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Electron Microscopic Observations on Intracellular Collagen Fibrils in Cotton Pellet-induced Granulomas

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Abstract

Intracellular collagen fibrils were detected by electron microscopy in experimental granulomas induced in the back of rats by cotton pellets. The banded intracellular collagen fibrils were observed in phagocytic vacuoles. Two types of collagen fibril-containing vacuoles were seen. In one type the vacuoles contained an electron-lucent material and the periodicity of the collagen fibrils was clearly seen. In the other type, the vacuoles contained an electron dense matrix and collagen fibrils were frequently not clearly evident. The cells that contained collagen fibrils had the features characteristic of fibroblasts actively engaged in collagen synthesis. These results suggest that these intracellular collagen fibrils had been phagocytosed by fibroblasts.

It is proposed that fibroblasts have the ability not only for the biosynthesis of collagen, but also for the phagocytosis of collagen fibrils during the rapid remodelling of connective tissue.

Key words : Cotton pellet granuloma, intracellular collagen fibrils, fibroblast

Introduction

The development of improved autoradiographic techniques, in particular electron micro-autoradiography, has led to a better understanding of collagen production by fibroblasts.[1,2]

Recently, in addition to their role in collagen biosynthesis, it has become evident that fibroblasts of various tissues also play an important part in collagen resorption through phagocytosis. The morphology of collagen degradation has received surpprisingly little attention, despite the existence of several adequate models for its study. Luse and Hutton (1964)[3] noted characteristic collagen fibrils within cytoplasmic vaculoles of fibroblasts in the involuting uterus of rats. Usuku and Gross (1972)[4] reported similar observations in the resorbing tails of metamorphosing bullfrogs.

Ten Cate (1972)[5] was the first to publish and clearly demonstrate the extensive distribution of intracellular collagen fibrils in oral connective tissue fibroblasts. Most recently, membrane-bounded intracellular collagen fibrils have been observed in fibroblasts of the periodontal ligament or gingiva of mice, dogs, and man.[6,7,8]

The aim of this paper is to illustrate and discuss the ultrastructural aspects of collagen fibril phagocytosis and degradation by fibroblasts using cotton pellet-induced granuloma as an experimental model.

Materials and Methods

Preparation of cotton pellet-induced granulomas

Male Wistar rats weighing 200g were used for experiments. Food and water were continuously avaiable. Cotton pellets were prepared as described by Winter, et al [9] The pellets were sterilized in an autoclave and implanted subcutaneously in the backs of rats under nembutal anesthesia. Six pellets were implanted in each animal. After implantation penicillin (200 unit/animal) was injected subcutaneously at a nearby site as an additional precaution against infection.

Electron microscopy

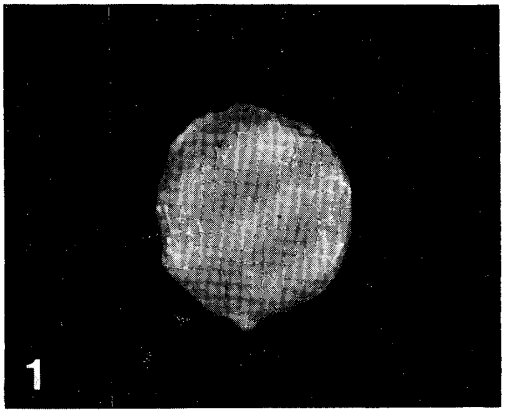
The granulomas were examined grossly every day ; and 6 days following implantation, the rats were sacrificed under light ether anesthesia by cardiac bleeding. Tissues, immediately after extraction, were fixed for 3 hrs at 4℃ in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH7.4. Most of these tissues were rinsed with 0.1M cacodylate buffer for 10 min and then postfixed for 2 hrs at 4℃ in 1% solution of cacodylate-buffered osmium tetroxide. They were then dehydrated with an ordinarily-graded ethanol series and embeded in Araldite 502. Initially, thick section were prepared from each block and stained with toluidine blue for light microscopic observation. Ultrathin section, cut on a LKB Ultratome V, were doubly stained with uranyl acetate and lead citrate.

