International Immunology, Vol. 19, No. 9, pp. 1063–1074
doi:10.1093/intimm/dxm079

© The Japanese Society for Immunology. 2007. All rights reserved.
For permissions, please e-mail: journals.permissions@oxfordjournals.org

Allorecognition of human neural stem cells by peripheral blood lymphocytes despite low expression of MHC molecules: role of TGF-β in modulating proliferation

Federica Ubiali[1], Sara Nava[1], Valeria Nessi[1], Simona Frigerio[2], Eugenio Parati[2], Pia Bernasconi[1], Renato Mantegazza[1] and **Fulvio Baggi[1]**

[1]Neurology IV and [2]Neurobiology and Neurorestorative Therapies, Neurological Institute Foundation 'Carlo Besta', Via Celoria 11, 20133 Milan, Italy

Keywords: NSC, one-way MLR, transplantation

Abstract

Neural stem cells (NSCs) transplantation has been proposed as a means of restoring damaged brain tissue, a possibility rendered more likely by reports of low NSCs immunogenicity in various experimental models because of low expression of MHC class I and II as well as co-stimulatory molecules. We investigated the immunogenicity of a human NSC line grown in normal culture conditions and in the presence of pro-inflammatory cytokines IFN-γ and tumor necrosis factor alpha by one-way mixed lymphocyte reaction (MLR) experiments with peripheral blood lymphocytes from eight HLA-incompatible donors. NSCs stimulated lymphocyte proliferation in almost all donors tested, with stimulation indices in the range of the low-end distribution curve of MLR between donors. The healthy subject that gave negative MLR results was the best compatible donor with respect to NSC haplotype. Since we observed low MLR responses overall, we studied if NSCs might exert any immunomodulatory activity. We detected transcription and release of the immunomodulatory molecule transforming growth factor beta (TGF-β)-1; moreover, the addition of TGF-β1 in MLR experiments down-regulated proliferative responses. To further confirm the immunological potential of human NSCs, we studied xenogeneic recognition of NSCs by immunocompetent cells derived from C57BL/6 mice, showing that NSCs can elicit an allo(xeno) response *ex vivo*. Our data indicate that NSCs have low but not negligible immunogenic potential that is sufficient to activate peripheral lymphocytes. Secretion of TGF-β1 might balance the immunogenicity of NSCs. Nevertheless, the possibility that allo-NSCs grafting might induce in the long term an immune activation, thus vanishing their therapeutical effect, should not be overlooked and deserves further investigation.

Introduction

The mammalian central nervous system (CNS) contains a population of undifferentiated multipotent self-renewing cells, known as neural stem cells (NSCs) (1–3). NSCs are well-defined cell precursors that can proliferate indefinitely *in vitro* as undifferentiated neurospheres or can be induced to differentiate into neurons, oligodendrocytes and astrocytes (4). NSCs transplantation has been proposed as a means of restoring damaged brain tissue (5–7), particularly in diseases of metabolic, genetic or inflammatory origin that result in focal neuronal degeneration. Improved motor function has been shown in CNS-damaged animals after NSCs transplant: the implanted cells migrated to the damaged area and differentiated into neurons and glia (8–10).

The CNS has been considered an immunologically privileged tissue (11), because the presence of the blood–brain barrier (BBB) that limits and regulates molecular exchanges between blood stream and neural tissue. In the BBB, complex tight junctions between adjacent endothelial cells that line cerebral microvessels represent a 'physical barrier' that causes most molecular traffic to occur through a transcellular route (12). Furthermore, naive T-cell activation in the brain could not take place due to absence of antigen-presenting cells (APCs) and/or insufficient expression of co-stimulatory molecules (13). However, the efficiency of this isolation varies between individuals, and neural graft rejection can occur (14). Graft rejection may be more rapid when the BBB is

Correspondence to: F. Baggi; E-mail: baggi@istituto-besta.it

Received 23 June 2006, *accepted* 11 June 2007

Transmitting editor: L. Steinman

Advance Access publication 28 July 2007

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

damaged or in degenerative conditions that give rise to an immunologically active microenvironment (15).

Recognition of class I and II MHC antigens expressed by grafted cells is thought to be the first line of host defense and one of the main mechanisms giving rise to allograft rejection. Methods to prevent the rejection of neural transplants have been mainly directed against MHC-restricted T cell-mediated mechanisms (14).

Several groups have recently investigated MHC expression by NSCs from mouse, rat and human, and have documented immunological effects, which, however, are generally considered to be negligible (16–20). In the present study, we have further investigated the potential immunological recognition of NSCs, performing one-way mixed lymphocyte reaction (MLR) experiments using NSCs (cultured in normal condition or in the presence of pro-inflammatory cytokines) as stimulator (S) cells challenging peripheral blood lymphocytes (PBLs) from healthy HLA-unrelated donors (21). MLR results were correlated with MHC class I and II expression and donor's HLA haplotype. Moreover, immunological recognition of human NSCs was also studied in xenoexperiments with C57BL/6 mice.

Our data suggest that NSCs can be recognized by donor PBLs notwithstanding low levels of MHC expression. This finding indicates that MLR experiments involving NSCs and recipient PBLs might provide information to predict host rejection of neural grafts, and hence be useful as an additional tool for defining host–donor histocompatibility in candidates for NSCs transplant (22).

To further assess the immunogenic potential of NSCs, we also investigated their production of immunomodulatory cytokines: IFN-γ and IL-2 (T_h1-type cytokines), IL-10 (T_h2-type cytokine), transforming growth factor beta (TGF-β)-1 (T_h3-type cytokine), finding that NSCs actively transcribe and release detectable levels of TGF-β1, which may contribute to their low immunogenicity when challenged with allo-PBLs.

Methods

Human NSC lines

Human NSCs were derived from 12-week-old healthy forebrains collected after elective routine abortions, following the ethical guidelines of the European Network for Transplantation (NECTAR, http://www.nesu.mphy.lu.se/nectar/index.html). The use of human CNS tissue was approved by the Ethics Committee of the Neurological Institute Foundation 'Carlo Besta' and of the Obstetric–Gynecological Clinic 'L. Mangiagalli', both of Milan. Brain tissue was treated with 0.1% type I collagenase (GIBCO–Invitrogen, Milan, Italy) and 1 U ml^{-1} DNase I (US Biological, Swampscott, MA, USA) in PBS for 20 min at 37°C in a humidified atmosphere containing 5% CO_2. The tissue was then dissociated mechanically, and a single-cell suspension seeded into 25-ml tissue culture flasks in the presence of human recombinant Epidermal Growth Factor (EGF) (20 ng ml^{-1}, Peprotech, London, UK) and Basic Fibroblast Growth Factor (bFGF) (10 ng ml^{-1}, Peprotech) in basal serum-free medium (DMEM-F12, GIBCO–Invitrogen) (23, 24). These conditions promote the formation of spherical clusters of cells (neurospheres) each derived from a single cell. Neurosphere cultures can be routinely propagated and cryopreserved, maintaining multipotentiality up to ~20 passages. They can be induced to differentiate into neurons, astrocytes and oligodendrocytes by culturing in differentiating medium, i.e. mitogen-free medium plus 2% fetal bovine serum (25).

Neurospheres of growing NSCs consisted of undifferentiated neural precursor cells lacking specific neural morphology and antigens except nestin, a CNS precursor marker. Their ability to differentiate into neurons, astrocytes and oligodendrocytes was maintained for up to 18–20 passages in culture, and was routinely checked in our experiments by culturing under appropriate conditions (data not shown).

Mixed lymphocyte reaction experiments

PBLs were isolated from the blood of eight unrelated healthy donors by centrifugation over a Ficoll-Paque™ Plus (Amersham Biosciences, Milan, Italy) gradient and re-suspended in RPMI 1640 medium supplemented with 5% human male serum type AB (Sigma–Aldrich, Milan, Italy), 1% Na-pyruvate, 1% non-essential amino acids, 1% L-glutamine, 1% penicillin–streptomycin (all from Euroclone Celbio, Milan, Italy) and 2×10^{-5} M 2-mercaptoethanol (2-ME) (BDH, Milan, Italy).

Unidirectional (one-way) MLRs were performed by co-culturing 2×10^5 PBLs [responder (R) cells] with the same number of irradiated (3000 rad) NSCs, autologous PBLs (for auto-MLR) or allogeneic PBLs as S cells in a U-bottom 96-well plate (Corning Celbio, Milan, Italy). NSCs were grown under basal conditions or treated with IFN-γ (100 U ml^{-1}, Sigma–Aldrich) or tumor necrosis factor alpha (TNF-α) (10 ng ml^{-1}, Roche, Milan, Italy) for 48 h prior to irradiation. After 5 days, 1 μCi [^{3}H]-thymidine ([^{3}H]TdR) (Amersham Biosciences) was added for a further 18 h. The amount of radioactivity incorporated into DNA was measured in a β-scintillation counter. The results were expressed as mean counts per minute (c.p.m.) from triplicates and as stimulation index (SI) to allow comparison of results between donors. MLR responses were considered positive when SI > 2.5.

