Human fetal neural precursor cells can up-regulate MHC class I and class II expression and elicit CD4 and CD8 T cell proliferation

R. Laguna Goya a,⁎, R. Busch b, R. Mathur c, A.J. Coles d, R.A. Barker a,d,e

a Cambridge Centre for Brain Repair, Forvie Site, Robinson Way, Cambridge, UK
b Department of Medicine, Addenbrooke’s Hospital, University of Cambridge, Cambridge, UK
c Department of Obstetrics and Gynaecology, Addenbrooke’s Hospital, Cambridge, UK
d Department of Neurology, Addenbrooke’s Hospital, Cambridge, UK
e Edith Cowan University, Perth, Australia

ARTICLE INFO

Article history:
Received 13 July 2010
Revised 29 September 2010
Accepted 7 October 2010
Available online 16 October 2010

Keywords:
Fetal neural precursor cells
Cell replacement
Major histocompatibility complex
Immunogenicity
Proliferation assay

ABSTRACT

The use of allogeneic fetal neural precursor cells (NPCs) as a cell replacement therapy in neurodegenerative disorders holds great promise. However, previous studies concerning the possibility of alloimmune rejection of the transplanted cells have been inconclusive. Here, we used flow cytometry to quantify the expression of major histocompatibility complex (MHC) molecules by human NPCs, obtained from the cortex or ventral mesencephalon of fetuses with gestational ages between 7 and 11 weeks. MHC class I was undetectable on the surface of freshly isolated primary fetal tissue from either location, but increased over time in proliferating NPC cultures; after 7 days in vitro, MHC class I was detectable on most cells. Following differentiation, MHC class I expression persisted on non-neuronal cells. MHC class II levels remained low at all time points but were inducible by pro-inflammatory cytokines, whereas the co-stimulatory molecules, CD80 and CD86, remained undetectable. Nonetheless, CD4+ and CD8+ T cells proliferated when peripheral blood mononuclear cells (PBMCs) were cultured with allogeneic NPCs. Weaker responses were obtained when NPCs were co-cultured with purified allogeneic responder T cells, suggesting that indirect allorecognition contributed significantly to PBMC responses. In conclusion, differentiating human NPCs are immunogenic in vitro, suggesting that they may trigger immune rejection unless transplant recipients are immunosuppressed.

© 2010 Elsevier Inc. All rights reserved.

Introduction

There are no licensed reparative therapies for neurodegenerative disorders such as Parkinson’s Disease (PD), but cell replacement therapies, in particular with human fetal neural precursor cells (NPCs), hold great promise (Goya et al., 2007). For the transplant to be successful, these cells must evade rejection by the host’s immune system, and the cell surface expression of class I and class II molecules encoded within the major histocompatibility complex (MHC) are critical in this regard. These molecules normally present self and foreign peptides for recognition by antigen receptors on T cells of the immune system. MHC molecules exhibit extensive genetic polymorphism, so that mismatches between the MHC class I and class II alleles of transplant donors and unrelated recipients are common and recognized by T cells as an interaction with non-self. CD4+ T cells recognize class II and CD8+ T cells recognize class I MHC molecules, and through this interaction an immune response is triggered, leading to rejection reactions (Felix and Allen, 2007). At least two mechanisms of allorecognition have been defined: a direct mechanism, in which donor MHC molecules and their bound peptides are presented by donor antigen-presenting cells (APCs) to the recipient’s T cells; and an indirect mechanism, in which material from donor cells, including the foreign MHC molecules, are captured and presented by the host’s APCs (Jiang et al., 2004).

