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Perifollicular, Perisinusal and Trabecular Myofibroblasts in the Human Fetal Spleen

Takao Fukuda

Department of Pathology, The Research Institute for Cancer, Tohoku University, Sendai, Japan

Summary. Electron microscopic studies of the human fetal spleen were performed on 22 embryos and fetuses from 31 days to 32 weeks of age. In the course of splenic development myofibroblasts were frequently observed in the perifollicular and perisinusal regions and within the trabeculae.

The perifollicular cells were flattened and markedly elongated in shape and had distinct bundles of microfilaments in their cytoplasm. The perisinusal cells under the sinal basement membrane had microfilaments found in particular beneath the plasmalemma. They were attached to each other and constituted a cellular network throughout the splenic cords.

The trabecular myofibroblasts, embedded among the abundant collagen fibers, had elongated and often indented nuclei. The cytoplasm contained well developed Golgi apparatus, abundant rough surfaced ER cisternae and massive bundles of myofilaments.

These cells showed cell-to-cell and cell-to-stroma connections and were assumed to play a role in the excretion of lymphocytes from the follicles and in pumping blood from the sinuses by their contraction.

Key words: Myofibroblasts – Spleen – Human fetus – Follicles – Sinuses – Trabeculae

It is well established that granulation tissue in healing wounds undergoes various degrees of contraction caused by the proliferation of contractile fibroblasts: myofibroblasts (refr. see Majno 1979). These cells show the characteristic features of both fibroblasts and smooth muscle cells, namely abundant cisternae of rough surfaced endoplasmic reticulum and bundles of microfilaments (Rudolph et al. 1977, Ryan et al. 1974). These myofibroblasts have been considered to be abnormal fibroblasts that do not exist within any organ under physiological conditions.

Here we show the presence of cells which resemble myofibroblasts in the human fetal spleen.

Offprint requests to: Prof. T. Fukuda, Department of Pathology, The Research Institute for Cancer, Tohoku University, 4-1 Seiryomachi, 980-Sendai, Japan

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Material and Methods

The splenic tissue examined was obtained from 22 embryos and fetuses (31 days – 32 weeks p.c.) which had been delivered by spontaneous or legal abortion or therapeutic hysterectomy. The age of embryos and fetuses in weeks post conceptionem (p.c.) was calculated according to Moore (1977) by measuring the crown-rump length.

The splenic tissue, taken within 1 h post mortem, was fixed in a mixture of 2.5 % glutaraldehyde and 2 % formaldehyde buffered to pH 7.2 with 0.1 M sodium cacodylate. The material was postfixed in 1 % osmium tetroxide, dehydrated in ethanol and finally embedded in Epon. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Observations

Perifollicular Myofibroblasts

In the fetal spleen lymphoid tissue was observed around the arteries from the 16th week p.c. and follicles were first found in the 18th week p.c. At first they were small in size and number and mostly composed of lymphoblasts, interdigitating cells, residential reticular cells and a few small lymphocytes. With the maturation of the fetus, follicles increased their size and number and small lymphocytes progressively increased.

From 20 to 32 weeks p.c. distinctive mesenchymal cells were found around the follicles (Figs. 1, 2, 3). These cells were flattened and markedly elongated in shape, as if compressed by lymph follicles. Their nuclei were a flattened oval in shape and the chromatin was finely dispersed with slight peripheral clumping. Occasional small nucleoli attached to the nuclear membrane were observed. The flattened cytoplasm contained a moderate number of polyribosomes, several mitochondria and microtubules and had indistinct Golgi apparatus. The cisternae of the rough surfaced endoplasmic reticulum (rER) were small in number but were sometimes distended and contained material of moderate electron density. (Fig. 3). The particular finding in these cells was the presence of abundant microfilaments of 40–80 Å in diameter and a few thick filaments of 120–180 Å in diameter. These microfilaments were randomly distributed, but at the periphery of the cytoplasm they were arranged parallel in bundles along the long axis of the cell (Fig. 2). Many electron dense areas were scattered in the bundles.

