Pathfinding during spinal tract formation in the chick-quail chimera analysed by species-specific monoclonal antibodies

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Summary

In order to analyse the spinal tract formation at early stages of development in avian embryos, chick-quail spinal cord chimeras were prepared and species-specific monoclonal antibodies (MAb) were developed. MAbs CN, QN and CQN uniquely stained chick, quail, and both chick and quail nervous tissues, respectively. All three antibodies appeared to bind to the same membrane molecule, but to different epitopes. Cord reversal revealed the features of axonal growth of both cord interneurons and dorsal root ganglion cells. Quail cord interneurons grew along an originally ventral marginal layer in the quail cord transplanted in a reversed position, then turned toward the ventral side at the boundary between the graft and the host, and grew along the host chick ventral marginal layer. Central axons of dorsal root ganglia were restricted to the ventrolateral region of the cord which originally formed the dorsal funiculus. These results suggest that cord interneurons and dorsal root ganglion cells actively select to grow along specific regions of the cord and that spinal tract formation appears to be determined by cord cells, and not by sclerotome cells.

Key words: pathfinding, chick-quail chimera, species-specific monoclonal antibody, spinal tract formation.

Introduction

Selective axonal growth of neuronal cells towards their target cells is considered to be one of the most important processes involved in the establishment of precise projection. In the spinal cord, Holder et al. (1987) demonstrated that axons from lumbar dorsal root ganglia (DRG) neurons selected predetermined pathways within the developing spinal cord. They rotated the thoracic spinal cord of tadpoles through 180° from dorsal to ventral and then the axonal growth of DRG neurons was examined by horseradish peroxidase as a tracer. DRG neurons selectively grew along dorsal column (somatic sensory tract) even at the rotated cord. This somatic sensory tract is also a pathway for ectopically transplanted retinal axons (Constantine-Paton and Capranica, 1976; Katz and Lasék, 1979; Fujisawa et al. 1989). We therefore examined intersegmental projection in the spinal cord of avian embryos to see if such selective axonal growth occurs in other spinal tracts.

In this study, we used chick–quail chimera embryos. Since chick lumbar motoneurons selectively innervate quail hindlimb muscles and vice versa (Tanaka and Landmesser, 1986) and spinal cord chimeras hatch and behave normally (Kinutani and Le Douarin, 1985; Kinutani et al. 1986), the putative chemical cues responsible for axonal guidance and the molecules recognizing them in motoneuron axonal growth (Landmesser, 1984) appeared to be identical in the two species. Therefore, it is reasonable to expect that axonal growth in the spinal cord occurs normally in chick–quail chimeras. The molecular compositions of chick and quail seem to be very similar, but not all epitopes in the two species are identical and species-specific antibodies have been reported (Lance-Jones and Lagenaur, 1987; Takagi et al. 1989). In this study, we developed such species-specific monoclonal antibodies and used them as markers of axonal growth.

Materials and methods

Generation of monoclonal antibodies

MAbs CN (chick-specific neuronal marker), CQN (neuronal marker of both chick and quail) and c-SC1 (chick-specific SCI) were obtained using the following immunization schedule: 6-week-old BALB/c mice were immunized intraperitoneally with a mixture of 6E (embryonic days) chick embryo spinal cord membrane fraction (ca. 10 cords per
Species-specific monoclonal antibodies

Species-specific general neuronal markers were obtained. These antibodies stained both central (i.e. spinal cord and brain except for the region that consisted of undifferentiated cells) and peripheral nervous tissues as shown in Fig. 1A. Although both cell bodies and fibers were stained by these MAbs, the staining intensity of fibers was much higher than that of cell bodies. MAb CQN bound to both chick and quail neural tissue (Fig. 1B). MAb QN bound only to quail but not to chick tissue (Fig. 1C) and MAb CN showed the opposite characteristic (Fig. 1D). Immunoblot analysis of a detergent, NP-40, extract of chick and quail brain and spinal cord confirmed the histological results. MAb CQN revealed identical major bands at 116 x 10^3 M_r in chick and quail. The minor bands at higher molecular weight were different in the two species (Fig. 1E). MAb QN and CN revealed only the quail and chick bands, respectively (Fig. 1E). Since all major bands revealed with these MAbs showed the same molecular weight and the staining patterns of these MAbs were identical, these MAbs appeared to bind to the same membrane protein but to different epitopes. These MAbs stained intact culture cells derived from embryonic spinal cords. Therefore, it was concluded that these MAbs bind to the cell surface epitopes. These species-specific antibodies were useful as markers for analysis of chimeras (Lund et al. 1985; Lange-Jones and Lagenaour, 1988; Takagi et al. 1989).

