Rapid Communication

On the Role of the Connective Tissue in the Patterning of the Chick Limb Musculature

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Summary. By modifying the temporal relationship between connective tissue and myogenic cell invasion during early limb bud development new evidence of the organizing role of the connective tissue was obtained.

Muscle cell-deprived wing buds were allowed to grow up to stages 22 to 27 of Hamburger and Hamilton, when they received a transplant of quail myogenic cells (somitic mesoderm or wing premuscular mass) into the dorsal face of their presumptive upper arm. Muscular arrangement in forearm and hand was analyzed 4 days later. In 8 out of 14 of those cases which had received a graft of premuscular mass before stage 25 of Hamburger and Hamilton, muscle development took place distally to the graft-site in accordance with the wing segment.

Key words: Chick and quail embryos - Limb bud musculature - Myogenic cells - Connective tissue

Introduction

The early limb but contains a heterogeneous population of cells comprised of somatopleural mesenchyme and skeletal muscle myoblasts. The latter constitute a distinct cell lineage (Kieny 1980) and originate from myogenic precursor cells which migrate from the somites into the limb-level somatopleural mesenchyme (Chevallier 1978). The dual origin of the limb musculature was demonstrated by heterospecific quail/chick grafting experiments (Chevallier et al. 1976, 1977; Christ et al. 1974, 1977), where for instance, wing level somites of the chick were replaced by quail somites. Under these circumstances the muscle cells of the resulting wing were of quail somitic origin, all the other tissues of the limb (Kieny et al. 1979), including the muscular connective tissues and tendons being of chick somatopleural origin.

Once in the limb bud the myoblasts congregate into dorsal and ventral muscle masses, which, by a process of sequential fissions, divide into discrete muscles (Shellswell and Wolpert 1977; Pautou et al. 1982). The heterogeneous constitution of the muscular tissue raises the question of the respective activity of somitic and somatopleural cells in the muscular patterning. There is already circumstantial evidence that the development of the limb muscle pattern is controlled by the connective tissue cells. Indeed somitic myogenic precursor cells are all equivalent along the cephalo-caudal axis with respect to their morphogenetic capacity (Chevallier et al. 1976, 1977); furthermore myogenic cells which have already conglomerated into premuscular masses inside the limb are still indifferent, at least up to the myoblast stage, regarding their region-specific determination (Mauger and Kieny 1980a, b).

Testing further the respective roles of muscle cells and connective tissue cells, we asked the question how long the premuscular connective tissue is able to exert its organizing role. Therefore we tried to let myogenic cells migrate directly into a muscleless limb bud, that is a limb bud which was previously experimentally, through local X-irradiation, deprived of any cells of somitic myogenic origin (Chevallier et al. 1978; Kieny and Chevallier 1979).

Material and Methods

The experiments were carried out on chick (Wyandotte X Rhode Island Red) and quail (Coturnix coturnix japonica) embryos. The procedure consisted in a two-step operation: 1) the creation of a limb bud which was deprived of its myogenic cells; 2) the introduction of quail myogenic cells directly into a chick muscleless limb bud.

Step 1. The somitic mesoderm of the wing level was destroyed on the right side of 2-day chick embryos (stages 12 to 18 pairs of somites) by local X-irradiation, which was performed under the same conditions as reported in previous papers (Chevallier et al. 1978; Kieny and Chevallier 1979; Lewis et al. 1981).

Step 2. Two or three days after X-irradiation, quail tissues were grafted into the dorsal face of the presumptive upper arm of the right wing buds of the irradiated chick embryos. The grafts were either pieces of 2-day (stages 12 to 22 pairs of somites) quail somitic mesoderm from the wing level with or without the adjacent neural tube, or portions of dorsal premuscular mass taken from 4-day quail wing buds (stages equivalent to stages 25-27 of Hamburger and Hamilton (1951) for the chick).

The operated embryos were sacrificed 4 days later, at 8 (stage 33 of Hamburger and Hamilton) or 9 (stage 34 of Hamburger and Hamilton) days of incubation. The wings were cut out, paraffin-embedded, 5 μm serially sectioned and stained according to Feulgen and Rossenbeck (1924). This procedure allows the identification of quail...
cells among chick cells (Le Douarin and Barq 1969). Histological analysis consisted in recording the distribution of quail cells in transverse sections of the recombinant wing.

**Results**

**A. Implantations Performed 2 Days after X-irradiation of the Wing Level Somitic Mesoderm**

The implantation of quail cells into the wing bud was performed when the X-rayed chick hosts had reached 4 days of incubation (stages 22 to 24 of Hamburger and Hamilton).

