Desmin Distribution in the Cardiac Outflow Tract of the Chick Embryo during Aortico-pulmonary Septation

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Summary. The appearance and distribution of desmin, a muscle type intermediate filament, was examined in the truncus arteriosus of the chick embryonic heart during AP septation by an indirect immunofluorescence method. Prior to septation, on the 4th day of incubation, staining with antidesmin antibody was observed in the AP septum anlagen in the distal truncus. No staining with the antibody was detected in the developing tunica media of the great arteries in the distal truncus at this stage. During septation, on the 5th day of incubation, intense staining with the antidesmin antibody was observed in the cell condensation of the AP septum. In the 6-day-old embryo, the staining in the AP septum was reduced, but fluorescence by staining with the antibody was observed in the developing tunica media. No fluorescence was detected in other mesenchymal cells in the truncal swellings.

Because of the time of appearance and the localization of antidesmin reactivity in the developing AP septum, it is suggested that muscle-type cells exist in the AP septum, and that these cells may perform an important function in septation.

During the development of the heart in higher vertebrates, the initial single arterial pole, the truncus arteriosus, is divided into two major vessels: the aorta and the pulmonary artery. Because truncal anomaly is one of the most frequently occurring congenital cardiac defects, much attention has been paid to the morphogenesis of the outflow tract (LOS, 1978; OKAMOTO, 1980; THOMPSON et al., 1983, 1985).

Before septation, the anlagen of the aortico-pulmonary (AP) septum appear as mesenchymal condensations in the distal part of the right and left truncal swellings. While the right and left swellings fuse with each other from a distal to proximal direction against the blood flow, the AP septum comes into recognition as a cell condensation between the aortic and pulmonary lumens (VAN MIELOP, 1969; LAANE, 1978, 1979).

Recently, phalloidin staining has demonstrated organized actin bundles with consistent orientation in the AP septum (SUMIDA et al., 1985a, b). Electron microscopy has further demonstrated that some of the cells within the AP septum have stress fiber-like bundles. It has been suggested that these cells play an important role in the division of the truncus arteriosus (THOMPSON et al., 1983, 1984, 1985). However, the characteristics of the cells with microfilaments within the AP septum still remain unclear. Thus, the following questions were raised: 1) Are the cells containing actin bundles within the AP septum muscular in nature? 2) Are these cells precursors of the tunica media of the great arteries?

To answer these questions, we examined the appearance and distribution of desmin, a muscle-type intermediate filament (LAZARIDES, 1980, 1982), in the chick out-flow tract using an immunohistochemical method.

MATERIALS AND METHODS

Fertilized White Leghorn chicken eggs were incubated at 38°C in a humidified atmosphere. The embryos were fixed at either 4, 5 or 6 days of incubation in 4% formaldehyde in 0.1 M phosphate buffer pH 7.4 for 4 hrs at room temperature. After staging according to HAMBURGER and HAMILTON (1951), the specimens were dehydrated with a graded series of ethanol and embedded in paraffin.

Immunofluorescent staining

Deparaffinized 6 μ m thick sections were incubated in 0.1% trypsin in 0.1% CaCl₂ for 30 min at 37°C. This treatment has been reported to increase sensitivity for the detection of antigens in tissues embedded in paraffin (HAUTZER et al., 1980). The following procedures were performed at room temperature: after rinsing in phosphate buffered saline (PBS), the sections were blocked with 20% normal goat serum in PBS for 20–30 min to avoid non-specific staining. This procedure was followed by incubation for 60 min with antidesmin rabbit IgG, diluted 50 times, raised against desmin from chicken gizzard (Bio-Science Products, Switzerland). For the control, non-immune rabbit IgG was employed in place of the antibody.

After rinsing in PBS, the sections were incubated with FITC-labeled goat anti-rabbit IgG (affinity purified, Bio-Yeda, or Tago, USA) for 30 min. The sections were rinsed in PBS, and mounted with glycerol-PBS (1 : 1) solution, and photographed under a fluorescence microscope (Olympus, BH2-RFK) with a B460 filter. The control specimens showed a negligible degree of fluorescence.

