参考文献

1) Report of the 1995 World Health Organization/ International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. Circulation 93:841-842,1996.

2) 池田義彦、河合祥雄、岡田了三、山口　洋：型不明型心筋症の多様性とその移行性。J Cardiol 31:91-98、1998.

3) 関口守衛、佐藤千美、厚地良彦：特発性心筋症とその類縁疾患－その診断と分類に関する試案－。厚生省特発性心筋症調査研究班昭和 49 年度研究報告集 1975；110-127.

4) 村尾覚、小山直、一安弘文、林輝美、小関克彦、諸岡成徳、坂本二哉、神坂和明、西山敬介：アルコール性心筋症の左室構造ならびに組織学的所見。厚生省特発性心筋症調査研究班昭和 50 年度研究報告集 1976；67-73.

5) 岡田了三、関口守衛、河村慧四郎、河合忠一、戸嶋裕徳、仁村泰治：心筋生検によるウィルス性ないし特発性心筋炎の病理診断基準－病理分科会共同研究－。厚生省特発性心筋症調査研究班昭和 63 年度研究報告集 1989；181-183.

6) 戸嶋裕徳、和泉徹、河合祥雄、河村慧四郎、楠川禮造、関口守衛、松森昭、 矢崎義雄、由谷親夫：日本循環器学会学術委員会報告　診断基準：慢性心筋炎の診断基準に関する研究(1991-1993 年度報告)。Jpn Circ J 1994;58(SuppleⅢ):1104-1110.

7) Yutani C, Imakita M, Ishibashi-Ueda H, Hatanaka K, Nagata S, Sakakibara H, Nimura Y: Three autopsy cases of progression to left ventricular dilatation in patients with hypertrophic cardiomyopathy. Am Heart J 1985;109:545-553.

8) 河村慧四郎，野田信一路，中山康，上村春男，山元章示，鷹津正：心筋生検における心筋線維の錯綜配列。厚生省特発性心筋症調査研究班昭和 55 年度研究報告集 1981；63-71.

9) 河合祥雄，河野浩章，貞刈鴨代，久岡英彦，岡田了三：心筋錯綜配列を伴う拡張型心筋症と肥大型心筋症における細胞浸潤－病理分科会共同研究－．厚生省特発性心筋症調査研究班平成 2 年度研究報告集 1991；119-122.

10) Franks AJ: Cardiac pathology in chronic myopathy, with particular reference to dystrophic myotonica. Pathology 1978;125:213-217

11) 相楽達男；筋緊張性ジストロフィーの心病変に関する臨床的研究。埼玉医科大学雑誌 1992；19(3)：329-341.

12) Tezuka F, Sato I, Mori H, Nomura M, Hort W: Quantitative analysis of fiber disarray developing in papillary muscle unloaded after mitral valve replacement. Acta pathol Japonica 1989;39:779-785.

13) 宝田明、錬寛之、南地克美、伊藤成規、森孝夫、林孝俊、藤本俊典、吉田浩、横田慶之、福崎恒：潜在性拡張型心筋症の臨床的検討。J Cardiol 1992；22：61-71.

14) Seiler C, Jenni R, Vassalli G, Turina M, Hess OM: Left ventricular chamber dilatation in hypertrophic cardiomyopathy: related variables and prognosis in patients with medical and surgical therapy. Br Heart J 74:508-516, 1995.

15) Ozawa T, Tanaka M, Sugiyama S, Hattori K, Ito T, Ohno K, Takahashi A, Sato W, Takada G, Mayumi B, et al.: Multiple mitochondrial DNA deletions exist in cardiomyocytes of patients with hypertrophic or dilated cardiomyopathy. Biochem Biophys Res Commun. 170: 830-836, 1990.

16) Sengers RC, Stadhouders AM, van Lakwijk-Vondrovicova E, Kubat K, Ruitenbeek W.:Hypertrophic cardiomyopathy associated with a mitochondrial myopathy of voluntary muscles and congenital cataract. Br Heart J. 54:543-71985.

17) Young TJ, Shah AK, Lee MH, Hayes DL.:Kearns-Sayre syndrome: a case report and review of cardiovascular complications. Pacing Clin Electrophysiol. 28:454-457, 2005.

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Matrix Pathobiology

Tenascin-C Regulates Recruitment of Myofibroblasts during Tissue Repair after Myocardial Injury

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Tenascin-C (TN-C) is an extracellular matrix molecule that is expressed during wound healing in various tissues. Although not detectable in the normal adult heart, it is expressed under pathological conditions. Previously, using a rat model, we found that TN-C was expressed during the acute stage after myocardial infarction and that α-smooth muscle actin (α-SMA)-positive myofibroblasts appeared in TN-C-positive areas. In the present study, we examined whether TN-C controls the dynamics of myofibroblast recruitment and wound healing after electrical injury to the myocardium of TN-C knockout (TNKO) mice compared with wild-type (WT) mice. In TNKO mice, myocardial repair seemed to proceed normally, but the appearance of myofibroblasts was delayed. With cultured cardiac fibroblasts, TN-C significantly accelerated cell migration, α-SMA expression, and collagen gel contraction but did not affect proliferation. Using recombinant fragments of murine TN-C, the functional domain responsible for promoting migration of cardiac fibroblasts was mapped to the conserved fibronectin type III (FNIII)-like repeats and the fibrinogen (Fbg)-like domain. Furthermore, alternatively spliced FNIII and Fbg-like domains proved responsible for the up-regulation of α-SMA expression. These results indicate that TN-C promotes recruitment of myofibroblasts in the early stages of myocardial repair by stimulating cell migration and differentiation. *(Am J Pathol 2005, 167:71–80)*

