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Ultrastructure of multinucleated giant cell apoptosis in foreign-body granuloma

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Abstract To elucidate the role of apoptosis in the disappearance of multinucleated giant cells from the granulation tissue in cases of foreign-body granuloma, we induced a foreign-body reaction by implanting a collagen sponge into the dorsum of the rat and observed apoptotic changes within the multinucleated giant cells using electron microscopy. Two types of multinucleated giant cells were identified presenting apoptotic characteristics morphologically. One was characterized by apoptosis of only one nucleus, followed by cytoplasmic changes, rupture of the plasma membrane and necrosis evoking an inflammatory reaction. The other showed typical apoptotic changes in the majority or in all of the nuclei, followed by phagocytosis of the apoptotic syncytia. The results of the present study suggest that apoptosis occurring within only one nucleus might be triggered by overexpression of the *p53* protein, because DNA abnormalities are confined to this single nucleus. In contrast apoptosis occurring simultaneously in the majority or all of the nuclei is most probably due to cell death caused by senescence.

Key words Foreign-body giant cells · Granulation tissue · Apoptosis · Ultrastructure · *p53* expression

Introduction

When an insoluble spongy biomaterial is implanted subcutaneously into an animal, the interstices within the artificial sponge are filled with cellular components consisting mainly of small vessel and inflammatory cells as well as fibroblasts [18, 22, 28]. This procedure induces a foreign-body granuloma, resulting in a chronic inflammatory reaction of the foreign-body type [10, 11]. Such granulomatous inflammation provides a mechanism for the efficient elimination of the foreign body. Multinucleated giant cells are always present in cases of foreign-body granuloma. The formation of multinucleated giant cells is a characteristic feature of the inflammatory response to a foreign body. As the inflammation runs its course, the foreign-body granuloma induced by implantation disappears due to involution of the granulation tissue, which is related to the ingestion and absorption of the foreign body. In addition, the number of multinucleated giant cells in the foreign-body granuloma diminishes, and they eventually disappear from the granulation tissue.

Multinucleated giant cells present in foreign-body granulomas were once regarded as an efficient disposal system for macrophages that are metabolically effete [19]. However, the current widely-held view is opposed to this inactivity theory, based on evidence showing that multinucleated giant cells are as active as macrophages in foreign-body granulomas, and that they exhibit Ia antigens [24] and several membrane antigens [34] on their surface. Thus, they play an essential role similar to that of other cellular components involved in the formation and development of foreign-body granulomas [15]. However, they are relatively short-lived, lasting for several days only [19, 26]. Very little has been reported about their fate in foreign-body granulomas.

Recently, Desmoulière et al. [4] reported that the number of vascular cells undergoing apoptosis increases as a wound closes and develops into a scar due to involution of granulation tissue. Apoptosis is a distinctive form of cell death [2, 12, 14, 39] that is thought to involve the expression of an endogenous endonuclease that cleaves DNA [3, 6, 9, 38]. The same morphological changes are observed consistently during apoptosis, including condensation and fragmentation of the nucleus and modification of the cytoplasmic organelles. Apoptotic cells are eliminated via phagocytosis, either by macrophages or by neighboring cells. Apoptosis presents in various physiological and pathological conditions [13, 32, 35]. However, whether or not cell death due to apoptosis is responsible for the disappearance of multinucleated giant cells from foreign-body granulomas during the involution process remains unclear.

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The present study utilizes an in vivo model in order to investigate the gradual disappearance of multinucleated giant cells from foreign-body granulomas. Collagen sponge, an insoluble spongy biomaterial, was implanted subcutaneously into the dorsum of each experimental animal in order to induce foreign-body granulomas. The elimination of multinucleated giant cells from the foreign-body granulomas was investigated by quantifying daily changes in the multinucleated giant cell population using electron microscopy. The results show that apoptotic cell death causes the disappearance of multinucleated giant cells in granulation tissue.

Materials and methods

Female rats of the Fisher 344 strain weighing 100–120 g were used throughout the investigations.

Collagen sponges (Koken, Japan) were reconstituted from bovine skin collagen by freezing in the presence of hexamethylene-diisocyanate, resulting in a porous, highly cross-linked, resilient matrix. All surgical procedures were performed under general anaesthesia by intraperitoneally injected Nembutal (pentobarbital sodium; Dainabot, Japan) (35 mg/kg). A 1 cm incision was made in the skin of the dorsum, and the skin was separated from the underlying fascia, creating a short subcutaneous tunnel. One sterile collagen sponge (1×1×0.5 cm) impregnated with phosphate buffered saline (pH 7.4) was inserted into the tunnel. The incision was closed with 3-0 sutures.

Groups of at least three animals were sacrificed every 5–10 days for 18 weeks beginning on 2nd day following implantation. The collagen sponges were dissected carefully from the surrounding tissues, and bisected using a scalpel. One portion of the sponge was fixed in 10% neutral formalin, and embedded in paraffin. For light microscopic examination, 4 μm thick sections were prepared and stained with haematoxylin and eosin. Three sections separated by 500 μm were collected from each sponge, and the number of giant cells containing three or more nuclei were counted in each section at a magnification of 100 by means of a graticule fitted into the eyepiece of the microscope.

The other portion of the sponge bisected for electron microscopic examination was fixed for 2 h in a chilled solution of 2.5% glutaraldehyde in 0.1 mol/l of phosphate buffer (pH 7.4). The prefixed tissue was washed in 0.1 mol/l of phosphate buffer (pH 7.4) and subdivided into 1 mm^3. These pieces were post-fixed at 4° C for 2 h in 1% osmium tetroxide buffer solution (pH 7.4), washed, dehydrated through a series of graded alcohols, and immersed in propylene oxide for 20 min. The pieces were then transferred into Epon 812 diluted with propylene oxide, embedded in pure Epon, and polymerized. Next, a selected area of each Epon block was trimmed, and serial ultrathin sections were cut and stained with uranyl acetate-lead citrate. The sections were examined using a Hitachi H-7100 electron microscope.

