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Olfactory Neural Cells: An Untapped Diagnostic and Therapeutic Resource

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Objective: **This is an overview of the cellular biology of upper nasal mucosal cells that have special characteristics that enable them to be used to diagnose and study congenital neurological diseases and to aid neural repair.** ***Study Design:*** **After mapping the distribution of neural cells in the upper nose, the authors' investigations moved to the use of olfactory neurones to diagnose neurological diseases of development, especially schizophrenia. Olfactory-ensheathing glial cells (OEGs) from the cranial cavity promote axonal penetration of the central nervous system and aid spinal cord repair in rodents. The authors sought to isolate these cells from the more accessible upper nasal cavity in rats and in humans and prove they could likewise promote neural regeneration, making these cells suitable for human spinal repair investigations.** ***Methods:*** **The schizophrenia-diagnosis aspect of the study entailed the biopsy of the olfactory areas of 10 schizophrenic patients and 10 control subjects. The tissue samples were sliced and grown in culture medium. The ease of cell attachment to fibronectin (artificial epithelial basement membrane), as well as the mitotic and apoptotic indices, was studied in the presence and absence of dopamine in those cell cultures. The neural repair part of the study entailed a harvesting and insertion of first rat olfactory lamina propria rich in OEGs between cut ends of the spinal cords and then later the microinjection of an OEG-rich suspension into rat spinal cords previously transected by open laminectomy. Further studies were done in which OEG insertion was performed up to 1 month after rat cord transection and also in monkeys.** ***Results:*** **Schizophrenic patients' olfactory tissues do not easily attach to basement membrane compared with control subjects, adding evidence to the theory that cell wall anomalies are part of the schizophrenic "lesion" of neurones. Schizophrenic patient cell cultures had higher mitotic and apoptotic indices compared with control subjects. The addition of dopamine altered these indices enough to allow accurate differentiation of schizophrenics from control patients, leading to, possibly for the first time, an early objective diagnosis of schizophrenia and possible assessment of preventive strategies. OEGs from the nose were shown to be as effective as those from the olfactory bulb in promoting axonal growth across transected spinal cords even when added 1 month after injury in the rat. These otherwise paraplegic rats grew motor and proprioceptive and fine touch fibers with corresponding behavioral improvement.** ***Conclusions:*** **The tissues of the olfactory mucosa are readily available to the otolaryngologist. Being surface cells, they must regenerate (called "neurogenesis"). Biopsy of this area and amplification of cells in culture gives the scientist a "window to the developing brain," including early diagnosis of schizophrenia. The "Holy Grail" of neurological disease is the cure of traumatic paraplegia and OEGs from the nose promote that repair. The otolaryngologist may become the necessary partner of the neurophysiologist and spinal surgeon to take the laboratory potential of paraplegic cure into the day-to-day realm of clinical reality.** ***Key Words:*** **Olfactory neurones, olfactory ensheathing glial cells, paraplegia cure, schizophrenia diagnosis prevention.**

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INTRODUCTION

Thank you very much for the honor and privilege of presenting the Ogura Lecture for the Triological Society for the year 2000. Joe Ogura died in 1983 when I was in my second year of advanced surgical training in otolaryngology. I had been a doctor for 6 years. Although new to the field of otolaryngology, I knew all about Joe, his influence as a surgeon and teacher and his role in the development of partial laryngeal surgery. This article concerns nasal physiology and anatomy, the diagnosis of schizophrenia and perhaps other neurological diseases, and the treatment of nerve injuries and traumatic paraplegia.

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This, on the surface, does not appear to have much in common with Dr. Ogura's academic interests. However, by the end of this article, I hope you will share the same wonder and excitement that I have in this research and that you will understand the critical role of the otolaryngologist in this research and probably in the future diagnosis and treatment of a wide array of neurological conditions. I hope this research reflects something of the ideals of scholarship Dr. Ogura promoted.

