adult bulbs, these cells continued favoring neurite extension and enfoldment. The *in vivo* role of these cells and the demonstration that they retained their properties in culture suggested that adult OB-OEG could be a good tool to foster axonal regeneration within the adult mammalian CNS (Ramon-Cueto and Valverde, 1995).

A region that closely resembles the PNS/CNS olfactory bulb transition zone is the dorsal root entry zone. However, in this region spontaneous axonal regeneration into the spinal cord is not possible (Carlstedt, 1985; Carlstedt et al., 1989; Ramón y Cajal, 1991). Thus, a "proof of principle" study could evaluate whether or not adult OB-OEG favored the entrance of injured root axons across this PNS-CNS interface. Indeed, these axons were able to cross the root-cord boundary, elongate within the CNS environment of the spinal cord and reach the laminae they innervate under normal conditions after OB-OEG grafting (Ramon-Cueto and Nieto-Sampedro, 1994). These positive results encouraged scientists to pursue the next challenge: to determine whether OB-OEG could also exert axonal growth-promoting effect and functional improvement after CNS damage. This would, in turn, determine the real prospect of adult OB-OEG transplants for nervous system repair in humans.

In this article we provide an overview of the legislation currently in force in Europe for cell-based therapies and how this may affect the clinical translation of therapies using OB-OEG grafts for neural repair. We also review the studies that have been carried out so far in animal models using adult OB-OEG as a therapeutic agent for neural repair. Finally we discuss whether or not adult OB-OEG transplants fulfill the guidelines for the translation into clinical application.

Regulatory European framework for cell-based therapies

All new medicinal products have to be validated and approved by an appropriate national regulatory agency. However, not all countries throughout the world have the same ethics and regulations. Consequently, this has resulted in several experimental treatments applied to humans whose safety and efficacy were not validated *in vitro* and in appropriate animal models. An international common regulatory body and/or legislation would safeguard in all nations equal rights for the patients, but this still has to come. The health regulatory agencies in the United States and in the European Community, the Food and Drug Administration (FDA) and the Committee for Medicinal Products for Human Use, respectively, follow similar general guidelines. They intend to protect the public from any risk or harm coming from an unsubstantiated treatment. In the European Community, all Member States have to follow the Directive of the European Parliament and of the Council on the Community code relating to medicinal products for human use (Directive 2001/83/EC). In this Directive, Part IV of Annex I regulates the specific requirements for "advanced therapy medicinal products" (ATMP) which are comprised of gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (SCMP) and tissue-engineered medicinal products (EMP). This Directive was amended by the Regulation EC No 1394/2007 in order to introduce additional provisions that better regulate the use of ATMP in humans. However, during the last years this field has grown exponentially and has been placed at the forefront of innovation, offering new prospects and hope for diseases that currently have no cure. This legislation had to be adapted again to meet the scientific and technical progress of this field. As a result, part IV of Annex I was replaced by the text of Directive 2009/120/EC accordingly (Schneider et al., 2010). This new legislation entered into force in April 2010.

Cell-based medicinal products (CBMP) may include somatic cell therapy (SCMP), gene therapy (GTMP) and also tissue-engineered products (EMP) from either autologous, allogenic or xenogenic sources (Schneider et al., 2010). According to this European pharmaceutical legislation (Directive 2009/120/EC), a cell-based therapy fulfill the criteria of a medicinal product when it "contains or consists of cells or tissues that have been subjected to substantial manipulation so the biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered." The following are not considered substantial manipulations: "cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilization, irradiation, cell separation, concentration or purification, filtering, lyophilization, freezing, cryopreservation, vitrification" (Annex I of Regulation (EC) No 1394/2007). A cell-based therapy is also a medicinal product when it "consists of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor." When cells have been genetically modified and are intended for regenerating, replacing or repairing human tissue they are included in the category of "tissue-engineered medicinal products" (Schneider et al., 2010). Moreover, they may also be considered "gene therapy medicinal products." Cells (autologous or others) that are subjected to any "substantial manipulation" to achieve the properties relevant for the intended therapeutic use are also

considered tissue-engineered products. All these cell-based therapies that are defined as medicinal products for human use have to comply with the European laws, regulations and administrative provisions related to the conduct of clinical trials (Directive 2001/20/EC). Cell therapies excluded from the definitions of cell-based medicinal products (CBMP) are not under this Regulation (Schneider et al., 2010).

How may the European legislation affect the clinical translation of OEG-based therapies?

Depending on the type of graft, how this is manipulated before transplantation and what are the properties of the cells, OEG grafts may or may not be considered medicinal products. What seems clear, according to the Regulation currently in force, is that OEG cultures whose procedures exclusively include maneuvers listed as non-substantial manipulations (cutting; grinding; shaping; centrifugation; soaking in antibiotic or antimicrobial solutions; sterilization; irradiation; cell separation, concentration or purification; filtering; lyophilization; freezing; cryopreservation; vitrification) are excluded from the definition of medicinal product (Regulation EC No 1394/2007; Directive 2009/120/EC). This seems to be the case for bulbar and mucosal OEG cultures (Fig. 1), including OEG expansion and purification (Gorrie et al., 2010; Ito et al., 2008; Kawaja et al., 2009; Lindsay et al., 2010; Miedzybrodzki et al., 2006; Murrell et al., 2008; Ramon-Cueto and Nieto-Sampedro, 1992; Richter et al., 2008; Rubio et al., 2008). In both cases, after bulb or mucosa extraction from the donor (Fig. 1A), the tissue is minced and dissected ("cutting") (Fig. 1B, C), then cell suspensions are obtained ("grinding" and/or "cell separation") and the cells cultured in antibiotics-containing culture medium ("soaking in antibiotic or antimicrobial solutions") (Fig. 1D). In the case of primate OB-OEG, their culture and expansion do not require the addition to the culture medium of any non-human material, growth factor, mitogen or any other reagent. These cells survive and divide in DMEM/F-12 basal media (a solution containing salts, glucose, non-essential amino acids, pyruvate and glutamine), antibiotics and serum (that may be human)(Rubio et al., 2008). When cultures reach confluence the cells are detached from the dishes or flasks, centrifuged and cultured again under the same conditions ("cell separation" "grinding", "centrifugation", "concentration" and again "soaking in antibiotic or antimicrobial solutions") (Fig. 1E). In addition, freezing and cryopreservation are accepted as non-substantial manipulations, which opens up the possibility of creating a bank of OEG for future use. The purification of bulb/mucosa OEG or p75-positive OEG from the cultures is not considered a substantial manipulation ("cell separation" and "purification") (Bianco et al., 2004; Krudewig et al., 2006; Ramon-Cueto and Nieto-Sampedro, 1994; Ramon-Cueto et al., 1998; Rubio et al., 2008).