Immunohistochemical staining was performed on the 4 μm thick sections using the avidin-biotin complex immunoperoxidase technique in order to detect the *p53* protein. The sections were deparaffinized in xylene, and rehydrated through a series graded alcohols. Endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxidase in methanol. After blocking non-specific reactions with 10% normal rabbit serum, the sections were incubated overnight with a mouse monoclonal antibody specific for both the wild and mutant types of the *p53* protein (DO-7; Dako, Carpinteria, Calif., USA). The sections were then incubated with biotinylated rabbit anti-mouse immunoglobulin for 30 min, and subsequently with avidin-horseradish peroxidase complex for 30 min. After colour development with 0.03% diaminobenzidine (including 0.01% hydrogen peroxide), the sections were counterstained with methyl green and mounted.

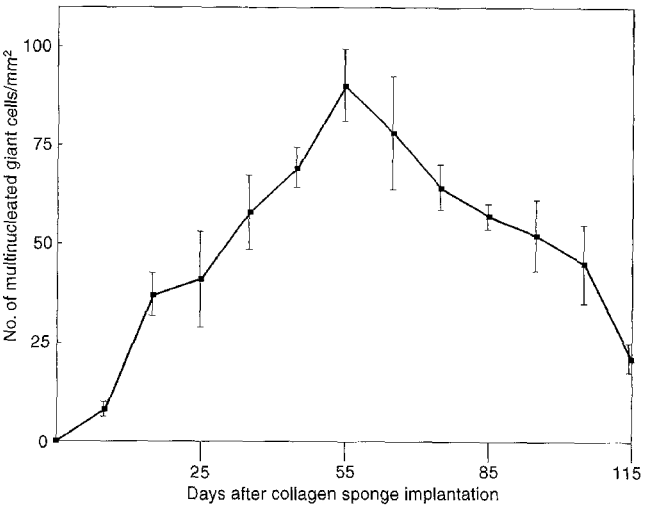


Fig. 1 Number of multinucleated giant cells in foreign-body granulomas induced by the implantation of collagen sponges. Results were expressed as mean counts per 1 mm^2 of each section from three sponges ±SE

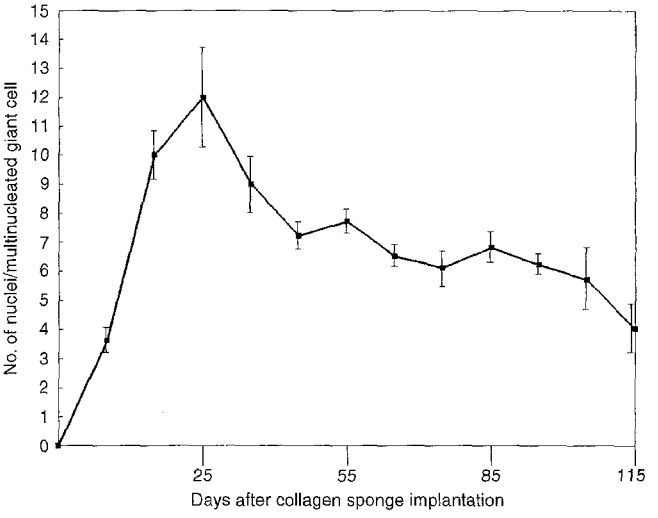
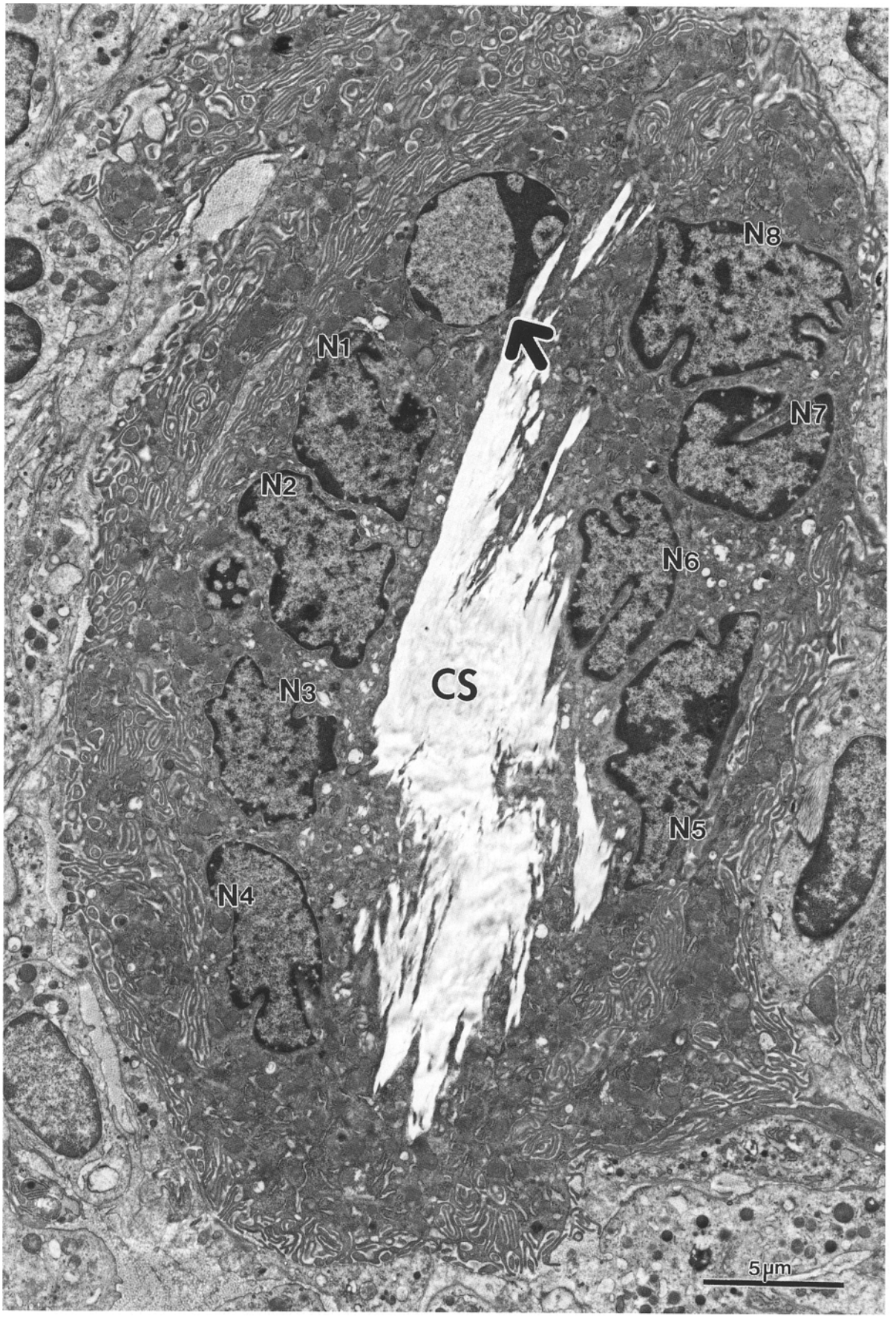