**Implantation of Somitic Mesoderm** (11 cases). The implanted quail cells remained grouped in the upper arm, where they always differentiated into connective tissue, dermis, cartilaginous nodules and sparse myotubes which were not organized into skeletal muscles. When neural tube had been grafted simultaneously with the somitic mesoderm, neural tissue formed too. Just as the upper arm, the forearm and hand of these experimental wings also lacked skeletal muscles, although several distal tendons were present in the digits. No quail cells were found distally to the elbow. The area between skeletal elements and integument was occupied by loose unorganized connective tissue (Fig. 1).

**Implantation of Wing Premuscular Mass** (14 cases). The grafted quail cells developed always inside the upper arm in one muscle mass, whose myocytes were of quail origin, and whose muscular connective tissue was mostly of quail and in places of chick origin. This mass was never connected to tendons. In one case a small cartilaginous nodule formed. Apart from these quail muscle differentiations, the upper arm lacked chick skeletal muscles. In forearms and hands no quail cells were found in 6 cases. They lacked muscles and had a loose unorganized connective tissue between integument and cartilage (cf. Fig. 1).

However, in 5 cases, quail cells were found distally to the elbow, where they were grouped in skeletal muscles in the forearm and also in 3 cases, in the hand (Figs. 2 and 3). These muscles had always a bispecific constitution: the myocytes were of quail origin, whereas the intramuscular connective tissue and the long distal tendons were of chick origin. Muscles were always present dorsally, but ventrally their presence was sporadic. As concerns the dorsally located muscles, their architecture was typical of that of the dorsal extensor muscles (Figs. 2–5), but was delayed by 12 to 24 h, namely at most by one splitting. Instead of six individuated extensor muscles in the mid region of the forearm at stage 33 of Hamburger and Hamilton (8 days), in the experimental forearms there were often only 4 muscle blocks, the spatial distribution of which corresponded to that of stage 30–31 of H.H. forearms (Fig. 4).

**B. Implantations Performed 3 Days after X-irradiation of the Wing Level Somitic Mesoderm**

The implantations of quail cells into the wing bud were performed when the X-rayed chick hosts had reached 5 days of incubation (stages 25 to 27 of Hamburger and Hamilton).

Whatever the graft type (somitic mesoderm with or without adjacent neural tube (7 cases), premuscular mass (19 cases)), the forearm and hand of the experimental wings were devoid of quail cells. The quail cells were restricted to the upper arm where they differentiated according to their origins, as described in the first experimental series where quail somitic mesoderm was implanted. All wings lacked skeletal muscles, although again autopodial tendons were present dorsally and ventrally in digits III and IV.

**Discussion and Conclusions**

This paper deals with the interaction between presumptive muscle cells and presumptive muscle connective tissue, with the role of somito-somatopleural migration of the myogenic precursor cells and in particular with the duration of the capacity of the somatopleural mesenchyme to organize the migrating muscle cells into a wing skeletal muscle pattern. The cells that give rise to the wing muscles migrate from the somites at early stages, long before the wing bud is bulging out, therefore the presumptive muscle cells are immediately involved in the developmental programme of the wing. For this reason we destroyed the host somites by X-rays before they contribute myogenic cells to the wing anlage and let the development of the chick host wing bud proceed to stages when normally migrating muscle cells are about to congregate or have already congregated into dorsal and ventral premuscular masses. Thereafter, we implanted quail myogenic cells into the dorsal face of the presumptive upper arm of these muscle cell-deprived wings. The grafts were of two types, portions of somitic mesoderm (with or without adjacent neural tube) or portions of premuscular masses, taken respectively before the myogenic cells had started their somito-somatopleural migration and after they had accomplished it.

In the cases of implantation in the upper arm of hosts of later stages (stages 25 to 27), whatever the quality of the graft, no muscle developed distally to the graft-site. The implantation in the presumptive upper arm of younger hosts (stages 22 to 24) gave different results depending on the type of the graft. The forearm and hand of the wings that had received a somitic mesoderm-graft (with or without neural tube) lacked skeletal muscles. But the majority (8 out of 14) of wings which had received a premuscular mass-graft were equipped with skeletal muscles. Their pattern was typical of the forearm, but was delayed by 12 to 24 h, which probably correspond to the time required for the grafted cells to invade the forearm and there constitute the premuscular mass(es). The experimental protocol and histological observation of the six remaining cases, where the graft did not participate in the formation of the skeletal musculature, revealed that the negative result was not due to the stage of the hosts at the time of grafting (which ranged between stage 22 to 24 included) nor to a partial mislocation of the implant into the chondrogenic area of the presumptive upper arm. These six negative cases may thus be explained by a lack of proper integration of the graft into the host tissue.

These results show that the patterning of the skeletal muscles is dependent on the migration of the myogenic cells. Somitic myogenic precursor cells, which are prevented from undergoing their normal somito-somatopleural migration, by being placed directly in contact with the presumptive muscular connective tissue, are unable to migrate away from the grafting site. These cells, apparently because they have not migrated, are not or do not become competent