The olfactory nerve or bulb is more an outgrowth of the brain than a cranial nerve. It is totally within the cranial cavity, receiving 20 bundles or fascicles of nerves surrounded by dura mater, pia, and arachnoid from the foramina of the cribriform plate. The periosteum of the ethmoid is continuous with this dura mater around these fascicles. The olfactory mucosa is classically described as occupying the upper 1 cm of the nasal cavity. This mucosa has Bowman's glands with olfactory neurones between them sending a dendrite to the surface and an axon centrally surrounded by electrically insulating olfactory-ensheathing glial cells. Between the neurones, on the basement membrane of the olfactory mucosa, sit immature neurones and stem cells. The axons of the olfactory neurones synapse centrally in the glomeruli of the olfactory bulb and are therefore, at least partially, within the brain.

Our studies of human olfactory mucosa[1] confirm that olfactory mucosa is present in the upper 1 cm of the nose. However, there is no clear line of demarcation between respiratory and olfactory mucosa. Rather, there is a fine patchwork of intermingling microscopic areas of respiratory and olfactory mucosa that are mostly olfactory in the upper zones but become respiratory the further down the nose you biopsy. There is a concentration of olfactory mucosal zones in the front of the middle turbinate but the higher and further back the biopsy is taken, the more likely one will find larger areas of olfactory mucosa present. Remarkably, olfactory mucosa is also found more anteriorly and inferiorly than previously appreciated, suggesting that it is present as a patchwork of microscopic sites over large areas of the septum and lateral wall.

Olfactory neural cells are the only surface neural cells of the body and must therefore withstand wear and tear and be able to replicate and regenerate. As said previously, part of these neurones is within the meninges and cranial cavity. At least some of the ultrastructural elements of the olfactory neurones, for example, β-tubulin3 and microtubule-associated protein-5, are otherwise only found in embryonal brain cells.

The growth and replication of neural cells is termed "neurogenesis." It is of great interest that after the age of 51/2 years, the areas of the mammalian brain known to undergo neurogenesis are areas associated with olfaction, the olfactory bulb, the hippocampus, and areas adjacent to the upper lateral ventricle. Cells that are produced near the lateral ventricle stream into the olfactory bulb, at least in laboratory animals. The flow of these cells in humans has not been established, but a recent study published in *The Proceedings of the National Academy of Sciences of the United States of America*[2] has demonstrated volume changes of the adult human hippocampus, which in its posterior segment has a function associated with spatial memory. This hippocampal growth occurs in London taxi drivers. The authors found large hippocampi in these taxi drivers and the longer a person has been driving taxis, the bigger the hippocampus becomes.

In the rat, mature neurones may be identified with antibodies to the olfactory marker protein (OMP), while immature neurones are identified with an antibody to growth-associated protein-43. In the rat, if the fascicles of the olfactory bulb are cut just above the cribriform plate, the mature neurones on the olfactory area will die. Immature neurones will send dendrites to the mucosal surface and axons centrally to the olfactory bulb, surrounded by olfactory-ensheathing glia. They will cross the cribriform plate and scar tissue and then these axons will implant into glomeruli within the olfactory bulb. Although this mass movement of axons may not result in implantation into exactly the correct glomerulus, it has recently been demonstrated that with lesser degrees of axonal regeneration, the penetrating reparative axon is highly accurate in finding the appropriate glomerulus to register the smell that that neurone is coded to register.[3]

Thus, the olfactory mucosa contains neurones at least partially present within the brain. The neurones have some features and structures normally associated with the embryonal brain. The axons of olfactory nerve-ensheathing glia are able to penetrate scar tissue and the central nervous system. This mucosa is readily available to the otolaryngologist to biopsy and study. Can this area be a window to brain disease, especially diseases of neural development? Can the central nervous system-penetrative properties of olfactory nerve-ensheathing glia be recruited to allow repair of central nervous system lesions? Our studies indicate they can.