While this is well described in solid organ transplantation, the mechanisms of rejection in the central nervous system (CNS) are less well understood, and are likely to be different in two major ways: the CNS is thought to be a relatively immunologically privileged site (Tepavcevic and Blakmore, 2005) and fetal neural tissue expresses low or no levels of MHC (Odeberg et al., 2005). Nonetheless, several studies, including clinical trials, suggest that human fetal neural allografts can elicit an inflammatory/immune response (Olanow et al., 2003). However, the situation may be different for NPCs, as opposed to primary neural tissue, as NPCs are more immature and in xenotransplantation studies have been shown to be less immunogenic (Armstrong et al., 2002). To delineate the mechanisms of immunogenicity of transplanted human NPCs, we asked if MHC up-regulation in human NPCs can occur and if NPCs can induce proliferative responses in T lymphocytes. Resolving this issue is becoming more urgent as we move into a new era of possible stem cell therapies for CNS disorders (Goya et al., 2007).
Murine NPCs express at most very low levels of MHC class I molecules constitutively, but their expression can be strongly up-regulated by pro-inflammatory cytokines (Hori et al., 2003; Mammolenti et al., 2004; McLaren et al., 2001; Yin et al., 2008). Similar results have been reported for human NPCs derived from fetal forebrain (Odeberg et al., 2005; Ubali et al., 2007), although these authors failed to detect substantial alloresponses to NPCs in vitro, in apparent conflict with the ability of NPCs to elicit immune responses following transplantation, discussed above. Other unresolved questions include whether NPCs derived from different brain regions differ with regard to MHC expression and immunogenicity, and how NPC differentiation influences immunogenicity.

Recent work suggests that MHC class I molecules may, in addition, have important non-immune roles in the integration of differentiating neurons into functional neural networks — another critical requirement for the therapeutic success of allogeneic NPC transplantation. The expression of MHC class I molecules has been shown to be up-regulated at times of increased neural activity, in the developing visual system and in the adult hippocampus after seizures (Corriveau et al., 1998). Furthermore, genetic manipulation of MHC class I expression alters activity-dependent synaptic plasticity in the visual cortex and the hippocampus (Huh et al., 2000). Thus, MHC protein expression on NPCs or their differentiated progeny may have both beneficial non-immune and deleterious immune consequences for successful NPC transplantation.

In this study we have, therefore, sought to examine (a) the constitutive cell surface expression of MHC class I and class II molecules in two different regions of the human fetal brain: cerebral cortex (CTX) and ventral mesencephalon (VM), (b) the changes in MHC molecule expression after expansion of NPCs derived from these locations in proliferating media, with and without pro-inflammatory cytokines and (c) after differentiation in vitro. Lastly, we re-examined (d) the ability of these NPCs to elicit proliferative responses by allogeneic CD4+ and CD8+ T cells. The experimental approach is summarised in Supplemental Fig. S1.

Materials and methods

Human tissue collection

NPCs were derived from the cerebral CTX and VM of 9 different human fetuses of gestational age 7 to 11 weeks, collected from routine termination of pregnancies under full ethical approval in line with Department of Health guidelines (LREC 96/085).

Cell culture

Tissue was dissected and subsequently dissociated with Accutase (PAA Laboratories, Sommerset, UK). NPCs were then grown as neurospheres in flasks containing 70% Dulbecco’s modified Eagle medium (DMEM; Invitrogen, UK), 30% Ham’s F-12 (Invitrogen), 2% B27 supplement (Invitrogen, UK), 100 U/ml Penicillin, 100 mg/ml Streptomycin, 250 mg/ml Amphotericin B and 20 ng/ml EGF and FGF-2 at 37 °C in a 5% CO2 atmosphere at a density of 2 to 5 × 105 cells/ml. The media was changed twice a week (proliferation media). The minimum purity of 93% CD3 positive cells. Cells were resuspended for labeling in PBS at a concentration of 3 × 106 cells/ml CFSE (Invitrogen, UK) was added at a concentration of 1.6 μM and incubated for 10 min at 37 °C in the dark. Labeling was stopped by adding the same volume of RPMI-1640 media containing 5% human AB positive serum, followed by centrifugation of the cells and resuspension in supplemented RPMI media/proliferation assay media.