FACS analysis for CD25, CD69 and CD86 molecules on CD4 and CD8 T-cell subsets was performed on MLR cultures at days 0, 2 and 5. Briefly, cultures were harvested by gentle pipetting, counted and re-suspended in PBS, 2% FCS and 0.1% Na azide (FACS buffer). Cells (3×10^5) in 50 μl were stained with mAbs to CD4 (PE–Cy5, clone RPA-T4), CD8 (FITC, clone SK1), CD25 (PE, clone 2A3), CD69 (PE, clone FN50) and CD86 (PE, clone 2331-FUN-1) (all from BD Biosciences, Milan, Italy). Aspecific staining was determined with appropriate Isotype Control IgG1/IgG2a (BD Biosciences). After 30 min incubation on ice, the samples were pelleted (200 g), washed with cold FACS buffer and analyzed immediately in a FACScan flow cytometer (BD Biosciences) equipped with CellQuest Pro software (BD Biosciences). At least 20 000 events were acquired for each sample. Non-viable cells were excluded by physical gating.

HLA typing of donor PBLs and NSCs

Donor PBLs or NSCs were re-suspended in digestion buffer (NaCl 0.1 M, Tris–HCl 0.01 M, pH 8.0 and EDTA 0.025 M,

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

pH 8.0, all from Carlo Erba Antibiotics, Milan, Italy), $NaDodSO_4$ 0.5% (BDH) and proteinase K 0.1 mg ml^{-1} (Euroclone Celbio) and incubated at 50°C for 12–18 h. Nucleic acids were extracted with an equal volume of phenol/chloroform/isoamyl alcohol (SEVAQ, BDH). The aqueous layer was transferred to another tube, and the genomic DNA precipitated with cold ammonium acetate and ethanol. The air-dried pellet was re-suspended in Tris-EDTA buffer (TE buffer). HLA haplotypes at low resolution and high resolution (for alleles in common between PBLs and NSCs) were determined using commercially available kits (Olerup SSP™ Kits, GenoVision, BioSpa, Milan, Italy).

Flow cytometric measurement of expression of MHC class I and II on NSCs

MHC expression was determined on three different NSC cell lines grown under basal conditions and after treatment with IFN-γ (100 U ml^{-1}) or TNF-α (10 ng ml^{-1}) (48 h, 37°C, 5% CO_2). The cytokines up-regulate several immune-related molecules even in non-immune cells, including human muscle cells (26) and NSCs (16, 20).

Neurospheres were dissociated by gentle pipetting, counted and re-suspended in PBS, 2% FCS and 0.1% Na azide (FACS buffer). NSCs (3×10^5) in 50 μl were stained with FITC- or PE-conjugated mAbs to MHC class I (anti-human HLA-A, B and C, clone G46-2.6, BD Biosciences) or to MHC class II (anti-human HLA-DR, clone L243, BD Biosciences). After 30 min incubation on ice, the samples were pelleted (200 g), washed three times with cold FACS buffer and analyzed immediately in a FACScan flow cytometer (BD Biosciences) equipped with CellQuest Pro software (BD Biosciences). At least 20 000 events were acquired for each sample. Non-viable cells were excluded by physical gating. Aspecific staining was determined after incubation of NSCs with FITC plus PE-conjugated Isotype Control IgG1/IgG2a (BD Biosciences). PBLs from healthy donors and EBV-lymphoblastoid cell lines (LCLs) were always stained and counted along with NSCs to serve as positive controls. Mean fluorescence intensity (MFI) was calculated and expressed as MFI of stained sample minus MFI of negative control.

Immunodetection of MHC class I and II molecules on NSCs

Neurospheres from three cell lines were dissociated into individual cells and plated onto normal glass slides or slides coated with adhesive substrate (allowing the cells to flatten out and reveal structure) (Matrigel Matrix, BD Biosciences), allowed to adhere for 60 min and fixed in acetone (50 and 100%, 1 min each). Acetone-fixed cells were processed to detect MHC class I molecules (mouse mAb to HLA-ABC, clone W6/32, Dako Cytomation, Milan, Italy, dilution 1:50) and MHC class II (mouse mAb to HLA-DR, clone DK22, Dako Cytomation, dilution 1:25) by incubating with primary antibody for 90 min at room temperature. After several washes, the cells were then incubated for 60 min with HRP-conjugated anti-mouse antibody (ENVISION® System-HRP, Dako Cytomation) as secondary antibody. After three washes, diaminobenzidine tetrahydrochloride solution (Vector DBA, Milan, Italy) was added, followed by counterstaining with Mayer's hematoxylin. On negative controls, primary antibodies were omitted. The slides were mounted with Bio-Mount (Bio-Optica, Milan, Italy) and examined under a Nikon Eclipse TE300 microscope (×10 original magnification).

Molecular analysis of cytokine transcripts by NSCs grown in basal conditions

Transcripts for IFN-γ, IL-2, IL-10 and TGF-β were assayed in three different NSC cell lines grown under basal conditions by conventional reverse transcription (RT)–PCR. Total RNA was extracted from 1×10^6–2×10^6 NSCs using RNAwiz reagent (Ambion, Woodward Austin, TX, USA) and treated with DNase I (Ambion). Random-primed cDNA was prepared using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions and stored at −20°C pending PCR amplification. A constant amount of cDNA (corresponding to 200 ng of total RNA) was amplified in a PCR reaction containing 1× PCR buffer (Finnzymes, Espoo, Finland), 0.1 mM each deoxynucleoside triphosphate (Applied Biosystems, Foster City, CA, USA), 1 U DynaZyme DNA polymerase (Finnzymes) and 1 mM each of primer pairs. Primer sequences were as follows—IFN-γ: forward 5′-AAGTTATATCTTGGCTTTTCAGC-3′ and reverse 5′-TCTTCGACCTTGAAACAGCATCT-3′ (amplified fragment length: 477 bp); IL-2: forward 5′-GAATGGAATTAATAATTAC-AAGAATCCC-3′ and reverse 5′-TGTTTCAGATCCCTTTAGT-TCCAG-3′ (amplified fragment length: 229 bp); TGF-β: forward 5′-CTGCGGATCTCTGTGTCATT-3′ and reverse 5′-CTCAGAGTGTTGCTATGGTG-3′ (amplified fragment length: 246 bp); IL-10: forward 5′-AACCTGCCTAACATGCTTCG-3′ and reverse 5′-GGTCTTGGTTCTCAGCTTGG-3′ (amplified fragment length: 202 bp) and β-actin: forward 5′-CAGGATTTA-AAAACTGGAACGGT-3′ and reverse 5′-GGAATGACTATTAAA-AAAACAACAA-3′ (amplified fragment length: 120 bp). Amplification conditions were as follows: 32 cycles at 94°C for 30 s, annealing for 30 s at temperatures depending on primer pair sequences and elongation at 72°C for 30 s. The PCR products were separated by 2% ethidium bromide-stained agarose gel electrophoresis and the images acquired with Quantity One software (Gel Doc EQ Gel Documentation System, Bio-Rad Laboratories, Hercules, CA, USA).

TGF-β1 assay and in vitro effect on MLR

TGF-β1 protein was measured in the supernatants collected from three cultured NSC lines using the Quantikine kit for human TGF-β1 (R&D Systems, Minneapolis, MN, USA). Briefly, dissociated NSCs (2×10^5 cells, 1 ml) were seeded into 24-well plates and cultured in basal serum-free medium (see above for composition) and in the presence of IFN-γ (100 U ml^{-1}) or TNF-α (10 ng ml^{-1}) for 3 and 5 days; supernatants were collected and stored at −70°C pending assay. ELISA assay was conducted according to the manufacturer's instructions; each sample was tested in duplicate, and latent TGF-β1 was acid activated for 10 min at room temperature and then neutralized. TGF-β1 was quantified from a standard curve and expressed as pg ml^{-1} of culture medium.

TGF-β1 effect on PBLs proliferation was assessed by MLR experiments from four healthy donors; briefly, 2×10^5 PBLs were co-cultured in triplicate wells with irradiated allogeneic

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

PBLs in the presence of human recombinant TGF-β1 (R&D Systems) in the range of 0.01, 0.1, 1 and 10 ng ml^{-1}. After 5 days, 1 μCi [^{3}H]TdR (Amersham Biosciences) was added for a further 18 h. The amount of radioactivity incorporated into DNA was measured in a β-scintillation counter. The results were expressed as mean c.p.m. from triplicates and as percentage of the MLR response in normal culture medium without hTGF-β1 (set as 100%).