Tenascin-C (TN-C), an extracellular matrix molecule expressed at high levels during embryonic development and cancer invasive fronts, as well as in response to injury, is known to influence various cell activities.[1–4] Each subunit of a hexameric glycoprotein consists of TA (tenascin assembly domain), epidermal growth factor (EGF)-like repeats, fibronectin type III (FN III)-like repeats, and a C-terminal fibrinogen (Fbg)-related domain. Alternative splicing results in several different forms of TN-C, containing variable numbers of FN III repeats. Accumulating results of *in vitro* studies point to each domain having specific functions, for example in the regulation of cell adhesion, migration, or growth.[1–4]

In the heart, TN-C is expressed at very early stages of embryonic development,[5] is not detected in normal adult myocardium, but is re-expressed in various pathological conditions.[6–13] After myocardial infarction, TN-C appears during the acute stages, at the interface between infarcts and intact myocardium.[7,8] We previously reported that TN-C may loosen the linkage between cardiomyocytes and connective tissue and thus helps with tissue remodeling at the edges of residual myocardium.[8] Furthermore, we found α-smooth muscle actin (α-SMA)-positive myofibroblasts in TN-C-positive areas and that deposition of TN-C precedes their recruitment.[8]

Myofibroblasts are specialized fibroblasts that share characteristics with smooth muscle cells expressing α-SMA. They play an important role in wound healing by synthesizing collagens and exerting strong contraction forces to minimize wound areas.[14–17] It is thought that residential interstitial fibroblasts at the edges of injured tissue differentiate into myofibroblasts and migrate into damaged areas. In the present study, we investigated whether TN-C contributed to myocardial tissue repair, with particular attention to recruitment of myofibroblasts. For this purpose TN-C knockout (TNKO) and wild-type (WT) mice were compared with regard to the healing processes after electrical injury to the myocardium. Fur-

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thermore, the effect of TN-C on cell proliferation, migration, and differentiation of cardiac fibroblasts into myofibroblasts was examined *in vitro*. Functional domains were also determined using recombinant fragments of murine TN-C.

Materials and Methods

TN-C Knockout Mice

The originally produced TN-C knockout mouse[18] was backcrossed with BALB/c inbred mice.

Myocardial Injury Model

TNKO and WT mice, 8 weeks old, were handled according to guidelines approved by the Mie University Animal Experiment and Care Committee. Animals were anesthetized by an intraperitoneal injection of 50 mg/kg of sodium pentobarbital and the left diaphragm was exposed through a midline incision in the abdomen. The ventricular wall was injured through the diaphragm by an electric pulse current (160 μA, 0.5 second) using an electric coagulator (OPERER II-F; J. Morita, Tokyo, Japan) that was delivered with a probe of 2 mm in diameter. This procedure caused a transmural injury at the postinferior wall of the left ventricle of ~2 to 3 mm in diameter. Mice were sacrificed 1, 2, 3, and 5 days after injury. The hearts were removed and fixed in a 4% paraformaldehyde solution at 4°C for 16 hours, embedded in paraffin, and cut into 4-μm-thick sections. To examine cell proliferation, some mice received an intraperitoneal administration of 20 mg/kg of 8-bromodeoxiuridine (BrdU) 30 minutes before sacrifice.

Immunohistochemistry

Production and characterization of an anti-TN-C polyclonal rabbit antibody and mouse monoclonal antibody clones 4F10TT (IBL, Gunma, Japan) and 4C8MS (IBL), as well as the immunostaining procedures with tissue sections, were previously described.[8,9,19] 4C8MS specifically recognizes the alternative splicing sites, whereas 4F10TT reacts with constitutive sites of TN-C molecules. Sections on slides were incubated with either rabbit polyclonal antibodies (1 μg/ml), 4F10TT (1 μg/ml), or 4C8MS (5 μg/ml), overnight at 4°C, and subsequently with peroxidase-conjugated anti-mouse or anti-rabbit IgG Fab' (1:500; MBL, Nagoya, Japan) for 1 hour. After washing, diaminobenzidine/H_2O_2 solution was used to demonstrate antibody binding. The sections were then lightly counterstained with methyl-green or hematoxylin to facilitate orientation. Incorporated BrdU in tissue sections was immunostained according to Yoshimura and colleagues.[20] The labeled cells excluding inflammatory cells in the injured areas near the border zone were counted in three different fields of ×40 objective, and the average of each sample (n = 5 for each) was calculated. Myofibroblasts were labeled by a direct immunoperoxidase method with anti-α-SMA antibody (EPOS; Dako Japan, Kyoto, Japan) and the α-SMA-positive cells in the injured areas were also counted. Double immunohistochemistry for TN-C and α-SMA was performed as previously described.[8]

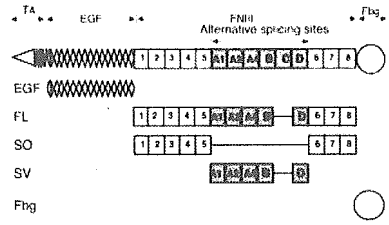


Figure 1. Diagram of mouse TN-C and its recombinant fragments. FL: FNIII repeats including both conserved (1 to 5, 6 to 9) and alternatively spliced repeats (A1, A2, A4, B, D). SV: Alternatively spliced FNIII repeats. SO: Conserved FNIII repeats. EGF: the EGF-like domain. Fbg: fibrinogen-like domain.

