

Possible mechanism for disposal of degenerative cardiomyocytes in human failing hearts: phagocytosis by a neighbour

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Abstract

The index case was a 51-year-old woman suffering from doxorubicin cardiomyopathy. In her endomyocardial biopsy specimen, we observed under electron microscopy six scenes in which degenerative cardiomyocytes were engulfed by neighbouring cardiomyocytes. The enclosed cardiomyocytes appeared more degenerative than the enclosing ones in every pair: the myofibrils were more severely damaged. At more degenerative stages, some desmosomes of the intercalated discs on the enclosed cardiomyocyte had disappeared. The membranes between the cardiomyocytes were occasionally disrupted, and there appeared to be sharing of cellular contents between the cells. One pair of such a phagocytosis-like figure was observed in one case with 5-fluorouracil cardiomyopathy (a 68-year-old man) among eight other chemotherapy-induced cardiomyopathies but none among 30 non-drug-induced dilated cardiomyopathies. The findings suggest a mechanism for disposal of degenerative cardiomyocytes in human failing hearts: phagocytosis by a neighbour, although alternative interpretations remain (e.g. giant autophagic vacuoles or two cardiomyocytes with degenerative intercalated discs).

Keywords Cardiomyopathy; Chemotherapy; Endomyocardial biopsy; Heart failure; Phagocytosis; Ultrastructure

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Introduction

The causes of heart failure progression are a reduction in the number of cardiomyocytes (quantitative alteration), deterioration of the cardiomyocytes (qualitative alteration), or both.^{1,2} In addition, changes in the extracellular matrix are also important indicators.³ Myocardial fibrosis, especially the replacement type, indicates past and/or ongoing dropout of cardiomyocytes. A key question yet to be answered is how are degenerated/dead cardiomyocytes disposed of within human failing hearts? During electron microscopic examination of an endomyocardial biopsy specimen from a patient with heart failure due to doxorubicin-induced cardiomyopathy (Patient 1), we made ultrastructural observations that may shed light on the answer to that question; we observed

severely degenerative cardiomyocytes engulfed by their neighbouring cardiomyocytes. Doxorubicin toxicity to cardiomyocytes is very specific in the molecular mechanisms and morphological aspects.^{4–6} We further investigated endomyocardial biopsies from other cases with heart failure and found another similar case (Patient 2). Here, we describe those findings, which appear to indicate a mechanism for disposal of degenerative cardiomyocytes in failing hearts.

Case report

Patient 1

Patient 1 (the index case) was a 51-year-old woman who had been diagnosed with uterine sarcoma with metastasis to the

liver and mesentery 3 years earlier. She was treated with doxorubicin and cisplatin, with the total dose of the former reaching 579 mg/m² of body surface. Eighteen months later, the patient complained of dyspnoea. Echocardiography revealed diffuse hypokinesis of the heart with a left ventricular ejection fraction of 33%, which suggested doxorubicin-induced cardiomyopathy. The coronary arteries were intact, and a left ventricular endomyocardial biopsy was performed to confirm the diagnosis. Histological findings were compatible with doxorubicin-induced cardiomyopathy—that is, many cardiomyocytes exhibited degenerative changes under light microscopy, including vacuolization and severe rarefaction of the myofibrils (*Figure 1*). The cardiomyocytes were hypertrophied, with a mean size of 21 ± 3.4 (mean \pm standard deviation) μ m. Fibrosis, including the interstitial, replacement, and subendocardial types, was also noted and occupied 22% of the area of the specimens (%fibrosis). On the other hand, there was no obvious inflammatory cell infiltration.

Under electron microscopy, many cardiomyocytes exhibited only scanty myofibrils remaining at the periphery (the so-called Adria cells) (*Figure 1C*). Moreover, several pairs of cardiomyocytes consisting of an enclosed cell

and an enclosing cell were detected (*Figures 2–5*). The cardiomyocytes in each pair were originally adjacent to one another, as intercalated discs remained between them, although one cell now nearly surrounded the other. In addition, the enclosed cardiomyocyte appeared more degenerative than the enclosing one in each pair—that is, the myofibrils were more severely damaged (*Figure 2*) or scarce (*Figure 3*). On the other hand, the cytoplasm of the enclosed cardiomyocytes was not shrunken, but was instead oedematous, and unlike with apoptosis, the nuclei did not contain condensed chromatin. The enclosed cardiomyocytes were at various stages of degradation. At more degenerative stages, the enclosed cardiomyocyte was nearly indistinguishable from a phagocytized cell or giant autophagic vacuole within the enclosing cardiomyocyte (*Figure 4*). The intercalated discs were dilated, and some desmosomes at the periphery of the enclosed cardiomyocyte had disappeared or become unilateral (*Figures 4 and 5*). The membranes between the cardiomyocytes were occasionally disrupted, and there appeared to be sharing of cellular contents between the cells (*Figure 5*). Totally, six phagocytosis-like figures were found in the specimen.