The effect of neutralizing mAb to TGF-β1 (clone 1D11.16.8, HB 9849 American Type Culture Collection, Manassas, VA, USA) was studied in MLR experiments between donor 8 (as R) and the NSC line 1. PBLs (2×10^5) were co-cultured in triplicate wells with NSCs in the presence of the neutralizing mAb at 1 and 10 μg ml^{-1}. After 5 days, 1 μCi [^{3}H]TdR (Amersham Biosciences) was added for a further 18 h. The amount of radioactivity incorporated was measured in a β-scintillation counter. The results were expressed as mean c.p.m. from triplicates (±SE).

Xenogeneic MLR experiment in C57BL/6 mice

Unidirectional (one-way) MLR experiments were performed by co-culturing single-cell suspension (2×10^5 cells) from spleen of naive C57BL/6 mice with an equal number of irradiated human NSCs (3000 rad) in a U-bottom 96-well plate (Corning Celbio). RPMI 1640 medium supplemented with 10% FCS, 1% Na-pyruvate, 1% non-essential amino acids, 1% L-glutamine, 1% penicillin–streptomycin (all from Euroclone Celbio) and 2×10^{-5} M 2-ME (BDH) was used. Control cultures were performed with autologous irradiated (3000 rad) splenic cells (for auto-MLR) or irradiated (5000 rad) EBV-LCLs as S cells. After 5 days of culture, 1 μCi [^{3}H]TdR (Amersham Biosciences) was added for a further 18 h. The results were expressed as c.p.m.

Ex vivo experiments were performed by injecting 5×10^5 NSCs (100 μl, injectable NaCl 0.9% solution, Fresenius Kabi Italia S.p.A., Verona, Italy) in the hind footpads of four female C57BL/6 mice 8 weeks of age (Charles River Italia, Calco, Italy); as controls, 8 mice were inoculated with 100 μl NaCl solution or with 5×10^5 irradiated (5000 rad) EBV-LCLs. Seven days later, draining lymph nodes and spleens were aseptically removed and processed into a single-cell suspension. Cells (2×10^5) were seeded in culture medium (see above for composition) with 2×10^5 irradiated NSCs, irradiated EBV-LCLs or irradiated autologous spleen cells in U-bottom 96-well plates (Corning Celbio). After 5 days of culture, 1 μCi [^{3}H]TdR (Amersham Biosciences) was added for a further 18 h. The results were expressed as c.p.m.

Statistical analysis

Analysis of variance followed by Bonferroni *post hoc* test and non-parametric Kruskal–Wallis test followed by Dunn's multiple comparison test were performed to assess statistical significance of results. Differences were considered significant when $P < 0.05$. Statview 5 for Macintosh (Abacus Concepts, Berkeley, CA, USA) and GraphPad Prism version 4.0 for Macintosh (GraphPad Software, San Diego, CA, USA) programs were used for data elaboration.

Results

One-way MLR experiments

Constitutive or cytokine-inducible expression of MHC class I and II molecules on NSC surfaces suggested to test whether these cells could activate immune-competent cells (PBLs) from allogeneic donors. This was assessed by measuring the proliferative responses of PBLs (R cells) from eight randomly selected healthy donors to a single line of irradiated NSCs (S cells) by one-way MLR. PBLs from a given donor was tested simultaneously with various donor PBLs as positive controls and with irradiated autologous PBLs to assess auto-MLR as negative control. Mean SI (±SE) from auto-MLR experiments was 1.1 ± 0.1; when PBLs were challenged against the NSC line, positive responses (SI > 2.5) were obtained in four of eight donors, with an overall mean SI (±SE) of 3.1 ± 0.8 (Fig. 1; supplementary Table 1 is available at *International Immunology* Online); this difference was not significant compared with auto-MLRs.

Increased expression of MHC class I and II molecules on NSCs might follow pro-inflammatory stimulation and result in greater MLR responses. Indeed, positive responses (SI > 2.5) to IFN-γ-treated NSCs were found in PBLs from five of eight donors (mean SI ± SE 3.5 ± 0.8; Dunn's multiple comparison test $P < 0.05$ compared with auto-MLR). Moreover, positive responses to TNF-α-treated NSCs occurred in the

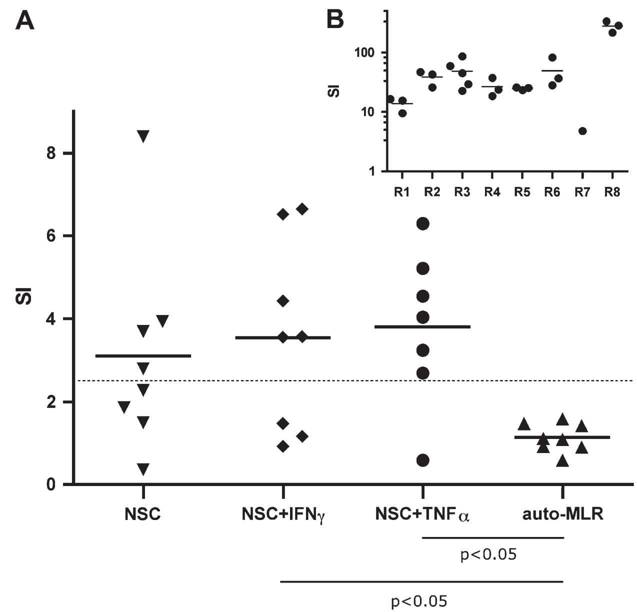


Fig. 1. One-way MLRs with NSCs. (A) NSCs grown in basal medium or pre-incubated with IFN-γ (100 U ml^{-1}) or TNF-α (10 ng ml^{-1}) for 48 h were cultured with PBLs from healthy donors 1–8 (NSC:PBL ratio = 1) for 5 days. [^{3}H]TdR was added for further 18 h. Autologous MLR (auto-MRL) are shown as negative control. Representative data from repeated experiments are expressed as SI to allow comparison between Rs and plotted as scattered symbols and mean. Dotted line represent cut-off value of SI = 2.5. Dunn's multiple comparison test was used for statistical analysis; MLR with NSC versus auto-MLR *P* not significant (ns); MLR with NSC + IFN-γ versus auto-MLR $P < 0.05$; MLR with NSC + TNF-α versus auto-MLR $P < 0.05$. (B) Allo-MLR experiments among Rs 1–8 were performed to assess the magnitude of response in the case of full competent immune cells.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

PBLs of six of seven donors (mean SI ± SE 3.8 ± 0.7; Dunn's multiple comparison test $P < 0.05$ compared with auto-MLR). These data are consistent with the increased levels of MHC class I molecules in TNF-α-treated NSCs, as well as the modest increase in MHC class II, found by FACS analysis (Fig. 2, see below).

Allogeneic MLR experiments were performed to assess the extent of immunological responses in our donors. As expected, strong proliferative responses to allo-PBLs were observed, with an overall mean SI ± SE 64.3 ± 17.8 (Fig. 1B; supplementary Table 2 is available at *International Immunology* Online). Magnitudes of allo-MLR responses were not comparable to those obtained with NSCs; however, low proliferative responses (SI < 10) were observed in some cases (R1 versus S5; R7 versus S2; Fig. 1B; supplementary Table 2 is available at *International Immunology* Online).

FACS analysis for CD25, CD69 and CD86 molecules, T-cell activation markers, were performed on one-way MLR cultures with allo-PBLs and with the NSC line 1, at days 0, 2 and 5 to further confirm NSC-induced MLR responses. Representative data of FACS analysis are reported in supplementary Figure 1 (available at *International Immunology* Online) for the CD4+ subset (panel A) and for the CD8+ subset (panel B). At day 0, the basal expression level of these markers (i.e. before co-cultures) were as follows: CD4+CD25+ 20.6%; CD4+CD69+ 0.6%; CD4+CD86+ 1.5%; CD8+CD25+ 0.4%; CD8+CD69+ 2.0% and CD8+CD86+ 0.4%. T-cell subset frequencies were found similarly increased in both MLR versus PBLs and in MLR versus the NCS line, with minor differences that might indicate a delayed expression during the co-cultures (supplementary Table 3 is available at *International Immunology* Online). Only CD4+CD25+ T-cell subset did not show any significant modification from basal level (T = 0).

Since the proliferative responses of allo-PBLs to NSCs were variable, from no MLR response to moderate SIs, we defined HLA haplotypes of the NSC line and the eight donors looking for shared HLA alleles. Interestingly, R1 and NSC shared three of six allele combinations (i.e. HLA-A*0201, HLA-B*1518 and HLA-DR*1103/1104). This donor did not show any response against the NSC line, grown in basal medium or in the presence of IFN-γ or TNF-α (MLR SI = 0.4, 0.9 and 0.6, respectively) (Table 1).