Purification of TN-C and Its Recombinant Fragments

TN-C was purified from conditioned medium of the U-251MG human glioma cell line.[21] Recombinant fragments of TN-C (Figure 1): FNIII repeats including the alternative splicing site (FL), FNIII repeats of the alternative splicing site (SV), FNIII repeats without the site (SO), the EGF-like domain, and the fibrinogen (Fbg)-like domain, were obtained from conditioned media of CHO K-1 cells permanently transfected with cDNAs encoding the respective domains, and purified.[19]

Cell Cultures

Primary cultures of cardiac fibroblasts were obtained from cardiac ventricles of either TNKO or WT mice. Five mice were used for each preparation. The mice were sacrificed by cervical dislocation and the hearts were quickly removed under sterile conditions. Ventricular tissue was excised, thoroughly minced, and digested with 0.1% collagenase (class II; Worthington, Freehold, NJ) in Dulbecco's phosphate-buffered saline. The isolated cells were collected by centrifugation, resuspended in Iscov's modified Dulbecco's medium (IMDM; Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Inc., Grand Island, NY), and plated on a 75-cm^2 Primaria culture flask (Becton Dickinson Labware, Franklin Lakes, NJ). After the cardiac fibroblasts had attached to the bottom surface of the flask, nonadherent cells (primarily myocytes, leukocytes, and endothelial cells) could be washed away. This approach enabled us to obtain virtually pure cultures of fibroblasts. The cells were grown in IMDM with 10% FBS at 37°C in a humidified incubator gassed with 5% CO_2. Experiments were performed using cells at the second passage. The effects of intact TN-C and recombinant fragments of TN-C on fibroblasts were examined using the cells from TN-C-null mice to exclude the influence of intrinsically synthesized TN-C.

BrdU Incorporation

Cells (1×10^5) of cardiac fibroblasts from TNKO mice were plated on each well of Falcon four-chamber culture slides (Becton Dickinson Labware), grown in IMDM/10% FBS for 12 hours, washed with serum-free medium, and then incubated in medium with 0.1% FBS for 24 hours. Thereafter, TN-C was added to a final concentration of 1 to 10 μg/ml. After incubation for 12 hours, the cells were labeled with BrdU (10 μg/ml) for 2 hours, fixed with 100% ethanol at −20°C for 30 minutes, and treated with 1 N HCl solution at room temperature for 20 minutes. Labeled nuclei were detected with monoclonal anti-BrdU antibody (Dako Japan) and peroxidase-conjugated goat anti-mouse IgG (MBL). The BrdU-positive nuclei and total number of nuclei (more than 500) were counted and percentage values were determined.

Migration Assays

We first compared the migration of cardiac fibroblasts from TNKO and WT mice, by a transwell migration assay using cell culture inserts (8-μm pore size, Becton Dickinson Labware). Cells (5×10^4 of either type) in 0.5% bovine serum albumin/serum-free IMDM were plated into the inner chamber. The medium, containing 5% FBS as a chemoattractant, was poured into the outer chamber (Falcon 24-well plate, Becton Dickinson Labware). To examine the effect of TN-C on cell migration, intact TN-C (0 to 10 μg/ml) or one of the recombinant fragments (10 μg/ml) was added to medium of the upper chamber of TN-C-null cells. The cells were allowed to migrate to the lower membrane surface for 8 hours. The cells remaining on the upper surface were then wiped off and the inserts were fixed with 100% ethanol and stained with 0.1% crystal violet (Sigma) in 10% ethanol. Stained cells on 1 mm^2 of the lower membrane surface were counted under a ×10 objective lens.

Expression of α-SMA

Cardiac fibroblasts from either TNKO or WT mice were plated on Falcon four-chamber culture slides (5×10^4 for each well) in IMDM/10% FBS for 3 hours, washed with serum-free medium, then incubated in medium with 0.1% FBS for 24 hours. Thereafter, intact TN-C (5 to 20 μg/ml) or one of the recombinant fragments (10 μg/ml) was added to TN-C-null fibroblasts. After incubation for 24 hours, the cells were fixed with 4% paraformaldehyde and labeled with antibodies against α-SMA (Sigma) for 1.5 hours, then exposed to secondary antibodies (fluorescein isothiocyanate-conjugated anti-mouse IgG, MBL) and rhodamine-phalloidin (Molecular Probes, Eugene, OR) for 1 hour. Cells were examined with a ×20 objective lens by epifluorescence microscopy (BX-50; Olympus, Tokyo, Japan). Polyclonal monospecific antibodies against either TN-C or calsequestrin were affinity-purified with antigen-conjugated columns. Calsequestrin is protein of the sarcoplasmic reticulum, and anti-calsequestrin antibody was used as a control IgG. Purified TN-C (10 μg/ml) was incubated with either antibodies (10 μg/ml) at room temperature for 30 minutes, and then added to the culture medium.

Collagen Gel Contraction Assay

Cellular collagen gel contraction assays were performed in Falcon 24-well plates. A collagen solution in IMDM with 0.1% FBS was prepared from porcine collagen I according to the manufacturer's instructions (Nitta Gelatin, Osaka, Japan) and combined with cardiac fibroblasts of either TNKO or WT mice at 2×10^5/ml. Five hundred μl of the collagen/cell mixture (final collagen concentration, 1.0 mg/ml) was plated into each well and allowed to polymerize at 37°C. After incubation for 24 hours, 0.5 ml of IMDM containing 0.1% FBS with or without TN-C (10 μg/ml) was added to each well, and the gels were carefully detached from the dishes. The mean size of the gel ($n = 3$ for each sample) was taken at each time point with the aid of a flatbed scanner (Epson, Tokyo, Japan). To estimate the contractility of the cells, the size of the gel was subtracted from the starting area and expressed as a percentage. This experiment was repeated three times.

Results

Wound Healing and Expression of TN-C in Injured Mouse Myocardium

In WT mice, coagulation necrosis of cardiomyocytes and edema around lesions were apparent 1 day after injury (Figure 2A), becoming more pronounced on day 2 (Figure 2B). On day 3, granulation tissues began to be formed in the border zone and eventually replaced necrotic masses (Figure 2C). TN-C began to be expressed within 1 day after injury at the border between intact myocardial tissues and necrotic areas (Figure 2D) and was gradually deposited in developing granulation tissue (Figure 2, E and F). Using the monoclonal antibody 4C8MS, it was confirmed that the deposited molecules contained the large splice variants (Figure 2G). In TNKO mice, myocardial healing appeared to proceed normally and there was no obvious difference from WT mice on routine histological analyses (Figure 3).