Figure 1 Light and electron micrographs of the endomyocardial biopsy from Patient 1. (A1 and A2) Cardiomyocytes are separated by a wide interstitium and exhibit severe degenerative changes, including vacuolization and rarefaction of the myofibrils. Haematoxylin and eosin-stained preparation. Panel (A2) is an expanded view of the area within the square in panel (A1). (B) Masson's trichrome staining shows interstitial, replacement, and endocardial fibrosis (blue area). (C) Electron microscopy revealed cardiomyocytes with scanty myofibrils remaining at the periphery (the so-called Adria cells). Lp, lipofuscin granules; Mf, myofibrils; N, nucleus. Scale bars: 200 μ m in panel (A1), 50 μ m in panels (A2) and (B), and 1 μ m in panel (C).

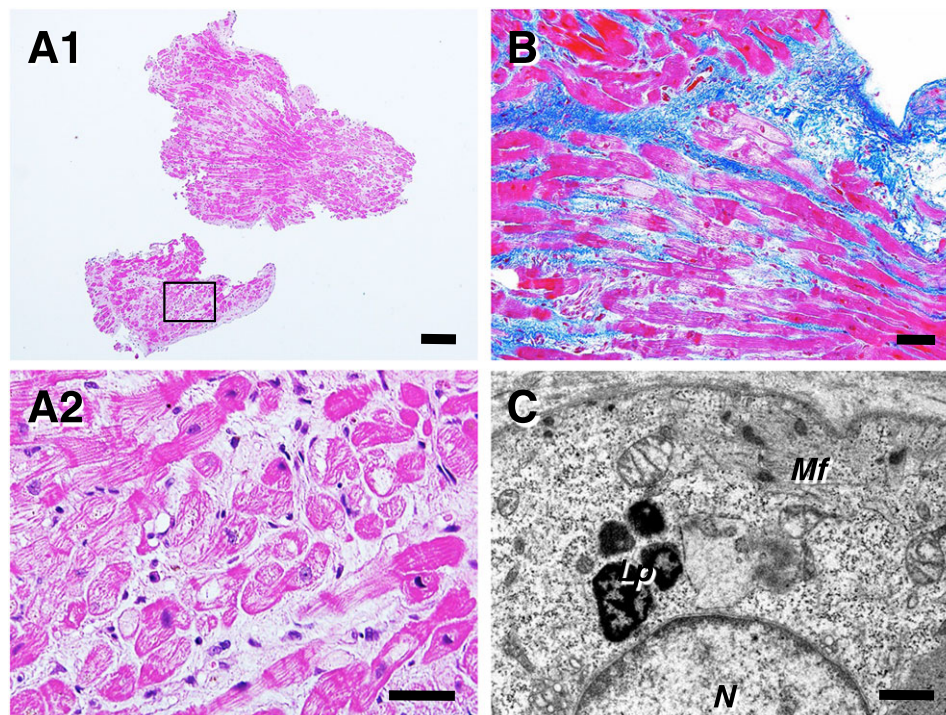
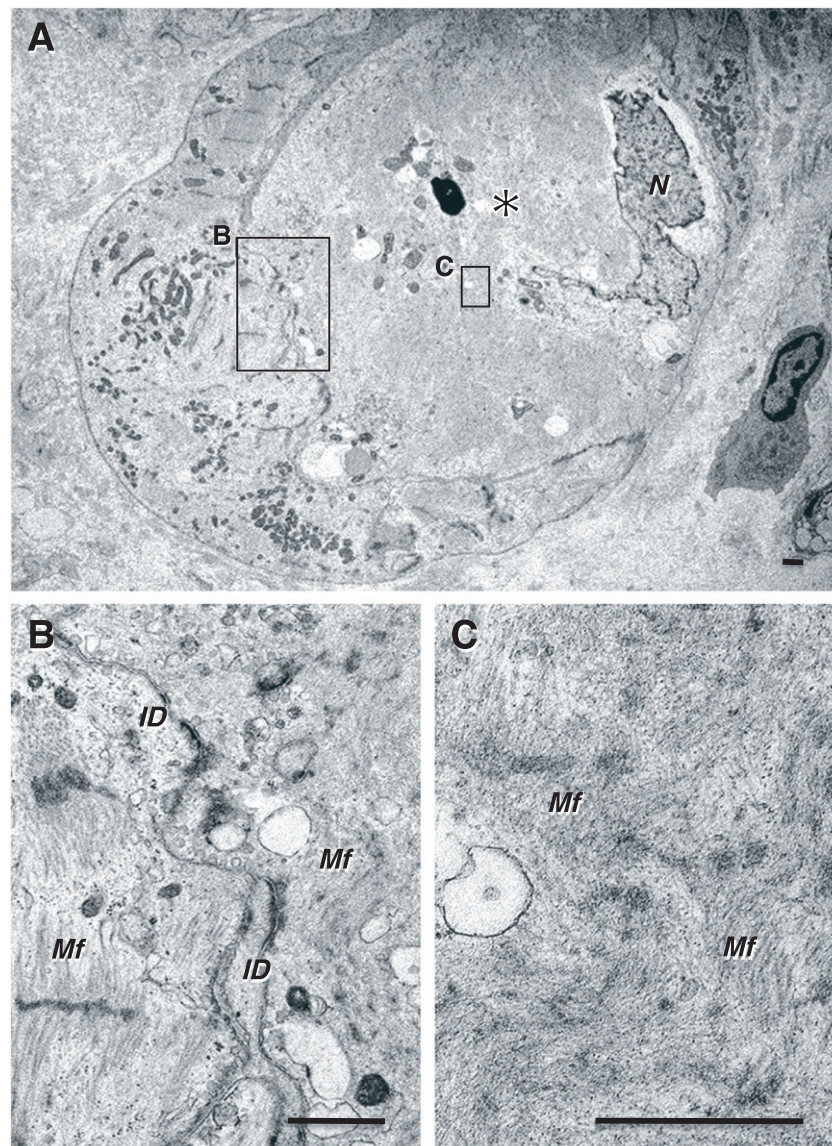