Diagnostic Resources in the Upper Nose

Schizophrenia is the most common psychosis, affecting approximately 1% of the population. There is undoubtedly a genetic component to the onset of this condition, although inheritance is probably polygenetic. The risk of disease is higher in affected families with a risk of approximately 50% if a monozygotic twin has the disease. Risk factors include several perinatal environmental variables such as mother's nutrition, winter birth, and city versus country childhood. Schizophrenics often have physical characteristics of a high-arched palate and other minor physical anomalies, suggesting factors operate during prenatal development. They are frequently strangely behaving young teenagers with few friends and then develop hallucinations and full-blown schizophrenia in the 18- to 25-year age group. It is now generally accepted that the brain lesion of schizophrenia starts early and is therefore probably a lesion involving the brain during the prenatal period. If olfactory neurones are similar to brain tissue and indeed have characteristics of a developing brain, can cellular biology techniques differentiate the olfactory neurones of the patient with schizophrenia from the normal control?

Our group reported on a small study of schizophrenics and normal control subjects whose olfactory neurones were grown in the laboratory from upper nasal biopsies

taken under local anesthetic as an office procedure.[4] Biopsy samples were sliced into 200-μg thicknesses and grown on fibronectin-coated glass slides immersed in a serum-free medium containing antibiotics and growth factors.

The biopsies of schizophrenic patients do not attach to fibronectin, a component of the normal epithelial basement membrane. Normal control biopsies in culture attached within 1 to 2 days; however, biopsies from schizophrenic patients would often not attach even after multiple attempts over 14 days after being taken. These differences in attachment were statistically significant. Additionally, there were statistically significantly more mitoses in the schizophrenics' cultures and a nonsignificant trend to more cell death or apoptoses. However, when dopamine was added to the culture, and most schizophrenic drugs are dopamine receptor blockers, there were marked differences between mitoses and apoptoses in the schizophrenic and control populations. Dopamine reduced the number of mitoses in both groups but decreased apoptoses in schizophrenics, whereas it increased apoptoses in control subjects. These were constant features and allow differentiation of the schizophrenic group from the control group.

Thus, a nasal biopsy has the potential to provide an objective and additional sign for the differential diagnosis of schizophrenia, possibly long before other firm diagnostic criteria become evident. Obviously the study, being only recently published in December 1999,[4] needs to be confirmed, and we are currently doing a second study. Early diagnosis of schizophrenia is important because early intervention with medication leads to a better prognosis and milder continuing symptoms.

With the olfactory area being a "window to the brain," other neurological diseases such as Alzheimer's disease or multiple sclerosis may, like schizophrenia, manifest themselves in olfactory tissues readily available for study in the upper nose.

Therapeutic Resources in the Upper Nose

The "Holy Grail" of neurobiology is the cure of traumatic paraplegia, and the olfactory mucosa may have a vital role to play.

Our group, and our collaborators at the Spinal Injury Research Unit at the University of New South Wales Department of Anatomy and at the Fourth Military Medical University, Xian, China, have been studying spinal cord regeneration using olfactory-ensheathing glial cells (OEGs) from the upper nasal cavity.[5] OEGs have been postulated to promote axon penetration of the central nervous system and even scar tissue within it. OEGs have both Schwann cell and glial cell characteristics. They are able to form myelin sheaths and promote axonal growth like Schwann cells. They can reside in both the central and peripheral nervous systems and penetrate an astrocyte-rich and oligodendroglial-rich central nervous system, which normally secretes compounds inhibitory to axonal penetration. For example, oligodendroglial cells secrete "Nogo," a 1200 amino acid protein that is inhibitory to axon growth, whereas astrocytes have been shown to secrete a number of similarly inhibitory compounds.[6,7]

A study of the literature to date highlights the pioneering work of Ramon-Cueto. Her 1994 experiment[8] involved the division of the dorsal sensory nerve root close to the spinal cord of the laboratory rat. The dorsal nerve root was rejoined to the spinal cord using microsurgical techniques. In some of these anastomoses, a suspension of OEG cells taken from allograft donor rats was injected into multiple sites on either side of the cut. In those animals, OEG implantation facilitated the regrowth of the sensory axons into the dorsal horn from the severed dorsal root. Similar regrowth was absent in the controls. However, there was no behavioral improvement in the denervated areas of these rats.

