Short Contribution

Role of early migratory neural crest cells in developmental anomalies induced by ethanol

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ABSTRACT The purpose of this work was to study the dispersion of early migratory neural crest cell (NCC) of chick embryos treated with ethanol concentration known to induce the Fetal Alcohol Syndrome (FAS). After a direct treatment with ethanol (250 mg/dl), there was a higher number of abnormal embryos than in the control group, showing neural and cardiac anomalies. After NC-1 immunostaining, ethanol-treated embryos showed smaller number of NCC at all neuraxis levels and presumptive NCC were frequently seen flowing towards the lumen of the neural tube. Present data support the view that ethanol impairment of migratory behaviour of NCC may explain certain anomalies of FAS such as those found at the cephalic end of the body, which is known to be largely derived from NCC.

KEY WORDS: *cell migration, ethanol induced anomalies, fetal alcohol syndrome, neural crest cells*

Fetal Alcohol Syndrome (FAS) is a teratogenic pathology caused in human beings by ethanol ingestion (Abel, 1984), or induced in animal models (Campbell *et al.*, 1986). Considering that the main feature of the FAS is the epigenetic craniofacial malformation, and that most of the cephalic structures are neural crest cell (NCC) derivatives (Le Douarin, 1982), the purpose of this work was to study the dispersion of early migratory NCC of chick embryos treated at early stages of development with ethanol at concentrations known to induce the FAS.

A higher number of abnormal chick embryos were seen in the ethanol-treated group (36/46) than in the control group (16/44) (χ^2= 14.51; p<0.0005). Serial sections allowed us to establish anomalies at different levels of the neuraxis (cranio-rachischisis, asymmetry of NCC distribution, presumptive NCC in the lumen of neural tube, and septal cardiac anomalies).

After NC-1 immunostaining, ethanol-treated embryos showed a small number of NCC at the pro- and mesencephalic levels. At the rhombencephalon, only few NCC were detected in the ethanol group in areas normally occupied by this cell population (Fig. 1). Truncal segments of ethanol-treated embryos showed scarce NCC, and groups of presumptive NCC were frequently seen flowing towards the lumen of the neural tube (Fig. 2).

Prevention of normal emigration of NCC towards lateral sides of neural tube and protruding to the neural lumen was shown after alteration of cell-matrix interactions under *in vivo* (Rovasio, 1982; Boucaut *et al.*, 1984; Thiery *et al.*, 1985; Bronner-Fraser, 1986) and *in vitro* conditions (Bilozur and Hay, 1989), as well as by the effect of HNK-1 antibody (Bronner-Fraser, 1987). In the present work, we have seen the same phenomenon and interpret it as the result of ethanol-induced alterations in the mechanism of NCC normal migration. On the other hand, the luminal translocation of NCC may be triggered by a higher cell density produced by their intrinsic proliferative behavior (Rovasio *et al.*, 1983; Rovasio and Thiery, 1987; Paglini and Rovasio, 1994).

Data from the present work support the view that treatment with ethanol – at presumptive or early migratory stages of NCC – was consistently followed by a lower number of migratory NCC at all neuraxis levels. These impairments of the migratory behavior of NCC may explain certain anomalies of FAS such as those found at the cephalic end of the body, which is known to be largely derived from NCC. It remains to be established whether direct contact with ethanol or its degradation products can derange subcellular structures, thus leading to the failure of the migratory capacity of NCC.

Experimental Procedures

Fertile chick eggs (Cobb line) were incubated for about 28 h at 38°C in a humidified incubator up to Hamburger and Hamilton (1951) stage 8, which is immediately previous to NCC migration. Afterwards, the whole egg was transferred to a shell-less culture system, and a flat ring of 2% agarose in phosphate buffer saline (PBS) was placed over the blastoderm. Thus, a container was formed around the embryo where 50 µl of ethanol solution (250 mg/dl) was dropped. A parallel series of control embryos was treated with PBS solution in the same way. After a post-incubation period of 48 h, embryos were fixed at H-H stage 11-12 with 4% formaldehyde in PBS, paraffin embedded, and serial sections were submitted to conventional staining and immunocytochemical reaction with the NC-1 monoclonal antibodies to locate NCC populations (Vincent and Thiery, 1984).

Abbreviations used in this paper: NCC, neural crest cell; FAS, fetal alcohol syndrome.