Perisinusal Myofibroblasts

From the 12th week p.c. splenic cords became distinct and were flooded with abundant erythrocytes and platelets. The sinus endothelial cells were situated on the basement membrane and had abundant microfilaments and pinocytotic vesicles in their cytoplasm.

The perisinusal cells situated on the cordal side of the basement membrane were stellate in shape and connected to each other. They formed a cellular network throughout the splenic cords (Fig. 4). Desmosome- and hemidesmosome-like connecting structures were occasionally observed. The nuclei of these cells were oval or indented and had indistinct nucleoli. The chromatin showed slight central and peripheral clumping. Compared with other cells, the cytoplasm of these cells were markedly electron lucent and contained a moderate number of mitochondria,

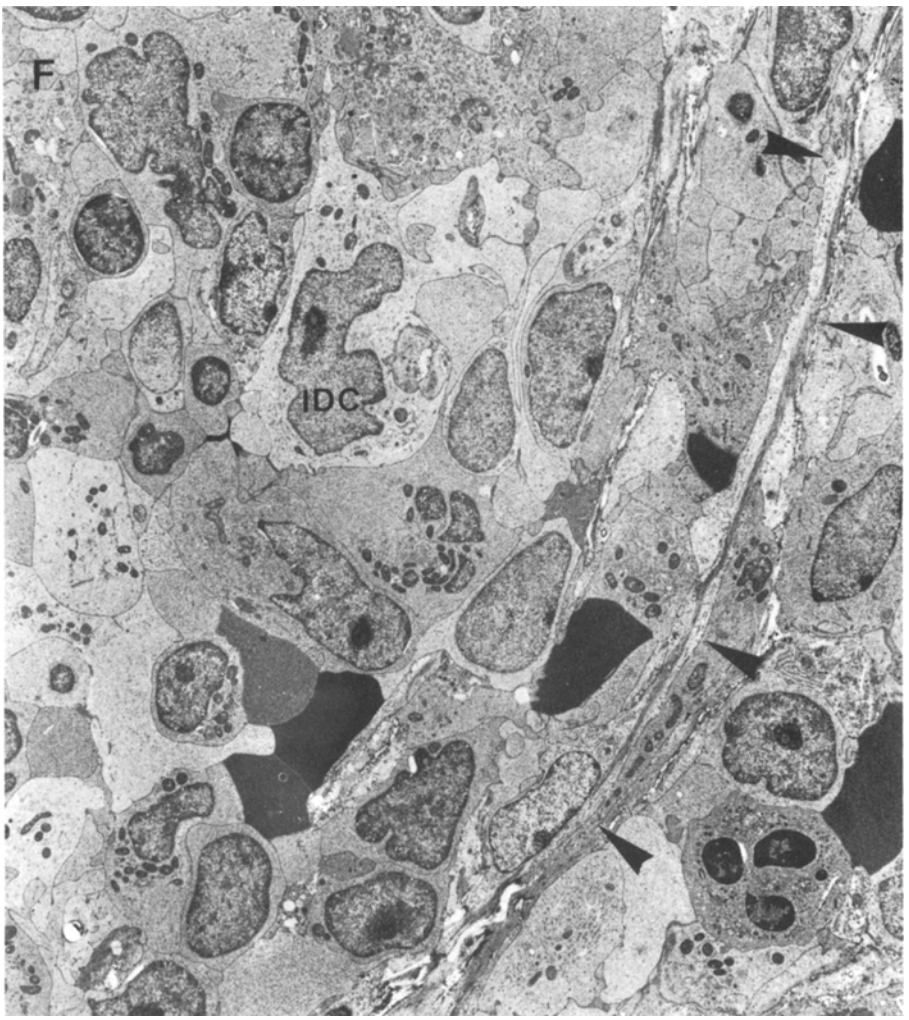

Fig. 1. Perifollicular myofibroblast (*arrows*). The compressed and distended cytoplasm contains distinct bundles of microfilaments. Lymph follicle (*F*), Interdigitating cell (*IDC*). × 5,300

rER cisternae, microtubules and distinct Golgi apparatus. Many pinocytotic vesicles were found on the plasmalemma (Fig. 5). Microfilaments were abundant particularly beneath the plasmalemma and these were attached to the basement membrane (Fig. 5). The microfilaments were arranged in bundles and many electron dense areas were present both in the bundles and immediately beneath the plasmalemma.

