Fine Structural Aspects of the Development of Ito Cells (Vitamin A Uptake Cells) in Chick Embryo Livers*

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Received April 27, 1983

Summary. The development of Ito cells in the chick embryo liver was studied using electron as well as fluorescence microscopes.

The collagen fibrils in the Disse’s space can already be seen in 6-day-old chick embryos. This space contains fibroblast-like cells which should be called primitive Ito cells. They are slender cells characterized by numerous free polyribosomes. The rough endoplasmic reticulum, Golgi apparatus and 10 nm microfilaments are also well developed.

The fluorescence of vitamin A and lipid droplets begin to appear in the primitive Ito cell at 9 days of incubation in both the control and vitamin A-treated animals. A special topographic relation between the lipid droplets and cell organelles is difficult to recognize. The primitive Ito cell in the Disse’s space acquires the ability to produce collagen fibrils earlier than that of taking up and storing vitamin A. The Ito cells containing lipid droplets increase in number with embryonic age, and about 40–50% of perisinusoid cells have droplets of vitamin A at 21 days of incubation. The droplets are usually less than 1 μm in diameter and do not fuse with each other.

Since the discovery of the fat storing cell in the perisinusoidal space of Disse in the liver by Ito (1951), numerous papers have been published dealing with the function and morphology of this cell. Recently it has come to be believed that this type of cell corresponds to the stellate cell in Kupffer’s original paper (1876) as pointed out by Wake (1971), and that vitamin A is taken up by this cell to be stored in the lipid droplet (Nakane, 1963; Wake, 1971; Hirokawa and Yamada, 1973; Kusumoto and Fujita, 1977). The fat storing cell, which is now called the Ito cell or vitamin A-uptake cell, is known to be transformed from the fibroblast-like cell taking up vitamin A (Takahashi et al., 1978; Sakano and Fujita, 1982), and both cells are believed to belong to the same kind of perisinusoidal cells (Wake, 1971). Fine structural aspects of the development of this cell have not yet been reported, though light microscopic findings of the ontogenesis of this cell in the domestic fowl have been described by Ito et al. (1960). The present study intends to clarify the development of the Ito cell in the chick embryo liver using electron as well as fluorescence microscopes.

*This study was supported by a grant (No. 57370001) from the Ministry of Education, Science and Culture.
MATERIALS AND METHODS

Livers of White Leghorn chick embryos and young chicks were used for this study. Thirty two embryos were injected with 5,000 I.U. of vitamin A (Chocola A, the Eisai Co., Ltd.) into the yolk sac one day before sacrifice at 6–21 days of incubation, and 32 animals were injected with the same dose of vitamin A at 5 days of incubation and sacrificed at 6–21 days. Twelve chicks were injected with 5,000 I.U. of vitamin A intraperitoneally one day before sacrifice at either 2, 3, 5, 6, 20, or 40 days after hatching. In addition to these, 32 chick embryos and 12 chicks at all the ages mentioned above served as the control without any treatment of vitamin A.

The embryos taken at 6 to 14 days of incubation were perfused with 2% glutaraldehyde solution buffered at pH 7.4 with Millonig’s phosphate, and the other animals older than 14 days of incubation with 3% glutaraldehyde.

For electron microscopy, the specimens perfused with glutaraldehyde solution were cut into small pieces, immersed in the same fixative mentioned above for 2 hr, and postfixed with 1% OsO4 solution for 1 hr. For block staining, the tissue blocks were rinsed with 10% sucrose solution and stained with 3% uranyl acetate solution for 1 hr. The materials were dehydrated in graded concentrations of ethanol and embedded in Epon. Ultrathin sections, cut on a Porter-Blum ultramicrotome and stained doubly with uranyl acetate and Millonig’s lead, were examined with a Hitachi H-500 type electron microscope.

For light microscopy, the tissue blocks fixed with the glutaraldehyde fixative for 2 or 3 days were dehydrated in graded concentrations of ethanol and embedded in a JB-4 Embedding Kit (Polysciences, Inc.). Thin sections were cut on a JB-4 microtome (Sorvall) at 2 μm thickness and stained with hematoxylin-eosin.

For fluorescence microscopy, the tissue blocks were perfused, immersed in 3% formaldehyde solution for 2 days, and then rinsed in 10% sucrose solution for 5 hr. Frozen sections were cut at 10 μm thickness, mounted in glycerol and examined with a Nikon Fluophot microscope. When excited by 328 nm ultraviolet light, vitamin A emits a yellow-green fluorescence which characteristically fades away within 30 to 60 sec.