Another criterion that disqualifies a cell therapy as a "medicinal product" is that the biological, functional and structural properties of the cells relevant for the clinical use must remain unaltered. In addition, the cells should be used in the host for the same essential function they have in the donor. Cultures of adult OEG fulfil both criteria. These cells maintain their characteristics and the ability to promote axonal elongation after purification from the bulb and mucosa. Indeed, adult primate OB-OEG, including humans, preserve the typical OEG phenotypic properties and do not become senescent for up to 2.5 months *in vitro* and up to 34 passages (Barnett et al., 2000; Lim et al., 2010; Miedzybrodzki et al., 2006; Rubio et al., 2008; Techangamsuwan et al., 2008) (Fig. 1D, E). Moreover, they keep their ability to promote axonal elongation in culture (Lim et al., 2010) and neural repair after transplantation into injured spinal cords (Barnett et al., 2000; Guest et al., 2008; Kato et al., 2000). Similarly, human

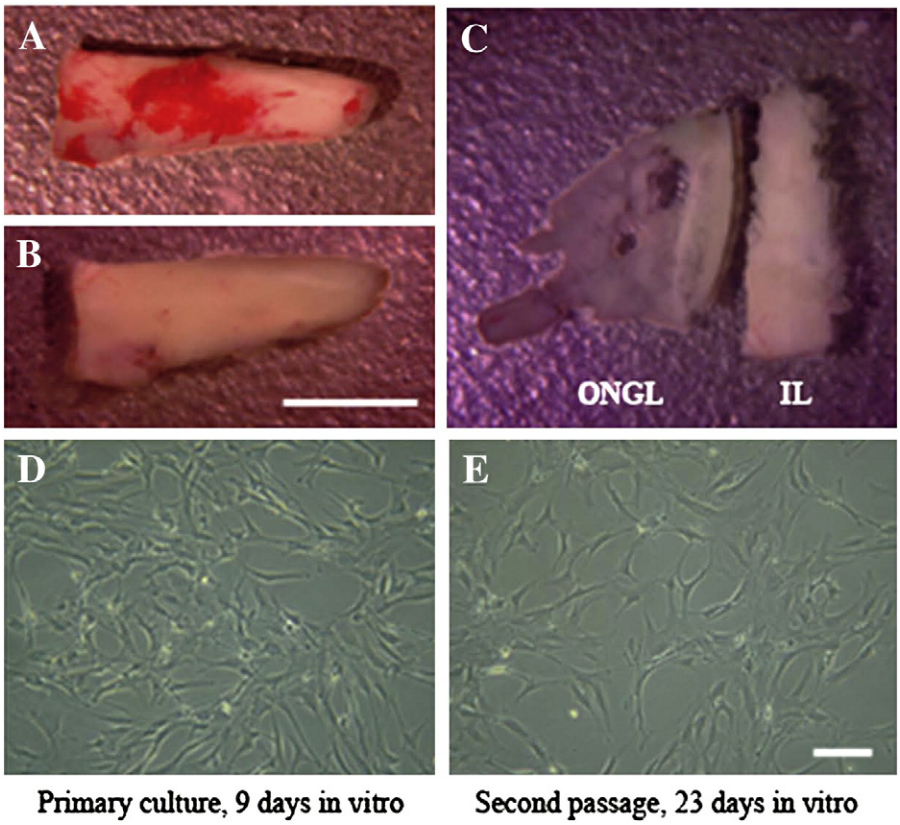

Fig. 1. Olfactory bulb obtained surgically from a living 60 year old woman, and subsequent culture of OEG from this bulb. A. Appearance of the olfactory bulb after surgical extraction. B. Appearance of the same olfactory bulb shown in A after removal of the piamater. C. Dissection of the olfactory nerve and glomerular layers (ONGL) from the inner bulb layers (IN). Magnification of A, B and C is equal. Bar = 4 mm. D. OB-OEG primary culture from the ONGL shown in C after 9 days in vitro. E. OB-OEG subculture of the same culture shown in D, after the second passage, and after 23 days in vitro. Magnification in D and E is equal. Bar = 100 micrometers.

mucosal OEG retain their biological and functional features in culture (Bianco et al., 2004; Choi et al., 2008; Ito et al., 2008; Kawaja et al., 2009) and their repair properties after being grafted into the injured CNS (Deng et al., 2006; Feron et al., 2005; Gorrie et al., 2010). In addition, the intended use for OEG is the promotion of axonal elongation in the nervous system, which is the same essential function these cells have in the olfactory system. Therefore, OEG-based therapies using exclusively these purified cells do not comply with the definition of advance therapy medicinal product under the current legislation (Table 1).

Some groups have used non-pure OB-OEG cultures for nervous system repair (Table 1). In this case, the properties of the accompanying cells will determine whether the therapeutic approach is considered a medicinal product. Grafts containing cells whose essential function in the donor and the host is not the same shall be considered "somatic cell therapy medicinal products" ((Schneider et al., 2010) and Directive 2009/120/EC) and must fulfil the requirements of the Clinical Trials Directive (Directive 2001/20/EC). This could be the case of OB-OEG grafts containing fibroblasts (Lakatos et al., 2003a,b; Li et al., 2003a,b, 1998, 2005; Teng et al., 2008) or stem cells (Amemori et al., 2010; Deng et al., 2008; Johansson et al., 2005; Morita et al., 2008; Murrell et al., 2005, 2008; Shukla et al., 2009; Srivastava et al., 2009; Tang et al., 2010; Wang et al., 2010) when the main function of the accompanying cells is different from the intended use in the recipient (i.e., neural repair). Fibroblasts are mesenchymal-derived cells that exert their role outside the CNS and whose function is not related to the promotion of neural repair. Moreover, after traumatic CNS injury, meningeal fibroblasts form an inhibitory fibrous scar at the injury site crating a decisive barrier to axonal regeneration (Klapka et al., 2005; Komuta et al., 2010; Niclou et al., 2003). This is opposite of the intended reparative function for the grafted cells in the host. An exemption may be, however, the case of fibroblasts derived from the piamater covering the olfactory bulb. Geoff Raisman and collaborators have proposed the participation of these particular fibroblasts in the reconstruction of channels that favour axonal growth through the PNS-CNS interface of the olfactory bulb (Li et al., 2005; Raisman et al., 2011; Raisman and Li, 2007). When OB-OEG and olfactory nerve fibroblasts are transplanted to repair plexus injuries they have the same essential function before and after transplantation (Ibrahim et al., 2009a,b).