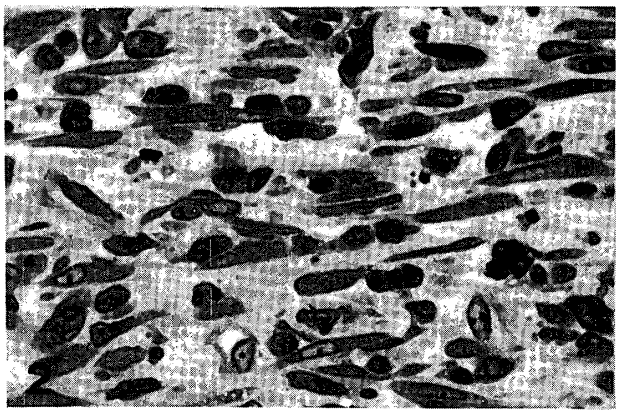
Observation was made with a HITACHI H-500 electron microscope at an accelerating voltage of 75 kV.

Results

Observation was restricted to 6-day-old granulomas only. Granulomas were elastic and 13.5 mm ± 2 in diameter (Fig. 1). Under light microscopic observations, a number of cells having oval-shaped nuclei and long cytoplasmic processes were seen. The long axis of many of these cells were oriented in the same direction. In addition to these cells, inflammatory cells were noted. Collagen fibers were demonstrated between cells (Fig. 2). By ultrastructural examination, the majority of cells were fibroblasts, although inflammatory cells—leukocytes, macrophages, and unclassified cells—were also observed. The extracellular components of granulomas were collagen fibrils and electron-dense amorphous material (Fig. 3). The collagen fibrils were distributed near fibroblastic cells.

Intracellular collagen fibrils were frequently observed in those cells containing a well-developed Golgi apparutus, abundant dilated sacs of granular endoplasmic reticulum, and prominent mitochondria (Fig. 4). These features are characteristic of fibroblasts actively engaged in collagen secretion as described by Ross, et al.[10] Commonly the collagen fibrils were found in the





