Foetal H-2K^b-tsA58 Transgenic Mouse Tissue Develops Normally When Grafted to Adult Mouse Brain

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INTRODUCTION

The aim of this preliminary study was to determine whether foetal neural tissue from the H-2K^b-tsA58 transgenic mouse /1/ is able to develop and differentiate in a manner similar to non-transgenic foetal tissue after transplantation into adult rodent brain, or whether it would proliferate under the combined influence of the H-2K^b-tsA58 transgene and i) local production of interferon by, e.g., astrocytes following the implantation and ii) a brain temperature of 38°C /2/, which is below the non-permissive temperature 39°C for the tsA58 gene and causes the grafted cells to proliferate into a tumour-like mass.

METHODS

To minimise MHC differences and problems with immunological rejection of the grafts, same strain F1 mice (CBA/Ca x C57/BL10) were used as hosts. Four weeks prior to grafting, the cholinergic innervation of the hippocampus (HC) of host mice was removed by excitotoxic (8mM S-AMPA) lesions of the medial septal area (MSA).

Foetal tissue for transplantation was taken from the ventral basal forebrain (VBF) region of E14-15 transgenic mice embryos. The tissue fragments were incubated in 0.1% trypsin solution for 20 minutes, gently triturated, washed and made into a single cell suspension to a density of 1 tissue piece/10 µl. Bilaterally, 1.5 µl of suspension was stereotactically implanted into the HC of lesioned host mice. Graft survival time was 2, 4, 8 and 15 weeks. Two-three mice were perfused at each time point with 4%

paraformaldehyde, the brains soaked in 25% sucrose and 20-30 µm sections through the graft cut on a cryostat.

Adjacent sections were stained for Nissl, AChE and immunocytochemistry. Antibodies used included GFAP (1:1000 - DAKO), MAP 1 91:100 - G10:- gift of Dr. R. Calvert); neurofilaments (NF) (1:100 - RT97, gift of Prof. B. Anderton; 1:50 -NFL & NFM - Amersham Int.). The antibodies were visualised using the biotin/streptavidin-fluorescein system (Amersham Int.).

RESULTS

The foetal H-1K^b-tsA58 transgenic tissue survived at all time points looked at, and no tumours were found. Graft placement was not always in the HC. It was often found on the cortex surface, within the corpus callosum or in the IIIrd ventricle. However, there was no noticeable difference in survival or morphology between grafts found in different sizes.

The graft morphology at 2 weeks showed AChE positive and well differentiated neurons between clumps of undifferentiated, AChE negative neuroblast-like cells with small dark nuclei. These are likely to be grafted cells in which the transgene remains active in so far as the cells have not fully progressed along their developmental pathway, and remain undifferentiated. At 4 weeks no neuroblast-like cells were seen. The grafted cells show a pattern often seen with VBF grafts with clumps of neuronal cells interspersed with glial cells.

GFAP reactivity was seen in the region of the graft; however, all astrocytes within the mouse HC were GFAP+, even in non-operated mouse

brain. It was difficult to distinguish between scarring around the graft mass and the level of GFAP reactivity seen in the normal control host HC. The NF antibodies showed similar staining patterns over the time course involved. Between 2 and 4 weeks there was a large increase in the number of NF+ fibres seen. Most of the fibres were within the graft mass either parallel with the cortex (for surface transplants), intra-graft or crossing the graft-host interface. It was not possible to determine whether these fibres were of graft or host origin.

The G10 antibody, which is specific for growing/regenerating axons, was negative, although there was some diffuse staining at 2 weeks within the graft.

CONCLUSION

Foetal neural tissue from the H-2K^b-tsA58 transgenic mouse will survive and differentiate in a manner similar to normal tissue after trans-

plantation into adult CNS with the grafted tissue showing many features of the normal VBF graft.

This transgenic mouse tissue will allow the generation of cell lines derived from neural tube and other CNS areas that contain cells that have not passed through their final development and had their fate determined. The cells are likely to survive and differentiate without forming tumours under the influence of the tsA58 oncogene.

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REFERENCES

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