Flow cytometry

MHC class I (HLA-A, -B, -C) expression was measured by flow cytometry using the W6/32-biotin antibody (1:10, Serotec). L243 antibody was used for detection of MHC class II (HLA-DR) (1:200, purified from hybridoma supernatant over protein A- sepharose). Anti-CD80-PE (1:20, BD Pharmingen) and anti-CD86-PE (1:50, BD Pharmingen) were used to detect co-stimulatory molecules. Background staining was evaluated using isotype controls. Primary tissue and neurospheres from CTX and VM were dissociated to single cell suspensions using Accutase and then resuspended in PBS, 0.1% BSA and 0.1% sodium azide. Cells were incubated with antibody for 30 min on ice, washed with PBS, 0.1% BSA and 0.1% sodium azide, incubated with streptavidin or secondary antibody for 30 min, and washed again. The cut-off for positivity was set so that isotype control staining was less than 1% positive.

In the proliferation assays, CFSE dilution was measured using flow cytometry. Cells were counterstained with anti-CD3-APC, and either CD4-PerCP or CD8-PerCP antibodies (BD Pharmingen) in PBS, 0.1% BSA and 0.1% sodium azide for 30 min on ice and then washed. Flow cytometric analysis was performed on a FACSDiVa flow cytometer (Becton Dickinson) using the FACSDiVa program. The proliferation
data was analysed using the proliferation wizard module of ModFit LT™. Lymphocytes were gated based on forward-scatter and side-scatter profiles, and CD3/CD4+ and CD3/CD8+ cells were analysed separately. The parent population was set individually for every day of analysis using an unstimulated control sample.

Cytokine quantification using ELISA

Cell-free culture supernatants were collected from the proliferation assays and frozen at −20 °C. An ELISA kit (R&D Systems) was used to detect TNF-α and IFN-γ and the concentration (average of duplicate wells) was measured in a plate reader (μQuant, Bio Tek).

Statistical analysis

SPSS software (version 14.0) and R software (version 2.8.0) were used for statistical analysis. The tests employed are specified in the figure legends.

Results

MHC class I expression increases in fetal NPCs over time in proliferating culture conditions

We studied the expression of MHC class I in human fetal NPCs obtained from CTX and VM at the time of harvest (primary tissue) and after in vitro culture in proliferation media. The percentage of cells expressing MHC class I increased from 1–12% in primary tissue to 70–100% after 2–3 weeks in proliferative NPC cultures, in line with previous studies (Odeberg et al., 2005) (Fig. 1). This high expression remained stable for up to 10 weeks of culture in proliferating media.

Throughout the time course, the percentage of class I-positive cells was slightly, but significantly greater with VM NPC than on CTX NPC (p = 0.0012), but the rate of increase of MHC class I expression was similar (p = 0.1497). The age of the fetus at termination had no substantial effect on the results.

IFNγ, one of several pro-inflammatory cytokines known to induce MHC class I molecules, was undetectable in NPC culture supernatants (data not shown).

NPCs become gliogenic over time in culture in proliferating conditions and maintain MHC class I expression levels upon differentiation

NPCs changed their differentiation potential over time in culture in proliferating media, in parallel with changes in MHC I expression. NPCs derived directly from the fetal CTX differentiated mainly into neurons (52% MAP2+ cells) but as the NPCs were maintained in culture more differentiated into glia (30% GFAP+ cells in passage 1, 57% in passage 2; p<0.001) (Fig. 2A). We therefore investigated MHC

Fig. 1. MHC class I up-regulation on cultured, proliferating NPCs. A. The percentage of NPCs expressing MHC class I (HLA-A, -B, -C) increased from close to 0 to almost 100% after 2–3 weeks in culture. There were no differences in the rate of increase between CTX and VM (p = 0.1497), but CTX reached a lower plateau than VM (p = 0.0012). Data obtained from nine different donors and analysed with a non-linear mixed-effects model. B. Representative flow cytometry dot plots demonstrating expression of HLA-ABC molecules (vertical axis) in NPCs from CTX and VM at different times in culture. A sensitive avidin/ biotin indirect staining procedure was used. Background fluorescence in VM (but not in CTX) was correlated with autofluorescence in the “FITC” channel (525 nm emission; horizontal axis). In the examples shown, CTX and VM differ with respect to the pattern of up-regulation, but this was not consistent between experiments.