Figure 2 Electron micrographs obtained from Patient 1 showing a pair of cardiomyocytes consisting of an enclosed cell (asterisk) and an enclosing cell. There is an intercalated disc between them. The enclosed cardiomyocyte appears more degenerative than the enclosing one, with severely disintegrated myofibrils and Z bands broken to pieces. Panels (B) and (C) are expanded views of the areas within the squares in panel (A). ID, intercalated disc; Mf, myofibrils; N, nucleus of the enclosed cardiomyocyte. Scale bars: 1 μ m.

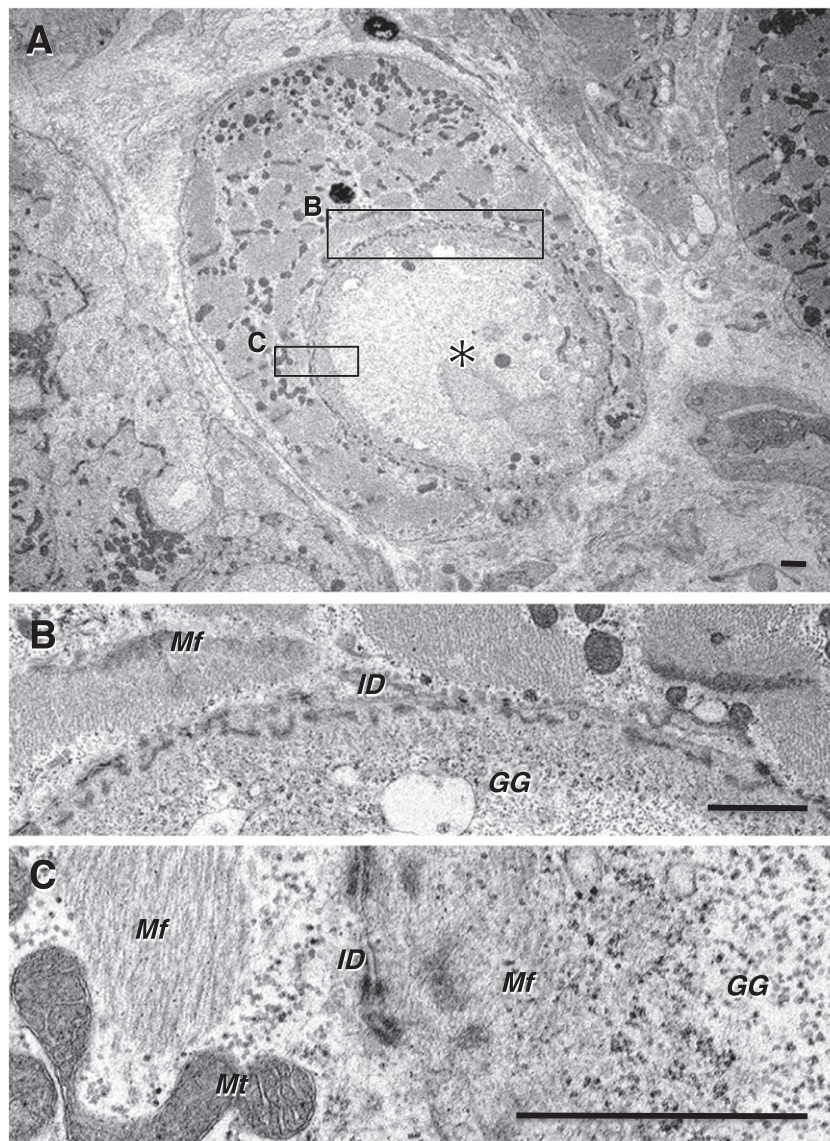


Patient 2

As controls for the index case Patient 1, we examined eight other patients diagnosed as chemotherapy-induced cardiomyopathy (Table 1), while a second control group consisted of 30 patients with non-drug-induced dilated cardiomyopathy (Table 1). The eight patients with chemotherapy-induced cardiomyopathy had been treated with anthracyclines such as doxorubicin ($n = 6$) or with 5-fluorouracil ($n = 1$) or paclitaxel ($n = 1$). The left ventricular ejection fraction was reduced, and

histological examination of the biopsies revealed cardiomyocyte hypertrophy, substantial interstitial fibrosis, and rare inflammatory cell infiltration. In the group consisting of patients with non-drug-induced dilated cardiomyopathy, the left ventricular ejection fraction was reduced to the similar degree as in the chemotherapy-induced cardiomyopathy group. Histological examination of the biopsies revealed cardiomyocyte hypertrophy, substantial fibrosis, and rare inflammatory cell infiltration (Table 1). No significant difference was noted in the size of the cardiomyocytes or amount of fibrosis

Figure 3 Electron micrographs from Patient 1 showing a pair of cardiomyocytes consisting of an enclosed cell (asterisk) and an enclosing cell. There is an intercalated disc between them. Apart from the degenerated myofibrils at the periphery, the cytoplasm of the enclosed cardiomyocyte contains almost exclusively glycogen granules. Panels (B) and (C) are expanded views of the areas within the squares in panel (A). GG, glycogen granules; ID, intercalated disc; Mf, myofibrils; Mt, mitochondria. Scale bars: 1 μ m.