Fig. 2 Number of nuclei of multinucleated giant cells in foreign-body granulomas induced by the implantation of collagen sponges. Results were expressed as mean counts per cell ±SE. At least 110 to 150 multinucleated giant cells were scored for each time point

Fig. 3 Electron micrograph of multinucleated giant cell with one apoptotic nucleus in 105-day-old granuloma. The nucleus *(arrow)* shows sharp line of demarcation between condensed chromatin and electron-lucent nuclear interior. However, other eight nuclei *(N1-N8)* show normal clumping of nuclear chromatin. Cell surface shows numerous long microvilli and intracytoplasmic labyrinth. No microvilli are present on cell surface in contact with collagen sponge material *(CS)* ▶


N8
N1
N7
N2
N6
CS
N3
N5
N4
5μm

Results

Two days after implantation, the granulation tissue primarily consisted of fibroblasts and mononuclear macrophages entering the periphery of the sponge. The pores in the centre of the sponge were filled with fluid consisting of a fibrinous network and numerous neutrophils. From the 4th day, capillaries began to appear in the periphery of the sponge, and in sponges that had been implanted for 25 days, infiltration by moderately vascular granulation tissue occupied most of the sponge except for a small central area. By the 55th day, the granulation tissue had completely saturated the sponge. From the 15th day, the granulation tissue contained few neutrophils and lymphocytes, and macrophages were the most predominant type of cell in the sponge.

Giant cells containing three or more nuclei began to appear as single elements in a few of the most peripheral trabecula of the sponges starting on the 4th day, increasing in quantity within the granulation tissue and penetrating further into the sponge thereafter. By the 55th day, multinucleated giant cells were observed throughout the sponge, and their number per unit area reached a maximum (Fig. 1). By the 115th day, the sponge was gradually absorbed by phagocytosis, and the number of multinucleated giant cells began to decrease in the granulation tissue of the foreign-body granulomas.

The number of nuclei in the multinucleated giant cells varied depending on the length of implantation. Initially, the giant cells were rather small, containing 5–6 nuclei. Later, they increased in size, in some cases containing 20–30 nuclei or more. By the 25th day, the mean number of nuclei in the multinucleated giant cells reached a maximum of 12 (Fig. 2). Subsequently, a steep decrease in the number of nuclei was observed. The mean number of nuclei per multinucleated giant cell fell to seven by the 45th day, and remained almost constant for the remainder of the experimental period. At the end of the experimental period (115–125 days), large phagocytes containing two or three nuclei were predominant in the scar tissue that developed from the foreign-body granuloma.

Foreign-body granulomas are pervaded by foreign-body giant cells with nuclei scattered throughout the cytoplasm, although a small number of Langhans-type giant cells with a peripheral ring of nuclei are observed as well. Ultrastructurally, the nuclear chromatin of each type of giant cell is morphologically similar. Several multinucleated giant cells are observed to have very thin peripheral rims of heterochromatin along the nuclear membrane in every nucleus, whereas in other multinucleated giant cells it was diffuse throughout the euchromatin in every nucleus. Microvillous structures are present to some extent on the cell surface of all multinucleated giant cells. Interestingly, in some cases the microvillous structures are very prominent, whereas in other cases they are extremely poorly developed. The intensive development of microvilli forms labyrinthine invaginations of the membrane with a direct continuation of the extracellular space. Such processes, described as an "intracytoplasmic labyrinth" [23], are commonly observed in foreign-body granulomas.