Trabecular Myofibroblasts

The major trabeculae appeared in the 16th week, mainly around the blood vessels. They gradually extended between the splenic cords and increased their width. At

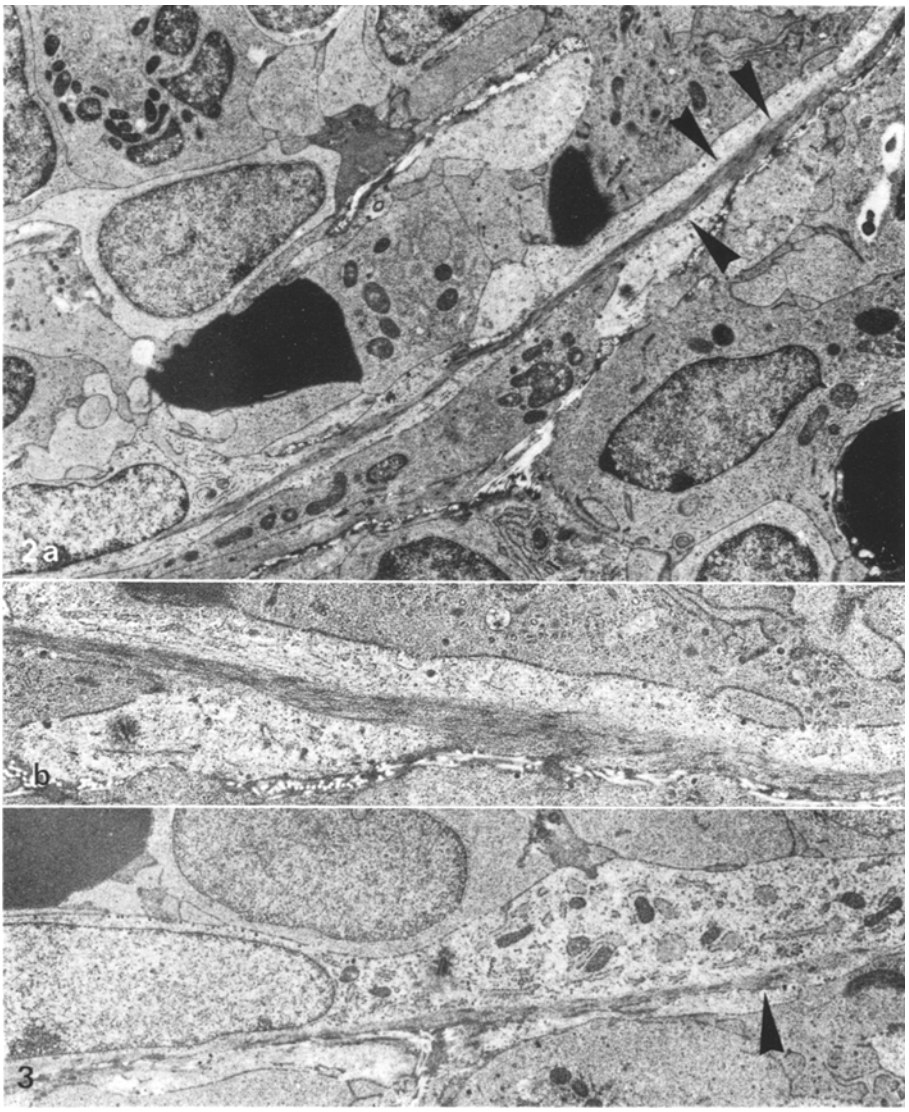

Fig. 2a and b. A part of the perifollicular myofibroblast shown in Fig. 1. (**a**) ×10,000. (**b**) ×22,000. Many dense areas can be seen in the bundle

Fig. 3. Perifollicular myofibroblast. The cytoplasm contains a moderate number of rER cisternae and a bundle of microfilaments (*arrow*). ×12,000

first they were composed of spindle shaped immature mesenchymal cells which have abundant polyribosomes and a moderate number of rER cisternae. With the maturation of the fetus collagen fibers increased in the intercellular space and most of the mesenchymal cells became fibroblasts. Among these fibroblasts typical myofibroblasts were frequently observed (Fig. 6).