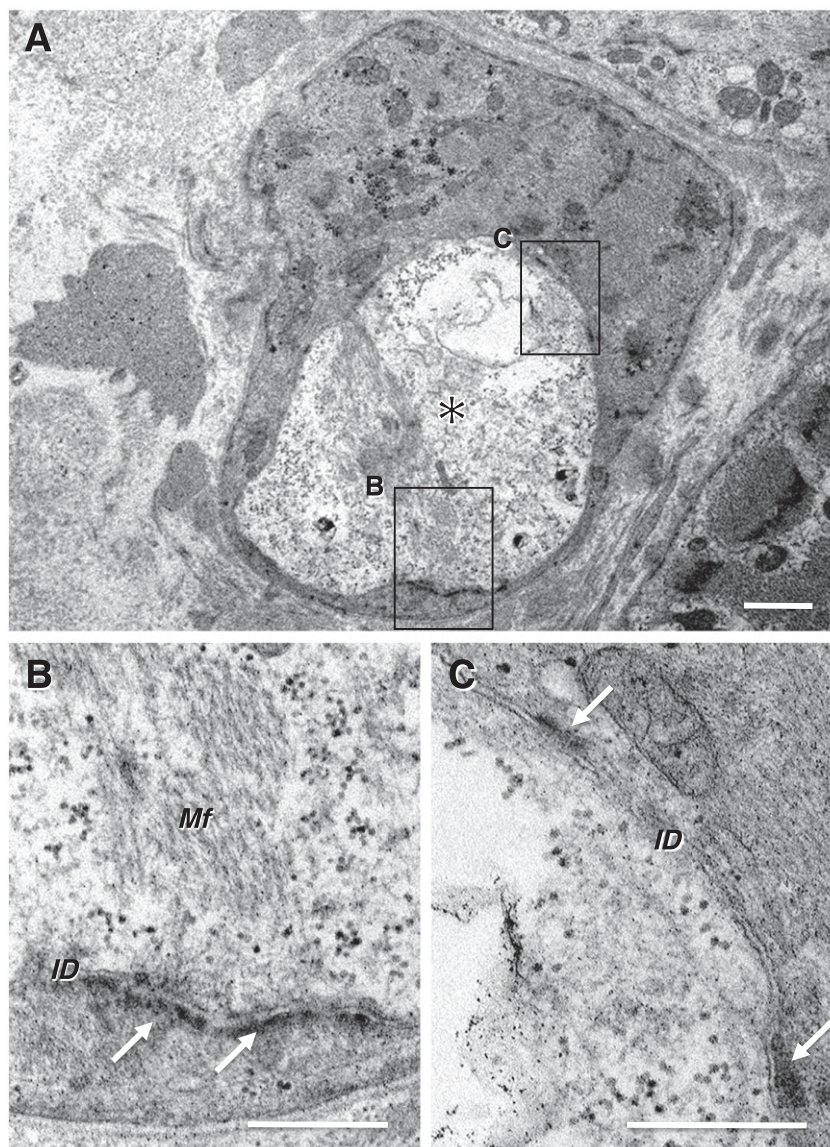


between the two groups, although inflammatory cell infiltration was more active in dilated than chemotherapy-induced cardiomyopathy.

Under electron microscopy, we detected one phagocytosis-like figure in one case of chemotherapy-induced cardiomyopathy (Patient 2), but we found none in the other drug-induced cardiomyopathies or in the dilated cardiomyopathies. Patient 2 was a 68-year-old man who had been diagnosed with gastric cancer and underwent a total gastrectomy 11 years earlier. Heart failure developed after

treatment with 5-fluorouracil for 8 years. Echocardiography revealed diffuse hypokinesis of the heart with a left ventricular ejection fraction of 26%. Histological examination of the left ventricular endomyocardial biopsy revealed the cardiomyocytes to be hypertrophied with a mean size of $33 \pm 6.6 \mu$ m, as well as interstitial, replacement, and subendocardial type fibrosis, which occupied 17% of the area of the specimens. No apparent Adria cells were seen, and inflammatory cells, including macrophages, were rare. Electron microscopic examination revealed a nearly intact

