Astroglial cells support the neuronal differentiation of immortalized neuroectodermal progenitors

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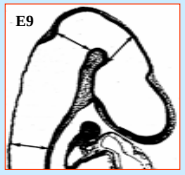
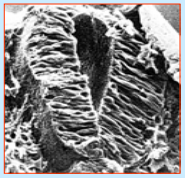
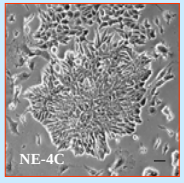
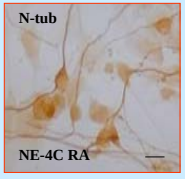
NE-4C neuroectodermal progenitor cells give rise to neurons and astroglia upon induction with *all trans* retinoic acid



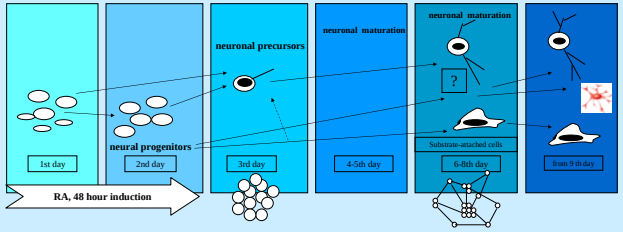




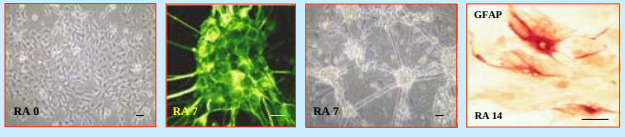


Neuroectodermal progenitor cells were isolated from the fore- and midbrain vesicles of 9-day old mouse embryos lacking functional "tumor suppressor" protein p53. One of the several single-cell cloned lines, NE-4C, was used in the present studies.
NE-4C cells give rise to neurons and astroglia upon induction with *all trans* retinoic acid (RA).
The RA induced neuronal differentiation of NE-4C cells proceeds through well reproducible steps of morphological and functional maturation (see refs. 5,6,7,8,9,10).

RA induced neuronal differentiation of NE-4C cells



Monolayer of apparently uniform, proliferating cells | Aggregate-formation; appearance of short processes | Migration; process elongation | Neuronal network-formation on monolayer of substrate-attached cells | Appearance of the first astroglial cells



The inducing effect of RA could not be replaced by addition of several growth factors like bFGF, EGF, IGF-1, Il-2, NGF. *All trans* RA, on the other hand, was effective in the initiation of neuron formation in a concentration range of 10^{-6}-10^{-8} M, even if applied for a short period, from 1h to 48 hours.
By the second day of RA treatment compact cell aggregates were formed. Aggregation was facilitated by increased cohesion of randomly encountering cells, emphasizing the role of RA in the alteration of cell surface properties (7). The first cells exhibiting neuron-like morphology appeared inside the aggregates. Aggregation alone, however, was insufficient to induce large-scale neuron-formation. The concerted actions of RA and cellular interactions were both necessary for neuron production (8).
RA-induced NE-4C cells expressed neuron-specific genes in a developmentally controlled manner and were characterized by complex electrophysiological and immunocytochemical properties of neurons (8,9,10).

References

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Acknowledgement

The authors are indebted to Prof. Duda E, Kósz E and Dr Domonkos Á. (Biological Research Center of Hung.Acad.Sci, Szeged) for the preparing and introducing the GFP-vector and Herberth B., Prof. Jaffredo T. (Institut de'Embryologie, College de France) for establishment of the PLAP/NE-4C subclone.

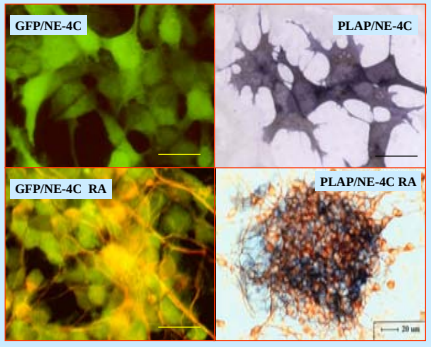
Introduction

All-trans retinoic acid (RA) was found to have important regulatory functions during embryonic development of the nervous system. *In vitro*, RA was shown to induce neuronal differentiation of several cell types, such as neural crest cells, PC12 cells, embryonal carcinoma (EC) cells and embryonic stem (ES) cells (12). In a new *in vitro* model system of neuronal differentiation, we show that astroglial cells can replace RA in induction of the neuronal phenotype.

Multipotential progenitor cells or stem cells within the *developing* and the *mature* brains are in close contact with *astroglial cells*. Astrocytes guide and regulate the migration of neuronal precursors during cortical development (1,2). Glial cells within the subventricular zone of adult animals seem to be involved in the maintenance of the non-differentiated neural stem cell population, or may serve as a source of new neurons themselves (3,4). Implanted neural progenitors or stem cells, which may – in theory - take part in the replacement of lost neuronal populations, encounter *reactivated astroglial cells* within the injured or diseased brain. Hence, the matter of glia/neural progenitor interaction is of great clinical importance.