These cells were bipolar and markedly elongated in shape and had long and often indented nuclei. The chromatin was finely dispersed with slight peripheral

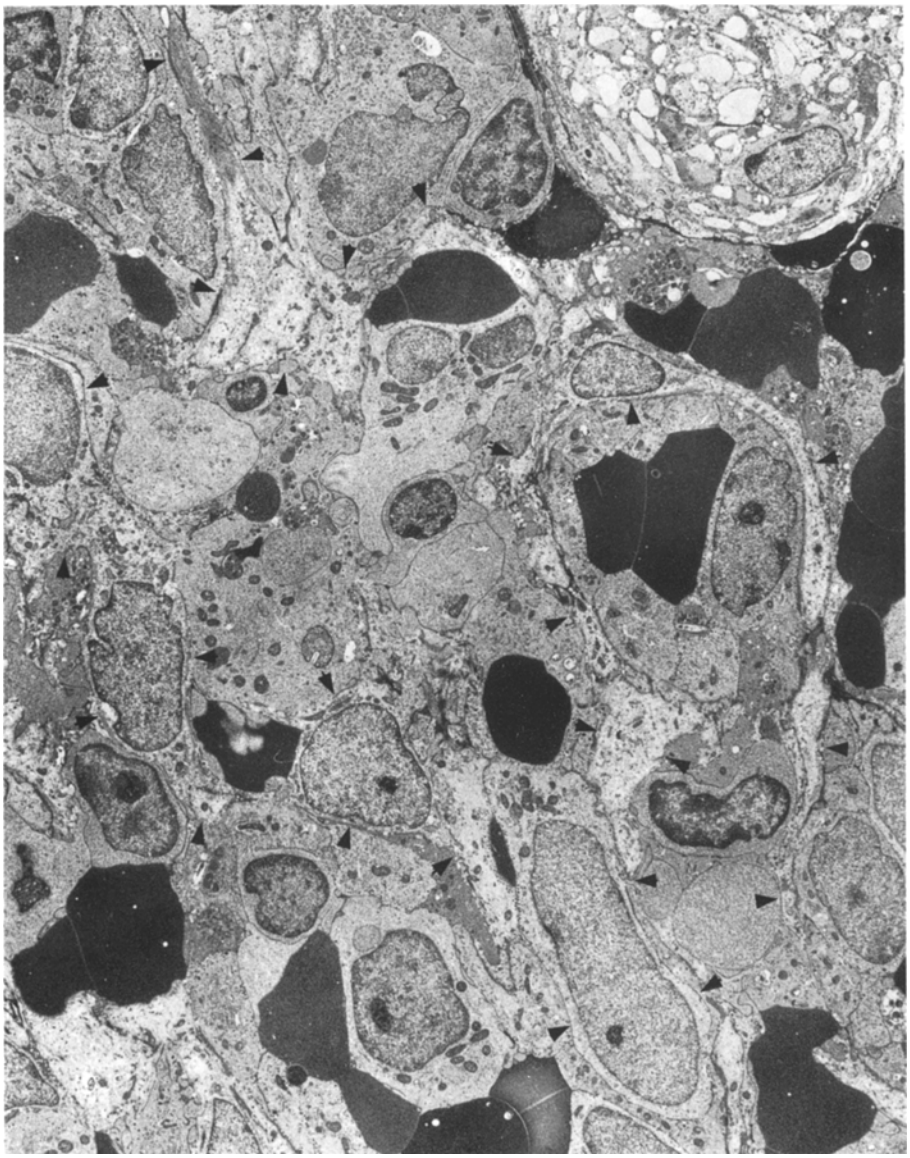

Fig. 4. Perisinusal myofibroblasts are connected to each other and constitute a cellular network throughout the splenic cords (*arrowheads*). ×6,000

clumping. The nucleoli were prominent in general. The cytoplasm contained several mitochondria, many microtubules, distinct Golgi apparatus and abundant rER cisternae, which were filled with material of moderate electron density. Many pinocytotic vesicles were found under the plasmalemma. The most prominent characteristic of these cells was the presence of abundant microfilaments. Both thin and thick filaments were arranged in bundles particularly beneath the cell membrane along the long axis of the cell. Many electron dense areas were found