Some authors have combined OEG with stem cells or stem -derived cells (Amemori et al., 2010; Deng et al., 2008; Johansson et al., 2005; Morita et al., 2008; Murrell et al., 2005, 2008; Shukla et al., 2009; Srivastava et al., 2009; Tang et al., 2010; Wang et al., 2010). However, bone marrow stromal cells, mesenchymal and embryonic stem cells do not have the same essential function in the recipient and the donor. Also, after their extraction, stem cells are normally subjected to substantial manipulations that alter their original biological properties and physiological functions. Either in culture or later after transplantation, stem cells usually lose their multipotency or pluripotency and differentiate into other cell types. Thus, OEG grafts containing stem cells are medicinal products according to the European Directive. Mesenchymal stem cells are also present in whole olfactory mucosa grafts (Guntinas-Lichius et al., 2002; Iwatsuki et al., 2008; Lima et al., 2006; Lu et al., 2001; Othman et al., 2005) and in non-purified mucosal OEG cultures (Guntinas-Lichius et al., 2001; Morita et al., 2008; Yamamoto et al., 2009) and thus, the same legislation should be applied. Moreover, non-purified mucosal grafts may also contain globose basal cells (neural precursor cells), sustentacular cells (non-neuronal supporting cells), fibroblasts, macrophages, pericytes, endothelial cells and Schwann cells (Lindsay et al., 2010; Murrell et al., 2005, 2008). These miscellaneous cell types have different roles in the donor tissue that are not always related to the intended use of neural repair in the host. Lastly, foetal olfactory bulb cells have been used for neural repair as well (Chen et al., 2010; Huang et al., 2008). Like the others, these grafts must also be included in the category of somatic cell therapy medicinal product because they contain a mixture of several cell types, including stem cells (Liu et al., 2010).

Genetically modified OEG for neural repair can be categorized as both a tissue-engineered medicinal product as well as a gene therapy medicinal product. According to the Regulation EC No 1394/2007 when a product falls within the definition of either somatic cell therapy or tissue engineered medicinal product and also gene therapy medicinal product, it shall be categorized as the latter. This is the case of OEG genetically-modified to express neurotrophic factors (Cao et al., 2004; Ma et al., 2010; Ruitenberg et al., 2005, 2002, 2003; Wu et al., 2008). These cells have been manipulated to enhance their properties so that they achieve better regeneration and repair, which consequently labels them as engineered cells. Also, as a result of the introduction of a recombinant nucleic acid sequence, these cells contain an active substance and, in addition, the expression of this sequence directly relates to their therapeutic effect (normative 2009/120/EC). OEG has also been genetically modified to increase the proliferative ability of these cells by expression of the catalytic subunit of telomerase (TERT) (Lim et al., 2010; Llamusi et al., 2011; Techangamsuwan et al., 2009). Although the gene product is not intended for therapeutic use, these cells have been subjected to a substantial manipulation: the target cells have been modified by a vector, the protein encoded and then expressed by gene transfer. Therefore, TERT-expressing OEG are considered a gene therapy medicinal product.

OEG administered in combination with products or devices shall be categorized as "Combined Advanced Therapy Medicinal Products" (Schneider et al., 2010) (Directives 90/385/EEC and 2007/47/EC) because they incorporate a drug, product or an "active implantable medical device" for human use. According to the Directive 90/385/EEC, a medical device is any appliance, instrument, material or other article that is necessary for the proper application of the therapeutic agent in human beings. An "active implantable medical device" is a device that is introduced into the human body by surgery or medically, and intended to remain after the procedure. Substances used in combination with OEG such as scaffolds, matrices, biomaterials, polymers, molecules, substrates, bridges or tubes (Bretzner et al., 2008; Deumens et al., 2006a,b; Li et al., 2010; Shen et al., 2010; Wang et al., 2007) are devices of this category and must satisfy the requirements of the legislation mentioned above.

In conclusion, according to the European Regulation, the use of suspensions of purified adult OEG from either the bulb or the mucosa, prepared with no other reagents considered medicines or non-human materials, do not fall under the definition of a medicinal product. In contrast, grafts containing OEG in combination with other cell types, contaminant cells or any product (matrices, biopolymers, scaffolds, growth factors, drugs, pharmaceutical compounds, etc) and genetically-modified OEG, shall be considered medicines. Only OEG-based therapies that are considered medicinal products will be subjected to the Regulatory European framework for Advanced Therapy Medicinal Products and, consequently to the European Clinical Trials Directive (Directives 2001/20/EC and 2009/120/EC). The guidelines from the European Medicines Agency addressing the development, quality control, non-clinical and clinical development of cell-based medicinal products that should be considered before entering into clinical trials can be found here: http://www.ema.europa.eu/pdfs/human/cpwp/41086906enfin.pdf. The interpretation of the European Regulation for cell- and OB-OEG-based therapies presented in this article has been reviewed and evaluated by experts in Civil Law and Bioethics who have approved its content (supplemental data 1).

Pre-clinical studies validating the usefulness of adult OB-OEG for neural repair

There are over 90 scientific articles reporting the beneficial effects of OB-OEG in the promotion of nervous system repair (Table 1), and just 11 showing no effects. The absence of positive results in the latter

Table 1
Types of OB-OEG grafts used experimentally with an indication of which definition of cell-based therapy medicinal product they comply.

Type of graft	CBMP	Type of CBMP	Reason for the classification	References
Purified OB-OEG	No	None	All manipulations for cell culture are included in Annex I of the Regulation EC 1394/2007. Thus, cells are not subjected to substantial manipulation Not genetically-modified cells Not engineered cells Cells with the same essential function in the donor and host: the promotion of axonal regeneration	Ramon-Cueto and Nieto-Sampedro (1994) Ramon-Cueto et al. (2000) Barnett et al. (2000) Verdu et al. (2001) Guntinas-Lichius et al. (2001) Takami et al. (2002) Smith et al. (2002) Lakatos et al. (2003b) Andrews and Stelzner (2004) Akiyama et al. (2004) Radtke et al. (2004) Lee et al. (2004) Riddell et al. (2004) Sasaki et al. (2004) Richter et al. (2005) Sasaki et al. (2006a) Sasaki et al. (2006b) Dombrowski et al. (2006) Andrews and Stelzner (2007) Dewar et al. (2007) Kubasak et al. (2008) Negredo et al. (2008) Lankford et al. (2008) Takeoka et al. (2009) Munoz-Quiles et al. (2009) Takeoka et al. (2010) Wu et al. (2011)
OB-OEG plus olfactory fibroblasts	No/yes	None or SCMP	Possibly not considered SCMP if fibroblasts have the same function in the donor than in the host (i.e. olfactory fibroblasts), otherwise SCMP	Li et al. (1998) Imaizumi et al. (1998) Li et al. (2005) Teng et al (2008)
Non-purified olfactory bulb cultures	Yes	SCMP	Grafts contain contaminant cells that have a different essential function in the donor than in the host	Kato et al. (2000) Imazumi et al. (2000a) Resnick et al. (2003) Lakatos et al. (2003a) Keyvan-Fouladi et al. (2003) Chuah et al. (2004) Boyd et al. (2004) Toft et al. (2007) Huang et al. (2008) Radtke et al. (2008) Giordana et al. (2010) Bohbot (2010) Chen et al (2010)
OB-OEG combined with other cell types	Yes	SCMP	Accompanying cells do not have the same function in the donor than in the host	Fetal mesencephalic : Agrawal et al. (2004) Johansson et al. (2005) Progenitors/Stem Cells: Deng et al. (2008) Srivastava et al. (2009) Shukla et al. (2009) Salehi et al. (2009) Amemori et al. (2010) Wang et al. (2010) Tang et al. (2010)
OB-OEG treated with pharmaceutical drugs	Yes	SCMP	Cells have been subjected to substantial manipulation	Wei et al. (2008)
OB-OEG purified using Dynabeads	Yes	CATMP	Dynabeads cannot be separated from OB-OEG. Thus, grafts contain Dynabeads (medical device)	Navarro et al. (1999) Pascual et al. (2002) Gomez et al. (2003) Verdu et al. (2003) Garcia-Alias et al. (2004) Polentes et al. (2004) López-Vales et al. (2004) Collazos-Castro et al. (2005) Lopez-Vales et al. (2006a) Lopez-Vales et al. (2007)
Genetically-modified (or engineered) OBOEG	Yes	GTMP or EMP	Cells subjected to substantial manipulation by gene expression The active substance consists of genetically-modified cells by a recombinant nuclei acid sequence	BDNF and NT3: Ruitenberg et al. (2003) GDNF: Cao et al. (2004) NT-3: Ruitenberg et al. (2005) Wu et al. (2008) Ma et al. (2010) GFP : Barakat et al. (2005) Pearse et al. (2007) Guest et al. (2008) TERT: Techangamsuwan et al (2009) Lim et al. (2010) Llamusi et al. (2011)