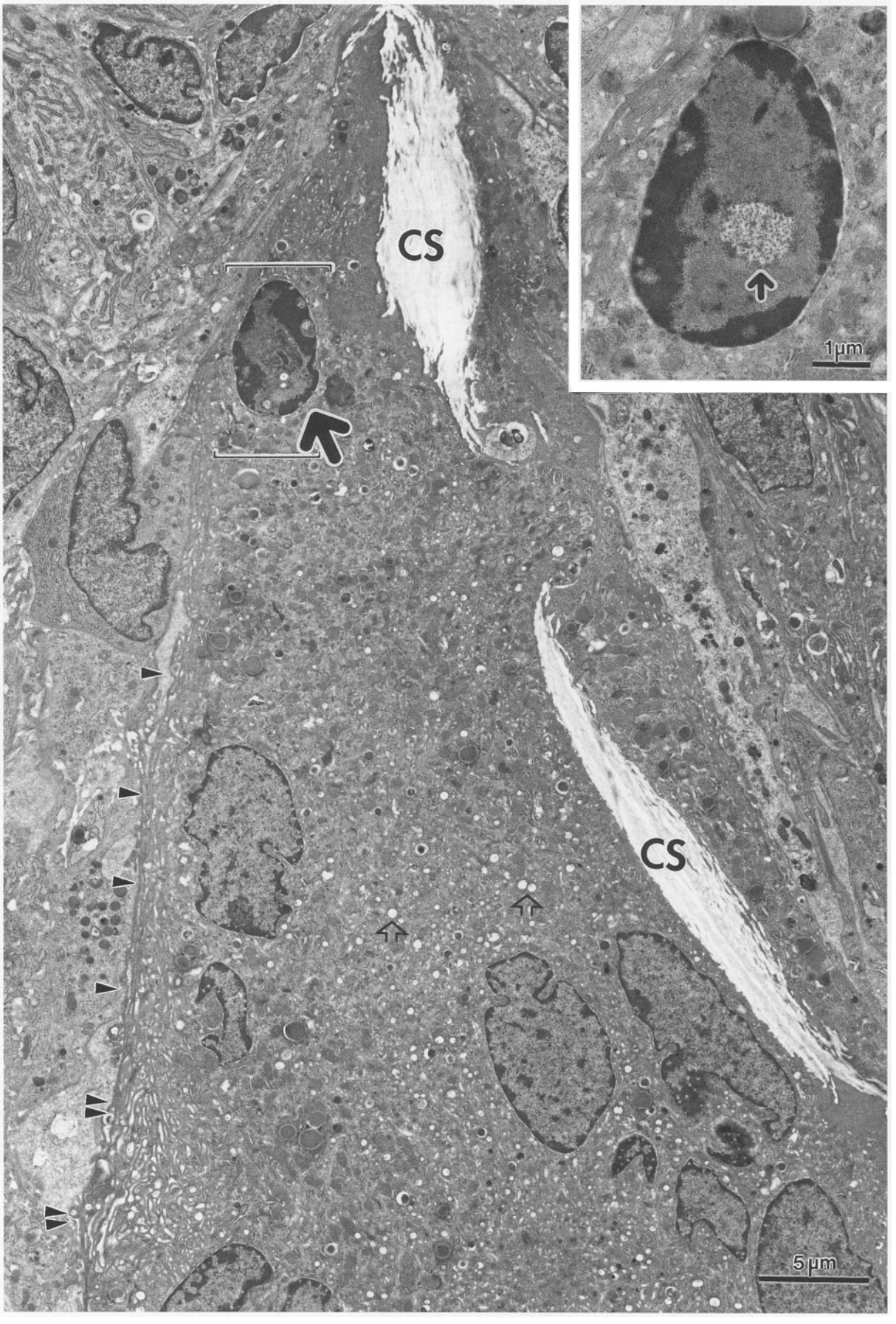
Sometimes a single nucleus of a multinucleated giant cell shows a condensed and marginated chromatin due to nuclear morphological changes associated with apoptosis, whereas all other nuclei in the cell present completely normal morphology, with no apoptotic chromatin condensation (Fig. 3). The contour of the condensed chromatin in the apoptotic nucleus either shows a pattern of localized to crescentic caps, or a pattern in which margination of the condensed chromatin is confluent throughout the nucleus.

Multinucleated giant cells containing a single nucleus with apoptotic chromatin condensation show an increased cytoplasmic density with condensed mitochondria. The cytoplasm of such giant cells contained numerous vacuoles (Fig. 4). Moreover, the collapse and disappearance of microvilli subsequent to the apoptotic changes was associated with dispersion of cytoplasmic vacuoles. Consequently, the intracytoplasmic labyrinth left a complex aggregation of microvillous remnants at the periphery of these cells. Degenerative cytoplasmic changes were observed in association with the apoptotic phenomenon in one nucleus, but no apoptotic changes were evident in any of the other nuclei. Subsequently, these degenerative changes lead to cell death associated with necrosis, similar to the secondary necrosis of apoptotic bodies as distinguished from cellular necrosis [39]. Multinucleated giant cells undergoing secondary necrosis thus contain one apoptotic nucleus presenting chromatin condensation and another nuclei presenting nuclear morphological changes associated with necrosis such as clumps of compacted chromatin (Fig. 5).

Multinucleated giant cells with single nucleus changes began to appear in the foreign-body granulomas 15 days after sponge implantation. Subsequently, such multinucleated giant cells appeared at a low rate (1–5%) throughout the experimental period until the 115th day, at which time the sponge is ingested by phagocytic cells and disappears from the granulation tissue as a result of involution (Table 1).

We observed another pattern of multinucleated giant cells with apoptotic chromatin condensation in the foreign-body granuloma. These cells presented with condensation and margination of nuclear chromatin as morphological evidence of apoptosis in more than half of the