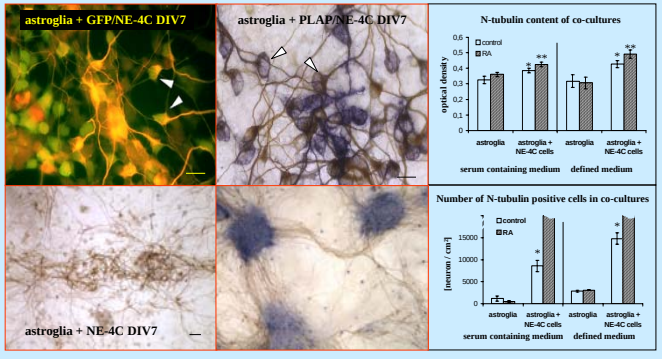
To examine the influence of astroglial cells on the fate of non-comitted neuroectodermal progenitors, we performed experiments *in vitro*. Neuroectodermal progenitor cells of the NE-4C cell line (developed in our laboratory [5,6,7,8,9,10]) were co-cultured with primary astroglial cells prepared from the brains of neonatal rats or mice.

Subclones of NE-4C cells, carrying histological marker genes display unchanged developmental potential



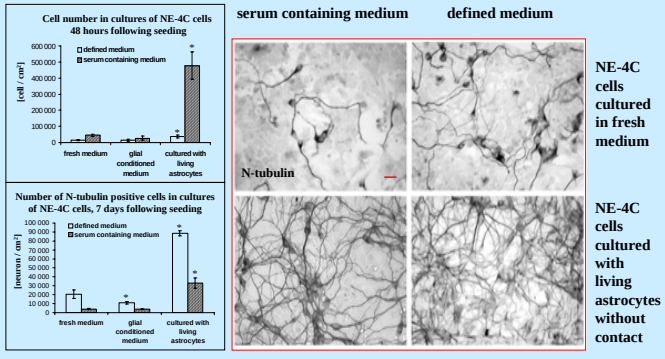
NE-4C cells were transfected with vectors encoding green fluorescent protein (GFP), or placental alkaline phosphatase enzyme (PLAP), respectively. The expression of the marker proteins did not alter the responsivity of the cells to *all trans retinoic acid*, neither in GFP-4C nor in PLAP-4C cell lines.

Astrocytes induce neuronal differentiation of the NE-4C neuroectodermal progenitor cells in "contact" co-cultures



The major characteristic feature of glia/progenitor co-cultures was the development of *foci of abundant neuron production*. Most of the neurons, identified by immunocytochemical detection of neuron specific III-type β-tubulin appeared in loose aggregates. By the 7th day of co-culture significant increase in the number of N-tubulin expressing cells was observed as it was shown either by in situ ELISA or direct cell counting. The rate of neuron formation supported by astroglial cells was always higher, if cells were cultured in serum-free defined medium.
Treatment with all-trans retinoic acid further increased the rate of neuron formation. Bundles of long neurites and compact aggregates of N-tubulin expressing cells were formed in the presence of RA.

Astrocytes induce the neuronal differentiation of the NE-4C progenitors through factor(s) released into the culture medium

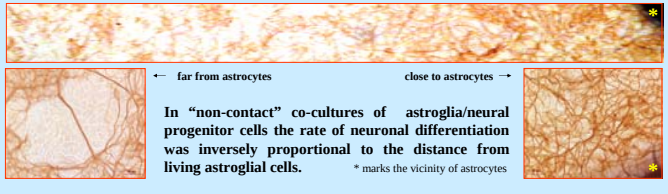


NE-4C cells cultured in fresh medium

NE-4C cells cultured with living astrocytes without contact

To see whether the neuronal differentiation observed in co-cultures was due to direct cell to cell signaling or the more to the action of secreted factors, we investigated the effects of glial conditioned medium (GCM). GCM was either transferred from pure astroglial cultures to NE-4C cells, or NE-4C progenitors were co-cultured with astrocytes in a common fluid environment, where physical contact of the two cell types was prevented.
Addition of GCM did not cause increase in the *overall cell number* of neural progenitor cells within 48 hours after plating. However, a significant increase in the cell number was observed, if neural progenitors were grown in the presence of living astrocytes. Similarly, the *rate of neuronal differentiation* was not raised by the addition of GCM, whereas a significant increase in total neuron number was observed in NE-4C cultures grown together with living astrocytes.
Both the rate of spontaneous neuron formation and the rate of astroglia-supported neurogenesis were significantly higher in serum-free culture medium - resulting in larger cell groups and in a denser neuronal network - than in medium containining serum.

gradient



In "non-contact" co-cultures of astroglia/neural progenitor cells the rate of neuronal differentiation was inversely proportional to the distance from living astroglial cells.

* marks the vicinity of astrocytes

Conclusion

Our results show, that **astroglial cells** derived from neonatal rat or mouse forebrains **support the neuronal differentiation of the neuroectodermal progenitor cells**. The data indicate, that the success of neuronal induction of neuroectodermal progenitors by astrocytes is **due to *a close cell to cell contact* and/or due to some *easily degradable, short range acting factors*** released into the medium by astroglial cells. All-trans retinoic acid is a potent candidate for such a role (11).