(continued on next page)

Table 1 (*continued*)

Type of graft	CBMP	Type of CBMP	Reason for the classification	References
OB-OEG from transgenic animals	Yes	EMP	Grafts contain engineered cells	Imaizumi et al. (2000b) Shi et al. (2010)
OB-OEG administered in combination with products or devices	Yes	CATMP	The graft contains any appliance, instrument, material, or other article used for therapeutic purposes and/or necessary for the proper application of the cells	Matrices: Smale et al. (1996) Li et al. (2003a) Li et al. (2003b) Li et al. (2004) Li et al. (2007) Ibrahim et al. (2009a,b) Bridging tubes: Ramon-Cueto et al. (1998) Verdu et al. (1999) Li et al. (2010) Biomatrix bridges: Deumens et al. (2006a,b)
OB-OEG administered in combination with drugs	Yes	CATMP	The graft is administered in combination with a pharmaceutical compound, drug or product	Methylprednisolone: Nash et al. (2002) Pearse et al. (2004) Fibrinogen: Plant et al. (2003) Chondroitinase ABC: Fouad et al. (2005) Vavrek et al. (2007) Fouad et al. (2009) FK506 López-Vales et al. (2006) COX2 inhibitors and iNOS: Lopez-Vales et al. (2006b)

CATMP: Combined Advanced Therapy Medicinal Product; CBMP: Cell-Based Therapy Medicinal Product; EMP: Engineered Medicinal Product; GTMP: Gene-Therapy Medicinal Product; SCMP: Somatic Cell Therapy Medicinal Product; OB-OEG: Olfactory bulb ensheathing glia.

is attributed to the source of the cells, the type of graft and the methodology used for transplantation (reviewed in (Franssen et al., 2007)). In 6 of the articles, OB-OEG were obtained from the bulbs of fetal (Boyd et al., 2004; Giordana et al., 2010; Piepers and van den Berg, 2010), and neonatal (Dewar et al., 2007; Richter et al., 2005; Riddell et al., 2004) donors. The remaining studies used adult OB-OEG and the lack of an effect after transplantation of these cells has a logical explanation. In two of the studies, OB-OEG were purified using magnetic beads (Collazos-Castro et al., 2005) and supramagnetic iron oxide nanoparticles (Lee et al., 2004) and the purification of OEG using magnetic particles affects their efficacy to support neuronal survival and axonal regeneration after spinal cord injury (Novikova et al., 2011). Moreover, in the former (Collazos-Castro et al., 2005) and in two more studies, OB-OEG were injected directly into the contusion site and they did not survive the pro-apoptotic environment (Resnick et al., 2003; Takami et al., 2002). This does not occur when OB-OEG are injected near the contusion, but not at the epicenter (Pearse et al., 2007; Plant et al., 2003). In the remaining study, OB-OEG were grafted in combination with a matrix poly (D,L)-lactide. This synthetic compound may have interfered with the normal behavior of adult OB-OEG (Deumens et al., 2006a,b).

Except for the five articles cited above, all the other studies using adult OB-OEG grafts (more than sixty) report neural restoration after transplanting these cells into the damage cord. Most of these studies were carried out in different animal models of spinal cord injury (Table 2). The International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) has established an international panel of leading researchers, clinical investigators and organizations, all aimed at supporting and providing guidelines for the translation of pre-clinical studies to clinical application in spinal cord injured patients (Adams and Cavanagh, 2004). According to ICCP, the desired validation pathway for the translation of any pre-clinical strategy to human application should meet the following requirements. 1) The initial results should be corroborated independently by one or more scientific groups. 2) The robustness of the finding has to be demonstrated: slight variations of the experimental treatment should provide similar results. 3) The fundamental nature of the intervention has to be demonstrated: more than one animal model of spinal cord injury should be used. Moreover, the intervention should be tested at least in two different species. 4) The relevance of the discovery to humans should be demonstrated by using animal models that mimic the human condition ((Fawcett et al., 2007; Lammertse et al., 2007; Steeves et al., 2007; Tuszynski et al., 2007); more information in http://www.icord.org/ICCP/).

Replication of the initial results with adult purified OB-OEG for spinal cord injury repair

The use of adult OB-OEG transplantation for spinal cord injury repair has undergone extensive investigation in animals, showing a consistent and repeatable effect (Table 2). The first study, published in 1994, reported the regeneration of selectively sectioned axons into the spinal cord of adult rats by purified OB-OEG (Ramon-Cueto et al., 1994). These results were later reproduced by other groups which, in addition, reported functional recovery after regeneration of root axons (Gomez et al., 2003; Li et al., 2004; Navarro et al., 1999; Pascual et al., 2002). The first time that pure adult OB-OEG were grafted into severely injured spinal cords was in 1998 (Ramon-Cueto et al., 1998). In this case, adult OB-OEG promoted long-distance regeneration of descending and ascending injured axons after complete spinal cord transection. Two years later, the recovery of motor and sensory functions of paraplegic rats was reported (Ramon-Cueto et al., 2000). This ability of adult OB-OEG to promote regeneration after complete spinal cord injury has also been replicated by other groups (Cao et al., 2004; Guest et al., 2008; Kubasak et al., 2008; Lopez-Vales et al., 2006a,b; Takeoka et al., 2010, 2009).