Fig. 2. Lineage-related MHC class I retention following differentiation of NPCs. A. Time of pre-culture in proliferating media affected differentiation potential. NPCs were differentiated for 7 days from primary tissue (P0) or after one or two passages (P1, P2), and stained for neuronal (MAP2) and glial (GFAP) markers (representative photomicrographs are shown at the bottom). The histogram shows a significant increase in the fraction of glial cells (mean±SEM shown) with increasing passage number prior to differentiation (p < 0.001 by one-way ANOVA), as well as a corresponding decrease in neurons (p < 0.05) in line with earlier work from our group (Jain et al., 2003). B. The percentage of MHC class I-positive cells was maintained over time following differentiation. HLA-ABC expression by primary tissue and NPCs is shown after different times of pre-culture in proliferating media and differentiation. Primary tissue and NPCs that had been cultured in proliferating media for 3, 6, 10–20 and 20–40 days were plated for 1, 7 and 14 days in differentiation media and their MHC expression measured by flow cytometry. Data analysed with one-way ANOVA for each of the times in proliferating media prior to differentiation. Data are mean ± SEM.
class I expression in NPCs that had been cultured for different lengths of time in proliferation media before being differentiated. Primary tissue and NPCs that had been cultured in proliferating media for 3, 6, 10–20 and 20–40 days were then plated out for 1, 7 and 14 days in differentiation media, and their MHC expression measured by flow cytometry. The percentage of MHC class I (HLA) positive cells remained similar, regardless of the time of differentiation (Fig. 2B), and was similar to that seen in the original proliferative NPC population being plated out.

Even though the temporal changes in MHC class I expression paralleled the gradual transition from a neurogenic towards a gliogenic differentiation pattern, the frequency of class I-expressing cells exceeded that of GFAP+ astrocytes, suggesting that other lineages might also express MHC class I molecules following NPC differentiation. To address this issue, we surface-stained differentiated NPC cultures for MHC class I, followed by fixation, permeabilization, and staining for several lineage markers, and analysed co-expression by immunofluorescence microscopy. Even though this analysis appeared to be less sensitive than flow cytometry, we were able to detect MHC class I expression consistently on astrocytes and, with less intensity, on NPCs and oligodendrocytes, but not on neurons (Fig. 3).

Fig. 3. MHC class I is expressed by neural precursor cells and glial cells, but not neurons. Differentiating cells were co-stained for lineage markers and MHC class I. Mature astrocytes (GFAP) exhibited greater levels of MHC class I staining per cell than did neural precursor cells (nestin), immature astrocytes (vimentin) and oligodendrocytes (O4). No W6/32 staining was detected on neurons (MAP2). Scale bar represents 20 μm in the O4 and MAP2 panels and 10 μm elsewhere.
Pro-inflammatory cytokines induce MHC class II expression on NPCs

MHC class II molecules are constitutively expressed by professional antigen-presenting cells (APCs), but can be induced by cytokines on other cell types. MHC class II (HLA-DR) expression on the cell surface of fetal NPCs was very low; however, after stimulation with recombinant IFN-γ, induced expression was clearly observed in expanded NPCs from both regions of the developing fetal brain (Fig. 4 and data not shown). Similar results were obtained using recombinant TNF-α (data not shown). In contrast, the co-stimulatory molecules CD80 and CD86 were not detected on the cell surface of VM and CTX NPCs, even after 48 h of stimulation with either IFN-γ, TNF-α or both (data not shown).