Figure 4 Electron micrographs from Patient 1 showing a pair of cardiomyocytes consisting of an enclosed cell (asterisk) and an enclosing cell at a degenerative stage. The enclosed cell is nearly indistinguishable from a phagocytized cell or giant autophagic vacuole within the enclosing cardiomyocyte. The intercalated disc is dilated to look like a simple double membrane, and desmosomes at the periphery of the enclosed cardiomyocyte have disappeared such that the structures are now unilateral (white arrows). Panels (B) and (C) are expanded views of the areas within the squares in panel (A). ID, intercalated disc; Mf, myofibrils. Scale bars: 1 μ m.



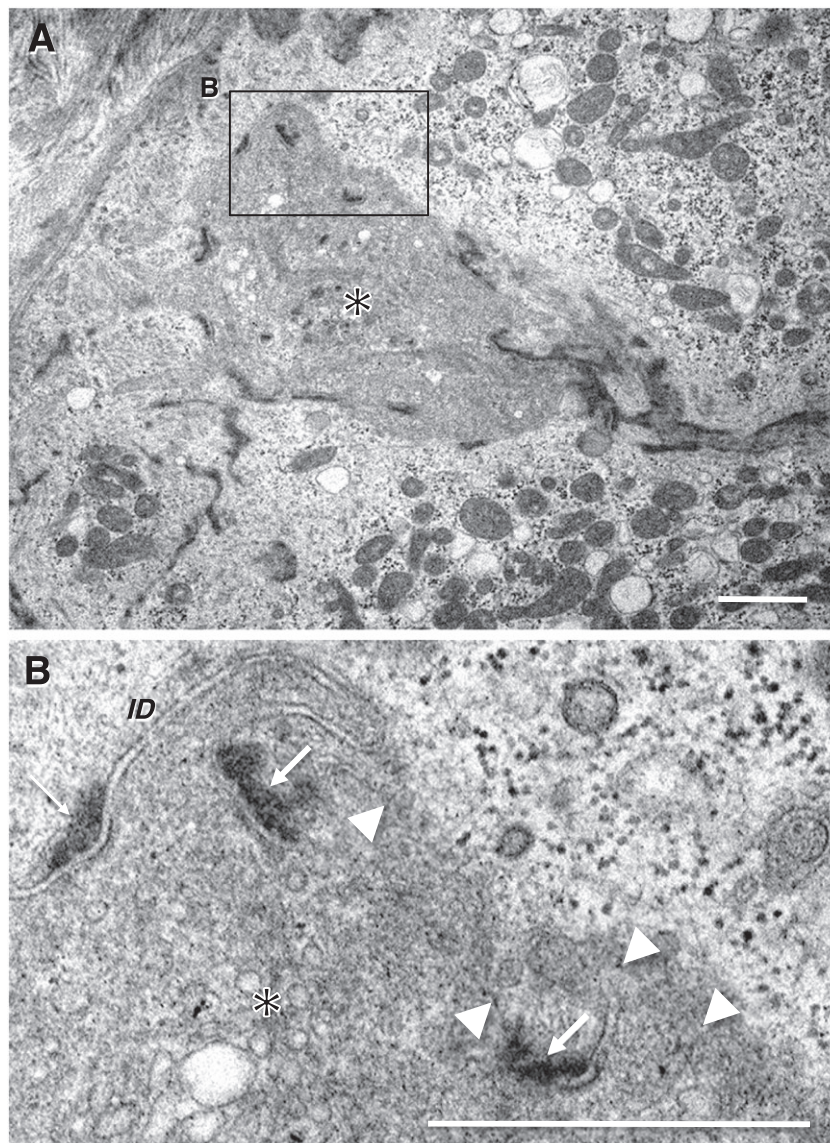
cardiomyocyte in close contact with a severely degenerated one such that the former appeared to enclose the latter (Figure 6). The degenerated cell had scanty myofibrils and structures reminiscent of Z bands. The intercalated disc between the cardiomyocytes possessed incomplete or unilateral desmosomes in one part (Figure 6B) and completely lacked of desmosomes in the other part (Figure 6C). Thus, cardiomyocyte phagocytosis by a neighbouring cardiomyocyte was found significantly more frequently in patients with chemotherapy-induced