Axonal growth of interneurons

We did not have any interneuron-specific markers for analysis of axonal growth from cord interneurons. However, species-specific antibodies, MAbs CN and QN, provided suggestive data on interneuron axonal growth. The first spinal tract during development is composed of interneuron axons and located at the ventral marginal layers (Oppenheim et al. 1988). When the quail cord was transplanted into the chick in normal orientation, quail axons entered the host chick cord (Fig. 2A). The quail axons were restricted only to the ventral marginal layers of the host chick cord. The host chick axons also entered the transplanted quail cord and these axons were restricted only to the ventral and
Fig. 1. Staining patterns of MAbs CQN, QN and CN. Transverse sections of chick (C) and quail (Q) embryos at stage 26 were stained with either MAb. (A) An enlargement of the chick section shown in B. Motoneurons, cord interneurons and dorsal root ganglion are stained. The staining intensity of spinal nerve, and ventral marginal layer and dorsal funiculus in the cord is much higher than that of motoneuron and dorsal root ganglion cell bodies. The ventricular zone in the cord, which consists of undifferentiated cells, is not stained. sc, spinal cord; d, dorsal root ganglion; nc, notochord; sn, spinal nerve. (A,B) MAb CQN, (C) MAb QN, (D) MAb CN. Bar, 50 μm for A and 200 μm for B–D. (E) Immunoblot analysis of membrane fraction of brain and cord of 9E–12E chick and quail embryos. C, chick sample; Q, quail sample; M, molecular weight markers (Sigma) stained with amido black. Arrowheads indicate the positions of β-galactosidase (116 000), phosphorylase b (97 400) and bovine serum albumin (66 000) from top to bottom.

ventrolateral marginal layers (Fig. 2D). These chick axons in the quail cord were not stained with MAb c-SC1, which selectively stained motoneurons and axons of DRG neurons in the cord (Tanaka and Obata, 1984), and it was therefore concluded that they extended from cord interneurons.

Interneuron axonal growth was investigated further in the chimeras with cord reversal. The quail neural primordium transplanted even in the dorsoventrally reversed position developed quite normally, as was shown by Narayanan (1970) and Jacob et al. (1976). In the quail cord, quail interneurons grew along originally ventral marginal layers which had been displaced to the dorsal margin of the reversed cord (Fig. 3A). These quail fibers grew in a ventral direction at the boundaries between the quail transplant and chick host (Fig. 3E), as demonstrated in the frog by Holder et al. (1987). They found that developing or regenerating axons of dorsal root ganglia grew across the two segments of frog tadpole spinal cord that had been rotated through 180° from dorsal to ventral. In the chick host cord, quail axons were restricted to the ventral marginal layers (Fig. 3G). These results indicate that quail interneurons actively select to grow along their own and host chick
Fig. 2. Intersegmental axonal projections between the host chick and transplanted quail cords. Quail cord was transplanted in normal orientation along the ventral–dorsal and anterior–posterior axes. The transverse sections of A and C are at the level of the host chick cord and B and D are of the transplanted quail cord. A and C are adjacent sections, as are B and D. A and B were stained with MAbs QN and basement membrane-specific M6715 (Obata and Tanaka, 1988) in order to show the outline of the cord. C and D were stained with MAb CN alone. Intersegmental quail axons are restricted to the ventral marginal layer of the host chick (arrows in A). Chick axons are distributed in the ventral and ventrolateral marginal layers of the quail cord (arrows in D). Bar, 100 μm.

ventral marginal layers, as represented schematically in Fig. 3H. Such selective axonal growth of cord interneurons was observed at both upper and lower boundaries of all three grafts examined. In one case, the graft adhered to the host cord at only one end, leaving the other end free. The characteristic axonal growth of interneurons was also observed at this adhered boundary. However, interneurons of the host cord did not grow well into the reversed graft, whereas they grew well into the quail cord transplanted in normal orientation (Fig. 2D). As shown in Fig. 3B, chick host fibers could be seen only sporadically in the transplanted quail cord.

Axonal growth of dorsal root ganglia
The dorsal root ganglia at the level of cord reversal were of quail neural crest origin, because these ganglia were QN(+) but CN(−) (data not shown). MAb M6764, which stained neural-crest-derived cells (Obata and Tanaka, 1988), stained the Schwann cells surrounding motoneuron axons (Fig. 4A arrows). As described by Weston (1963), these results indicated that quail neural crest cells migrated in a dorsal direction after transplantation. The axons of motoneurons and dorsal root ganglia formed spinal nerves and grew toward the periphery. The central fibers of dorsal root ganglia were restricted to very narrow regions within the spinal cord (arrowheads in Fig. 4A, arrows in Fig. 4B and Fig. 3A). These regions seemed to correspond to the original dorsal funiculus-forming areas. Therefore, it was concluded that the central fibers of dorsal root ganglia grew selectively toward the correct region of the cord and that the region of dorsal funiculus formation was determined by the cord tissue itself and not by the surrounding tissue.