In addition, adult purified OB-OEG has been used to remyelinate lesions of the adult rat spinal cord in animal models of demyelinising diseases and the first studies were published in year 2000 (Barnett et al., 2000; Kato et al., 2000). In both cases, the authors observed remyelination of dorsal column axons after transplantation of human OB-OEG into rodent spinal cords. Their results were confirmed later in the same injury model but using OB-OEG from rats (Lankford et al., 2008; Sasaki et al., 2006a,b), transgenic rats (Akiyama et al., 2004), dogs (Smith et al., 2002) and transgenic pigs (Radtke et al., 2004).

In conclusion, the initial results obtained after adult purified OB-OEG transplantation in animal models of both, traumatic and demyelinising spinal cord injuries have been corroborated independently by several groups.

Demonstration of the robustness of the finding obtained using adult OB-OEG for spinal cord injury repair

Variations on the way that adult OB-OEG are applied or on the type of OB-OEG-based experimental treatment have provided similar positive effects in injured spinal cords (Table 2). These effects include axonal regeneration, survival and tissue sparing, stimulation of

Table 2
Pre-clinical validation of the effectiveness of adult OB-OEG for neural repair.

Type of lesion	Type of graft	Specie		References
		Donor	Host	
Dorsal rhizotomy				
	Purified OB-OEG	Rat	Rat	Ramon-Cueto and Nieto-Sampedro (1994)
				Navarro et al. (1999)
				Pascual et al. (2002)
				Gomez et al. (2003)
	Not-purified OB-OEG embedded in a matrix	Rat	Rat	Li et al. (2004)
				Ibrahim et al. (2009a,b)
Avulsed ventral root				
	Not-purified OB-OEG embedded in a matrix	Rat	Rat	Li et al. (2007)
Complete spinal cord injury				
Segment removal	Pure OB-OEG, SC bridges	Rat	Rat	Ramon-Cueto et al. (1998)
	plus chondroitinase ABC	Rat	Rat	Fouad et al. (2005)
				Vavrek et al. (2007)
				Fouad et al. (2009)
Transection	Purified OB-OEG	Rat	Rat	Ramon-Cueto et al. (2000)
				Cao et al. (2004)
				Kubasak et al. (2008)
				Negredo et al. (2008)
		Primate	Rat	Guest et al. (2008)
		Rat	Rat	Munoz-Quiles et al. (2009)
				Takeoka et al. (2009)
Incomplete spinal injury				
Unilateral CST lesion	Not purified OB-OEG	Rat	Rat	Li et al. (1998)
Dorsal column transection	Purified OB-OEG	Pig	Rat	Ruitenberg et al. (2005)
		Rat	Rat	Imaizumi et al. (2000a,b)
				Toft et al. (2007)
	Purified plus NP	Rat	Rat	Wang et al. (2010)
Photochemical lesion	Purified OB-OEG	Rat	Rat	Verdu et al. (2001)
				Verdu et al. (2003)
				Garcia-Alias et al. (2004)
				Lopez-Vales et al. (2004)
	Plus COX-2 and iNOS	Rat	Rat	Lopez-Vales et al. (2006b)
Dorsal column transection	Purified OB-OEG	Rat	Rat	Ruitenberg et al. (2003)
				Sasaki et al. (2004)
				Sasaki et al. (2006b)
Hemisection				Polentes et al. (2004)
	Purified plus MP	Rat	Rat	Nash et al. (2002)
	Not purified plus: matrix produced by OEG	Rat	Rat	Li et al. (2003a)
Heat lesion	Not-purified OB-OEG	Rat	Rat	Keyvan-Fouladi et al. (2003)
		Dog	Dog	
Contusion	Purified OB-OEG	Rat	Rat	Plant et al. (2003)
				Jeffery et al. (2005)
				Wu et al. (2008)
	NT3-expressing OB-OEG			Ma et al. (2010)
	Purified plus SC, MP, IL-10	Rat	Rat	Pearse et al. (2004)
Dorsal funiculus crush	Purified OB-OEG	Rat	Rat	Andrews and Stelzner (2004)
				Andrews and Stelzner (2007)
Spinal cord compression				Amemori et al. (2010)
Demyelinating lesions				
	Purified OB-OEG	Human	Rat	Barnett et al. (2000)
		Dog	Rat	Smith et al. (2002)
		Pig	Monkey	Radtke et al. (2004)
		Rat	Rat	Akiyama et al. (2004)
				Sasaki et al. (2006a)
				Lankford et al. (2008)
	Not-purified OB-OEG	Human	Rat	Kato et al. (2000)
Other CNS lesions				
Thalamus	Purified OB-OEG	Rat	Rat	Perez-Bouza et al. (1998)
Optic nerve transection	Not purified plus: matrix produced by OEG	Rat	Rat	Li et al. (2003b)
Parkinson's disease model	Not purified plus NP	Rat	Rat	Agrawal et al. (2004)
				Johansson et al. (2005)
				Shukla et al. (2009)
Nigrostriatal transection	Not-Purified OB-OEG	Rat	Rat	Teng et al. (2008)
CA3 hippocampal lesion	Not purified plus NP	Rat	Rat	Srivastava et al. (2009)
PNS lesions				
Sciatic nerve	Purified OB-OEG	Rat	Rat	Verdu et al. (1999)
				Dombrowski et al. (2006)
	Not-purified OB-OEG	Rat	Rat	Radtke et al. (2008)
Olfactory nerve transection				Wei et al. (2008)

MP: methylprednisolone; NP: neural precursors; NSC: neural stem cells; OB-OEG: olfactory bulb ensheathing glia; SC: Schwann cells.

angiogenesis, immunomodulation, remyelination, neuroprotection, recovery of motor and sensory functions, and improvement of autonomic activity. A comprehensive state-of-the-art review about these OEG effects has been compiled in this Special Issue in several articles (Babiarz et al., 2011; Chuah et al., 2011; Higginson and Barnett, 2011; Mackay-Sim and John, 2011; Plant et al., 2011; Raisman et al., 2011; Wewetzer et al., 2011; Wu et al., 2011; You et al., 2011). The first variation of the initial treatment using adult OEG was published by Li et al in 1997 (Li et al., 1997). In their paradigm these authors administered adult OB-OEG in combination with fibroblasts to repair selectively injured corticospinal axons after electrolytic lesion, and this was the first contribution demonstrating the robustness of the finding.