Ramon-Cueto's 1998 study[7] entailed a laminectomy in 300-g Wistar white rats and excision of 4 mm of spinal cord in the T8–T10 segments. A 6-mm plastic tube containing a medium rich in Schwann cells was placed as a bridge between the cut ends and a suspension of OEGs microinjected into the cut ends of the cord. In this study, OEG grafts facilitated axonal penetration across the plastic tube and gel bridge and into the distal spinal cord stump. The regenerating axons tended to cross peripherally and probably did not join appropriate tracts on the opposite side. Once again, there was no behavioral improvement in the OEG injected rats; they remained paraplegic.

Two further studies from 1998 are worth reviewing. Studies of Li et al.[9] from London and Imaizumi from Yale[10] showed that OEG transplants could facilitate remyelination after localized lesions in the corticospinal tracts and dorsal columns.

The most exciting study to date on the axonal regeneration properties of OEGs was once again performed by Ramon-Cueto and published in February 2000.[11] Here, rats were made paraplegic by laminectomy and open, complete transection of the spinal cord at T8. A suspension of OEGs was made from olfactory bulbs and injected into the cut ends of the spinal cord. OEG grafts stimulated regrowth of axons across the scar and in some rats significant behavioral improvement occurred, including coordinated locomotion, weight bearing, touch, and proprioceptive functions. This study was hailed as a milestone in paraplegia cure.

In 1999, our collaborators similarly cut the spinal cords of 18 rats by open laminectomy at T10. Half of these animals had pieces of donor rat olfactory mucosal lamina propria inserted between the cut ends and half had respiratory mucosa lamina propria inserted. The lamina propria of the olfactory area is rich in OEGs. Another group of six similarly transected rats received injections into the injured cord of OEGs purified from olfactory lamina propria. A further six received saline injections. These rats were killed at 8 to 10 weeks after surgery, compared with 7 months in Ramon-Cueto's series. Even after only 10 weeks recovery, there was significant behavioral improvement in the nasal OEG-grafted rats, whereas the controls were left completely paraplegic.

Histologic cross-sections of the sectioned spinal cord showed the formation of a syrinx commonly found in human paraplegia. The OEGs entered the spinal cord rostrally and caudally and descending motor axons penetrated the distal spinal cord. Fluororuby placed caudally

was identified in brainstem neuronal cell bodies, and serotonin projections from the brainstem were identified distal to the transection site. Therefore, it appears that OEGs could be harvested from a patient's own upper nasal mucosa and be used to promote healing and axonal penetration of spinal cord lesions, at least in rats. This exciting breakthrough needs to be extended toward humans.

From Rats to Humans

Previous studies in rats have universally used OEGs harvested from the embryonic or adult olfactory bulb, which is a rich source of these cells, but the harvesting of OEGs from the olfactory bulb is not practical in humans. It requires an open craniotomy with all the attendant morbidity and mortality. It would involve significant reduction or loss of the sense of smell in a human patient who is already paralyzed from the waist down from previous multi-trauma, which may have already affected the sense of smell. With loss of mobility and possibly sexual function associated with paraplegia, the sense of smell enhances the enjoyment of food and beverages and is a major part of the enjoyment of life for the paraplegic patient. Endangerment of life with a craniotomy and endangerment of the sense of smell by the harvesting of olfactory bulb tissues makes OEGs harvested from olfactory bulbs difficult to study in the human clinical experimental situation. The harvesting of the same cells from a regenerating upper nose under local anesthetic by biopsy is a more feasible and justifiable proposition for further experiments.

We have been studying the upper nasal OEGs in normal subjects undergoing routine nasal surgery. As expected, OEGs become more numerous the higher up one goes in the nasal submucosa. Human OEGs are more densely intertwined and more difficult to separate than those of rats. Neurones and mucosal epithelial cells can be separated off the lamina propria containing OEGs by enzymatic dissolution of the basement membrane. Numerous cocktails of collagenases and proteases have been used to dissociate the OEG cells into a suspension. Techniques of immuno-panning and binding magnetic beads with anti-p75 receptor antibody, which selectively bind to OEGs, have allowed the separation and purification of these cells. We have been able to grow OEGs in culture, and by using a serum-free medium we have been able to kill fibroblasts and macrophages while keeping an OEG culture alive and amplifying.