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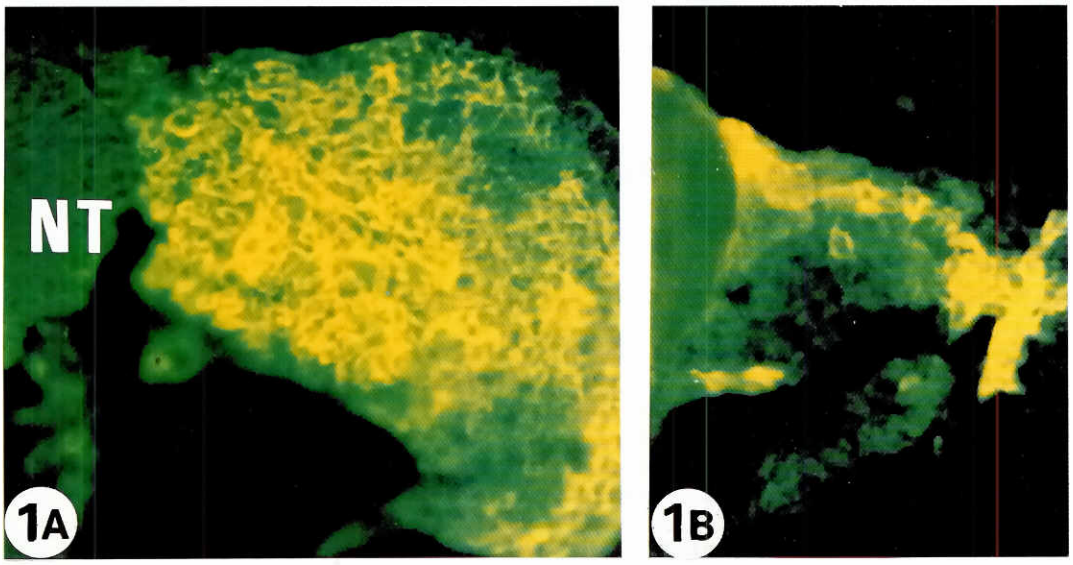

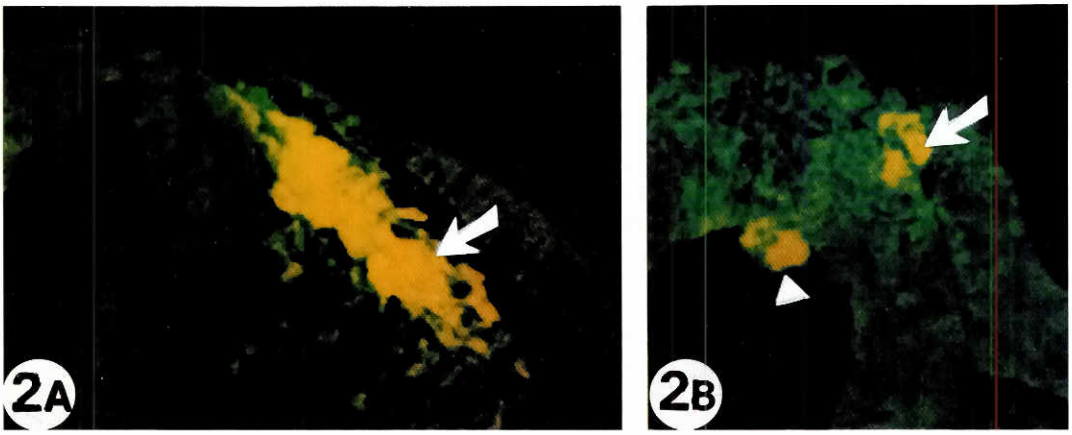

Fig. 1. Rhombencephalic segment of stage 12 control embryo (A) *shows abundant NC-1 positive NCC occupying most of neural tube (NT) lateral space. In an equivalent section of ethanol-treated embryo* **(B)** *only few NCC emigrate from the neural tube towards the lateral pathway. x350.*

Fig. 2. Trunk level *of stage 12 ethanol-treated embryo where the NCC were scarce* (**B**, arrow) *compared with equivalent level of control embryo* **(A)**. *Groups of presumptive NCC expressing NC-1 epitope are seen flowing towards the neural tube lumen of ethanol-treated embryos* (**B**, arrowhead). *x350.*

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