Adult OB-OEG have been administered into the damaged cords as cell suspensions (Andrews and Stelzner, 2004; Barnett et al., 2000; Guest et al., 2008; Kato et al., 2000; Kubasak et al., 2008; Li et al., 1997, 1998; Lopez-Vales et al., 2006a,b; Munoz-Quiles et al., 2009; Negredo et al., 2008; Ramon-Cueto et al., 2000; Ramon-Cueto and Nieto-Sampedro, 1994; Ramon-Cueto et al., 1998; Sasaki et al., 2006a,b) as well as embedded in a matrix of their own production (Ibrahim et al., 2009a,b; Li et al., 2004, 2003a,b, 2007). In addition, several types of adult OB-OEG-based treatments have been developed and tested in the injured spinal cord with positive results. Purified OB-OEG have been combined with Schwann cell-filled guidance channels (Fouad et al., 2009, 2005; Ramon-Cueto et al., 1998; Vavrek et al., 2007). They have also been applied in grafts containing other cell types such as cells from the olfactory bulb and olfactory fibroblasts from the piamater (Ibrahim et al., 2009a,b; Imaizumi et al., 2000a,b; Kato et al., 2000; Keyvan-Fouladi et al., 2003; Li et al., 2004, 2003a,b; Li et al., 1998), neural progenitors, and stem cells (Amemori et al., 2010; Salehi et al., 2009; Wang et al., 2010). Animals with spinal cord injury have been treated with adult OB-OEG transplants in combination with drugs such as methylprednisolone (Nash et al., 2002), methylprednisolone and IL-10 (Pearse et al., 2004), fibrinogen (Plant et al., 2003), chondroitinase ABC (Fouad et al., 2009; Fouad et al., 2005; Vavrek et al., 2007) and COX2 inhibitors and iNOS (Lopez-Vales et al., 2006a,b). Also, genetically-modified adult OB-OEG expressing BDNF (Ruitenberg et al., 2003), NT-3 (Ma et al., 2010; Ruitenberg et al., 2005; Wu et al., 2008), and GDNF (Cao et al., 2004) have been transplanted into injured spinal cords. Adult OB-OEG from H-transferase transgenic pigs have also been transplanted (Imaizumi et al., 2000a,b; Radtke et al., 2004).

There are also reports about the efficacy of adult OB-OEG in other injured regions of the CNS as well as in the PNS (Table 2). These cells promoted regeneration of sectioned axons in the fimbria fornix (Smale et al., 1996), the thalamus (Perez-Bouza et al., 1998), the optic nerve (Li et al., 2003a,b) and the nigrostriatal pathway (Teng et al., 2008). In the PNS, they promoted axonal regeneration and functional improvement after resection of the sciatic nerve (Dombrowski et al., 2006; Li et al., 2010; Radtke et al., 2009, 2010; Verdu et al., 1999) and olfactory nerve lesion (Wei et al., 2008). Adult OB-OEG have effectively been used in other animal models of CNS damage such us cognitive dysfunction by lesioning hipppocampal CA3 (Srivastava et al., 2009), intracerebral haemorrhage (Tang et al., 2010), and Parkinson's disease (Agrawal et al., 2004; Johansson et al., 2005; Shukla et al., 2009).

In conclusion, spinal cord injury repair by adult OB-OEG is a robust treatment option with positive results using numerous different techniques and therapeutic approaches. Moreover, promotion of axonal regeneration by OB-OEG extents to other CNS regions and also the PNS, providing further evidence of the robustness of this therapeutic strategy. The therapeutic potential of adult OB-OEG transplants is not just for spinal cord injuries; its beneficial properties can also be used in other nervous system disorders, thus opening the door for treating a myriad of different pathological conditions.

Demonstration of the fundamental nature of the therapeutic effect of adult OB-OEG on injured spinal cords

The beneficial histological and functional effects of adult OB-OEG have been demonstrated in a broad number of spinal cord injury animal models (Table 2). These cells have been proven to be effective after dorsal rhizotomy at thoracic, lumbar and cervical levels (Gomez et al., 2003; Li et al., 2004; Navarro et al., 1999; Pascual et al., 2002; Ramon-Cueto and Nieto-Sampedro, 1994) and ventral root avulsion (Ibrahim et al., 2009a,b; Li et al., 2007), after unilateral electrolytic lesion (Li et al., 1997, 1998) or transection (Ruitenberg et al., 2005) of the corticospinal tract, after complete removal of one spinal cord segment at T8 (Fouad et al., 2009; Fouad et al., 2005; Ramon-Cueto et al., 1998; Vavrek et al., 2007), after complete spinal cord transection at T8–T9 (Cao et al., 2004; Guest et al., 2008; Kubasak et al., 2008; Munoz-Quiles et al., 2009; Ramon-Cueto et al., 2000; Wang et al., 2010), after dorsal column transection at T9, T11 (Imaizumi et al., 2000a,b; Sasaki et al., 2006a,b, 2004; Toft et al., 2007) and C2–C4 (Nash et al., 2002; Ruitenberg et al., 2003), after dorsal funiculus crush at T8–T9 (Andrews and Stelzner, 2004; Andrews and Stelzner, 2007), after hemisection at the cervical level (Li et al., 2003a,b; Polentes et al., 2004), after contusion at thoracic level (Ma et al., 2010; Pearse et al., 2004; Plant et al., 2003; Wu et al., 2008), after photochemical injury at T12-L1 (Garcia-Alias et al., 2004; Lopez-Vales et al., 2006a,b; Verdu et al., 2001, 2003), after unilateral heat lesion at C1 (Keyvan-Fouladi et al., 2003), after demyelinating lesions of the dorsal columns at the thoracic level (Akiyama et al., 2004; Barnett et al., 2000; Kato et al., 2000; Lankford et al., 2008; Radtke et al., 2004; Sasaki et al., 2006a,b; Smith et al., 2002).

There is controversy about whether, in the case of spinal cord injury, primates should be used to corroborate the findings observed in rodent models (Courtine et al., 2007). But what seems clear is that in order to demonstrate the fundamental nature of a specific experimental therapy in other species the beneficial effects should be corroborated in at least two species. In the case of adult OB-OEG for spinal repair, there are two aspects to be examined: (i) the appropriateness of OB-OEG cultures for cell therapy and, (ii) the efficacy of OB-OEG grafts for spinal cord injury repair. The preservation of axonal-growth promoting properties, the reliability and safety of adult OB-OEG cultures for cell therapy have been confirmed in dogs (Krudewig et al., 2006; Techangamsuwan et al., 2008), non-human primates (Guest et al., 2008; Rubio et al., 2008) and humans (Barnett et al., 2000; Lim et al., 2010; Miedzybrodzki et al., 2006). In addition, adult OB-OEG from different species have been transplanted into the spinal cords of the same or other species and have effectively promoted histological repair and functional improvement. Adult porcine OB-OEG grafts fostered the regeneration and remyelination of transected dorsal funiculus axons in rodents (Imaizumi et al., 2000a,b). Moreover, these cells have also remyelinated the dorsal columns after transplantation into the spinal cords of non-human primates (Radtke et al., 2004). Similarly, canine adult OB-OEG grafted into rodent injured spinal cords promoted histological repair (Smith et al., 2002). These cells have also been autologously transplanted into paraplegic dogs (Jeffery et al., 2005). The efficacy of non-human primate and human adult OB-OEG for spinal cord repair has also been demonstrated. The former were transplanted into nude rodents with complete spinal cord transection and the animals obtained functional recovery (Guest et al., 2008). Human OB-OEG, grafted into the demyelinised spinal cords of rats, promoted remyelination of their dorsal columns (Barnett et al., 2000; Kato et al., 2000).