cardiomyopathy than in those with dilated cardiomyopathy (2 out of 9 vs. 0 out of 30, $P = 0.0486$).

Discussion

We report here electron microscopic findings of degenerative cardiomyocytes enclosed by neighbouring cardiomyocytes in endomyocardial biopsies from two patients with heart failure. Detection of such figures implies a mechanism for

Figure 5 Electron micrographs from Patient 1 showing a pair of cardiomyocytes consisting of an enclosed cell (asterisk) and an enclosing cell at a degenerative stage. Many desmosomes on the intercalated disc have become unilateral (white arrows). The border between the cardiomyocytes is sometimes disrupted (white arrowheads), and the cellular contents appear to be shared between the two cells. Panel (B) is an expanded view of the area within the square in panel (A). ID, intercalated disc. Scale bars: 1 μ m.



disposal of degenerative/dead cardiomyocytes in failing hearts. *Figure 7* summarizes the hypothesized disposal mechanism, 'phagocytosis by neighbours', deduced from the present cases. A severely degenerative/dead cardiomyocyte is enclosed by a neighbouring one. The intercalated disc then degenerates and becomes dilated, while desmosomes at the periphery of the enclosed cardiomyocyte disappear such that they become unilateral. The intercalated disc ultimately fades away, and the cellular contents are shared between the cells. Although we detected only two apparent instances of this process in this

study, we believe these findings are novel and biologically significant. Having that said, we recognize that our electron microscopic images could be alternatively interpreted other than phagocytosis. For example, they might correspond to different stages of the autophagic process in which the giant vacuoles are enclosing degenerating cellular components or they might be images of two adjacent degenerating cardiomyocytes with alteration in the intercalated discs. Single point observation is difficult to resolve the issue. To test our hypothesis, a detailed chronological analysis would be necessary using animal

Table 1 Profile of patients and their endomyocardial biopsies

Patient no.	Clinical data				Pathological data		
	Age (y)	Gender (M/F)	DOX amount (mg/m ²)	LVEF (%)	Myocyte size (μm)	Fibrosis (%)	Inflammatory cells (/HPF)
Chemotherapy-induced cardiomyopathy							
1 (Patient 1)	51	F	579	33	21	22	0
2	44	F	412	46	28	18	0.4
3	64	F	284	22	26	5	0.2
4 (Patient 2)	68	M	NA (5-FU)	26	33	17	0
5	75	M	405	42	24	4	0
6	42	F	NA (carboplatin)	20	30	12	0
7	73	F	480	26	26	25	0.2
8	64	F	306	37	16	9	0.4
9	55	M	564	37	31	38	0.4
Mean ± SD	60 ± 12	3/6	433 ± 116 (n = 7)	32 ± 9.2	26 ± 5.3	17 ± 11	0.2 ± 0.2
Non-drug-induced dilated cardiomyopathy							
1 to 30	35 to 78		NA	18 to 43	14 to 40	3 to 30	0 to 2.0
Mean ± SD	58 ± 13	19/11	0	33 ± 7.8	26 ± 5.9	16 ± 7.3	0.9 ± 0.7*

DOX, anthracyclines including doxorubicin; LVEF, left ventricular ejection fraction; 5-FU, 5-fluorouracil.