Fig. 4 Electron micrograph of degenerated multinucleated giant cell with one apoptotic nucleus in 105-day-old granuloma. Nucleus *(arrow)* shows chromatin condensation and margination, with vacuoles and granular masses in central, more lucent area. Higher-power view of serial section of framed area in Fig. 6 shows another view of nucleus detailed in *insert;* dissociation of fibrillar components *(small arrow)* is also observed in central area. Other nuclei show normal profiles. Compact cytoplasm contains numerous lucent vacuoles *(open arrows),* condensed mitochondria, and lipid droplets. Note that decreased microvilli *(arrowheads)* aggregate side-by-side parallel to cell surface, and that labyrinthine invagination of membrane *(double arrowheads)* is obviously moribund. Implanted collagen sponge material (CS) is also visible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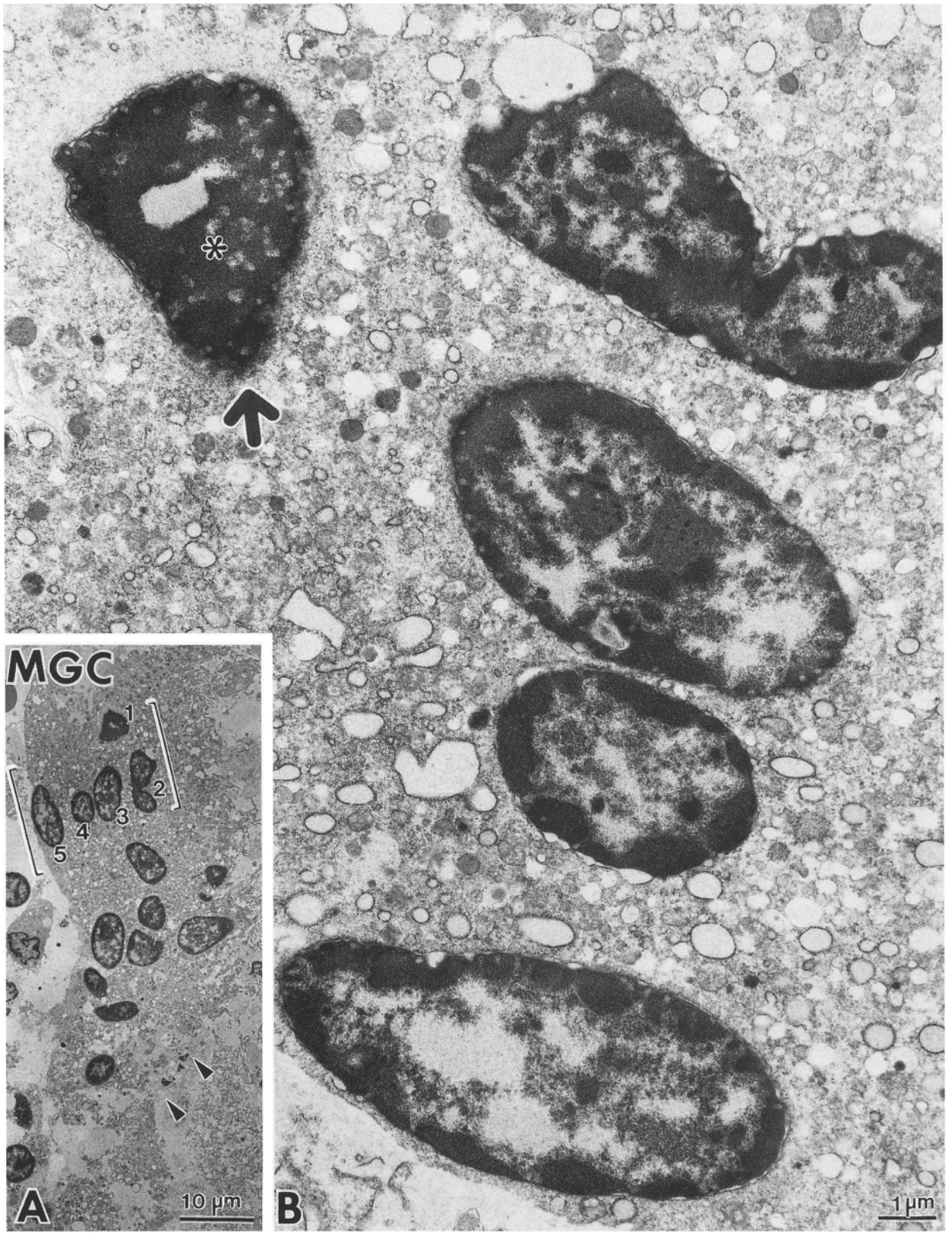

Fig. 5 **A** Electron micrograph of necrosis of multinucleated giant cell (MGC) in 55-day-old granuloma. Cell membrane is disrupted *(arrowheads),* and dissolution of cytoplasmic organelles is observed. **B** Higher-power view of framed area in **A** showing five nuclei (1–5) of multinucleated giant cell. One *(arrow)* of five nuclei displays condensed marginated chromatin *(asterisk)* in apoptosis, whereas others show masses of condensed chromatin more typical of necrotic death

Table 1 Electron microscopic findings of differential patterns of multinucleated giant cell apoptosis in foreign-body granulomas at various intervals after the implantation of collagen sponges. (Results were reported according ot the following semi-quantitative grading system: absent cells −, 1 to 5 cells +, >5 cells ++ per 100 multinucleated giant cells containing three or more nuclei in 21 to 33 Epon blocks of three animals for each time point)

Patterns of apoptotic changes within multinucleated giant cells	Days after collagen sponge implantation							
	15	25	35	55	75	95	105	115
Cells with only one apoptotic nucleus	+	+	+	+	+	+	+	+
Cells with more than two apoptotic nuclei	−	+	+	+	+	+	++	++

nuclei, and sometimes in all of the nuclei (Figs. 6, 7). Therefore, it seems appropriate to designate these cells as "apoptotic giant cells" in order to distinguish these cells from multinucleated giant cells containing only one apoptotic nucleus.

After the initial appearance of apoptotic giant cells in the granulation tissue on the 25th day, the rate of their occurrence remained low (1–5%). In the involution phase of the foreign-body granuloma during days 105–115, when multinucleated giant cells disappear from the granulation tissue, the rate of apoptotic giant cell detection increased (Table 1).

Dense masses of chromatin aggregate beneath the nuclear membrane of the apoptotic giant cells (Fig. 6). This is the earliest ultrastructurally recognizable stage of the apoptotic process. However, apoptotic giant cells showed a considerable diversity of apoptotic nuclear changes. In some nuclei, condensed chromatin was permeated throughout the cut surface or was arranged in crescentic caps. In other cases, all of the nuclei had developed both convolution of the nuclear outline and segregation of the condensed chromatin in a sharply circumscribed manner.

Early changes in the chromatin of multinucleated giant cells include nuclear chromatin condensation and margination during the early stage of apoptosis. The subsequent progress of these changes leads to extensive protrusion or blebbing of the cell surface. Protuberances on the cell surface give rise to membrane-bound apoptotic bodies of varying size and composition (Fig. 7). Some apoptotic giant cells convert into a number of small apoptotic bodies and a single, large apoptotic body. Some apoptotic bodies contain one or more nuclei, whereas in others, convolution of the cell and nuclear outlines is not always observed simultaneously or successively.

Thus, apoptotic giant cells that show condensation of the cytoplasm and production of apoptotic bodies following cell fragmentation appear to be phagocytosed by macrophages, and thereby eventually disappear from the foreign-body granuloma.

Immunoreactivity for *p53* was observed in only one nucleus among the multinucleated giant cells (Fig. 8). The staining intensity was generally weak, although positive staining within the nucleus sometimes showed accentuation around the nuclear border. The frequency of *p53*-positive detection in multinucleated giant cells was very low at each observation time after collagen sponge implantation (1–2%). In cases of apoptosis in the majority or all of the nuclei the immunoreaction of *p53* was negative.