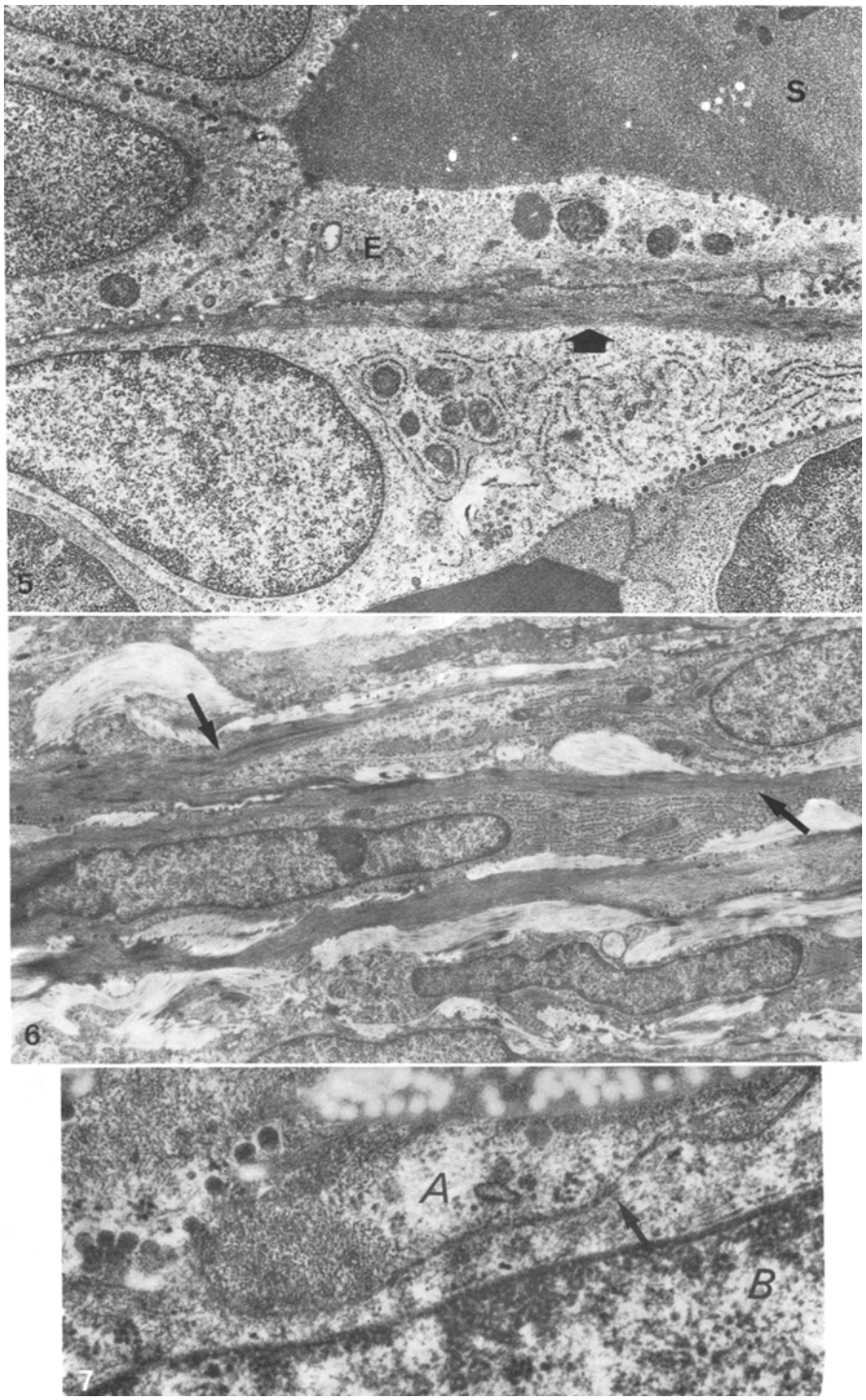

Fig. 5. Perisinusal myofibroblast. Sinus (*S*), Endothelium (*E*), Bundle of microfilaments (*arrow*). ×23,000