MHC class I and II expression by NSCs

The levels of MHC class I and class II molecules expression by the three different NSC lines, cultured in basal condition or in the presence of pro-inflammatory cytokines IFN-γ or TNF-α, were characterized by FACS analysis. Profiles of expression of MHC class I and class II molecules on NSCs at passage 11 are shown in Fig. 2(E and F), respectively; expression profiles of positive controls (PBLs and EBV-LCLs) are shown in Fig. 2(A–D). It is evident that NSCs did not express MHC class II molecules (Fig. 2F), but that a small proportion of NSCs expressed MHC class I molecules (Fig. 2E). MFI values for MHC class I were as follows: 16 for NSCs, 1540 for PBLs and 1816 for EBV-LCLs. MHC class I and II expression was also checked in the same NSC line at passage 6 and 16 in culture and found that class I expression and lack of class II expression did not differ from that found at passage 11 (data not shown). Expression of MHC class I and class II molecules on NSCs, studied by FACS, was confirmed by immunocytochemistry on acetone-fixed NSCs grown in basal conditions (Fig. 3). NSCs expressed MHC class I molecules (Fig. 3B and E), while MHC class II molecules were absent (Fig. 3C and F), as compared with negative controls (Fig. 3A and D).

To complete the characterization of our NSCs and compare them with those used by other groups (16, 17, 20), we incubated them with pro-inflammatory cytokines (IFN-γ and TNF-α), known to modulate the expression of MHC and other co-stimulatory molecules (e.g. CD80 and CD86) on non-professional APCs. When NSCs were treated with 100 U ml^{-1} of IFN-γ for 48 h, MHC class I expression increased slightly (Fig. 2G), compared with NSCs without IFN-γ (Fig. 2E). MHC class II molecules remained unexpressed (Fig. 2H). When NSCs were incubated with TNF-α (10 ng ml^{-1}) for 48 h (Fig. 2I), MHC class I expression increased considerably (MFI = 128) compared with NSCs without TNF-α, while MHC class II increased slightly (MFI = 7, Fig. 2J) compared with NSCs cultured in basal conditions and in IFN-γ (Fig. 2F and H, respectively).

The shift in the profile for MHC class II expression by NSCs (Fig. 2J) is closely similar to that observed for MHC class II expression in PBLs (Fig. 1B) except that NSCs did not express high levels of MHC class II molecules (MFI $>10^2$), as observed for PBLs (Fig. 2B).

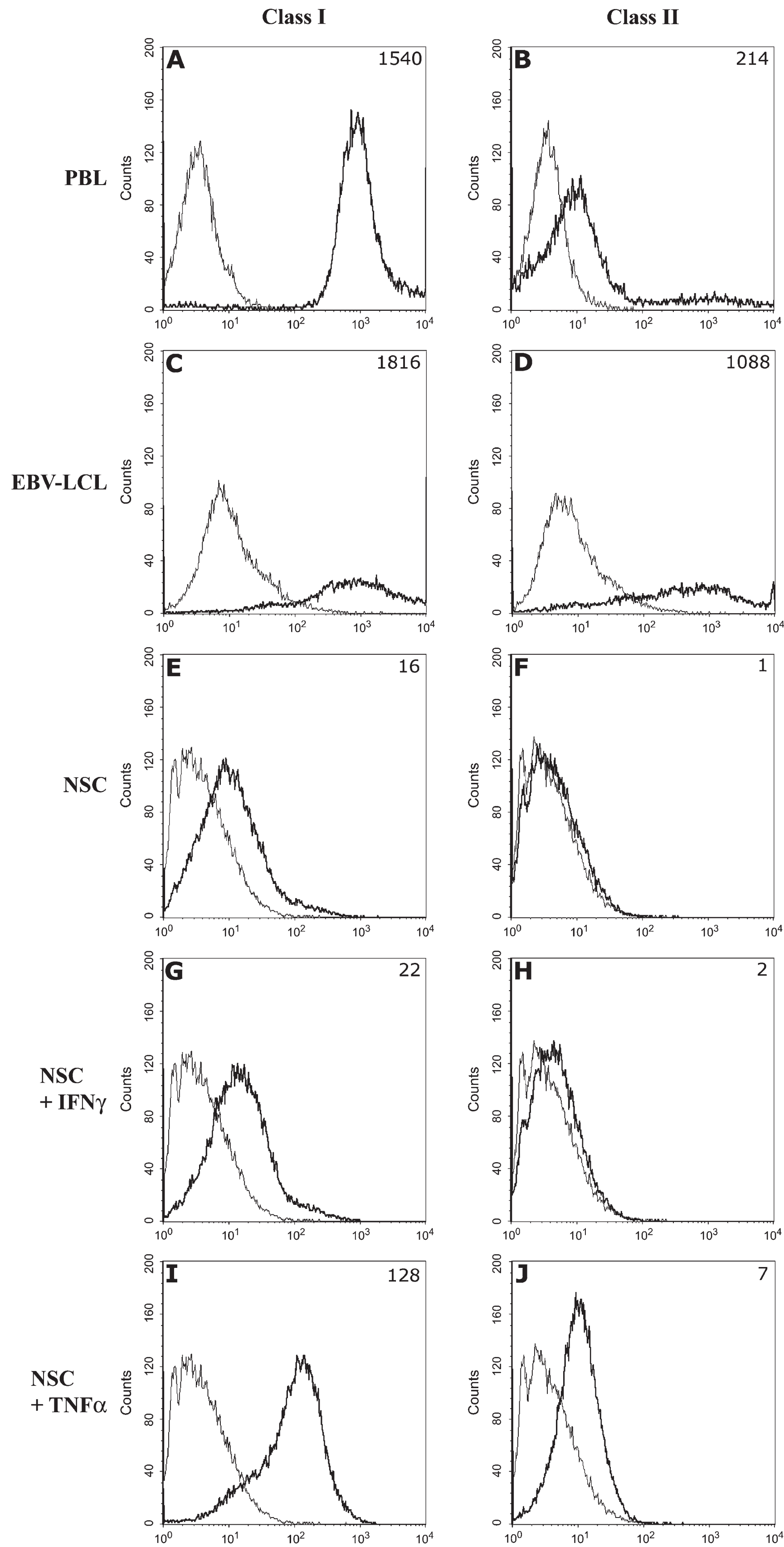
Production of TGF-β1 by NSCs as measured by RT–PCR and ELISA

We next wondered the possibility that NSCs might transcribe and release soluble mediators (cytokines) able to interact with immune-competent cells, such as PBLs involved in proliferative MLR responses. We tested this hypothesis by investigating IFN-γ-, IL-2-, TGF-β1- and IL-10-specific transcripts by RT–PCR analysis in cultured NSCs (Fig. 4). We detected only TGF-β1 mRNA in the all three NSC lines object of the present study (lanes 2–4). We did not detect transcripts for IFN-γ, IL-2 or IL-10. We then assessed by ELISA the presence of TGF-β1 in culture supernatant of NSC lines after 3 and 5 days of culture in basal medium. As shown in Fig. 5(A), TGF-β1 can be detected in NSC cultures either at day 3 (empty bars, mean pg ml^{-1} ± SE 53.9 ± 3.5 NSC L1, 56.0 ± 14.0 NSC L2 and 23.1 ± 6.3 NSC L3) and at day 5 (filled bars, mean pg ml^{-1} ± SE 91.0 ± 7.0 NSC L1, 82.6 ± 18.2 NSC L2 and 56.7 ± 3.5 NSC L3). We also measured TGF-β1 in cultures of IFN-γ- or TNF-α-treated NSCs (Fig. 5B), showing a marked reduction of cytokine release both at day 3 (mean pg ml^{-1} ± SE 30.8 ± 2.8 IFN-γ treatment and 11.9 ± 6.3 TNF-α treatment) and at day 5 (mean pg ml^{-1} ± SE 48.5 ± 5.1 IFN-γ treatment and 38.5 ± 4.9 TNF-α treatment).