Discussion

We induced foreign-body granulomas by implanting collagen sponges subcutaneously and observed apoptotic changes in the multinucleated giant cells of the foreign-body granulomas by electron microscopy. We observed that characteristic nuclear changes associated with apoptosis do not necessarily occur in a uniform manner in each nucleus within a multinucleated giant cell. This dissimilarity among the nuclei indicates that two types of multinucleated giant cells show different specific gene expression in apoptosis. One type presents apoptotic changes only in one of the nuclei contained within the cell; the other presents apoptotic changes occurring in a similar manner in the majority or all of the nuclei. The observation that in some cases apoptotic characteristics are manifest only in a single nucleus within the multinucleated giant cell is worthy of careful consideration.

The most convincing method of substantiating this observation is to obtain evidence that apoptotic characteristics are absent in all nuclei except the single apoptotic nucleus. Such conclusive evidence can be obtained by electron microscopic scanning of serial ultrathin sections of the multinucleated giant cell. The difficulty of this method is the prohibitive size of each cell, which reportedly often exceeds 200 μm [23, 30], making it technically impossible to prepare serial ultrathin sections of the entire cell.

Therefore, the procedure in the present study was to prepare serial ultrathin sections measuring 95 to 105 nm in thickness from the Epon block whenever we observed a multinucleated giant cell with a single apoptotic nucleus in an ultrathin section. We then compared the apoptotic nucleus and the non-apoptotic (normal) nuclei on the electron micrographs of these serial sections, and were

Fig. 6 Electron micrograph of early apoptosis of multinucleated giant cell (MGC) in a 115-day-old granuloma. Nuclei show sharply delineated masses of condensed chromatin *(arrows)* abutting nuclear envelope. Cytoplasm contains several inclusion bodies, such as a needle-like structure *(open arrow 1)*, irregular dense material *(open arrow 2)*, crystalloid material *(open arrow 3)* and amorphous material *(open arrow 4)*. Note that cell surface is characterized by complex microvillous profile with collapsed irregular morphology ▶