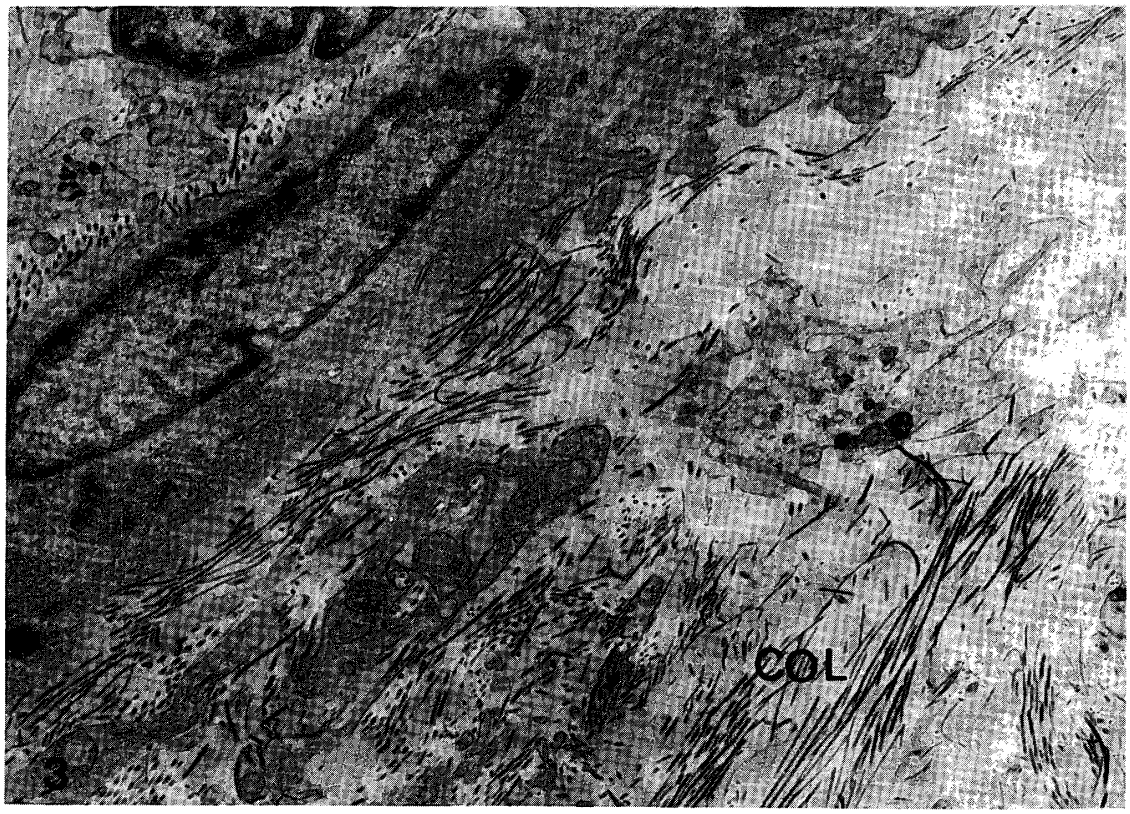
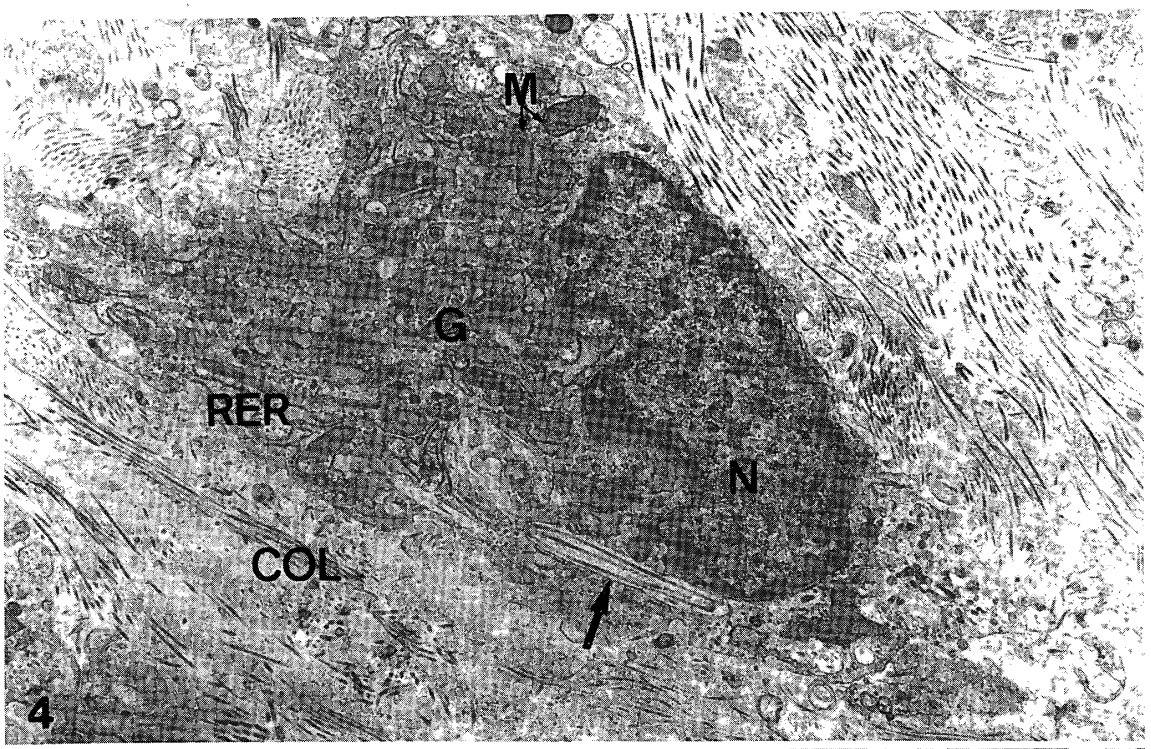


Fig. 1 Extracted cotton pellet-induced granuloma (6 days after implantation).