Effect of hTGF-β1 on one-way MLR

It has been reported that human bone marrow stromal cells are able to interfere with T-cell response by secreting immunomodulatory molecules (27). To investigate whether TGF-β could exert any control on allogeneic T-cell response that

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024



Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

Table 1. HLA haplotypes of the NSC line used in one-way MLR experiments and of the donors (R1–8)

	HLA haplotype[a]			MLR versus NSC[b]
	HLA-A	HLA-B	HLA-DR	
NSC	02/32 (0201)	15/38 (1518)	11/- (1104)	—
R1	02/24 (0201)	15/57 (1518)	11/13 (1103)	0.4, 0.9, 0.6
R2	03/24	08/51	03/05	2.3, 1.5, 4.0
R3	02/29 (0201)	44/51	07/11 (1104)	3.7, 4.4, 6.3
R4	26/-	08/49	03/11 (1101)	1.9, 1.2, 5.2
R5	02/33 (0205)	14/58	01/16	3.9, 6.6, 4.5
R6	01/11	52/55	11/15 (1104)	1.5, 3.6, 2.7
R7	02/02 (0201/0205)	40/50	10/11 (1101)	2.8, 3.6, n.d.
R8	01/-	37/57	01/11 (1103)	8.4-6.5-3.2

[a]HLA haplotype assessed at low resolution, except for shared alleles among NSCs and PBLs that were sub-typed at high resolution and are indicated in parenthesis.
[b]MLR responses to NSCs [grown in normal culture medium, in the presence of IFN-γ or TNF-α, see also supplementary Table 1 (available at *International immunlogy* Online)] expressed as SI. n.d. = not done.

occurs in MLR experiments, different amounts of hTGF-β1 (in the range comparable to that secreted by cultured NSC) were added to one-way MLRs from four healthy donors. As shown in Fig. 6, hTGF-β1 was able to reduce T-cell proliferation with maximal effect at the concentration of 0.1 ng ml^{-1}, where MLR responses were 56–86% of those obtained in normal medium.

In order to further demonstrate that hTGF-β1 modulates PBL's responses to NSCs, PBL's proliferation (from donor 8) to NSCs was studied in the presence of anti-TGF-β1-neutralizing antibody (clone 1D.11.16.8) (Fig. 7). Proliferative responses were higher (4606 ± 1048 c.p.m. ± SE, filled bar) than those from MLR versus NSC grown in normal culture medium (1989 ± 701 c.p.m. ± SE, empty bar). Neutralization of TGF-β1 effect was not observed when 1D.11 antibody was added at the concentration of 1 μg ml^{-1} to co-cultures (gray bar). Auto-MLR (as background) was 100 ± 15 c.p.m. ± SE; SI were 19.9 (in MLR versus NSC) and 46.1 (MLR versus NSC in the presence of 10 μg ml^{-1} of anti-TGF-β1 mAb).

Human NSCs induce xenogeneic-MLR in mice

To further characterize immunological recognition of NSCs, we performed xenogeneic-MLR experiment with immune-competent cells from C57BL/6 mice. Spleens from naive C57BL/6 mice were aseptically removed and single-cell suspensions were cultured as R cells in xenogeneic-MLR experiments with irradiated human NSCs at equal cell ratio for 5 days. Data are shown in Fig. 8(A), alongside with irradiated EBV-LCLs (to estimate maximal response—positive control) or irradiated autologous spleen cells (auto-MLR—negative control). A low proliferative MLR response induced by irradiated NSCs was observed (filled bar, mean c.p.m. ± SE 4867 ± 798), not different with control cultures of spleen cells alone (background (bkg), mean c.p.m. ± SE 2690 ± 316) and to auto-MLR cultures (mean c.p.m. ± SE 3151 ± 307). Xenogeneic-MLR with human EBV-LCLs as S cells gave a robust proliferative response, corresponding to a SI of 4.7 (mean c.p.m. 14928 ± 1376 SE, $P < 0.05$).

We then investigated if human NSCs, injected in the footpads of C57BL/6 mice (classical route for immune sensitization in animals), were able to boost the mouse immune response. Dissociated human NSCs (5×10^5) were injected in four naive mice, and after 7 days, spleens and draining lymph nodes were removed. C57BL/6 mice injected with irradiated EBV-LCLs were used as control group. xenogeneic-MLR experiments were then performed with spleen cells, while from lymph nodes we did not recover sufficient cells to perform individual MLR experiment. Proliferative responses were now observed when NSCs were used as S in all the four NSC-treated mice (Fig. 8B, filled bar, mean c.p.m. ± SE 9598 ± 2510); this difference was significantly different from auto-MLR results (mean c.p.m. ± SE 3297 ± 548, $P < 0.01$) with a SI = 2.9. Spleen cells from NSCs-injected mice tested with EVB-LCLs as S gave as result a mean c.p.m. ± SE 17 060 ± 907 (SI = 5.1), not statistically different to that obtained with EVB-LCL-challenged spleen cells from normal mice (Fig. 8A). Spleen cells from EBV-LCL-injected C57BL/6 mice did not show any proliferative response *in vitro* to NSCs (Fig. 8C) due to an unspecific activation of the mouse immune system as a consequence of the xeno-injection of an irrelevant human EBV-LCLs.

Discussion

Neuronal degeneration resulting from metabolic, genetic or inflammatory pathological brain conditions can be, at least partially and temporarily, controlled by the local injection of NSCs that are able to differentiate into neurons, oligodendrocytes and astrocytes. NSC injection improved motor function in CNS-damaged animals in which injected cells migrated to the damaged area and differentiated into neurons and glia

Fig. 2. Flow cytometric analysis of MHC class I and II expression by NSCs. MHC class I and II molecules expression was studied on NSC lines cultured in normal growing medium (NSC, panels E and F) and in the presence of IFN-γ (100 U ml^{-1}, 48 h) (NSC + IFN-γ, panels G and H) or TNF-α (10 ng ml^{-1}, 48 h) (NSC + TNF-α, panels I and J). MHC class I and II expression in PBLs (panels A and B) and in EBV-LCLs (panels C and D) are shown as positive controls. Background staining (isotype-matched IgG) is shown with light lines; dark lines represent the expression profile for MHC class I and class II molecules. The graphs are representative of three independent experiments. The numbers in the upper right corner indicate the mean fluorescence intensities of the MHC-stained sample minus the background.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

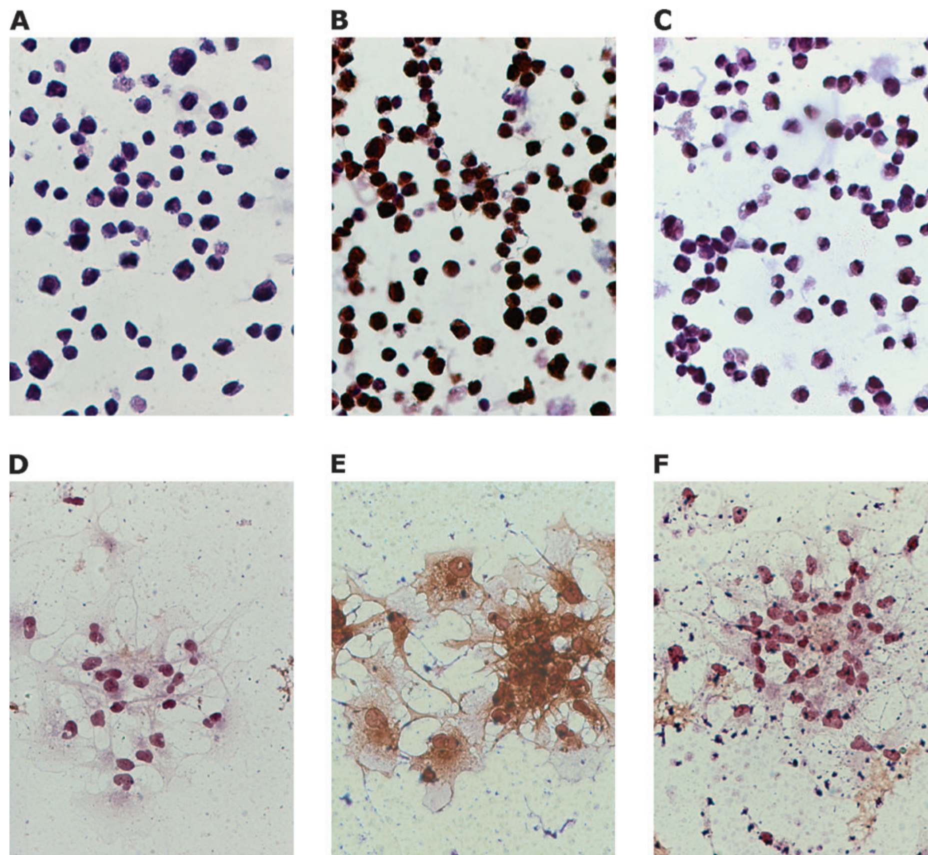


Fig. 3. Expression of MHC class I and II molecules on NSCs by immunocytochemistry. Single-cell suspensions were plated onto uncoated glass slides (A–C) or Matrigel-coated wells (D–F), allowed to adhere for 60 min and immunostained to reveal MHC class I or II molecules. Intense brown staining for MHC class I molecules is evident on NSCs (B and E) but not staining for MHC class II molecules (C and F). Negative control cultures, in which the primary antibody was omitted, are shown in A and D.