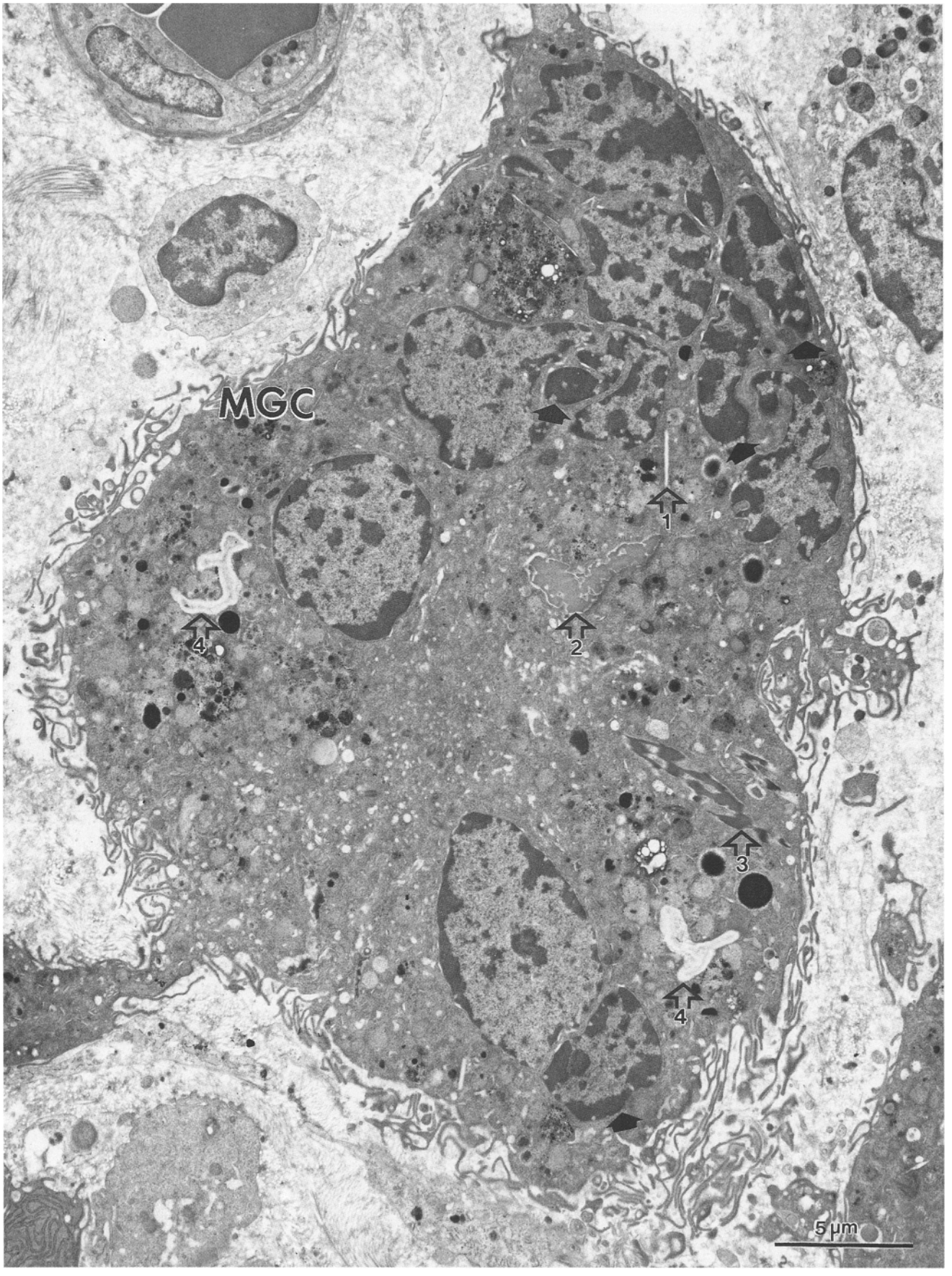

Fig. 6

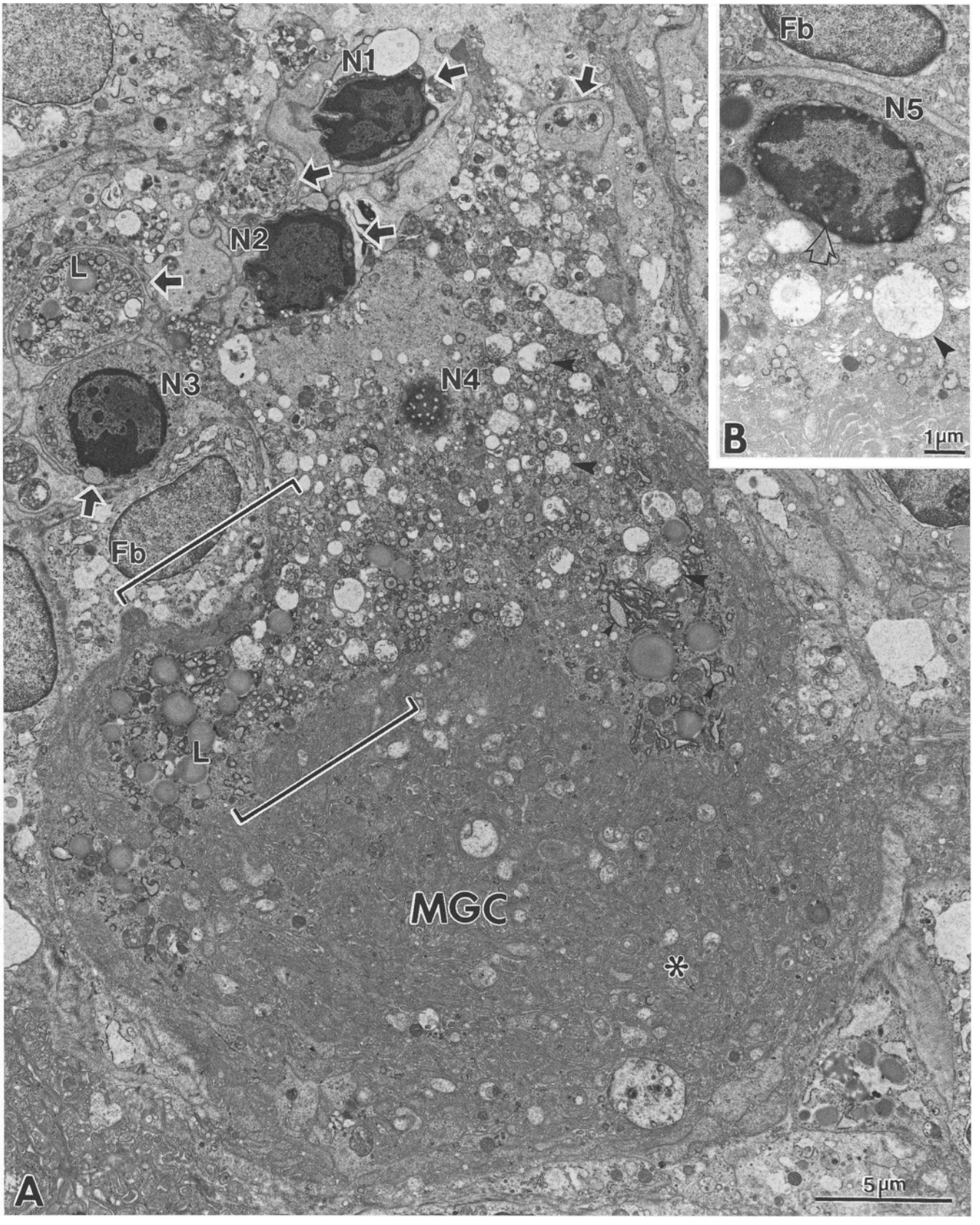

Fig. 7

◀ **Fig. 7** **A** Electron micrograph of apoptosis of multinucleated giant cell (MGC) in a 115-day-old granuloma. MGC undergoing apoptosis has led to formation of many small apoptotic bodies *(arrows)*. Whereas some of these bodies contain nuclei *(N1,N2 and N3)* with characteristically condensed and marginated chromatin, others consist of cytoplasm only, with lipid droplets (*L*). This apoptotic MGC with disappearance of microvilli and compact aggregation of intracytoplasmic labyrinth *(asterisk)* shows overall condensation, crowding of cytoplasmic organelles with gross swelling of mitochondria *(large arrowheads)* and distended cisternae of rough endoplasmic reticulum *(small arrowheads)*, and contains part of a nucleus (*N4*) with apoptotic features in this plane. **B** Electron micrograph of serial section of framed area in **A** shows enlarged view of affected cell with a cytoplasm that also contains the 5th apoptotic nucleus (*N5*) featuring a prominent nucleolus *(open arrow)*. A fibroblast (*Fb*) comes into contact with MGC in both **A** and **B**

able to confirm that the other nuclei in the same field of view did not present any apoptotic characteristics. Thus, we were able to confirm that such multinucleated giant cells contained only one nucleus undergoing apoptotic changes. Multinucleated giant cells containing a single apoptotic nucleus manifested in the foreign-body granulomas at a nearly constant rate throughout the experimental period.

Ultrastructural and cell-kinetic studies [19] as well as the addition of various cytokines [1, 7, 8, 15, 20, 36] have demonstrated conclusively that macrophage fusion is the most important process in multinucleated giant cell formation. However, in theory, such multinucleated giant cells could also be generated by cell fusion and/or by nuclear division without consecutive cytoplasmic separation [5]. Synthesis of DNA in the component neclei of multinucleated giant cells is usually synchronous, but in some cases is asynchronous [19, 25]. The cause of asynchronous DNA synthesis among the nuclei of a multinucleated giant cell is unclear. Thus, some nuclei contained in a multinucleated giant cell are likely to exhibit chromosomal abnormalities [19, 21], probably arising from the fusion of resident macrophages containing nuclei with chromosomal damage [27]. Either synchronous or asynchronous synthesis of DNA occurs in these multinucleated giant cells. The incidence of the discrepancy of DNA synthesis between the nuclei may be subject to a specific ratio. Thus, such chromosomally abnormal nuclei are occasionally present in nuclear division.