Fetal NPCs elicit a delayed proliferative response in allogeneic T cells

We studied the potential of human fetal primary tissue and expanded NPCs to elicit a proliferative response in allogeneic T cells in vitro. First, we showed that NPCs survived and differentiated under co-culture conditions (Supplemental Fig. S2A). We then co-cultured primary tissue or expanded NPCs with CFSE-labelled PBMCs and obtained proliferative T-cell responses, measured by CFSE dilution, which were comparable in magnitude to the mixed lymphocyte reaction (MLR) response (representative histograms are shown in Supplemental Fig. S2B). The majority of the NPC cultures were immunogenic, with 84% of the CTX and 78% of the VM cultures eliciting a proliferative response from allogeneic T cells (16/19 CTX and 18/23 VM experiments; Fig. 5A). Proliferation of both CD4+ and CD8+ T cells against CTX and VM derived cells was significant (p < 0.001), with no difference between regions (CD4 p = 0.26, CD8 p = 0.673) or T cell subtype (CTX p = 0.643, VM p = 0.394) (Fig. 5A). The onset of proliferation in these co-cultures was observed between 8 and 17 days post stimulation, delayed by an average of four days when compared with the MLR (Fig. 5B). In each experiment, anti-CD3/CD28 beads, used as a positive control, triggered early proliferation (measured at 3–4 days) of CD4+ and CD8+ T cells (Supplemental Fig. S2B).

NPC pre-culture in proliferating media does not increase immunogenicity

Given that MHC class I expression increases in NPCs in culture in proliferating conditions, we investigated if this increases their immunogenicity. Primary tissue and NPCs expanded in vitro for 5, 15 and 28 days in proliferating media were co-cultured with PBMCs. However, we detected no systematic effect of the duration of NPC pre-culture on either the magnitude of the proliferative responses (peak PI, not shown) or on the day of onset of that proliferative response (Supplemental Fig. S3).

Low-level proliferation of purified allogeneic CD4 and CD8 T cells in response to NPCs

In the preceding experiments, whole PBMCs were used as a source of responder T cells, which allows cross-presentation of NPC-derived allogeneic peptides by host antigen-presenting cells, as well as contributions from other cell lineages to the microenvironment. In order to examine whether NPCs are capable of stimulating T cells directly, NPCs were co-cultured with purified allogeneic T cells (≥ 95% CD3+). We found that 1 of 5 (20%) CTX and 4 of 6 (66%) VM NPC cultures triggered detectable blast transformation and proliferation of isolated CD4+ T cells (Fig. 6 and Supplemental Fig. S4); isolated CD8+ T cells responded to 1 of 5 (20%) allogeneic CTX and 2 of 6 (33%) allogeneic VM cultures. Proliferation of both CD4+ and CD8+ T cells against VM-, but not CTX-derived NPCs, was statistically significant (p < 0.05). These data are consistent with direct recognition by T cells of allogeneic NPC class I and class II molecules on NPCs, especially on NPCs derived from VM. However, the proliferative responses from isolated T cells were less frequent and generally much weaker (as judged by the proliferation indices and precursor frequencies calculated using ModFit) than from whole PBMCs. Our data therefore suggest that a mixture of direct and indirect allorecognition accounts for the immunogenicity of NPCs, with indirect allorecognition perhaps being the dominant route for immune recognition and T cell response.

The proliferation of purified CD4+ T cells in this system was unexpected, because no deliberate steps were taken to induce MHC
class II molecules on the allogeneic NPC stimulator cells. However, when NPCs were cultured with supernatants of proliferation assays, we observed up-regulation of MHC class II on a minority of NPCs (Fig. 7), which may have been induced by the release of inflammatory cytokines in the supernatant. Indeed, using ELISA, TNF-α secretion was detected in a significant proportion of T-cell/NPC co-cultures in which T cells had proliferated, whereas little TNF secretion was observed when there was no T-cell proliferation (Fig. 8). The same trend was observed, albeit less pronounced, for IFN-γ release. Differentiation of NPCs during co-culture with T cells (Fig. S2A) may also have contributed to MHC class II up-regulation.