After observation under a fluorescence microscope, the coverslips were removed in PBS, and the sections were stained with hematoxylin and eosin.

RESULTS

In the present study, we modified LOS's terminology (1978) concerning the developing heart as follows: "Truncus arteriosus" indicates the whole arterial pole, "distal truncus," the area including the "aortic sac," and "proximal truncus," the area including the "conus cordis" or "bulbus" or "infundibulum." The "distal truncus" denotes the region of the aortic arches of the embryo at a later stage. "AP septum anlagen" signifies condensed cell masses in the truncal swellings.

In the four-day-old chick embryo (stages 22–24), the truncus arteriosus was not divided, and a single lumen was seen in cross sections (Fig. 1a). The right and left truncal swellings were developing between the endocardium and the myocardium of the truncus. On both the left and right swellings, the anlagen of the AP septum were recognizable as condensed cell masses (Fig. 1a). A fluorescence micrograph (Fig. 1b) showed that these cell masses had reacted to antidesmin. No fluorescence was seen in the other cell group in the swellings.

At this stage, the cardiac muscle which strongly reacted to the antidesmin antibody surrounded the truncus arteriosus (Fig. 1a). The atrium reacted strongly to

Fig. 1. A cross section of the distal truncus of the 4-day-old chick embryo. **a.** Hematoxylin and eosin staining, **b.** Immunofluorescence micrograph of the same field as in **a.** The anlagen of the AP septum are seen as cellular condensations (surrounded by arrowheads in **a**). Reactivity of the antidesmin antibody is recognizable in the region corresponding to the anlagen of the AP septum (**b**). The myocardium surrounding the atrium (AM) and truncus arteriosus (MC) shows intense staining. No fluorescence is seen in the developing tunica media (arrows). L lumen of the truncus, TW thoracic wall. $\times 400$

the antidesmin antibody. In a region which lacked the myocardium in the distal truncus, no fluorescence was observed (Fig. 1a, b). Hematoxylin and eosin staining showed that the tissue of this region was the tunica media of the great arteries.

In the five-day-old chick embryo (stages 25–27), the right and left truncal swellings were in the process of fusing from distal to proximal, dividing the lumen into the aortic and pulmonary lumens. The AP septum appeared as a dense cell mass in the sparsely scattered mesenchymal cells of the swellings (Fig. 2c). This cell mass strongly reacted to the antidesmin antibody, other mesenchymal cells in the swellings not being stained with the antibody (Fig. 2d).

The tunica media of the great arteries was developing toward the proximal truncus (Fig. 2c). In the distal truncus, the arteries were completely divided into the aorta and pulmonary artery, and a condensed cell layer of the tunica media had developed around each lumen (Fig. 2a). At this stage, however, the developing tunica media in the distal truncus was not stained with the antidesmin antibody (Fig. 2b).

The myocardium surrounding the truncus arteriosus and the atrium strongly reacted to the antidesmin antibody, but the thoracic wall and the epicardium failed to do so (Fig. 2c, d).

In the six-day-old chick embryo (stages 28–30), the developing tunica media appeared as a concentric layer around the distal and proximal arterial lumens (Fig. 3a, c); at this stage the tunica media of the distal truncus was not stained with the antidesmin antibody (Fig. 3b).

The AP septum still remained a condensed cell mass (Fig. 3a, c), and reacted to the antidesmin antibody, though reactivity was weaker than that seen on the previous day.

The myocardium reacted strongly to the antibody.

Fig. 2. Cross sections of the distal (**a**, **b**) and the proximal truncus (**c**, **d**) in the 5-day-old chick embryo. **a** and **c**. Hematoxylin and eosin staining. **b** and **d**. Immunofluorescence micrographs of the same fields as in **a** and **c**, respectively. The section (**c**, **d**) is just distal to the division of the truncus into the aortic and pulmonary lumens. The truncus more proximal to the level of **c** is not divided. No fluorescence is seen in the developing tunica media (arrows) nor in the epicardium (large arrowheads). In the AP septum (arrowheads), the reactivity of antidesmin is apparent. The myocardium surrounding the atrium (*AM*) and truncus arteriosus (*MC*) shows intense staining. *A* aortic lumen, *P* pulmonary lumen, *TW* thoracic wall. $\times 200$

DISCUSSION

Our findings in this study are summarized as follows: 1) The cellular condensation in the AP septum and its anlagen reacted to the antibody raised against desmin, but the other cells in the truncal swellings did not; 2) the developing tunica media of the great arteries was not stained with the antibody until the 6th day of incubation.