Discussion
Chick–quail chimera and nuclear marker have provided successful analyses of neural crest development (Le Douarin, 1982). Since the nuclear marker cannot be used for analysis of axonal growth, we developed species-specific neuronal markers (Lund et al. 1985; Lance-Jones and Lagenaur, 1987; Takagi et al. 1989). MAbs CN and QN were shown to be very useful markers for tracing axonal growth in chimeras. Cord reversal experiments revealed important features of spinal tract formation. One of these was axonal growth of cord interneurons. Intersegmental projections have been observed within the ventral and ventrolateral marginal zones from early developmental stages (Oppenheim et al. 1988). In our chick–quail
Fig. 3. Intersegmental axonal projections between the host chick and quail cord transplanted in a reversed position along the ventral–dorsal axis. The transverse sections of A and B are at the level of the transplanted quail cord, C–F are around the boundary between host chick and transplanted quail, and G is of the host chick. A, C, E and G were stained with MAb QN. B, D and F were stained with MAb CN. Intersegmental axons of quail cord grow along the originally ventral marginal layer (A and C) and at the boundary between the host chick and quail, turn toward the ventral side (arrows in E) and then grow along the ventral marginal layer of the host chick cord (arrows in G). In contrast, host chick axons do not grow well into the transplanted quail cord. Only a few axons can be seen in the quail cord (arrow in B). These results are illustrated in H together with axons of motoneurons and dorsal root ganglia. Bar, 100 μm. T, transplant; H, host; d, dorsal side; v, ventral side.
chimera with transplants in normal orientation, chick axons growing into the quail graft were restricted to the ventral and ventrolateral marginal layers of the quail cord, and the same was observed for quail axons in the host chick cord, in agreement with the results of Oppenheim et al. (1988). Quail interneurons grew along the originally ventral marginal layer in the reversed cord. This result supports the conclusion that spinal tract formation appears to be determined by cord cells. At the boundaries between the chick host and reversed quail cords, quail fibers turned and grew into the chick ventral marginal layers, but chick fibers did not (Fig. 3).

Since chick axons grew into the quail cord transplanted in normal orientation, if chick axons confront the quail ventral marginal layer, they will grow into the quail cord. Therefore, the reason why chick interneurons do not grow dorsally at the boundary is unknown, but one possibility is insufficiency of the chemotropic factor for the chick commissural interneurons (Tessier-Lavigne et al. 1988) produced by floor plate cells in the small piece of transplanted quail cord.

The other result regarding spinal tract formation is the dorsal funiculus formation. Central axons of dorsal root ganglia were restricted to very narrow regions of the reversed cord (Fig. 4), which seemed to correspond to the areas that normally formed the dorsal funiculi. This finding indicates that central axons of dorsal root ganglia reach the correct region of the cord through an unusual pathway in the sclerotome and then grow along the originally dorsal marginal layer of the reversed cord. Therefore, this result again supports the conclusion that spinal tract formation seems to be determined by cord cells, and not by the sclerotome cells surrounding the cord. The mechanism by which dorsal root ganglion axons find the entrance of the cord is another problem that needs to be investigated. Since, following cord reversal, the relative positions of migrating neural crest cells, cord and dorsal root ganglia, the differentiation of which was influenced by a neural tube signal (Kalcheim and Le Douarin, 1986), were almost identical with the normal situation, neural crest cell migration might have some role in determining the growth direction of dorsal root ganglion axons.

In this study, neural tube was rotated at stage 14. At this stage, the differentiation of spinal cord cells seems to be already determined. Cord interneurons and DRG neurons grew along original ventral marginal layers and dorsal funiculi, respectively. Quail motoneurons developed in the dorsal side of the rotated cord and grew out from the cord via dorsal root (Figs 3A and 4A). No quail motoneurons were observed at the ventral side in the rotated quail transplants. At the boundary between chick host and quail transplant, motoneurons were sometimes observed in both ventral and dorsal portions of the cord by MAb SC1 (Tanaka and Obata, 1984) staining (not shown). However, MAb chick-specific SC1 staining showed that only the ventral motoneurons were chick and that, therefore, the dorsal ones were quail. Based on these results, motoneurons, cord interneurons and the cells forming regions of dorsal funiculus and ventral marginal zone appeared to

Fig. 4. Staining patterns of MAbs M6764 and SC1 on transverse sections of chick embryos in which the spinal cord was replaced by quail cord in an upside-down position. (A) MAb M6764 staining shows the distribution of cells of neural crest origin such as DRG (d), sympathetic ganglion (s) and Schwann cells surrounding the motoneuron axons (arrows). Distribution of DRG central axons in the cord is indicated by arrowheads. In this case, bleeding is observed at the positions of motoneuron cell bodies, but such bleeding is not usual. (B) MAb SC1 staining. Another example of the distribution of DRG central axons in the cord (arrows). Original roof plate cells (rp) of the quail are SC1(+). Bar, 100 μm for A; 60 μm for B.
develop normally after the rotation at stage 14. These results support the conclusion that the morphogenesis and histogenesis of the cord are unaffected by neural tube rotation (Narayanan, 1970; Jacob et al. 1976) and, therefore, the results in this study reveal the pathfinding activity of cord interneurons and DRG cells. At developmental stages earlier than stage 14, probably notochord and somite cells have influences on differentiation of neural tube cells and the results of neural tube rotation at such stages will differ from those of this study. However, such differences should be considered as a problem of induction, not of pathfinding.

References