**Discussion**

In this study we found that neural tissue derived from primary fetal human cerebral CTX and VM express very low levels of cell surface MHC class I proteins. However, when these cells are cultured in proliferating media, in the presence of EGF and FGF, the expression of MHC class I rapidly increases, in line with previous reports studying human fetal forebrain and spinal cord NPCs (Odeberg et al., 2005). This increased expression may relate to a change in precursor cell type within the culture possibly shifting from a more neuronal to a glial phenotype as the differentiation data would imply, and in line with our previous work (Jain et al., 2003). Indeed, further analysis indicated that MHC class I molecules were expressed on the surface of NPCs, glial progenitors and mature astrocytes and oligodendrocytes, but not on neurons. This suggests that the absence of MHC class I on the surface of NPCs prior to differentiation may be useful as a marker of neurogenic potential.

Previous studies have reported that human T cells do not proliferate markedly in response to allogeneic fetal NPCs (Odeberg et al., 2005; Ubiali et al., 2007). In contrast, we have now shown, for the first time, that NPCs from both human fetal CTX and VM can elicit proliferative T cell responses upon co-culture with allogeneic PBMCs and, to some extent, with isolated T cells. The onset of proliferation is delayed, compared to the allogeneic MLR (response to unrelated allogeneic T-depleted PBMCs), but the peak magnitude of these responses is comparable. The delay is not explained by the time required for MHC up-regulation, because primary tissue and NPCs, differing in their initial class I expression, elicited T-cell responses with similar kinetics. This suggests that other factors are rate-limiting for T-cell activation in this system. We considered the possibility that the delay could be due to the delayed death of NPCs during co-culture.

**Fig. 5.** Allogeneic NPCs elicit proliferative T-cell responses in PBMC co-cultures. A. Peak proliferation indices (PI, day 8–17 post stimulation) are shown for individual CTX and VM NPC co-cultures with CFSE-labelled PBMCs. Responses were scored positive if PI > unstimulated PI mean + 3 SD and if blast transformation was seen by forward scatter analysis (forward scatter correlates with cell volume). In all experiments, T-cell responsiveness was verified by co-culture with anti-CD3/CD28 beads and/or allogeneic, T-depleted PBMCs (not shown). Proliferation of both CD4+ and CD8+ T cells against CTX and VM NPCs was significant (Wilcoxon signed ranks test vs. unstimulated, \( p < 0.001 \)), with no significant differences between NPCs from different brain regions or between T cell subsets. The bar represents the median PI. B. Delayed onset of CD4 T cell proliferation in CFSE-labelled PBMCs stimulated by allogeneic VM and CTX NPCs, compared with PBMCs stimulated by allogeneic, T-depleted PBMCs (Mixed Lymphocyte Reaction (MLR)). The mean day of onset (horizontal bars) was day 7 in MLRs and day 11 in PBMC/NPC co-cultures for the start of the proliferation (\( p < 0.05 \), one-way ANOVA). Onset of proliferation was defined as the first day at which a positive response of gated T cells was observed. The CD8 response was similar (data not shown).

**Fig. 6.** Weak responses of purified CD3+ T cells to allogeneic NPCs in the absence of syngeneic antigen-presenting non-T cells. A. Peak proliferation indices of CD4+ and CD8+ T cells are shown for individual VM or CTX NPC co-cultures with immunomagnetically purified CD3+ T cells (purity >93%). There was proliferation of both CD4+ and CD8+ T cells against NPCs in some cultures, which was significant for VM (\( p < 0.05 \), Wilcoxon signed ranks test). PI values were low, due to low precursor frequencies, i.e. small subsets of T cells responding, but blast transformation was seen in the FSC/SSC plot. The bars represent median PIs.
with T cells, with necrotic debris subsequently providing the critical antigenic or pro-inflammatory stimulus. However, NPCs differentiated, but did not die during co-culture with PBMCs. In any case, the delayed response may explain why in the two previous studies, in which T cell proliferation was measured by 3H-thymidine uptake after only 5 days of co-culture, responses to allogeneic NPCs were not detected (Odeberg et al., 2005; Ubiali et al., 2007). Proliferative responses were accompanied by the secretion of pro-inflammatory (Th1-type) cytokines, suggesting that the responding T cells differentiated into effector cells. Thus, our data indicate NPCs are, in fact, able to prime alloresponses in vitro, and this may provide an explanation for the apparent immune rejection of transplanted NPCs in vivo.