Fig. 2 Light micrograph of 6-day-old granuloma. Several types of cells and collagen fibers are noted. T.B.×300

Fig. 3 Electron micrograph of 6-day-old granuloma. Most of the extracellular material is represented by irregular collagen fibrils (COL) and electron-dense material. ×8500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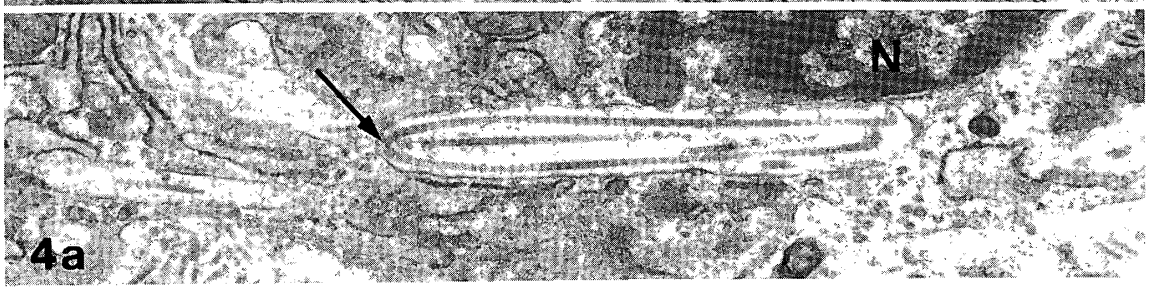
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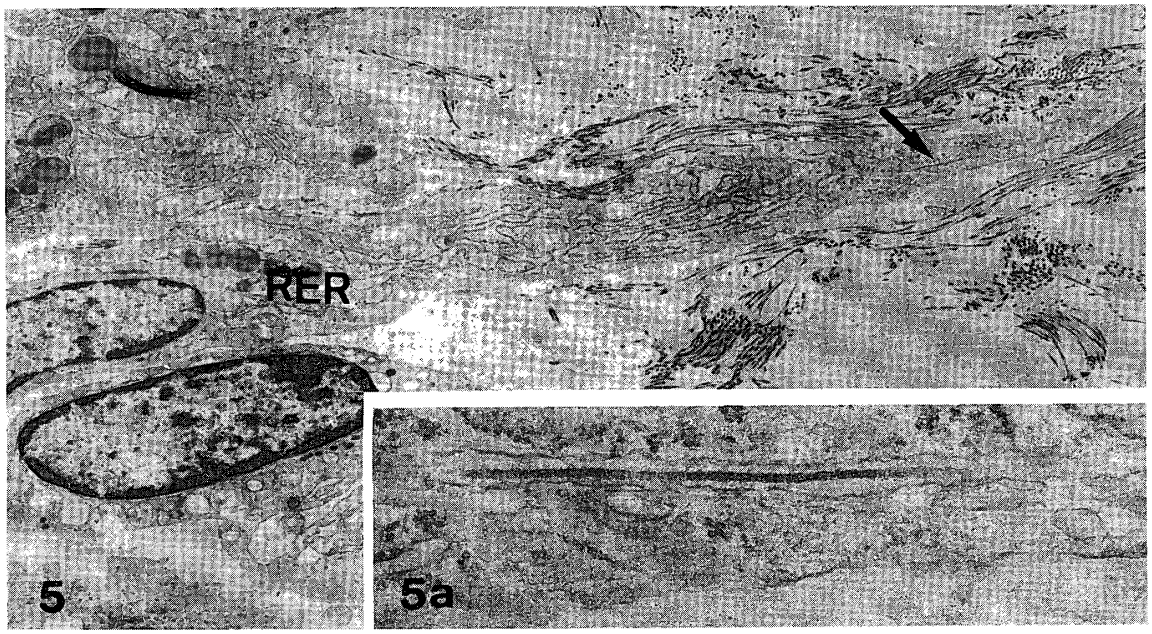
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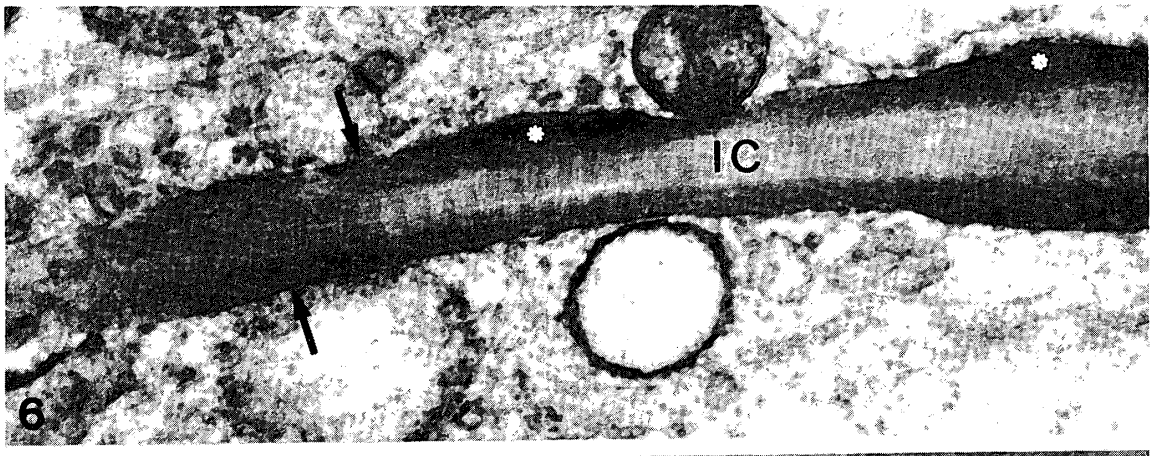