In conclusion, the healing effect of adult OB-OEG is consistent and repeatable in different injury models of spinal cord injury and also in different species, which demonstrates the fundamental nature of this therapeutic intervention.

Demonstration of the relevance of adult OB-OEG to human application

The human condition should be mimicked in order to demonstrate the real prospect of an experimental therapy for clinical application. In the case of cell-based therapies for spinal cord injury repair the following situations should be reproduced in animal models: (i) the type of injury; in humans the most frequent is the contusion; (ii) the reliability and safety of the cells; (iii) timing of the experimental treatment ("window opportunity"); (iv) the surgical procedures should be compatible with human practice; and v) the functional outcome measure should be similar to those used in humans.

Type of injury

Traumatic spinal injuries are the most appropriate model mimicking the human condition and, among these, crushes or contusions are the most frequent. The efficacy of adult OB-OEG grafts, with no other accompanying treatment, has been demonstrated after traumatic transection of axons leading to either complete (Cao et al., 2004; Guest et al., 2008; Imaizumi et al., 2000a,b; Kubasak et al., 2008; Munoz-Quiles et al., 2009; Ramon-Cueto et al., 2000, 1998; Takeoka et al., 2009) or incomplete (Li et al., 2003a,b; Polentes et al., 2004; Ruitenberg et al., 2005, 2003; Sasaki et al., 2006a,b, 2004; Toft et al., 2007) spinal cord injury, and also after crush (Sasaki et al., 2004; Andrews and Stelzner, 2007) and after contusion (Ma et al., 2010; Pearse et al., 2004; Plant et al., 2003; Wu et al., 2008). In all these paradigms, adult OB-OEG transplants promoted the regeneration of injured axons and functional recovery of the animals.

Reliability and safety of the cells

The reliability of adult olfactory bulbs as a source of OEG has been tested in non-human primates and in humans. These cells preserve the typical OEG phenotypic properties and do not become senescent for up to 2.5 months *in vitro* and up to 34 passages (Barnett et al., 2000; Lim et al., 2010; Miedzybrodzki et al., 2006; Rubio et al., 2008; Techangamsuwan et al., 2008). Moreover, the culture conditions do not alter the ability of adult human OB-OEG to promote axonal growth of CNS axons *in vitro* (Lim et al., 2010) and repair after transplantation (Barnett et al., 2000; Kato et al., 2000). Primate and human adult OB-OEG do not spontaneously immortalize or transform for up to 6 months in culture, which guarantees safety of the cultured cells for at least that period (Lim et al., 2010; Rubio et al., 2008; Techangamsuwan et al., 2008). During the non-senescent period, adult OB-OEG divide *in vitro* at a normal rate (approximately 2.5 population doublings per week) and then they started slowing down and stop dividing after the fourth month *in vitro*, at which point they become senescent. One single primate olfactory bulb provides, in a short term (three weeks), around one and half million cells, and during the pre-senescent period (2.5 months) an additional 20 billion (Rubio et al., 2008). Thus, just one bulb yields enough number of OB-OEG for transplantation into the same individual (autologous) and for storage for future use in other patients. Autologous grafting should be the option for safety reasons and best graft integration. However, in the case this is not possible and the cells had to be administered heterologously, the patients should be treated with immunosuppressants to avoid graft rejection.

The preservation of the properties of adult OB-OEG after long-term culture (up to 2.5 months), the cessation of division, and the absence of spontaneous immortalization, support that adult olfactory bulbs represent a reliable and safe source of OB-OEG for cell therapy. In addition, none of the *in vivo* studies reported any side effect of OB-OEG after transplantation (Table 2). Moreover, transplants of either non-human primate or human adult OB-OEG into injured spinal cords did not cause any sign of neoplastic transformation or any side effect in the paraplegic host (Barnett et al., 2000; Guest et al., 2008; Kato et al., 2000). Therefore, the pre-clinical safety of OB-OEG has already been established *in vitro* and *in vivo* not only for rodent cells, but also for non human primate and human cells.

Compatibility of timing, surgical procedures and functional assessment

In the case of spinal cord injuries, the most probable situation is a poly-traumatized patient that requires urgent stabilization after the accident. Consequently, a cell-based therapy administered at the acute stage does not seem realistic because this may not be safe for the patient. Given that many patients take several weeks or even months to stabilize, a delayed transplant will be more practical. In addition, if autologous transplantation is the choice, cell preparation requires time. Hence, an intervention several months after spinal cord injury seems more realistic. However, after the third month post-injury, the histopathology of the spinal cord is considered chronic because, with the absence of further manipulation, no more cellular or molecular changes occur (Hill et al., 2001; Velardo et al., 2004). Thus, the most clinically relevant model of the human condition is the application of the graft at either subacute or chronic stages of the injury. The efficacy of adult OB-OEG for spinal cord injury repair has been demonstrated at both subacute and chronic stages after traumatic injury, including contusion (Lopez-Vales et al., 2007; Munoz-Quiles et al., 2009; Plant et al., 2003). After 2 weeks *in vitro* the cells obtained from just one adult bulb yield enough OB-OEG not only for transplantation but also for storage (Rubio et al., 2008). Therefore, a therapy using adult OB-OEG satisfies an adequate "window of opportunity" required for a therapeutic application in patients with spinal cord injury. In addition, a surgical procedure compatible with human practice for the transplantation of OB-OEG grafts into injured spinal cords has already been developed in rodents (Kubasak et al., 2008; Munoz-Quiles et al., 2009; Ramon-Cueto et al., 2000, 1998) and later tested in primates for safety and feasibility (Santos-Benito et al., 2006). The surgical procedure to obtain one olfactory bulb was designed in non-human primates (tested in nine *macaque* monkeys) using methodology compatible with humans (Rubio et al., 2008). In two of the monkeys, the surgeries were performed in a surgery room fully equipped and similar to those of human use and two neurosurgeons confirmed the appropriateness of this methodology for humans (unpublished). The functional outcomes obtained after adult OB-OEG transplantation have also been measured with assessments similar to those used in humans, which include electrophysiological recordings and kinematic analyses. Functional regeneration of root axons was demonstrated by measuring nerve conduction and spinal reflexes (H response and withdrawal reflex) evoked by stimulation of afferents of the sciatic nerve (Navarro et al., 1999). Functional recovery after incomplete spinal cord injury was measured by motor- and somatosensory-evoked potentials (Garcia-Alias et al., 2004; Toft et al., 2007; Verdu et al., 2003). In the case of complete spinal cord transection, the recovery of motor function was assessed using kinematic analyses (Kubasak et al., 2008), motor-evoked potentials and electromyography during motion (Ziegler et al, submitted). In conclusion, the demonstration of the beneficial effects of adult OB-OEG in injuries mimicking the human condition, the demonstration of the appropriateness of these cells for cell therapy, and the use of procedures and outcome measures which are compatible with human practice, confirm the relevance of this therapeutic approach to human application.