**P* < 0.05 vs. chemotherapy-induced cardiomyopathy.

models of heart failure—for example, a chronic doxorubicin cardiomyopathy model, where immunoelectron microscopic studies should be performed targeting for the cell-to-cell junction-related and autophagy-related proteins.

Patient 1 suffered from heart failure due to doxorubicin-induced cardiomyopathy. With doxorubicin-induced cardiomyopathy, cardiomyocytes reportedly die via apoptosis or necrosis.^{6–8} Irrespective of the cause of death, the dying/dead cells must be eliminated from the myocardium, a task usually performed by macrophages. In general, however, macrophages are not frequently present within the myocardium in cases of doxorubicin-induced cardiomyopathy.⁵ The disposal process glimpsed here would enable efficient disposal of unnecessary cells from the myocardium despite the lack of phagocytosis by macrophages.

Patient 2 had a clinical diagnosis of 5-fluorouracil-induced cardiomyopathy. Although doxorubicin and 5-fluorouracil both induce cardiomyopathy, the two ailments differ substantially from one another. Whereas doxorubicin directly damages cardiomyocytes to bring about severe rarefaction of the myofibrils, 5-fluorouracil most frequently causes coronary vasospasm that induces ischaemic injury.^{9,10} 5-Fluorouracil can also induce myocarditis leading to non-ischaemic dilated cardiomyopathy, but that occurs less frequently.^{11,12} Like that of Patient 1, the heart of Patient 2 exhibited dilated cardiomyopathy with rare inflammatory cells.

Although a significantly higher incidence of the phagocytosis-like figures was noted in patients with chemotherapy-induced cardiomyopathy than in those with dilated cardiomyopathy, the disease specificity remains undermined because of the small number of patients studied. In addition, the causative agents differed in the two cases. Consequently, whether the hypothesized disposal mechanism is specific to the present cases or to chemotherapy-induced cardiomyopathy, or is more universal in hearts

failing for various reasons, remains unclear. Our sample is too small to draw a conclusion. However, our findings provide a rationale for a large-scale, prospective or retrospective, ultrastructural analysis of endomyocardial biopsies from patients with chemotherapy-induced and other cardiomyopathies.

Phagocytic ability is not commonly associated with cardiomyocytes. However, we previously reported that cultured cardiomyocytes actively phagocytized apoptotic cells,¹³ indicating cardiomyocytes may acquire phagocytotic functionality under some circumstances. Apoptotic cells present 'eat-me' signals on their surface to facilitate phagocytosis by neighbouring cells.¹⁴ However, no apoptotic cardiomyocytes were seen in the present electron microscopic examination. Nonetheless, we would speculate that the engulfed degenerative cells presented a similar signal.

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Conflict of interest

None declared.

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None.

Figure 6 Electron micrographs obtained from Patient 2 showing a pair of cardiomyocytes closely contacting each other. The nearly intact cell looks as if it is enclosing a severely degenerated cell (asterisk) containing scanty myofibrils and structures reminiscent of Z bands. The intercalated disc between the two cardiomyocytes possesses incomplete or unilateral desmosomes in some areas (panel (B)), and it completely lacks of desmosomes in other areas (panel (C)). Panels (B) and (C) are expanded views of the areas within the squares in panel (A). GG, glycogen granules; ID, intercalated disc; Lp, lipofuscin; Mf, myofibrils; Mt, mitochondria; N, nucleus of the enclosing cardiomyocyte. Scale bars: 1 μ m.