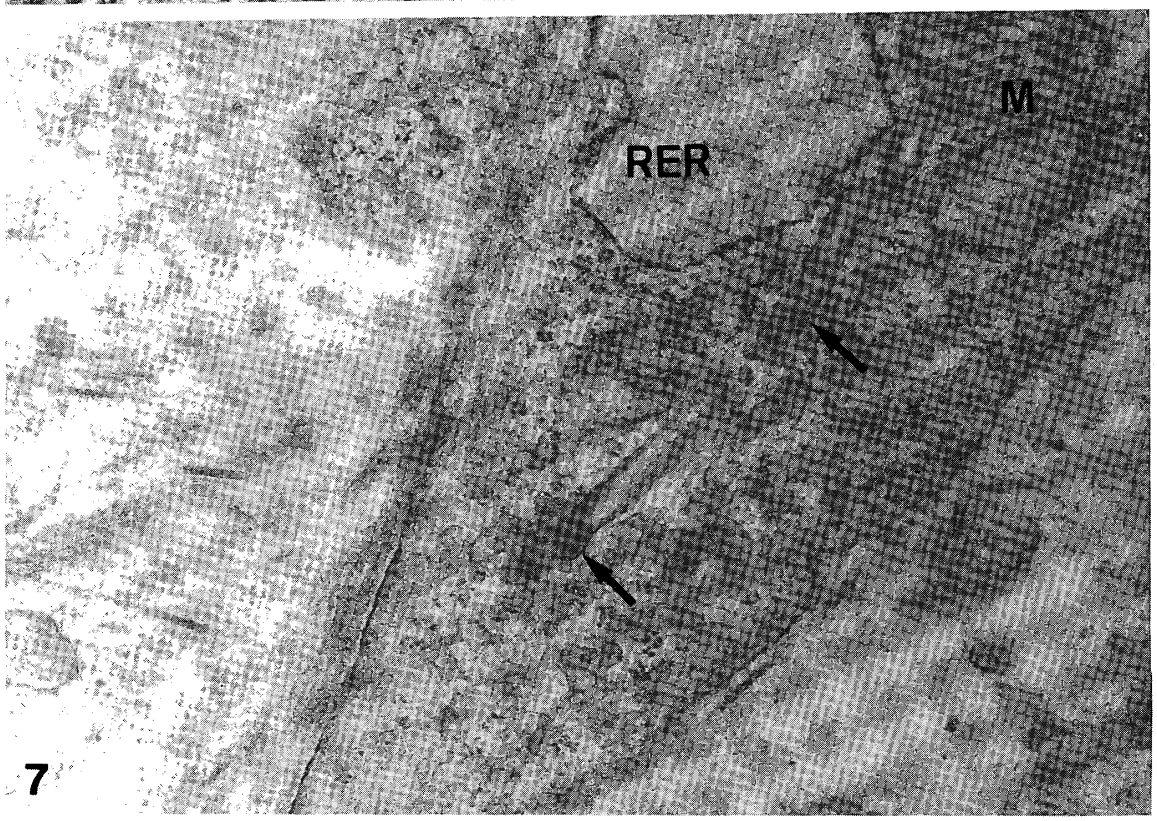

Fig. 4 Electron micrograph of granuloma fibroblast. The cytoplasm contained well-developed mitochondria (M), endoplasmic reticulum (RER), Golgi apparatus (G) and nucleus (N). Note the intracellular collagen fibrils (arrow). ×10000

Fig. 4a High power magnification of area indicated by arrow in Fig.4. Intracellular collagen fibrils form loops (arrow) and are surrounded by a smooth membrane. ×30000

Fig. 5 Electron micrograph of fibroblastic pseudopodal processes containing collagen fibril (arrow). ×7000

Fig. 5a High power magnification of fibril indicated by arrow in Fig.5. ×30000

Fig. 6 Electron micrograph showing an intracellular collagen fibrils (IC) within smooth-surfaced membrane (arrows), the fibrils being surrounded by an electron-dense material (*). ×46000

Fig. 7 Electron micrograph showing intracellular collagen fibril (arrows). Their periodicity is not clear. ×34000

long cytoplasmic extensions of such cells rather than in the central protion near their nuclei (Fig. 5). All collagen fibrils within cells were contained in well-defined smooth membrane-bound vacuoles (Figs. 4a, 5a). Collagen fibrils were never seen within the rough endoplasmic reticulum.

Two types of collagen-containing vacuoles were seen. One type displayed fibrils with a regular periodicity of 64nm which closely resembled collagen fibrils located extracellularly, and the vacuole matrix was electron-lucent (Figs. 4a, 5a). The other type of vacuoles containing collagen fibrils were filled with an electron-dense material, and often the 64nm periodicity of the collagen fibrils was not clearly seen (Figs. 6, 7).

Discussion

Intracellular collagen fibrils displaying characteristic periodic banding have been observed in a wide variety of biological systems as well as in pathological conditions[3, 4, 11]. The presence of collagen fibrils within fibroblasts has been a disputed point for many years despite the fact that others had reported the presence of phagocytosed collagen fibrils in fibroblasts.