Recruitment of Myofibroblasts in Granulation Tissue of the Heart

Immunostaining of α-SMA of WT mice showed that on day 1, only vascular walls were positive and few myofibroblasts were present, although TN-C deposition was clearly detectable (Figure 2D). On day 2, myofibroblasts emerged in TN-C-positive areas of the interstitium of the border zone (Figure 2E), and increased in number on day 3, moving into necrotic areas. The number of myofibroblasts per optic field in WT mice on days 1, 2, and 3 were 2.2 ± 0.3, 16.6 ± 6.2, and 30.6 ± 0.3, respectively (Figure 4). In TNKO mice, they were 0.7 ± 0.6, 4.9 ± 3.3, and 33.2 ± 3.5. On days 1 and 2, myofibroblasts in WT

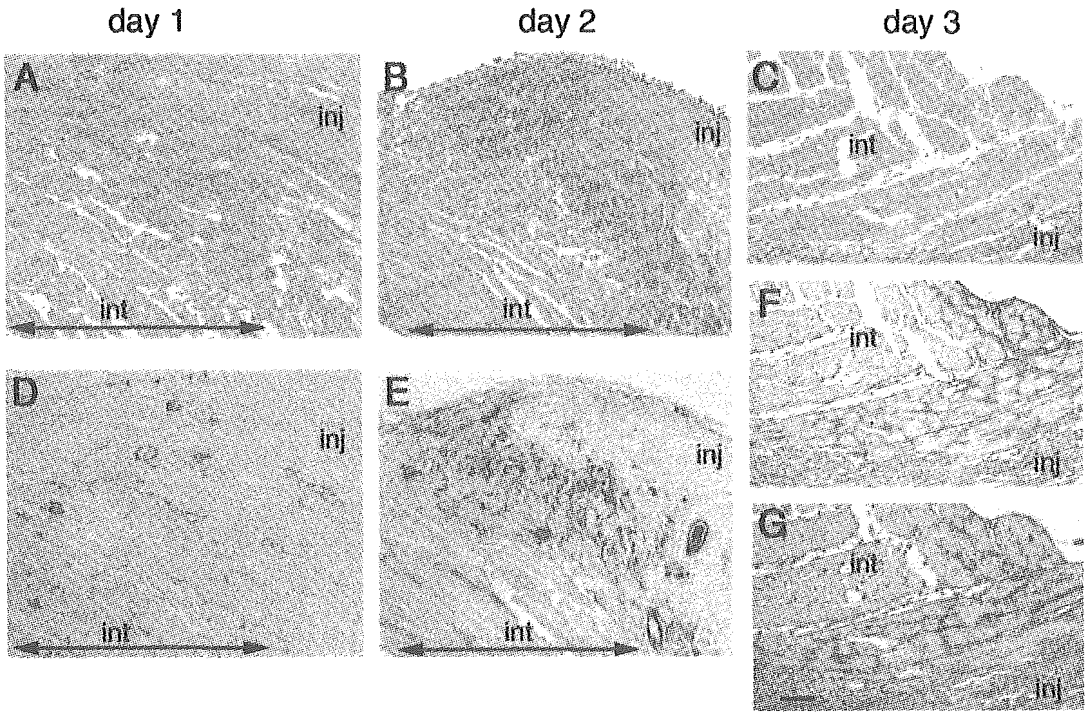


Figure 2. Tissue healing and TN-C expression after electrical injury of the myocardium at day 1 (**A**, **D**), day 2 (**B**, **E**), and day 3 (**C**, **F**, **G**). **A–C:** H&E staining; **D**, **E:** double immunolabeling for α-SMA (blue) and TN-C (brown). **F** and **G:** Immunolabeling with the antibody clone 4F10TT reacting with constitutive sites of TN-C (**F**), and 4C8MS that specifically recognizes an alternative spliced repeat of TN-C (**G**). On day 1, TN-C deposition is clearly detectable in the interstitial spaces of border zone myocardium, but only vascular walls are positive for α-SMA (**D**). **E:** On day 2, myofibroblasts are apparent in TN-C-positive areas. **G:** Note staining for large splice variants of TN-C. int, intact area; inj, injured area. Scale bar, 50 μm.

mice were significantly more numerous than in TNKO mice ($P < 0.01$), but the difference disappeared on day 3 (Figure 4).

Cell Proliferation in Granulation Tissue of the Heart

Since it is well known that TN-C enhances proliferation of some cell types, we examined DNA synthesis in granulation tissue by BrdU incorporation assay. The percentages of BrdU-labeled nuclei in WT mice on days 1, 2, and 3 were 5.7 ± 3.4%, 17.5 ± 2.7%, and 24.2 ± 5.9%, respectively, whereas in TNKO mice they were 6.1 ± 2.9%, 17.7 ± 2.8%, and 26.6 ± 7.0%, with no significant differences between the two animal groups (Figure 5A).

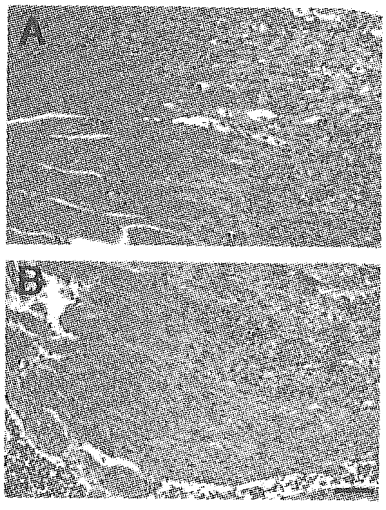


Figure 3. Comparison of the histopathology of myocardial repair in a WT (**A**) and TN-C knockout mouse (**B**) 5 days after electric injury. H&E staining. Scale bar, 50 μm.