(8–10, 28). Several laboratories investigated the immunological and immunogenic properties of NSCs derived from different species, suggesting that they can be 'tolerated' by the host's immune system because the BBB efficiently isolates the brain from immunological surveillance (at least in non-inflammatory conditions), and because of the absent or very low expression of MHC and accessory/co-stimulatory molecules (17, 19, 20, 29). Because of the protected brain environment and the particular immunological features of NSCs, the potential immunogenicity of NSCs is expected to be low.

We therefore investigated the capacity of NSCs from human fetal forebrain, grown in basal conditions and in the presence of pro-inflammatory cytokines, to induce an immune response. To address this issue, we performed MLR experiments with PBLs from eight unrelated healthy donors challenged with a single NSC line. Four of eight donor cultures developed a low but non-negligible response to NSCs (mean SI ± SE 3.1 ± 0.8 for PBLs versus NSC line MLR experiments and 1.1 ± 0.1 for auto-MLR experiments) grown in basal conditions (Fig. 1; supplementary Table 1 is available at *International Immunology* Online). The response was greater when NSCs were pre-treated with IFN-γ or TNF-α (Fig. 1; supplementary Table 1 is available at *International Immunology* Online) cytokines simulating an inflammatory environment such as may occur in neurodegenerative conditions.

The analysis for CD25, CD69 and CD86 molecules, markers of T-cell activation, further confirms the MLR responses, measured by [^{3}H]TdR incorporation experiments. Representative data are reported in supplementary Figure 1 and Table 3 (available at *International Immunology* Online): increased T-cell subset frequencies were found in response to the NSCs, at both time points considered (T = day 2 and T = day 5), and the differences in the percentages of expression suggest a delayed T-cell activation in response to NSCs, when data are compared between allo-PBL-induced MLR and NSC-induced MLR. CD4+CD25+ T-cell subset did not show any modification from basal level.

These data are consistent with the increased levels of MHC molecules expressed by NSCs treated with inflammatory cytokines, examined by immunofluorescent FACS (Fig. 2). Our FACS and immunocytochemical data (Fig. 3) showed that MHC class I (but not class II) molecules were expressed on our NSCs. These findings, similar to those by Poltavtseva *et al.* (29), differ from those of Odeberg *et al.* (20), who reported that NSCs expressed both MHC class I and II molecules. Incubation of NSCs with IFN-γ showed a slightly increased expression of MHC class I molecules but had no effect on class II whereas incubation with TNF-α considerably increased MHC class I and slightly increased MHC class II expression.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

Since our NSCs did not express MHC class II molecules in basal conditions or in the presence of IFN-γ, we suggest that in our experiments, PBLs response induced by NSCs is probably mediated by MHC class I molecules. However, we did not assess the roles of specific immune cell types (CD4+, CD8+ and NK) in MLR responses to NSCs in this study.

Considering that the proliferative response of allo-PBLs to NSCs was variable (from absent to moderate), we defined HLA haplotypes of the NSC line and of the eight donors to seek a relationship between the MLR responses and common HLA alleles at the HLA-A, HLA-B and HLA-DR loci (Table 1). We do not observed a correlation between allele differences at HLA loci of donors or NSCs and the degree of MLR responses. Donor 1 was particularly noticeable, since he was not able to produce a positive response to NSCs stimulation in basal or inflammatory conditions, but shared three of six allele combinations for MHC class I (i.e. HLA-A*0201 and HLA-B*1518) and MHC class II molecules (i.e. HLA-DR*1103/1104) with the NSC line.

As mentioned above, our MLR findings differ from those of Odeberg *et al.* (20) who investigated PBLs responses to a human NSC line, finding that PBLs were unresponsive when cultured with NSCs both in basal conditions and after treatment with IFN-γ. However, they used PBLs from two incompatible donors as positive controls that happened to react strongly with each other (20). We used a panel of incompatible donor PBLs and found that the extent to which these stimulated each other was highly variable (Fig. 1B; supplementary Table 2 is available at *International Immunology* Online), but lower values were comparable with that induced by NSCs (Donor 8 versus NSC cultured in basal condition and with IFN-γ; Donor 5 versus NSC cultured with IFN-γ). MLRs between donor PBLs are clearly useful as positive controls, since the complete array of stimulatory and co-stimulatory molecules expressed by these cells often leads to a striking proliferative response. Such a striking response is probably not expected from NSCs, but the low responses that these cells often provoked imply that they can stimulate the immune system. Similar studies have been performed with muscle cells and myoblasts to assess their capacity to act as non-professional APCs (26, 30–32): expression of MHC and co-stimulatory molecules increased after exposure to pro-inflammatory stimuli, and myoblasts could be efficient APCs. Nevertheless, myoblasts provoke low proliferative PBLs responses (much lower than those between allo-PBLs) in MLR experiments (31). It is also noteworthy that when a human muscle cell line (rhabdomyosarcoma TE671), transfected with cDNA coding for the relevant MHC class II molecule, was challenged with a CD4+ Acetylcholine receptor

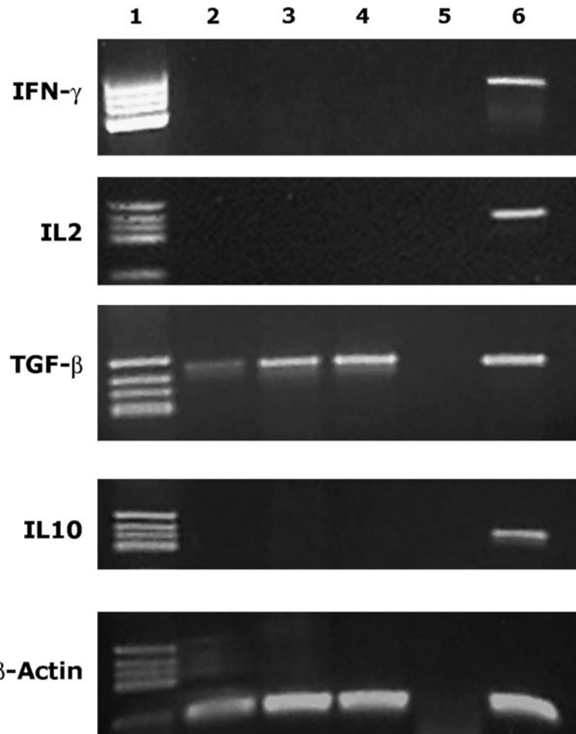


Fig. 4. Cytokine expression by NSC. Transcripts for IFN-γ, IL-2, TGF-β, and IL-10 were investigated by PCR in three NSC lines cultured in basal conditions (lanes 2–4). TGF-β mRNA was detected in NSCs. RT–PCR for β-actin was an internal control. NSCs did not produce detectable levels of IFN-γ, IL-2 or IL-10. Lane 1, molecular weight marker; lane 5, no cDNA template and lane 6, positive control (ConA-stimulated PBLs).

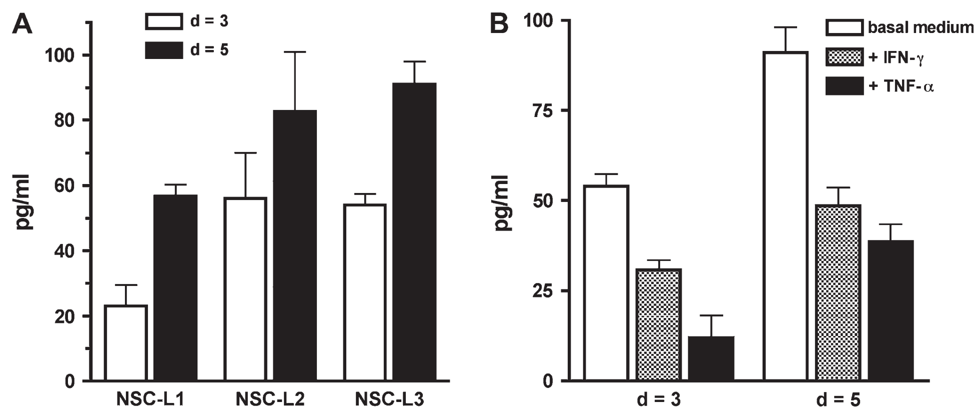


Fig. 5. ELISA detection of TGF-β1 in culture supernatants. Panel A: three NSC lines were cultured in basal medium (2×10^5 cells, 1 ml) and supernatant collected after 3 and 5 days for measurement of TGF-β1. Results are expressed as histograms (mean + SE, pg ml^{-1}) for 3 days (empty bars) and 5 days (black bars) of culture. Panel B: kinetics of TGF-β1 release was also defined in IFN-γ (100 U ml^{-1}, dotted bars) or TNF-α-treated NSCs (10 ng ml^{-1}, black bars), and cultured for 3 and 5 days, as indicated in the graph.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

