LEGENDS

Figure 1

Histological characterization of OECs and ONFs in the EPI and LP layers of the WM (A,B) and the glomerular (GL) and nerve fibre layers (N) of the OB (C,D). S100 (red) and p75 (green).

Cultures all 4 sources: OB (E 1,5, 9), WM (C 2, 6, 10), EPI (E 3, 7, 11) and LP (E 4, 8, 12). p75 (green), FN (red), and overlay. Scale bars, 100 µm (A-D) 200 µm (E).

Figure 2

Cultures of OB (A 1-3) and WM (C 1-3) S100 (red) and Thy1 (green). Comparison of the two ONF markers (FN, red and Thy1, green) in cultures of samples from OB (B 1-3) and WM (D 1-3). Evolution of changes in culture (OB) from 14DIV (E 1-3) to 28DIV (F 1-3). p75 (green); FN (red), and overlay. Abnormal, large cells in long term cultures (G, p75, green and H, Thy1, red). Progeny of cells from OB samples (I,K) and WM samples (J,L) sorted on the basis of p75 (I,J) and Thy1 (K,L) and cultured for a further 4 DIV. The p75 purified cells from the WM sourced cultures (J), but not the OB cultures (I), have generated a further small population of doubly labeled cells (J, white arrowheads), identified by the deposition of FN (red) which overlaps with the p75+ cells (green). Neither OB nor WM sourced cells purified on the basis of Thy1 (K,L) generate a p75+ population. p75, green; FN, red; DAPI, blue. Scale bars, 200 µm (A-F and I-L) and 100 µm (G,H).

Figure 3

Cytograms showing evolution of the 4 antigenic subpopulations (p75+Thy1-, p75-Thy1+, p75-Thy1- and p75+Thy1+) in samples of OB and WM at 0, 7, 10 and 14 DIV and EPI and LP at 7,10 and 14

DIV. X-axis, intensity of AF488 labelling for p75; y-axis, the intensity of PE labeling for Thy1. Cell density from blue (low) to red (high). The cytograms confirm the immunocytochemical observation of the development of distinct, non-overlapping populations of p75+Thy1- (OECs) and p75-Thy1+ (ONFs), as well as a double negative (p75-Thy1-) population and in the WM cultures, a population of double positive (p75+Thy1+) cells.

Figure 4

Changes in the cell populations at 7, 10 and 14 days in culture shown by flow cytometry.

Data points show the mean ± SD of 5 repeats for each of the four tissue sources (OB, WM, EPI and LP). Total number of samples = 60.

Figure 5

Flow cytometric bivariate simultaneous antigenic and cell proliferation assay

A-D show an example of the sequence of FACS analysis of one sample for p75+ cells.

A, Cellular aggregates-debris discrimination based on the forward (x-axis) and side (y-axis) scatter signals from each particle that is present. The gating discriminates live cells from debris and dead cells. This gated population is then used for cytometric analysis.

B, Cytometric analysis of OECs (abscissa) and ONFs (ordinate) based on p75 and Thy1 fluorescence, delineated with gatings. Negative control is cells stained with only fluorophore conjugated antibody. Positive control for anti-p75 is Schwann cell line RT4-D6P2T, stained for only anti-p75 and anti-IgG AF488 (Hai et al., 2002). For anti-Thy1.1, the positive control is LP cells cultured for DIV17, stained for only anti-Thy1.1-PE. Each of the sub-populations is subsequently used for singleton-doubleton discrimination.

C, DNA measurement and singletons-doubletons discrimination. The gating selects for only singletons, excluding doubletons or aneuploid cells. The singleton population is then used for cell cycle kinetic analysis.

D, Cell cycle kinetic analysis, based on EdU Pacific Blue fluorescence intensity (y-axis) and total DNA content CellCycle 633-red intensity (x-axis), illustrating a typical horseshoe distribution. For the negative control cells from the same batch were stained as above without incubation with EdU. The left lower gated aggregate represents quiescent cells in the non-dividing G1/G0-phase, which do not incorporate EdU. The upper gated population represents the S-phase cells which incorporated EdU during their DNA multiplication. The right lower population represents EdU-negative cells which had reached their G2/M-phase of doubled DNA content prior to the administration of EdU.

Figure 6

Flow cytometric simultaneous antigenic and cell cycle analysis at 7, 10 and 14 days in culture for the p75+Thy1- and p75-Thy1+ subpopulations in all four tissue sources, and the double labelled (p75+Thy1+) population (found only in the WM source).

Each point is the mean ± SD of 3 repeats of the proportion of the population in G0/1-phase (dashed line, squares), S-phase (solid line, triangles), G2/M (dotted line, circles).

Figure 7

Global correlation of the proportions of all OECs and ONFs in all samples with corresponding percentages of cells in S-phase

Figure 8

Partial correlations for each of the OB, WM, EPI and LP samples of the proportions of all OECs and ONFs in all samples with corresponding percentages of cells in S-phase.

Table 1

Evolution of the proportions of OECs, ONFs, and double negative and double positive cells in the 4 cultures over time.

Five replications for all phenotypes from all sources at 7-14 DIV, except 3 for WM p75+Thy1+, and 2 OB and WM at DIV0.

Table 2

The proportions of cells in S-, G0/1- and G2/M-phases in 36 OB, WM, EPI and LP samples at 7, 10 and 14DIV (3 replications of each sample at each time point).