Effect of TN-C on Proliferation, Migration, and Differentiation of Cultured Cardiac Fibroblasts in Vitro

To confirm the *in vivo* results, we first investigated the effect of TN-C on cell proliferation of cultured cardiac fibroblasts from TNKO mice by BrdU incorporation assay. Addition of TN-C in various concentrations to the fibroblasts was without significant effect (Figure 5B). In transwell migration assays, the number of TN-C-null fibroblasts that migrated through insert membranes was significantly lower than those from the WT mice (28.8 ± 3.7 cells/mm^2 versus 42.0 ± 3.6 cells/mm^2, $P < 0.01$). Addition of TN-C to TN-C-null cells at the concentration of 5 and 10 μg/ml, significantly increased the number of migrated cells to 50.8 ± 12.3 cells/mm^2 and 53.1 ± 5.2 cells/mm^2, respectively ($P < 0.01$ and $P < 0.001$, Figure 6).

Differentiation to myofibroblasts was assessed by expression of α-SMA (Figure 7) and collagen gel contraction assay (Figure 8). Cardiac fibroblasts in culture from TNKO mice showed well developed stress fibers and some cells expressed α-SMA (Figure 7A). However, the percentage of α-SMA-positive cells was significantly lower than that of WT cardiac fibroblasts (35.4 ± 2.03%

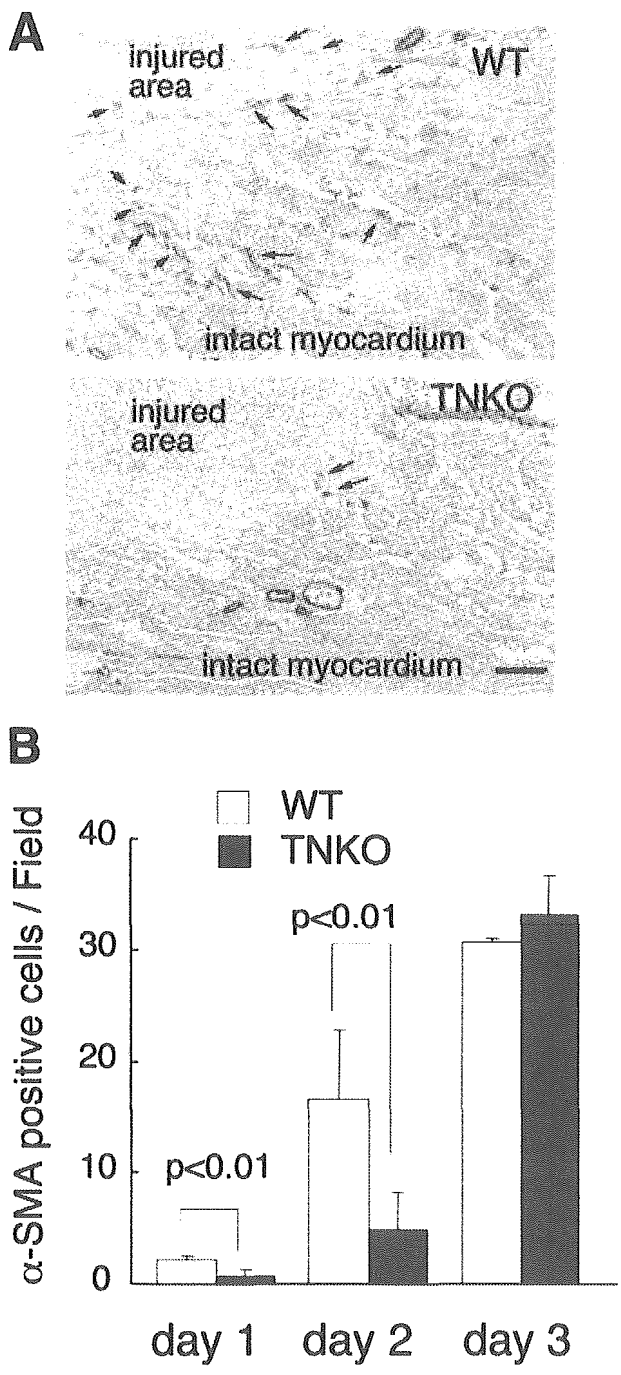


Figure 4. Myofibroblasts in the border zones of necrotic cardiac tissue. **A:** Myofibroblasts labeled with anti-α-SMA antibody. On day 2, α-SMA-positive cells are evident (**arrows**) in the myocardial interstitium near the necrotic area in a WT mouse. Fewer cells are apparent in a TN-C knockout (TNKO) mouse. **B:** α-SMA-positive cells were counted in three fields of view under a ×40 objective and the average of each sample was calculated. On days 1 and 2, the myofibroblasts in WT mice were more frequent than in TNKO mice ($P < 0.01$), but there was no difference on day 3. The data are averages and SDs of results from five animals. Scale bar, 50 μm.

versus 50.8 ± 3.7%, $P < 0.01$). Addition of TN-C significantly increased the α-SMA-positive ratio of the TN-C-null fibroblasts in a dose-dependent manner (Figure 7B). To confirm this result, we tested whether addition of an antibody against TN-C blocked this effect and found that treatment with the TN-C antibody (95 ± 5%) restored the