The proliferative responses were generally much weaker, or undetectable, when purified T cells were used in place of PBMC. This observation suggests an important role for professional APCs present amongst the PBMCs, which are removed during T-cell isolation. Their presence may be required because the NPCs lack detectable expression of co-stimulatory molecules, such as CD80, and CD86, even when stimulated with pro-inflammatory cytokines. The responder APCs, which do express these molecules (data not shown), may either provide third-party co-stimulation during direct recognition of foreign NPCs by T cells, or take up NPC-derived allo-peptides and present them on their own MHC molecules alongside CD80 and CD86. The possibility of direct allore cognition of foreign MHC on NPCs is consistent with our finding of low-level proliferation in some co-cultures with purified T cells. In vivo, indirect presentation and co-stimulation by resident or infiltrating host APCs may well contribute to rejection of transplanted NPCs.

Several recent reports have suggested that MHC class I molecules may be important in activity-dependent synaptic remodelling (Datwani et al., 2009; Huh et al., 2000; Oliveira et al., 2004) during development. Therefore, in the context of cell grafting, the expression of MHC class I may be vital if NPCs are to differentiate and fully integrate at the synaptic level into the recipient brain, and therapeutic approaches which aim to silence the expression of MHC class I molecules directly may adversely affect the behaviour of these transplanted cells. However, this remains

![Fig. 7. MHC class II up-regulation in VM and CTX NPCs induced by supernatants obtained from proliferating NPC/PBMC co-cultures.](image-url)

**Fig. 7.** MHC class II up-regulation in VM and CTX NPCs induced by supernatants obtained from proliferating NPC/PBMC co-cultures. MHC class II expression was increased on the surface of two different NPC cultures (VM711 and CTX713) when cultured with the supernatant of proliferation assays. Isotype control antibodies were used as specificity controls. Analysis of NPC cultures with supernatants from five other different co-cultures gave a similar result.

![Fig. 8. Pro-inflammatory cytokines are detected in the supernatants of the proliferation cultures in which PBMCs responded to NPCs.](image-url)

**Fig. 8.** Pro-inflammatory cytokines are detected in the supernatants of the proliferation cultures in which PBMCs responded to NPCs. Supernatants were collected from proliferation cultures and tested for TNF-α and IFN-γ in duplicate using ELISA. Beads and allo-PBMCs induced both TNF-α and IFN-γ production. When PBMCs proliferated in response to NPCs (+), there was cytokine release in some cases. However, when PBMCs did not proliferate in response to NPCs (−), there was no cytokine release. Similarly, TNF-α and IFN-γ were not present when PBMCs and NPCs were cultured separately. The bar represents the median cytokine secretion.
unproven especially as the only cells that did not express MHC in our system were the neurons differentiating out of the NPCs.

In summary, we have shown that human fetal NPCs derived from the VM and CTX can express MHC class I and, to a lesser extent, MHC class II molecules. This appears to allow priming of alloimmune T-cell response in vitro. Further work will be required to explore the implications of these findings for neural progenitor cell transplantation as a therapy for neurodegenerative diseases, but does at least suggest that the clinical adoption of cells of this type will require the use of some form of immunosuppression.

Supplementary materials related to this article can be found online at doi: 10.1016/j.nbd.2010.10.008.

Acknowledgments

We thank Pam Tyers for technical assistance, Patrick Bose, Chhaya Prasannan-Nair, and Diane Moore for provision of fetal tissue, Michael Zandi for comments on the manuscript, and our volunteers for donating blood for PBMC isolation. This work was supported by the Medical Research Council, a Senior Research Fellowship of the Arthritis Research Campaign (#18543, to R.B.) and an NIHR Biomedical Research Centre award to the University of Cambridge and Addenbrooke’s Hospital.

References