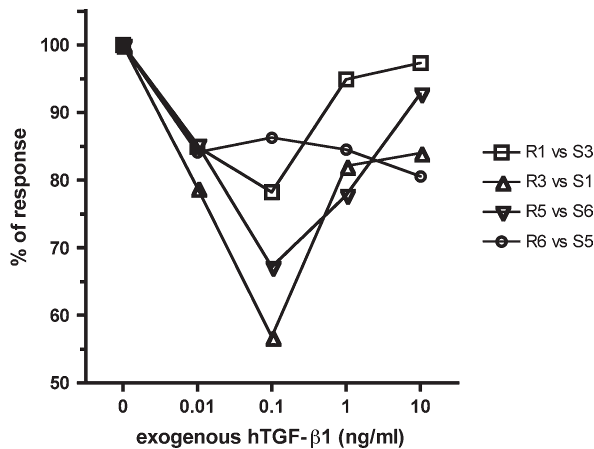


Fig. 6. Effect of hTGF-β1 on T-cell proliferation. R and irradiated S cells from four healthy donors were cultured at 1:1 ratio for 5 days (one-way MLR) in normal culture medium or in the presence of increasing amount of hTGF-β1 (0.01, 0.1, 1 and 10 ng ml^{-1}). [^{3}H]TdR was added for further 18 h. Results are expressed as percentage of the MLR response in normal culture medium without hTGF-β1 (set as 100%).

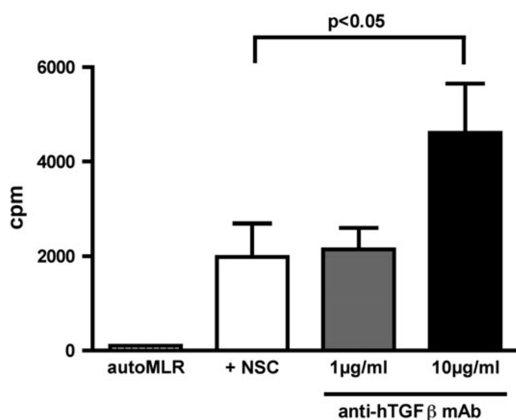


Fig. 7. Effect of neutralizing anti-TGF-β1 antibody on NSC-induced T-cell proliferation. PBLs from R8 were co-cultured with the NSC line 1 in a classical one-way MLR experiment (empty bar) and in the presence of two different amounts of neutralizing anti-TGF-β1 mAb clone 1D.11.16.8 (1 and 10 μg ml^{-1}, gray and black bars, respectively). PBLs proliferation to NSCs was found to be higher (4606 ± 1048 c.p.m. ± SE, black bar) as compared with MLR versus NSC in normal culture medium (1989 ± 701 c.p.m. ± SE, empty bar). Auto-MLR (as background) was very low (100 ± 15 c.p.m. ± SE). SI were 19.9 (MLR versus NSC) and 46.1 (MLR versus NSC in the presence of 10 μg ml^{-1} of anti-TGF-β1 mAb).

(AChR)-specific T-cell clone, a low proliferative response was observed, but a marked rhabdomyosarcoma cell death occurred, presumably mediated by the production of cytokines by activated AChR-specific T cells (33).

To examine thoroughly the ability of NSCs to induce immunoreactivity, we studied whether human NSCs could generate xenoreaction in mouse immune-competent cells. Human NSCs were injected into hind footpads of C57BL/6 mice, and splenic mouse cells were challenged with NSCs in a one-way MLR experiment. Significant cell proliferation was observed from mice that were exposed to human NSCs; on the contrary a very low reactivity was seen in splenic cells from untreated mice (Fig. 8). These preliminary data show that human NSCs transplantation into mice footpads can activate immune-competent cells within the spleen of recipient mice. In this regard, Grinnemo *et al.* (34) investigated xenoreactivity of human mesenchymal stem cells in experimentally induced ischemic rat myocardium and reported that a transplant reaction occurred in their xenogeneic model. In a recent paper, Muraoka *et al.* (35) investigated the immunologic response of the host tissue between autologous and allogeneic NSCs transplantation in adult rats. Their results indicated that NSCs survival, migration and neuronal differentiation were higher for autologous than for allogeneic transplantation, and immunoreactivity in the host brain tissue strongly influences NSCs transplantation.

We suggest that NSCs may be able to partially modulate immune recognition on the basis of our finding that all three NSC lines produced TGF-β1 transcripts in basal conditions and that TGF-β1 itself was detected in the culture medium at 3 and 5 days of culture. Di Nicola *et al.* (27) reported that human bone marrow stromal cells are able to produce TGF-β1 and suppress T-cell proliferation. We therefore added hTGF-β1 to allogeneic MLR experiments between four unrelated donors to investigate whether modulation of T-cell proliferation could be mediated by a soluble factor. Our MLR results (Fig. 6) indicate that hTGF-β1, at concentrations comparable to that released by NSCs cultured in basal conditions, exerts an inhibitory effect on T-cell proliferation, and suggest that TGF-β1 plays a role in modulating their potential immunogenicity. This hypothesis has been further confirmed by the effect of anti-TGF-β1-neutralizing antibody on NSC-induced MLRs: higher proliferative responses were associated to the presence of 1D.11 mAb during co-cultures (Fig. 7). The critical role of TGF-β1 in the control of T-cell responses has been also reported by others: Holzer *et al.* (36) showed that this cytokine was suppressive on human antigen-specific T_h1 clones, while on T_h2 clones only marginal effects were seen; Sung *et al.* (37) report that TGF-β1 enhances anti-CD3-stimulated proliferation in memory/effector CD4+ cells from BALB/c mice and on the contrary, TGF-β1 inhibits anti-CD3-stimulated proliferation in naive CD4+ T cells.

Nevertheless, our findings suggest that if the low—but not negligible—immune responses against NSCs were to occur *in vivo*, particularly in the context of an inflammatory microenvironment, they might be sufficient to activate other immune-mediated mechanisms that eventually result in late rejection of the transplanted NSCs. Our results are drawn from *in vitro* experiments only, but we cannot exclude that NSCs leakage might accidentally take place in the case of NSCs transplant in brain areas, particularly when the BBB is broken as in multiple sclerosis where pro-inflammatory cytokines are present in the brain lesions making BBB more permeable and NSCs can be more easily recognized by the immune system. This raises the possibility that allo-NSCs grafting might induce in the long term an aggressive immune activation, thus vanishing their therapeutical effect, particularly in the prospect of repeated NSCs administrations.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

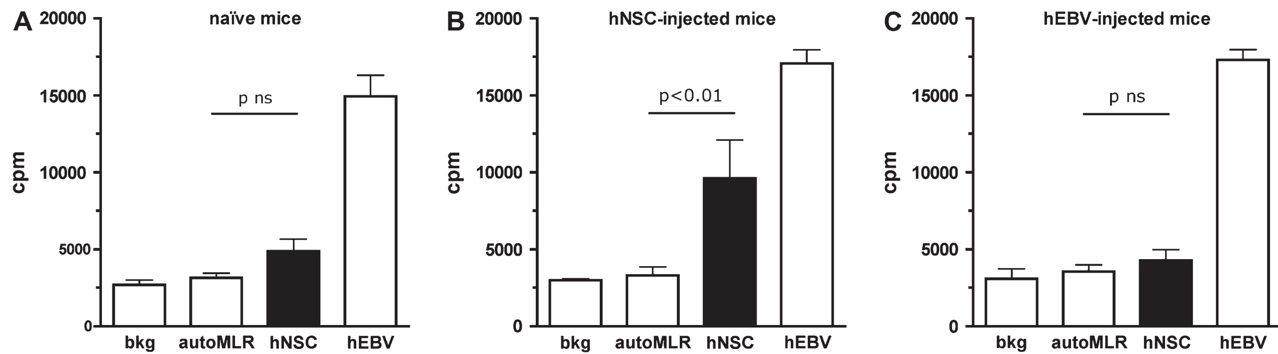


Fig. 8. Xeno-recognition of human NSC. Splenic cells from naive mice (panel A), from hNSC-injected mice (panel B) and from hEBV-injected mice (panel C) were co-cultured with the following irradiated S cells (1:1 ratio): autologous splenocytes (auto-MLR), hNSC line (the same used for *in vivo* treatment) and hEBV-LCL (as positive control), for 5 days. [^{3}H]TdR was added for further 18 h. The data are expressed as mean c.p.m. ± SE (from triplicate wells).