Prior to septation, the anlagen of the AP septum appear as condensed cell columns in the swellings across the truncus arteriosus (see Fig. 1) (LAANE, 1978; LOS, 1978). Our first finding is that the cells in the anlagen of the AP septum are immunohistochemically desmin positive. As desmin is found predominantly in muscle cells, it has been thought that desmin is characteristically expressed in muscle cells with the exception

Fig. 3. Cross sections of the distal (**a, b**) and proximal truncus (**c, d**) in the 6-day-old chick embryo. **a** and **c**. Hematoxylin and eosin staining. **b** and **d**. Immunofluorescence micrographs of the same fields as in **a** and **c**, respectively. In the developing tunica media (arrows) of the distal truncus, weak reactivity of the antidesmin antibody is recognizable. In the AP septum (arrowheads), reactivity to the antidesmin antibody is positive but weaker than that seen on the previous day. *A* aortic lumen, *P* pulmonary lumen, *AM* myocardium surrounding the atrium, *MC* myocardium surrounding the truncus arteriosus. $\times 200$

of some cells (LAZARIDES, 1982). Therefore, the cells within the AP septum and its anlage are referred to as muscle-type cells.

Condensed cells within the AP septum were believed to derive from the extracardiac area (VAN MIELOP, 1969). Recent studies have shown that these cells derive from the neural crest (KIRBY et al., 1983, 1985; AKIMOTO et al., 1986). On the other hand, loose mesenchymal cells in the truncal swellings derive from the endocardium (MARKWALD et al., 1975, 1977; FITZHARRIS, 1981; FITZHARRIS and MARKWALD, 1982). Thus, the appearance of the desmin positive and negative cells in the truncal swellings may reflect their derivation.

The AP septum develops from the distal truncus toward the ventricles. Similarly, the tunica media of the great arteries develops in a distal to proximal direction (FITZHARRIS et al., 1979; THOMPSON and FITZHARRIS, 1979). The neural crest cells migrate into the tunica media of the distal truncus as well as into the AP septum, but

these cells do not migrate into the tunica media of the proximal truncus at the level of the proximal region of the AP septum (AKIMOTO et al, 1986). This finding suggests that the cells in the AP septum and in the tunica media of the great arteries are of different origin. Furthermore, the developing tunica media of the truncus did not react to the antidesmin antibody at the stage when the AP septum was intensely stained with the antibody (see Fig. 1 and 2). The tunica media of the great arteries was stained with the antidesmin antibody in the 6-day-old chick embryo. It was at this stage that, contrarily, staining in the AP septum was decreasing. From these results, it is suggested that the muscle-type cells in the AP septum are neither developing smooth muscle cells nor their precursor cells of the tunica media of the great arteries.

ICARDO (1985) reported that the AP septum in the chick embryo was rich in fibronectin during septation. After septation in the 6-day-old chick embryo, fibronectin reactivity within the AP septum decreased. We demonstrated that the reaction to the antidesmin in the AP septum decreased in the 6-day-old chick embryo. It was shown that F-actin bundles with consistent orientation were localized in the AP septum (SUMIDA et al., 1985a, b). Although a close association between fibronectin and actin fiber has been described (HYNES 1981), the relationship between fibronectin and desmin is still unclear. Elucidation of their relationship will be necessary.

In the truncal swellings, the muscle-type cells already appeared within the AP septum anlagen prior to septation. This finding suggests that the muscle-type cells may functionally contribute to the division of the truncus arteriosus. The muscle-type cells might have both mechanical strength and contractile ability. This characteristic of the muscle type cells may be required in order to divide the single lumen into the aortic and pulmonary lumens against the direction of the blood flow.

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