Fig. 6. Trabecular myofibroblasts. The cytoplasm contains abundant rER cisternae and prominent bundles of microfilaments (*arrows*) can be seen under the cell membrane. ×7,000

Fig. 7. Trabecular myofibroblasts. Nexus-type junction (*arrow*) between two adjacent cells (A, B). ×65,000

within the bundles. The trabecular myofibroblasts were used to be surrounded by basal lamina-like material and so-called microtendons (Ryan et al. 1974) were occasionally observed. The neighboring myofibroblasts were often connected to each other by desmosome or nexus-type junctions (Fig. 7).

Smooth muscle cells in groups were occasionally found from the 25th week, within both the capsule and the trabeculae.

Discussion

The electron microscopic studies of the human fetal spleen confirmed the presence of many mesenchymal cells which have distinct bundles of microfilaments in (1) perifollicular, (2) perisinusal and in (3) trabecular regions, even though there was some deviation between their cytological features.

The typical myofibroblasts described by Majno and his school (Gabbiani et al. 1976; Majno 1979; Ryan et al. 1974) were found in the trabeculae. Perifollicular and perisinusal cells had a smaller number of rER cisternae than myofibroblasts and their nuclei rarely showed typical indentations. But presence of microfilament-bundles which have many dense areas in their cytoplasm, seemed to be sufficient to consider these cells as myofibroblasts. Some of the splenic reticulum cells are known to have intracytoplasmic microfilaments (Burke, Simon 1970).

The myofibroblasts are often found in the connective tissue of various pathological conditions, e.g. healing wounds (Gabbiani et al. 1971; Majno et al. 1971; Rudolph et al. 1977; Ryan et al. 1974), certain fibrotic diseases (Gabbiani and Majno 1972; Gould and Weinstein 1976; Wirman 1976), cirrhosis of the liver (Bhathal 1972), carcinoma (Ohtani and Sasano 1979, 1980; Seemayer et al. 1979) and various mesenchymal tumors (Bhawan et al. 1979; Stiller and Katenkamp 1975; Taxy and Battifora 1977; Vasudev and Harris 1978). These myofibroblasts have been considered to be abnormal fibroblasts that are not found within any organ under physiological conditions (Majno 1979, Seemayer et al. 1979). However, Güldner et al. (1972) found similar cells in duodenal villi of the rat and Bressler (1977) considered the myoid cells of the adrenal cortex to be myofibroblasts.

Pictet et al. (1969) described intermediate forms between fibroblasts and smooth muscle cells in the splenic trabeculae of rats and suggested the possibility of transformation of one form to the another.

There are three important questions concerning these cells. (1) What cause fibroblasts to modulate into myofibroblasts? (2) How long can these cells remain as myofibroblasts? (3) Can these modulated contractile fibroblasts: myofibroblasts change back into fibroblasts?

From experiments in granulating wounds Rudolph et al. (1977) showed that the contractile myofibroblasts rapidly proliferate within 2 weeks and disappear 8–16 weeks after the operation. The wound then ceases to contract. This fact indicates that the life span of myofibroblasts is generally from 6 to 8 weeks, but the most important question remains unanswered.

The presence of myofibroblasts was first described here in human fetal spleen under physiological conditions. The perifollicular and perisinusal myofibroblasts were not seen before the appearance of the follicles and the construction of the

splenic sinuses but they appeared immediately after the establishment of these structures. This fact suggests that the pressure of enlarging follicles and of distending sinuses modulate the surrounding fibroblasts into contractile fibroblasts: myofibroblasts. The cells are connected to each other or to the stroma and seem to play a role in the excretion of lymphocytes from the follicles and in pumping the blood from the sinuses by their contraction.

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