[3, 4, 12] Cullen[13] gave three possible explanations for intracellular collagen fibrils : 1) the collagen fibrils are actually located extracellularly, but because of the plane of section they appear to be intracellular, 2) the collagen fibrils within cells are produced by intracellular synthesis, and 3) they have been phagocytosed and ingested by cells: However, fine structural studies utilizing acid and alkaline phosphatase have provided strong evidence for collagen degradation following phagocytosis by fibroblasts.[14]

Subsequence work with serial sections of fibroblasts cultured with collagen fibrils from the periodontal ligament of the monkey has demonstrated the continuty of the membrane surrounding the collagen fibril-containing vacuoles.[15] Futhermore, phagocytosis and degration of collagen fibrils in cultured human gingival fibroblasts were domonstrated by 16mm microcinematography by Minamide.[16] The results of all these studies indicate that fibroblasts have the capacity not only to synthesize collagen, but also to phagocytose it.

The results of this study demonstrate the frequent occurrence of intracellular collagen fibrils with the periodic banding (64nm) typical of native collagen. Collagen fibrils in cells appeared singly or in bundles in vacuoles which were surrounded by a limiting membrane. There were also noted banded materials which had apparently fused with vacuoles containing a dense lysosomal-like matrix as well as vacules which contained intact collagen fibrils with periodic banding. It is proposed that collagen-containing vacuoles may fuse with lysosomes for the degradation of the collagen fibrils through lysosomal activity in the vacuoles. By this mechanism enzyme-containing vacuoles could account for the observation of vacuoles filled with a electron dense material. It is suggested that fibroblasts have the ability not only for the biosynthesis of collagen, but also for phagocytosis of collagen fibrils in tissues.