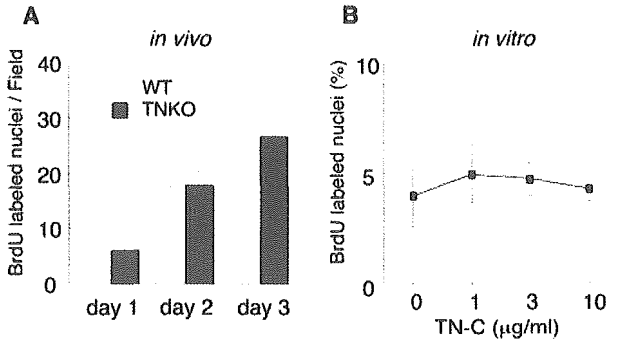


Figure 5. Effect of TN-C on proliferation of cardiac fibroblasts *in vivo* and *in vitro*. **A:** BrdU-labeled cells in the injured areas of WT and TNKO mice. BrdU-positive nuclei were counted in three fields of view of a ×40 objective and the average of each sample was calculated. The data are expressed as averages and SDs of results from five animals. Note the lack of significant differences between WT and TNKO. **B:** Cells isolated from TNKO mice were plated and grown on culture glass slides. After serum starvation for 24 hours, TN-C was added. Cells labeled with BrdU were visualized by immunocytochemistry and BrdU-positive and total number of nuclei (more than 500) were counted and percentage values generated. The data are averages and SDs from three independent experiments. Note the lack of any increase with TN-C treatment.

α-SMA-positive cells to the levels in fibroblasts that were cultured without TN-C (100 ± 5%), while addition of control IgG had no effect (117 ± 8%). Both TN-C-null and WT cells contracted collagen gels, but contraction by TN-C-null cells was significantly weaker than those by WT cells. Addition of TN-C significantly increased the gel contraction of TN-C-null fibroblasts (Figure 8).

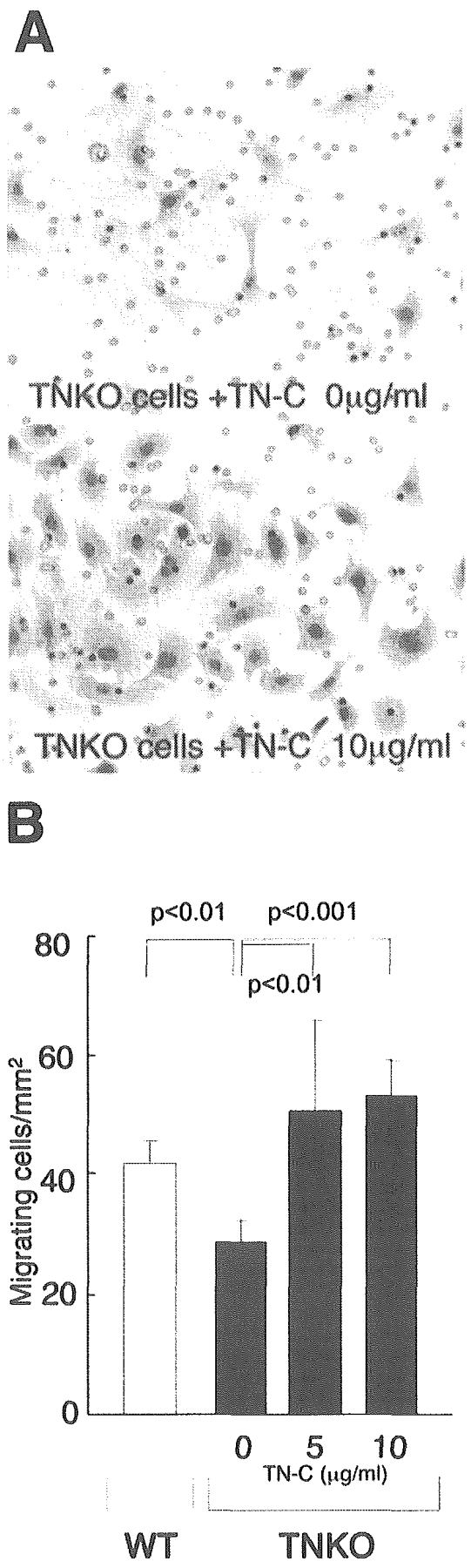
Functional Domains of TN-C on Migration and α-SMA Expression of Cardiac Fibroblasts

Having shown that TN-C promoted migration and α-SMA expression, we tried to determine the responsible functional domains of TN-C, using the following recombinant fragments: FNIII repeats including alternative splicing sites (FL), conserved FNIII repeats (SO), alternative splice sites (SV), the EGF-like domain, and the fibrinogen (Fbg)-like domain (Figure 1). On addition to fibroblast cultures, FL and SO increased the numbers of migrating cells to 151 ± 28% and 151 ± 18%, respectively, of the control value. The Fbg-like domain also caused a 165 ± 32% elevation, while addition of neither EGF nor SV fragments had significant effects (Figure 9). Therefore, the conserved FNIII repeats and the Fbg-like domain may have the ability to promote migration of fibroblasts.

FL also augmented the proportion of α-SMA-positive fibroblasts (123 ± 15%) as compared with the controls (Figure 10). Although SO did not exert any apparent influence, SV also caused a significant increase, along with the Fbg-like domain (118 ± 16%). Addition of EGF was without effect. Therefore, alternatively spliced FNIII repeats and the Fbg-like domain may thus induce differentiation from fibroblasts to myofibroblasts.

Discussion

TN-C has been proposed to play significant roles in wound healing in various tissues because of its dramatic



increase after injury.[22] Although TN-C knockout mice were initially reported to be phenotypically normal,[18] several reports have documented abnormal tissue restoration after injury to the cornea,[23] skin,[24] or after habu venom-induced glomerulonephritis.[25,26] Using different models, others have reported no significant differences in the wound healing on morphological analysis,[27,28] but deposition of fibronectin was found to be reduced in the granulation tissue of skin wounds in TN-C-null mice.[27] *In vitro*, TN-C promotes epithelial cell migration and proliferation,[19] which could be partly responsible for abnormal repair of epithelial tissue in TNKO mice.