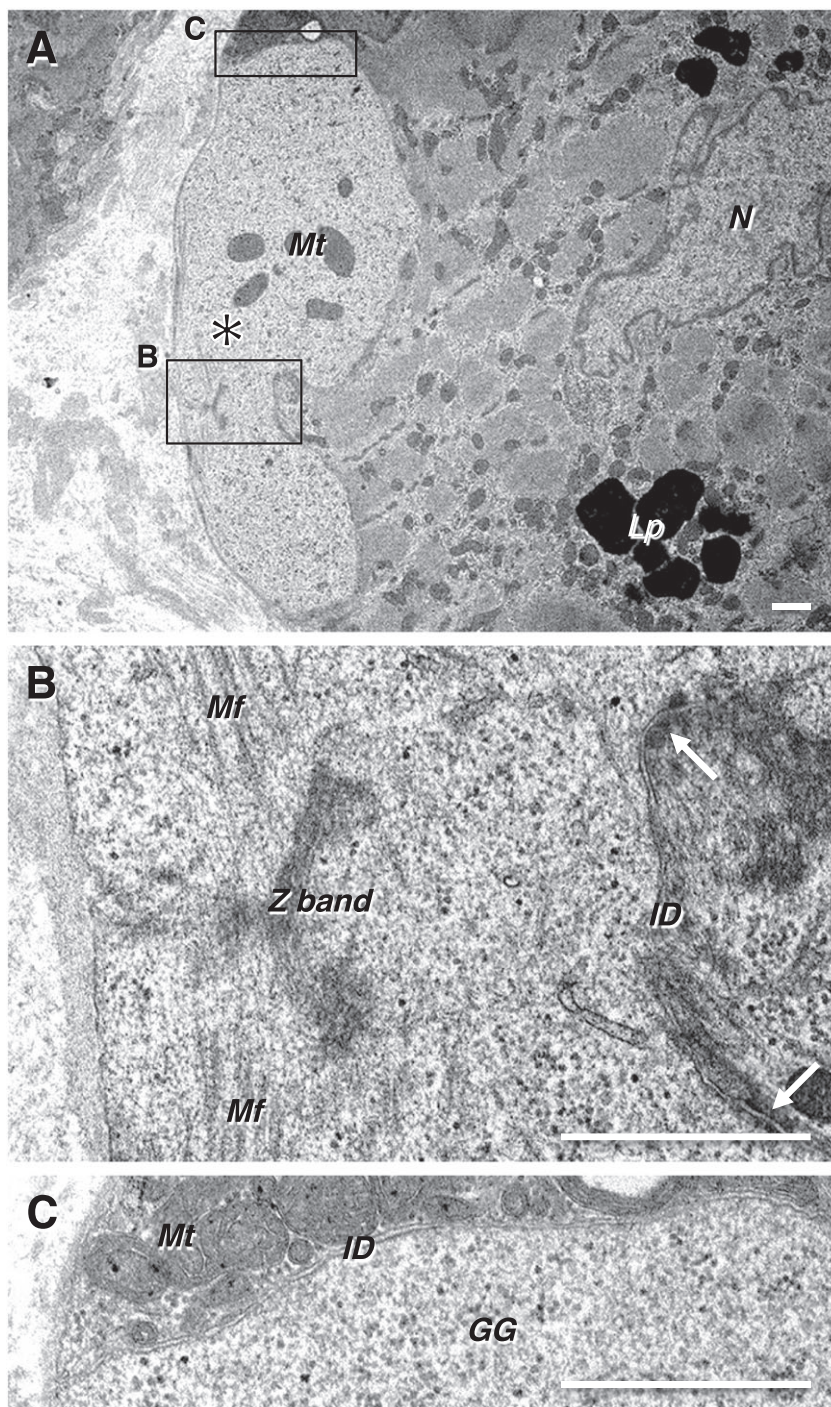
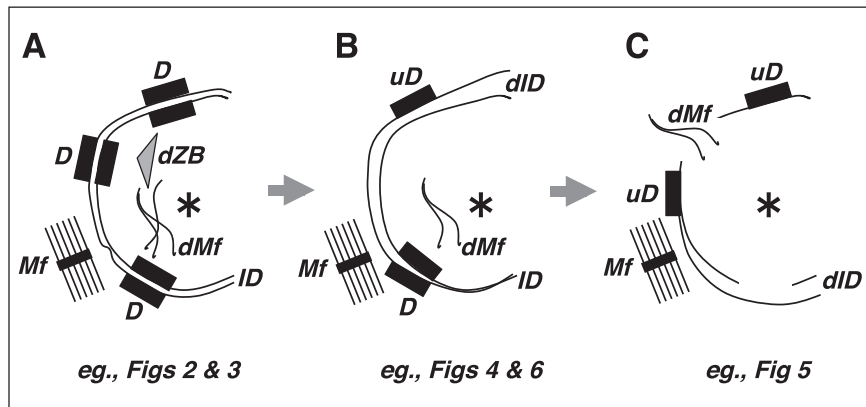


Figure 7 Hypothesized mechanism for disposal of degenerative/dead cardiomyocytes in heart failure deduced from the present cases. (A) A severely degenerative/dead cardiomyocyte (asterisks) is enclosed by a neighbouring one (left side). (B) The intercalated disc then degenerates and becomes dilated (dID), while desmosomes at the periphery of the enclosed cardiomyocyte disappear such that they become unilateral (uD) (middle panels). (C) The intercalated disc ultimately fades away, and the cellular contents are shared between the two cells (right panels). D, desmosome; dID, degenerated intercalated disc; dMf, degenerated myofibrils; dZB, degenerated Z band; ID, intercalated disc; Mf, myofibrils; uD, unilateral desmosome.



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