OBSERVATIONS

1. Orientation
The hepatic sinusoidal wall consists of endothelial cells and Kupffer cells. Between these cells and hepatocytes is a connective tissue space, named Disse’s space. It contains fat storing cells, fibroblast-like cells ("empty" fat-storing cells), macrophages, collagen fibrils (reticular fibrils) and tissue fluid. In the present paper, fat-storing cells and fibroblast-like cells having the ability to store lipid droplets are called Ito cells, and the fibroblast-like cells lacking in this ability in the early embryo are named primitive Ito cells. Hematopoietic cells could hardly be seen in the Disse’s space in all the chick embryo livers used.

2. Six–8 day-old embryos (stage 29–34)
No fluorescence of vitamin A was observed in the liver tissues of all the 6–8 day-old embryos treated with or without vitamin A.
Development of Ito Cells

By electron microscopy, all the fibroblast-like cells located at the perisinusoidal space, and which should be called primitive Ito cells, were slender cells spreading out along the endothelial lining. They appeared in sections as fusiform elements with long tapering processes (Fig. 2, 3a). No lipid droplets could be recognized in the cytoplasm of these cells in the control as well as in vitamin A-treated animals. Numerous free polyribosomes, distributed throughout the cytoplasm, and flattened cisterns of rough endoplasmic reticulum were well developed and widely distributed (Fig. 2, 3a). Mitochondria, smaller in size than those of hepatocytes, were scattered in the cytoplasm, and a marked Golgi apparatus consisting of stacks and vesicles was located near the nucleus (Fig. 2b). In addition to these findings, a large number of cytoplasmic filaments about 10 nm in diameter ran randomly or sometimes parallel to one another among the cell organelles and in the cytoplasmic processes. Small vesicles about 30–100 nm in diameter, some coated, were sometimes seen near the Golgi apparatus and the plasma membrane. In the Disse's space, a few collagen fibrils running randomly already existed at 6 days of incubation (Fig. 2a).

3. Nine-day-old embryos (stage 35)
A few Ito cells started to manifest a few small lipid droplets in their cytoplasms. The fluorescence characteristic for vitamin A was observed by fluorescence microscopy in a few cells throughout the hepatic tissue of both the control and vitamin A-treated animals (Fig. 1a). By electron microscopy, lipid droplets, about 0.3–0.6 μm in diameter, were rarely seen in the cytoplasm without a special topographic relation with the cell organelles (Fig. 3b). No marked changes were recognized in the fine structure of the cell organelles of Ito cells as compared with those of the 6–8 day-old embryos.

4. Ten–13 day-old embryos (stage 36–39)
The Ito cells containing lipid droplets slightly increased in number with embryonic age (Fig. 4). The fine structure of the cell organelles and the number of the collagen fibrils in the Disse's space were not so markedly changed.

5. Fourteen–15 day-old embryos (stage 40–41)
From this stage the Ito cells containing lipid droplets began to increase in number

Fig. 1. Fluorescence micrographs of chick embryo livers. a. A 9-day-old embryo without any treatment. ×400. b. A 16-day-old embryo treated with vitamin A. ×400. c. A 17-day-old embryo treated with vitamin A. ×200
Fig. 2. Primitive Ito cells in a 7-day-old embryo treated with vitamin A. a. No lipid droplets are seen in the cytoplasm. Elements of rough endoplasmic reticulum are well developed and collagen fibrils (arrows) are distributed in the Disse's space. C capillary lumen, N nucleus of Ito cell, H hepatocyte. ×11,000. b. A part of the cytoplasm of the Ito cell. Note the well-developed Golgi apparatus (G), small vesicles, profiles of rough endoplasmic reticulum (R), and cytoplasmic filaments. ×23,000. c. A part of the process of the Ito cell extending in the Disse's space between the hepatocyte (H) and endothelium (E). Note numerous cytoplasmic filaments in the cytoplasmic process. C capillary lumen. ×17,000
rapidly (Fig. 5a). About 5-10% of the Ito cells contained lipid droplets. The number of the droplets in a cell was mostly 1-5 in the thin sections.

6. Sixteen-21 day-old embryos (stage 42-46)

About 40-50% of Ito cells contained lipid droplets in 21-day-old embryos. The intensity of the fluorescence in each cell became stronger (Fig. 1b, c). There were 1 to 20 droplets in the thin section, with some of them gathered into clusters (Fig. 5b). The

Fig. 3. a. A primitive Ito cell in an 8-day-old embryo without any treatment. No lipid droplets are observed. Profiles of rough endoplasmic reticulum (R) and numerous free ribosomes are seen. H hepatocyte, N nucleus of primitive Ito cell, C capillary lumen. x 17,000. b. An Ito cell in a 9-day-old embryo treated with vitamin A. Two lipid droplets (L) are seen. G Golgi apparatus, N nucleus of Ito cell. x 16,000
limiting membrane of each droplet was not a unit membrane but a leaflet of membrane as that of other kinds of fat droplets. The droplet was round in shape, electron lucent, and 0.3–1.0 μm in diameter. The fine structure of the cell organelles was almost the same as those in the younger embryos, though cytoplasmic filaments were less distinct. There were no marked differences in number and size of the lipid droplets and in the intensity of vitamin A fluorescence in the Ito cells between the vitamin A-treated group and the control group.

