

# Recovery in Function in Olfactory Ensheathing Cell Transplanted Rat Spinal Cord After Stopping the Injection of Cyclosporine

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## Abstract

The study aimed to check whether animals can retain directed forepaw reaching (DFR) function after stopping cyclosporine which is used to prevent immunorejection in xenotransplant of Olfactory Ensheathing cells (OECs) in Albino Swiss Strain (AS) rat corticospinal tract lesion. The Hematoxylin & Eosin (H&E) staining and immunohistochemistry techniques have been performed on the cryostat sections of corticospinal tract (CST) lesion post-surgery to check the immunorejection and the existence of OEC transplanted tissue before and after injection of cyclosporine. The DFR function have been checked after each stage to see whether animals retain the DFR tasks after stopping cyclosporine. The results show that the daily injection of 100ml diluted cyclosporine avoids immune attack and reduces inflammatory cells infiltrate in delayed CST ipsilateral rat model and also our study proved that stop of cyclosporine injection would not prevent DFR function and the regenerated fibres can still be visualised after 6 months post-operation period. Despite the fact that so many methods such as animal genetic engineering is used to avoid immunorejection after xenotransplantation in animal model, daily cyclosporine injection for some period after surgery seems to be effective and effortless method to not only deter immunorejection in host animal but it also preserve function such as DFR for a long period post-surgery.

**Keywords:** AS Rat, Xenotransplant, GFP, DPX, Olfactory Ensheathing Cells.

## Introduction

Generally in order to elude immunorejection in preclinical model various methods have being used, such as transgenic pig expressing the human complemented inhibitor, hCD59 in rat transacted spinal cord which shows axonal regeneration 1. In our study we use the daily injection of 100 ml of diluted cyclosporine. Whether the OEC transplanted AS rats can survive ipsilateral forepaw reaching after stopping cyclosporine for up to 6 months is controversial issue which was investigated specifically at this study. As this study shows the animals continue recovering after transplantation of OECs despite the halt of cyclosporine.

## Material and Methods

In our study animals were used in accordance with the UK Home Office regulations for the care and use of laboratory animal, the Uk Animals (Scientific Prosecures) Act 1989, with the ethical approval of the University College London, Institute of Neurology.

In order to study whether there is any immunorejection against OECs xenotransplant at grafted tissue, we divided 15 adult female rats (180-210 g body weight) of a locally inbred Albino

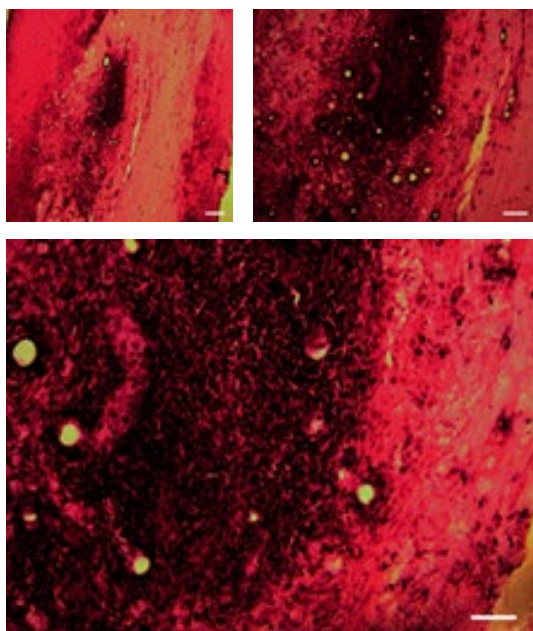
Swiss Strain (AS) in 5 groups. OECs labelled by green fluorescent protein (GFP). The operation was performed. Animal was perfused and tissue was prepared. Animals were divided in 5 groups with the survival time of 4 days, 1week, 2 weeks, 3 weeks, 4 weeks, 5 weeks for group 1 (n=2), group 2 (n=2), group 3 (n=4), group 4 (n=4) and group5 (n=3) respectively. To study the immunorejection of the grafted tissue at all groups the sections were stained by Hematoxylin & Eosin (H&E). To look at the GFP labeled OECs cells, sections were counterstained by Sytox Orange (Invitrogen, Uk) and viewed by confocal microscope. Firstly, Slides were stained in hematoxylin to confirm that chemical binding sites are saturated and later on additional staining is removed by controlled leaching in an alcoholic acid solution 2. Leaching of the stain is arrested to a basic environment (20 sec) in Ammonia solution whereby the stain becomes blue and is permanently fixed to cell structure. Finally sections were counterstained by eosin and mounted by DPX after dehydration. The result shows surviving transplant cells only at group 1 and 2 where the animal survival time was 1 week and 2 weeks respectively. No GFP labelled OEC could be observed at group 2, 3, and 5 where the survival time is over 2 weeks. Therefore the results prove that the animals need to be under immunosuppressant reagent after xeno-transplantation by mouse

OECs in order to avoid immune rejection by leaving the animals under trial over 2 weeks' time.