Because of the limited ability for regeneration of cardiomyocytes, myocardial wound healing mostly depends on interstitial fibroblasts, which form and contract granulation and scar tissue via multiple cell activities.[29] Especially, myofibroblasts, specialized fibroblasts, are key players in the myocardial repair.[30–32] In the present study, we found that their appearance in injured myocardium was clearly delayed in TN-C knockout mice, indicating that TN-C promotes recruitment of myofibroblasts.

On tissue injury, primarily in response to mechanical stress, interstitial fibroblasts evolve into proto-myofibroblasts with a contractile microfilamentous apparatus, then, with various stimuli, into differentiated myofibroblasts characterized by expression of α-SMA. These then migrate into the injured areas, generate contraction forces, synthesize, organize, and degrade collagen and other ECMs, and finally results in a shortening of collagen matrix with corresponding wound closure.[14,15,33,34]

A close relationship between TN-C and myofibroblasts in cancer stroma or normal tissue has been reported.[35–37] In cancer stroma, myofibroblasts have often been identified as the source of TN-C deposits[38] and it is supposed that they may provide a proinvasive signal to cancer cells mediated with TN-C.[39] By careful sequential observation in a rat model after myocardial infarction, we have found that, initially α-SMA-negative interstitial cells in the border zones express TN-C, and then myofibroblasts appear in the TN-C-positive areas.[8] Similar results were observed in mouse myocardial wound healing in this study. It seems likely that interstitial cells synthesize TN-C, which, in an autocrine and paracrine manner, then causes interstitial cells to change their phenotype to differentiated myofibroblasts and promotes migration into damaged areas.

Our *in vitro* findings support this possibility. Cardiac fibroblasts from TNKO mice showed lower cell migration and α-SMA expression than WT cells that synthesize TN-C in culture. Addition of TN-C to TN-C-null cells recovered both cell migration and α-SMA expression. Expression of α-SMA is a critical step for myofibroblast

Figure 6. Effects of TN-C on migration of cardiac fibroblasts in a transmigration assay. Cells isolated from cardiac ventricles of either TNKO or WT mice were plated on the culture inserts, treated with TN-C (5 or 10 μg/ml) or without TN-C, and allowed to migrate for 8 hours. **A:** Cells migrating through the membrane were stained with 0.1% crystal violet. **B:** Migration of TN-C-null cells was significantly lower than that of WT-cells, and addition of TN-C significantly enhanced cell migration in a dose-dependent manner. Cells were counted in three fields of view of 1 mm^2 in each insert, and the data are averages and SDs of results from three independent experiments.

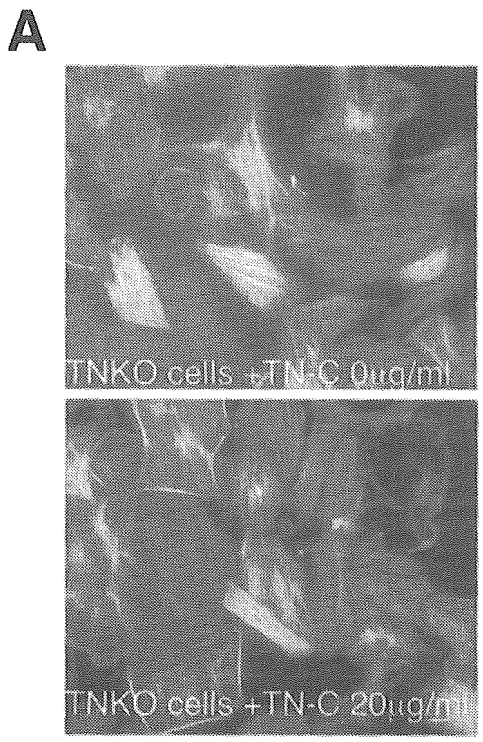


Figure 7. Effects of TN-C on α-SMA expression in cardiac fibroblasts. Cells isolated from cardiac ventricles of either TNKO or WT mice were plated and grown on the culture glass slides. After 24-hour serum starvation, TN-C (0 or 20 μg/ml) was added to TN-C-null cells and incubated for another 24 hours. **A:** α-SMA expression was detected by indirect immunofluorescent staining with fluorescein isothiocyanate-conjugated secondary antibody, and all cells were counterstained with rhodamine-phalloidin. The α-SMA-positive ratio of TN-C-null cells was significantly lower than that of WT cells. **B:** Addition of TN-C significantly up-regulated α-SMA expression in a dose-dependent manner. The α-SMA-positive and total cells (more than 200) were counted to allow generation of percentage values. The data are averages and SDs of results from three independent experiments.

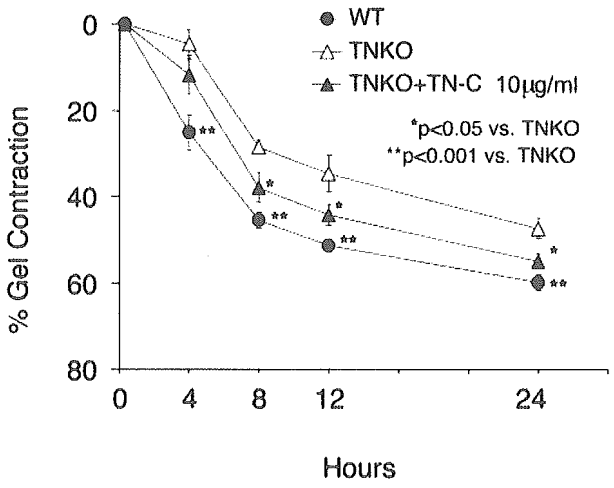


Figure 8. Effects of TN-C on collagen gel contraction by cardiac fibroblasts. Cardiac fibroblasts of either TNKO or WT mice were combined with collagen gel mixture and allowed to polymerize. After 24 hours medium with or without TN-C (10 μg/ml) was added, then the gels were detached from the dish. Percentages of the contraction were measured at 4, 8, 12, and 24 hours. Contraction by WT cells (**closed circle**) was significantly stronger than that by TN-C-null cells (**open triangle**) at each time point. Addition of TN-C (**closed triangle**) significantly increased the gel contraction of TNKO fibroblasts at 8, 12, and 24 hours. The data are expressed as averages and SDs of triplicate samples.