7. Two-6 day-old chicks

The population density of Ito cells containing lipid droplets in the liver tissue was con-
sistently higher than that in the oldest embryos. The droplets in each cell amounted to 1–20 in the thin sections in both the control and vitamin A-treated animals. They were in contact with each other, forming a cluster in some Ito cells. Cytoplasmic filaments which could be clearly seen in the perinuclear cytoplasm and cytoplasmic processes in young embryos decreased in number and were difficult to recognize after hatching. On the other hand, collagen fibrils increased in number around the Ito cells.

8. Twenty and 40 day-old chicks

In both 20 and 40 day-old chicks, most perisinusoidal cells showed intense fluorescence of vitamin A in the control and vitamin A-treated animals. In each cell, lipid droplets 5–30 in number were seen in thin sections of the Ito cells (Fig. 6). Though the droplets were in contact with one another, no signs of their fusion were recognized. The droplets usually did not exceed 1 \( \mu \text{m} \) in diameter in this animal as noted by Ito (1978).

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**Fig. 5.** a. A part of the Ito cell in a 15-day-old embryo treated with vitamin A. A cytoplasmic process of the Ito cell extending in the Disse’s space (D) contains numerous free ribosomes, rough endoplasmic reticulum (R) and a lipid droplet. H hepatocyte, C capillary lumen. \( \times \)38,000. b. An Ito cell in a 16-day-old embryo treated with vitamin A. Note many lipid droplets (L) which do not fuse with each other. \( \times \)10,000
DISCUSSION

The present study demonstrated fibroblast-like cells with numerous polysomes, well developed rough endoplasmic reticulum, and Golgi apparatus in the Disse’s space in 6-day-old embryos. No other cell types can be seen in this region. This fibroblast-like cell should be called a primitive Ito cell. In embryos at the same stage, collagen fibrils are already present in this space. Ito et al. (1960) also reported the existence of reticular fibers by silver impregnation and azan staining at this stage of embryonic develop-
ment. These findings suggest that the fibroblast-like cells of this stage are capable of producing exportable protein: tropocollagen. However, vitamin A is not detectable anywhere in the liver tissue by fluorescence microscopy, and lipid droplets are not found by electron microscopy in the perisinusoidal cells of this stage. Neither vitamin A fluorescence nor lipid droplets appear at 6–8 days of incubation, even if excess vitamin A is given to the animals.

At 9 days of incubation, both the fluorescence of vitamin A and electron-microscopically definable lipid droplets appear in the primitive cells. It can therefore be postulated that primitive Ito cells begin to differentiate into the vitamin A uptake cells at 9 days of incubation, and accordingly should now be called the fat storing cells of Ito. It was also revealed in the present study that the differentiation of the cells to produce collagen fibrils precedes the appearance of the ability to take up and store vitamin A. The mechanism for the synthesis of tropocollagen in this cell might be different from that for the uptake of vitamin A.

Even though excess vitamin A is given, the advent of lipid droplets in the cytoplasm of the Ito cell does not occur earlier than in the control group. During all stages of development, there are no marked differences in number and size of the lipid droplets and intensity of vitamin A fluorescence in the Ito cell between the vitamin A-treated group and the control group. It is known that the yolk sac usually contains 360–400 I.U. of vitamin A, and so the excess vitamin A injected is considered to be ineffective in facilitating the development of Ito cells. On the other hand, vitamin A administration has been reported to markedly increase the lipid droplets in the Ito cells of adult rats and fish (Kobayashi and Takahashi, 1971; Takahashi et al., 1978; Sakano and Fujita, 1982). In the primitive Ito cells, the administration of vitamin A does not induce the ability to take up and store vitamin A, while cytoorganelles such as the rough endoplasmic reticulum, Golgi apparatus and mitochondria are already well developed in 6-day-old embryos. In addition, the lipid droplet appears in the cytoplasm without any special topographic relation with the cell organelles at 9 days of incubation. The ability to take up and store vitamin A in this cell seems not to be related to the development of these cell organelles. The mechanism for the uptake of vitamin A in this type cell is a problem for future inquiry.

In the early developmental stage, Ito cells are relatively rich in intracellular filaments of 10 nm. Fujita et al. (1980), and Sakano and Fujita (1982) reported the existence of desmosomes between Ito cells and other kinds of cells, and of intracellular filaments in Ito cells in some teleosts which almost lack collagen fibrils in the Disse's space. The intracellular filaments are considered to be necessary for supporting the Ito cell shape in the Disse's space where collagen fibrils are not so rich.

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