One of the difficulities using granuloma tissue has been the classification of the cells, in particular those cells which contain collagen fibrils. Granuloma formation by irritants is one of

the most frequently used procedures for the induction of inflammation. In fact, we have observed leukocytes and macrophages in 6-day-old cotton pellet-induced granulomas. Macrophages are known to be responsible for the phagocytosis and resorption of collagen fibrils that occurs during involution of the post-partum uterus of the rat[17] and in the peritoneum of the mouse.[18] The most striking features of the fibroblasts compared with macrophages were the presence of an extensive dilated granular endoplasmic reticulum and a well-developed Golgi apparutus. In addition the these features, fibroblasts also contained enlarged mitochondria with irregular small cistae. In contrast the macrophages contained numerous vacuoles, occasionally full of ingested matter, and irregular dense amorphous masses, and characteristically the cisternae of the endoplasmic reticulum were short and narrow.[10, 19, 20]

The cytological features of the cells which contained collagen fibrils as seen by electron microscopy in this study confirm the classification of these cells as fibroblasts. The majority of the cells in granuloma tissue appear to be fibroblasts. Similar findings have been made in carrageen-induced granulomas by Pérez-Tamayo.[12]

The mechanism of connective tissue breakdown under physiological conditions is not well understood. Connective tissue remodelling involves a change in shape or structure of a tissue without impairment in function.[21] The existence of a steady state situation regarding connective tissue maintenance clearly depends on whether there is a balance between synthesis and degradation of collagen. Phagocytosis of collagen fibrils by fibroblasts were found in regions in which there was continuous breakdown or remodelling of connective tissue.[3~6] It is known that the cotton pellet-induced granuloma forms mucopolysaccharides and collagen rapidly and then degrades these components.[22] Assuming this to be the case, the presence of the intracellular collagen fibrils encountered in the present study would seem to indicate that the collagenous component of the lesion undergoes rapid remodelling and turnover while the granulomatous lesion is progressively expanding. The function of collagen phagocytosis by fibroblasts may be associated with a high rate of collagen turnover.

Conclusions

1. Experimental granulomas were induced in the backs of rats by cotton pellets.
2. Electron microscopic examination of 6-day-old granuloma cells revealed a frequent occurrence of intracellular collagen fibrils in smooth membrane vacuoles.
3. The cytological features of the cells which contained collagen fibrils in this study confirm the classification of these cells as fibroblasts.

These results suggest that intracellular collagen fibrils were phagocytosed by fibroblasts. It is proposed that fibroblasts have ability not only for biosynthesis of collagen, but also for phagocytosis of collagen fibrils.

Acknowledgements

The authors express sincere appreciation to Prof. M. Kumegawa (Dept. of Oral Anatomy, Josai Dental University) for his encouragement during the study and to Assistant Prof. M. Hiramatu (Dept. of Dental Pharmacology, Josai Dental University) for kindly providing samples of granulomas. Thanks are also due to Mr. T. Ito (Laboratory of Electron Microscopy, Higashi-Nippon-Gakuen University) for photographic assistance.

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コットン・ペレット肉芽腫において見られた細胞内コラーゲン原線維の電子顕微鏡的観察

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抄　　録

細胞内コラーゲン原線維の検索のためにコットン・ペレット法を用いてラット背部に肉芽腫を発症させ電子顕微鏡を用いて観察をおこなった。その結果，細胞の小体内にコラーゲン原線維が認められるものが存在した。

細胞内コラーゲン原線維は，その形態から２つのタイプが観察された。その１つは，コラーゲン原線維を含む小体内部が明調で，コラーゲン原線維の横紋構造が明らかに認められるもの。もう１つのタイプは，小体内が暗調で，コラーゲン原線維の横紋構造が不明瞭か，もしくは認められないもの。

これらの所見は，細胞により取り込まれ，細胞内で消化をうける一連の過程で生じるものと思われ，またコラーゲン原線維を含む細胞は，その細胞小器官の検索から，特徴的な線維芽細胞の形態を備えていた。

以上のことから，線維芽細胞の機能は，コラーゲン合成のみならず，コラーゲン線維の崩壊にも役割をはたしていることが示唆される。

受付：昭和57年10月12日