Supplementary data

Supplementary Figure 1 and Tables 1–3 are available at *International Immunology* Online.

Funding

The Italian Ministry of Health (2001/95).

Acknowledgements

We thank C. Antozzi, G. Alessandri and M. Gelati for critical discussion of the manuscript and Don Ward for help with the English. This work was presented in part at the Federation of Clinical Immunology Societies (FOCIS) Annual Meeting 2006, June 1–5, 2006, San Francisco, CA, USA.

Abbreviations

AChR	acetylcholine receptor
APC	antigen-presenting cell
BBB	blood–brain barrier
CNS	central nervous system
IFN-γ	interferon gamma
MHC	major histocompatibility complex
MLR	mixed lymphocyte reaction
Ns	not significant
NSC	neural stem cell
PBL	peripheral blood lymphocytes
R	responder
S	stimulator
SI	stimulation index
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha

References

1 Gage, F. H. 2000. Mammalian neural stem cells. *Science.* 287:1433.
2 Galli, R., Gritti, A., Bonfanti, L. and Vescovi, A. L. 2003. Neural stem cells: an overview. *Circ. Res.* 92:598.
3 Parati, E. A., Pozzi, S., Ottolina, A., Onofrj, M., Bez, A. and Pagano, S. F. 2004. Neural stem cells: an overview. *J. Endocrinol. Investig.* 27 (Suppl.6):64.
4 Bez, A., Corsini, E., Curti, D. *et al.* 2003. Neurosphere and neurosphere-forming cells: morphological and ultrastructural characterization. *Brain Res.* 993:18.
5 Isacson, O. 2003. The production and use of cells as therapeutic agents in neurodegenerative diseases. *Lancet Neurol.* 2:417.
6 Lindvall, O., Kokaia, Z. and Martinez-Serrano, A. 2004. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat. Med.* 10 (Suppl.):S42.
7 Martino, G. and Pluchino, S. 2006. The therapeutic potential of neural stem cells. *Nat. Rev. Neurosci.* 7:395.
8 Riess, P., Zhang, C., Saatman, K. E. *et al.* 2002. Transplanted neural stem cells survive, differentiate, and improve neurological motor function after experimental traumatic brain injury. *Neurosurgery.* 51:1043.
9 Imitola, J., Raddassi, K., Park, K. I. *et al.* 2004. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. *Proc. Natl Acad. Sci. USA.* 101:18117.
10 Wennersten, A., Meier, X., Holmin, S., Wahlberg, L. and Mathiesen, T. 2004. Proliferation, migration, and differentiation of human neural stem/progenitor cells after transplantation into a rat model of traumatic brain injury. *J. Neurosurg.* 100:88.
11 Barker, C. F. and Billingham, R. E. 1977. Immunologically privileged sites. *Adv. Immunol.* 25:1.
12 Abbott, N. J., Ronnback, L. and Hansson, E. 2006. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* 7:41.
13 Pachter, J. S., de Vries, H. E. and Fabry, Z. 2003. The blood-brain barrier and its role in immune privilege in the central nervous system. *J. Neuropathol. Exp. Neurol.* 62:593.
14 Tambur, A. R. 2004. Transplantation immunology and the central nervous system. *Neurol. Res.* 26:243.
15 Duan, W. M., Westerman, M. A., Wong, G. and Low, W. C. 2002. Rat nigral xenografts survive in the brain of MHC class II-, but not class I-deficient mice. *Neuroscience.* 115:495.
16 McLaren, F. H., Svendsen, C. N., Van der Meide, P. and Joly, E. 2001. Analysis of neural stem cells by flow cytometry: cellular differentiation modifies patterns of MHC expression. *J. Neuroimmunol.* 112:35.
17 Modo, M., Mellodew, K. and Rezaie, P. 2003. *In vitro* expression of major histocompatibility class I and class II antigens by conditionally immortalized murine neural stem cells. *Neurosci. Lett.* 337:85.
18 Mammolenti, M., Gajavelli, S., Tsoulfas, P. and Levy, R. 2004. Absence of major histocompatibility complex class I on neural stem cells does not permit natural killer cell killing and prevents recognition by alloreactive cytotoxic T lymphocytes *in vitro*. *Stem Cells.* 22:1101.
19 Imitola, J., Comabella, M., Chandraker, A. K. *et al.* 2004. Neural stem/progenitor cells express costimulatory molecules that are differentially regulated by inflammatory and apoptotic stimuli. *Am. J. Pathol.* 164:1615.
20 Odeberg, J., Piao, J. H., Samuelsson, E. B., Falci, S. and Akesson, E. 2005. Low immunogenicity of *in vitro*-expanded human neural cells despite high MHC expression. *J. Neuroimmunol.* 161:1.
21 Mickelson, E. M., Longton, G., Anasetti, C. *et al.* 1996. Evaluation of the mixed lymphocyte culture (MLC) assay as a method for

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024

selecting unrelated donors for marrow transplantation. *Tissue Antigens.* 47:27.

22 Petersdorf, E. W., Anasetti, C., Martin, P. J. and Hansen, J. A. 2003. Tissue typing in support of unrelated hematopoietic cell transplantation. *Tissue Antigens.* 61:1.

23 Vescovi, A. L., Parati, E. A., Gritti, A. *et al.* 1999. Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp. Neurol.* 156:71.

24 Pagano, S. F., Impagnatiello, F., Girelli, M. *et al.* 2000. Isolation and characterization of neural stem cells from the adult human olfactory bulb. *Stem Cells.* 18:295.

25 Parati, E. A., Bez, A., Ponti, D. *et al.* 2002. Human neural stem cells express extra-neural markers. *Brain Res.* 925:213.

26 Mantegazza, R., Gebbia, M., Mora, M. *et al.* 1996. Major histocompatibility complex class II molecule expression on muscle cells is regulated by differentiation: implications for the immunopathogenesis of muscle autoimmune diseases. *J. Neuroimmunol.* 68:53.

27 Di Nicola, M., Carlo-Stella, C., Magni, M. *et al.* 2002. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 99:3838.

28 Pluchino, S., Quattrini, A., Brambilla, E. *et al.* 2003. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature.* 422:688.

29 Poltavtseva, R. A., Marey, M. V., Aleksandrova, M. A., Revishchin, A. V., Korochkin, L. I. and Sukhikh, G. T. 2002. Evaluation of progenitor cell cultures from human embryos for neurotransplantation. *Brain Res. Dev. Brain Res.* 134:149.

30 Goebels, N., Michaelis, D., Wekerle, H. and Hohlfeld, R. 1992. Human myoblasts as antigen-presenting cells. *J. Immunol.* 149:661.

31 Bernasconi, P., Confalonieri, P., Andreetta, F., Baggi, F., Cornelio, F. and Mantegazza, R. 1998. The expression of co-stimulatory and accessory molecules on cultured human muscle cells is not dependent on stimulus by pro-inflammatory cytokines: relevance for the pathogenesis of inflammatory myopathy. *J. Neuroimmunol.* 85:52.

32 Curnow, S. J., Willcox, N. and Vincent, A. 1998. Induction of primary immune responses by allogeneic human myoblasts: dissection of the cell types required for proliferation, IFNgamma secretion and cytotoxicity. *J. Neuroimmunol.* 86:53.

33 Baggi, F., Nicolle, M., Vincent, A., Matsuo, H., Willcox, N. and Newsom-Davis, J. 1993. Presentation of endogenous acetylcholine receptor epitope by an MHC class II-transfected human muscle cell line to a specific CD4+ T cell clone from a myasthenia gravis patient. *J. Neuroimmunol.* 46:57.

34 Grinnemo, K. H., Mansson, A., Dellgren, G. *et al.* 2004. Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infarcted rat myocardium. *J. Thorac. Cardiovasc. Surg.* 127:1293.

35 Muraoka, K., Shingo, T., Yasuhara, T. *et al.* 2006. The high integration and differentiation potential of autologous neural stem cell transplantation compared with allogeneic transplantation in adult rat hippocampus. *Exp. Neurol.* 199:311.

36 Holzer, U., Rieck, M. and Buckner, J. H. 2006. Lineage and signal strength determine the inhibitory effect of transforming growth factor beta1 (TGF-beta1) on human antigen-specific Th1 and Th2 memory cells. *J. Autoimmun.* 26:241.

37 Sung, J. L., Lin, J. T. and Gorham, J. D. 2003. CD28 co-stimulation regulates the effect of transforming growth factor-beta1 on the proliferation of naive CD4+ T cells. *Int. Immunopharmacol.* 3:233.

Downloaded from https://academic.oup.com/intimm/article/19/9/1063/665363 by guest on 20 April 2024