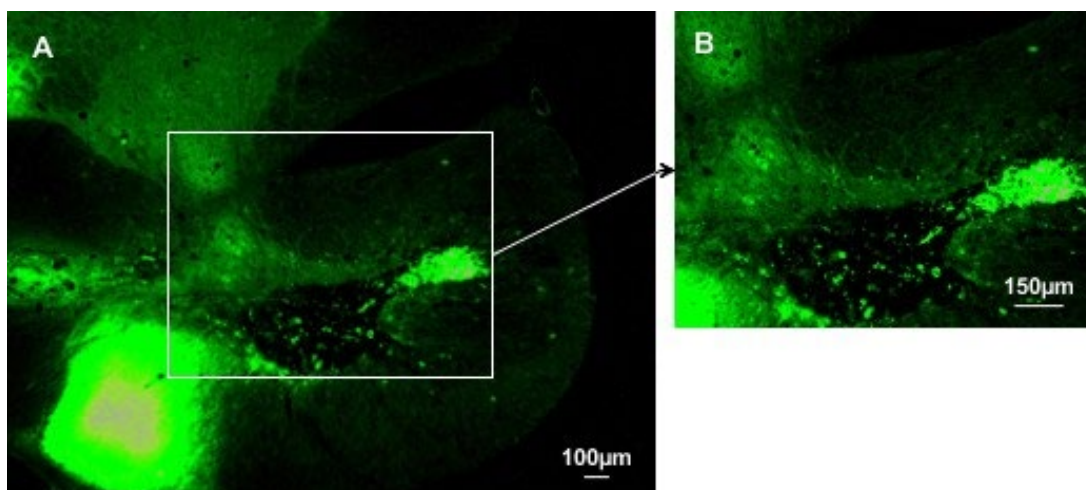
### Result

The histology images show that the animals after 10 days start immunorejecting the xenotransplanted mice OECs in grafted tissue. Among five groups of animals the one who had survival time of 4 and 1 weeks did not show any immunorejection and the GFP labeled OEC cells can be visualised and there is not inflam-

mation sign. However the histology images of groups 3, 4 and 5 animals with the survival time of 10-14 days, 3, 4 and 5 weeks show immune reaction to xenograft transplant of OEC degenerated cells and the central necrosis and lymphocyte infiltration in lesion area (Fig 1). Moreover, the images of postoperative xenotransplanted OECs under daily subcutaneous injection of 100 ml of a dosage of 6 mg/kg/day avoid immunorejection (Fig2) which shows the misplace of GFP labeled transplanted OECs with the survival time of 4 weeks and there is no sign of inflammation.



**Figure 1:** Histological assessment of spinal cord tissue immune reaction to xenogenic transplant of OEC without the injection of immunosuppressant agent, the image shows the degenerated cells and the central necrosis and lymphocyte infiltration after 1 week post surgery in lesion area. Survival time: 10 days, Scale bar: 200  $\mu\text{m}$ .



**Figure 2:** The 20  $\mu\text{m}$  thickness coronal section of transplanted CST lesion failure. A. The image shows the GFP labelled grafted OECs at the dorsolateral side of lesion. B. The enlarged view of transplanted side show the distance between OEC transplanted and the lesion. Surviving time: 4 weeks; scale bar: A, 100  $\mu\text{m}$ ; B, 50  $\mu\text{m}$ .

Following the stop of cyclosporine after 4 weeks postsurgery the animals were tested for the paw reaching tasks for 6 months, the results indicated that despite the halt of cyclosporine the animals preserved pawreaching for over 6 months survival time.

### Discussion

The histology analysis (Fig 1) shows that the animals after two

weeks start immunorejecting the xenotransplanted mice OECs in grafted tissue. Among five groups of animals group 1 and 2 with the one which had survival time of 4-7 days did not show any immunorejection and the GFP labeled OEC cells can be visualised and there is not any inflammation sign. However the histology images of groups 3, 4 and 5 animals with the survival time of 10-14 days 3, 4 and 5 weeks show immune reaction to

xenograft transplant of OEC degenerated cells and the central necrosis and lymphocyte infiltration in lesion area. Moreover, the images of postoperative xenotransplanted OECs under daily subcutaneous injection of 100 ml of a dosage of 6 mg/kg/day of cyclosporine avoid immunorejection (Fig 2) which shows the misplace of GFP labelled transplanted OECs with the survival time of 4 weeks and there is no sign of inflammation. In general in preclinical model different approaches has being used to avoid immunorejection, such as genetically engineered pig expressing the human complemented inhibitor, hCD59 in rat transacted spinal cord which shows axonal regeneration 1, 3. Our study uses the daily injection of 100 ml of diluted cyclosporine. However, the greater survival of groin flaps and reduces in calcineur in inhibitor drug toxicity by combination therapy of triptolide and cyclosporine A than recipients treated with cyclosporine A only 4, 5. Therefore, the mice xenograft model of 10 AS rats delayed OEC transplant show the regeneration of damaged ipsilateral corticospinal tract and DFR function return, and also the result shows that the daily injection of 100ml diluted cyclosporine avoids immune attack and reduces inflammatory cells infiltrate in delayed CST ipsilateral rat model and also our study proved that stop of cyclosporine injection would not prevent DFR function in over 6 months AS rats and the regenerated fibres can still be noticed.

### Conclusion

There was concern whether animals retain DFP function after stopping the injection of cyclosporine after xenotransplantation surgery. The AS rats have gone under CST lesion surgery and mice OECs have been transplanted in to CST lesions and DFR function have been tested in 10 days, 4 weeks and 6 months post-surgery. The results shows that DFR function of AS rats under transplantation of OEC in corticospinal tract lesion and regenerated fibres can be visualised despite the stop of daily injection of cyclosporine even after 6 months post-surgery. Although there are other techniques such as genetically engineering animals to avoid immunorejection after xenotransplantation, the injection of cyclosporine for few days post-surgery have proved to be convenient and efficient method to avoid immunorejection and retain the function of xenotransplanted animals [1-6].

### Ethical Approval and Consent of Participate

In our study animal were used in accordance with the UK Home Office regulations for the care and use of laboratory animal, the UK Animals (Scientific Procedures) Act 1989, with the ethical approval of the University College London, Institute of Neurology.

### Consent for Publication

Not applicable

### Availability of Data and Material

The data used and analyzed in this study are available from the corresponding author upon reasonable request.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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### Contributors

YL and GR contributed to the supervision of project both heretically and technically.

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