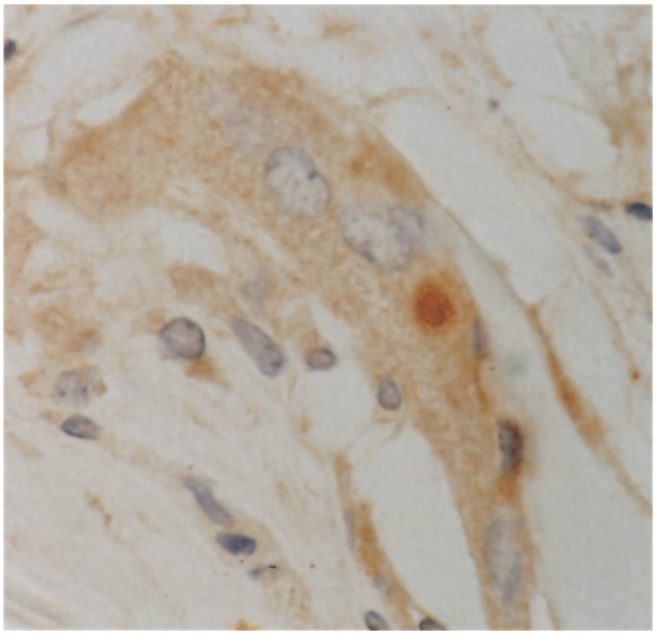

Fig. 8 Immunohistochemical staining for *p53* protein shows faint reactivity in only one nucleus of multinucleated giant cell, whereas all other nuclei in this giant cell show no *p53* immunoreactivity. Immunoperoxidase stain; original magnification ×930

The *p53* gene, a tumour suppressor, has been demonstrated to induce apoptosis in cells in response to DNA damage, and thereby plays an important role in eliminating damaged cells [16, 40]. Recently, Wiethege et al. [37] reported that excessive expression of the *p53* tumour suppressor gene was detected in one or two nuclei within the investigated multinucleated giant cells, representing only 2–3% of the multinucleated giant cells derived from rat alveolar macrophages in vitro. In the present study, using the avidin-biotin complex immunoperoxidase technique on formalin-fixed, paraffin-embedded tissue sections, we detected *p53* protein accumulation in only one nucleus among the multinucleated giant cells. Moreover, we observed ultrastructurally single nuclei with apoptotic features in the multinucleated giant cells. In such cases, apoptosis was probably initiated in a macrophage prior to its fusion to the polykaryon. Such results appear to suggest that multinucleated giant cells are selectively eliminated from foreign-body granulomas when one nucleus shows chromosomal abnormality resulting from the induction of apoptosis associated with the *p53*-dependent response to DNA damage.

The morphological changes of apoptosis occur in three phases [2, 12, 14, 35, 39]. In the first phase, reduction in nuclear size and condensation of chromatin into toroids or crescentic caps at the nuclear periphery is observed. In the second phase (which sometimes occurs concurrently with the first), blebbing at the cell surface and crenation of the nuclear outline is observed. However, multinucleated giant cells with a single apoptotic nucleus show no convolution of cell and nuclear outlines suggesting blebbing on the cell surface or of membrane-bound apoptotic bodies despite characteristic chromatin condensation. Moreover, in the contracted cytoplasm, degenerative changes such as prominent vacuolization and the appearance of condensed mitochondria were occasionally observed. Although the non-apoptotic nuclei retain their morphologically normal configuration in such cells, the fate of the cell itself is necrosis resulting from cytoplasmic degeneration and rupture of the plasma membrane. As this necrotic process develops, if these multinucleated giant cell are not rapidly phagocytosed by macrophages, an acute inflammatory response may occur as the monocytes and neutrophil leukocytes emigrate from blood vessels to the involved area in order to contribute to multinucleated giant cell formation.

However, the apoptotic giant cells in which early chromatin changes were observed the majority or all of the nuclei usually presented with convolution of the cell and/or nuclear outlines. In the next phase, extensive budding occurs with separation of protuberances that develop on the cell surface, resulting in the production of membrane-bound apoptotic bodies accompanied by the disappearance of complex microvilli. Ultimately, the apoptotic bodies are phagocytosed by macrophages and disappear from the granulation tissue of the foreign-body granuloma. This sequence of ultrastructural changes in apoptotic giant cells has been observed in many normal organs and in tumours [13, 35].

Several reports [19, 26] have indicated that multinucleated giant cells in foreign-body granulomas have short lifespans, estimated at approximately 7 days. Thus, the granulation tissue of foreign-body granulomas may be the site of multinucleated giant cell formation, resulting from the fusion of macrophages when not all of the nuclei are at the same stage of DNA synthesis [19, 26]. The most probable course of these multinucleated giant cells is that after surviving for approximately 1 week, they age and reach cell death. Neutrophilic leukocytes derived from peripheral blood tend to senescence when they are released from the bone marrow into the peripheral blood. Furthermore, it has been demonstrated that neutrophils [31], eosinophils aged in culture [33], and senescent megakaryocytes in bone marrow [29] undergo apoptosis. Thus, apoptosis is likely to be a mechanism of cell death for senescence of the multinucleated giant cell as a community unit, because in the present study, all of the nuclei in the apoptotic giant cells showed synchronous chromatin condensation. This phenomenon is also attributed to the fact that the earliest unequivocal morphological evidence of the onset of apoptosis in syncytia induced during in vitro HIV infection is detected simultaneously in all of the nuclei [17].

The present study has demonstrated that apoptosis of multinucleated giant cells in foreign-body granulomas differs in some respects from the typical apoptotic process. Apoptosis of multinucleated giant cells involves a discrepancy in the apoptotic changes occurring in the individual nuclei. We have concluded that two types of multinucleated giant cells present the morphological characteristics of apoptosis. One type of multinucleated giant cell contains only one apoptotic nucleus, whereas all of the other nuclei possess normal morphology. In the other type of multinucleated giant cell, the majority or all of the nuclei exhibit apoptotic changes simultaneously. Our results suggest that in multinucleated giant cells showing apoptosis within only one nucleus, the process might be triggered by overexpression of *p53* due to DNA abnormalities confined to this nucleus. In contrast apoptosis occurring simultaneously in the majority or all of the nuclei is most probably due to cell death caused by senescence.

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