An experiment on monkeys in collaboration with surgeons at the Fourth Military Medical University in Xian (unpublished observations) demonstrated the difficulty of using a primate species in transit to humans. Young Macaque monkeys (*Macaca mulatta*) were anesthetized and underwent a laminectomy and spinal cord hemisection at T11. A hemisection was chosen as the most humane and ethical method and the most technically feasible because the animals retained bladder and bowel control and some mobility. Before this, biopsies had been taken from the olfactory mucosa and the lamina propria containing OEGs was isolated. Small pieces of lamina propria were then placed in the hemisected spinal cord. Five months later, the two OEG-grafted animals were walking normally. A control monkey was hemisected but received no graft. This animal also walked normally. The two experimental animals were re-anesthetized and a second hemisection was made at T9 either contralaterally or ipsilaterally to the first cut. Two months later, both animals had recovered. Histologic and immunochemical assessment of all four lesions in these animals showed nerve fibers crossing the lesion sites, those with and those without lamina propria grafts.

Thus to Humans

The microinjection of a pure OEG autograft suspension in the paraplegic human is feasible without excessive risk of harm. Experiments on the usefulness of these cells should be able to begin shortly. Because crush and devitalization lesions are much more common in humans, OEGs would probably be needed to be instilled over a wide area of the spinal lesion. The remyelination properties of OEGs in spinal injuries raise interesting possibilities. The property of the promotion of axonal penetration of OEGs is ready for human study, especially autograft OEGs harvested by a minimally invasive technique in the constantly regenerating upper nose.

CONCLUSION

The olfactory mucosal cells, the neurones, and the olfactory-ensheathing glia offer remarkable possibilities in the diagnosis of neurologic and psychiatric disorders and in the healing of central nervous system lesions. Within the near future, the otolaryngologist and neurophysiologist may be the necessary partners of the psychiatrist and spinal surgeon to bring this experimental evidence for neurologic diagnosis and paraplegia cure into the realm of clinical, everyday reality.

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BIBLIOGRAPHY

1. Feron F, Perry C, McGrath J, Mackay-Sim A. New techniques for biopsy and culture of human olfactory epithelial neurones. *Arch Otolaryngol Head Neck Surg* 1998;124: 861–866.
2. Maguire E, Gadian D, Johnsrude I, et al. Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci USA* 2000;97:4398–4403.
3. Gogos J, Osbourne J, Nemes A, et al. Genetic ablation and restoration of the olfactory topographic map. *Cell* 2000; 103:609–620.
4. Feron F, Perry C, Herning M, McGrath J, Mackay Sim A. Altered adhesion, proliferation and death in neural cultures from adults with schizophrenia. *Schizophr Res* 1999; 40:211–218.
5. Lu J, Feron F, Ho S, Mackay-Sim A, Waite P. Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats. *Brain Res* 2000;889:344–357.
6. Chen M, Huber A, Van Der Haar M, et al. Nogo-A is a

myelin-associated outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* 2000;403:434–438.
7. Ramon-Cueto A, Plant G, Avila J, Bunge M. Long distance axonal regeneration in the transected adult rate spinal cord is promoted by olfactory ensheathing glia transplants. *J Neurosci* 1998;10:3803–3815.
8. Ramon-Cueto A, Nieto-Sampedro M. Regeneration to the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. *Exp Neurol* 1994;127: 232–244.
9. Li Y, Field P, Raisman G. Regeneration of rat corticospinal axons induced by transplanted olfactory ensheathing cells. *J Neurosci* 1998;24:10514–10524.
10. Imaizumi T, Lankford K, Waxman S, Greer C, Kocsis J. Transplanted olfactory ensheathing cells remyelinate and enhance axonal conduction in the demyelinated dorsal columns of the rat spinal cord. *J Neurosci* 1998;16: 6176–6185.
11. Ramon-Cueto A, Cordero M, Santos-Benito F, Avila J. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. *Neuron* 2000;25:425–435.