Therefore, the use of adult OB-OEG for spinal cord injury repair meets the ICCP validation pathway required for a proper translation of any pre-clinical strategy to human application.

Autologous OB-OEG transplantation for neural repair

The safest scenario for a cell-based therapy occurs when patients receive their own non-modified differentiated cells. In this case, the risk of graft rejection is eliminated, the possibility of cell transformation is reduced, and there is no need to treat patients with immunosuppressant drugs throughout their lives. A cell-based

autologous therapy requires previous experimental demonstration on the effectiveness, innocuousness, and availability of the cells intended for grafting. However, these experimental studies should be carried out in cells having the same age as the patients for whom the experimental treatment is being investigated. The incidence of traumatic and demyelinating lesions of the nervous system is higher in young adults and adults. Thus, to evaluate whether OB-OEG can be applied autologously for neural repair their efficacy, safety and availability should be verified using adult cells.

In the previous section we provided evidence about the beneficial effects exerted by adult OB-OEG grafts on a variety of nervous system injuries, with broader documentation on spinal cord repair (Table 2). The feasibility of autologous transplantation using OB-OEG has already been demonstrated in paraplegic dogs (Jeffery et al., 2005). None of the studies using adult OB-OEG for neural repair have reported any undesired effect of OB-OEG grafts or any complication in the transplanted animals. Adult OB-OEG are naturally located in the CNS and they are terminally differentiated cells with the role of supporting axonal elongation within the olfactory bulb. Thus, it is reasonable that these cells integrate properly within the host parenchyma after transplantation. In fact, they infiltrate the lesion site, interact favorably with astrocytes and are able to migrate (Fairless and Barnett, 2005; Lakatos et al., 2003a,b, 2000; Ramon-Cueto et al., 2000). The repair properties of adult OB-OEG were not altered after delaying transplantation of these cells at either subacute (Lopez-Vales et al., 2007; Munoz-Quiles et al., 2009; Plant et al., 2003) or chronic stages of spinal cord injury (Munoz-Quiles et al., 2009). This time frame provides enough time for patient stabilization before surgery and for OEG preparation, thus making an autologous transplantation a highly feasible approach. OB-OEG can be obtained from the olfactory bulbs of adult primates including humans and their reliability, safety and availability for cell therapy has already been reported (described in 4.4.2). Moreover, adult primate OB-OEG, including human, can be cultured in medium containing just serum (Rubio et al., 2008) (Fig. 1) which, in turn, guarantees a complete autologous therapy as the serum can be obtained from the patient. As these cells are not sensitive to hyperoxic culture conditions, grafts can be prepared in standard incubators, facilitating cell preparation for transplantation.

Other important issues to be addressed in patients with spinal injuries are the safety of the surgical procedures in them and the appropriate moment for the interventions. In the case of autologous cell therapies, patients have to receive two surgeries: one to obtain the cells and another to graft them into the spinal cord. For any patient (either with or without spinal injuries) both surgeries have to reach safety standards and should preferably be minimally invasive. Accordingly, we have developed minimally invasive procedures for bulb removal and OEG injection and tested their safety in individuals closely related to humans, such as *Macaca mulatta* monkeys (Rubio et al., 2008; Santos-Benito et al., 2006). The latter was carried out in paraplegic monkeys, thus, demonstrating safety of the stereotaxic procedure to inject cells in this clinical condition (Santos-Benito et al., 2006). The methodology for unilateral bulbectomy was also carried out in human cadavers to determine feasibility (unpublished). The olfactory bulb is a structure located far from the site of injury, does not interfere with basic spinal cord functions and, during the surgical procedure for its extraction, there is no manipulation of the neck, thorax or abdomen of the patient. Hence, in patients with spinal cord injuries this surgery can be carried out with the patient completely stabilized and with less risk of destabilization. Moreover, the olfactory bulb is a very accessible structure allocated in a groove at the cranial base, medial to the orbit, separated from the brain, and covered by its own duramater sack. For the extraction there is no need to open the duramater of the brain and thus, no need to perform intracranial surgery (Rubio et al., 2008). We have already obtained OB-OEG from living patients (Fig. 1), and hence, harvesting of OB-OEG is feasible.

In patients with spinal cord injuries the most appropriate and safest moment for an operation is once their clinical condition is stable and not immediately after the accident. This usually occurs after the third month post-injury. Hence, the two surgeries associated with any cell-based autologous therapies are recommended beyond this point, what implies that the cells should be effective at this time post lesion. We demonstrated that there was no efficacy decay when OB-OEG were transplanted four months after injury (Munoz-Quiles et al., 2009). Therefore, bulbectomy, OB-OEG culture and OEG transplantation, can be carried out after the third month and once the patients are already stabilized.

Concluding remarks

OB-OEG-based therapies may or may not be defined as Medicinal Products. In the case they are, they have to be validated and approved by an appropriate regulatory agency before human application. Grafts consisting of suspensions of purified adult OB-OEG used for the promotion of axonal regeneration in the CNS do not comply with the definitions provide by the European Medicines Agency to be considered a Medicinal Product. Other OB-OEG-based therapies used in combination with other cell types, drugs, products or devices, or therapies using genetically-modified OB-OEG shall be considered Medicinal Products.

Pre-clinical studies using adult OB-OEG for neural repair have corroborated the positive results obtained with these cells and have demonstrated the robustness of the findings, the fundamental nature of the intervention, and the relevance of the results to humans. More importantly, OB-OEG can be used autologously for neural repair, offering a safe approach for patients. As a result, the use of adult OB-OEG for spinal cord injury repair meets the ICCP validation pathway required for a proper translation of any pre-clinical strategy to human application.

Acknowledgments

We thank Ms Yasmin Ebrahimi Rahmani from our laboratory for her contribution to improve the grammar and syntaxes of the article and Prof Josefina Alventosa del Rio from the Department of Civil Law at the University of Valencia, for supervising the European Regulation reflected in the present article. We are grateful to the neurosurgeon Dr. Juan Barcia for obtaining for us the human olfactory bulb shown in Fig. 1A. The preparation of this article was supported by Fundación Investigación en Regeneración del Sistema Nervioso (IRSN).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.expneurol.2010.10.001.

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