

Fluorescence-activated sorting of fetal human oligodendrocyte progenitor cells

This plot shows the result of dual-color FACS of a 23 week human fetal ventricular zone dissociate, after concurrent immunostaining for both A2B5 (*green*) and PSA-NCAM (*red*). The FACS plot on the left (**A**) illustrates a matched but unstained 23 week dissociate. On the right, **B** shows the same VZ dissociate, sorted after dual immunolabeling for A2B5 (FL1, *y* axis) and PSA-NCAM (FL2, *x* axis). The A2B5⁺/PSA-NCAM⁻ fraction in R1R3, comprising 16.5% of the dissociate, corresponded to glial progenitor cells. Although these were able to generate both astrocytes and oligodendrocytes, they were preferentially oligoneogenic when derived at this gestational age, and were thus designated as oligodendrocyte progenitor cells (OPCs). In contrast, the R1R5 fraction, defined by the antigenic phenotype A2B5⁻/PSA-NCAM⁺, generated largely neurons in vitro (*not shown*), and was therefore defined as a neuronal progenitor pool.

C-D shows, at lower and higher magnification respectively, the presence of O4⁺ oligodendrocytes generated from the A2B5+/PSA-NCAM- fraction four days post-FACS. In contrast, the R1R5 fraction, defined by the antigenic phenotype A2B5-/PSA-NCAM-, generated largely neurons in vitro (*not shown*), and was therefore defined as a neuronal progenitor pool.

E and **F** plot the phenotypic composition of sorted fetal oligodendrocytic and neuronal progenitor cells, respectively defined by the antigenic phenotypes A2B5⁺/PSA-NCAM⁻ (**E**) and A2B5⁻/PSA-NCAM⁺ (**F**), and sorted within the R1R3 and R1R5 fractions of 1B. Each isolate was immunostained for oligodendrocytic O4, neuronal βIII-tubulin, astrocytic GFAP, and nestin as a marker of uncommitted and immature phenotypes. Scale: **C**, 60 μm; **D**, 30 μm.