differentiation and functionally important for force generation.[40,41] Although gel contraction could be regulated by various factors including differentiation of myofibroblasts, cell-matrix adhesion, and maturation of cytoskeletal contractile apparatus,[34,42–44] the increment of contraction by addition of TN-C in our experiment should partly reflect the up-regulation of α-SMA expression. Although TN-C is well known to stimulate migration of various types of cells, it has also been suggested to modulate differentiation of cells.[45–47]

Functional domains responsible for differentiation into myofibroblasts were mapped to alternative spliced FNIII repeats, but not the conserved repeats, and the Fbg-like domain of TN-C. In contrast, the responsible domain for

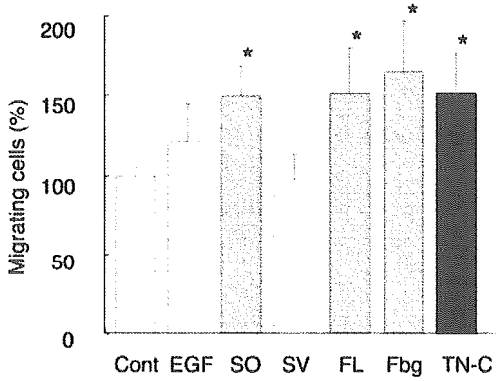


Figure 9. Determination of functional domains promoting cell migration using recombinant fragments of TN-C. TN-C-null cardiac fibroblasts were plated on culture inserts and treated with TN-C (10 μg/ml) or TN-C fragments (10 μg/ml), and allowed to migrate for 8 hours. The cells were counted in three fields of view of 1 mm^2 in each insert, and the data are averages and SDs of results from six independent experiments, relative to the control without TN-C. $^*P < 0.01$.

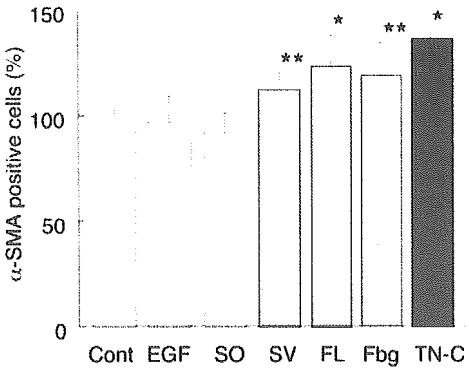


Figure 10. Determination of functional domains up-regulating α-SMA expression using recombinant fragments of TN-C. TN-C-null cardiac fibroblasts were plated and grown on the culture glass slides. After 24-hour serum starvation, they were treated with TN-C (10 μg/ml) or TN-C fragments (10 μg/ml), and incubated for 24 hours. α-SMA expression was detected by immunofluorescence. α-SMA-positive cells and total number of cells (more than 200) were counted and the percentage values were generated. The data are averages and SDs of results from six independent experiments, relative to the control without TN-C. $^{*}P < 0.01$, $^{**}P < 0.05$.

promoting migration of cardiac fibroblasts was mapped to the conserved FNIII repeats and the Fbg-like domain. Several receptors might bind to the respective domains of TN-C and transmit multiple signals that could trigger various cellular functions.

Interestingly, using the same recombinant fragments and the same assays, our previous study demonstrated that the alternative spliced FNIII domains promoted migration of breast cancer cells, but the conserved repeats and Fbg domain did not.[19] The conserved repeats of FNIII 3 and 6 have similarly been demonstrated to be involved in glioma migration.[48] It has also been reported that the Fbg-like domain, but not the entire FNIII repeats, can mediate migration of aortic smooth muscle cells on a TN-C substrate.[49] Conversely, a recombinant fragment of alternatively spliced FNIII A to D, but not the conserved FN III repeats or the Fbg domain, was found to enhance migration of endothelial cells.[50] Therefore, cell-type specificity is apparent. A number of molecules, such as members of the integrin family, annexin II, EGF receptor, cell adhesion molecules (CAMs), syndecan, and phosphacan/receptor-type protein tyrosine phosphatase ζ/β, have been reported as receptors for TN-C.[3] Moreover, TN-C may also regulate cell behavior by direct or indirect modulation of other ECM proteins. For example, it has been reported that TN-C may inhibit fibronectin initiating signaling by interfering fibronectin-syndecan 4 binding.[44,51,52] Therefore, functional activities provided by TN-C could occur through different mechanisms, possibly using separate receptors depending on the cell.

In this study, we demonstrated that TN-C plays critical roles in stimulating myofibroblasts in response to injury. However, after 3 days there was no longer any difference in myofibroblasts in TN-C-null and control mice, and myocardial healing did not show distinct morphological differences so that a compensatory mechanism must exist. Indeed, TN-C is not the only factor that controls behavior of myofibroblasts. For example, the transition between proto-myofibroblasts and differentiated myofibroblasts is known to be stimulated by many factors, such as various cytokines, growth factors, endothelin 1, angiotensin II, and newly synthesized extracellular matrix proteins.[16] It is well established that the combined action of the splice variant of cellular fibronectin containing ED-A and transforming growth factor-β is important in this regard.[53,54] In cancer tissues, TN-C is often co-expressed with fibronectin containing ED-A,[37,55] and also can enhance the functions of transforming growth factor-β in cancer cells.[56] Therefore, TN-C might control behavior of myofibroblasts in a complex way, collaborating and interfering with many other factors.

Nevertheless, it is clear that TN-C is a major factor for recruitment of myofibroblasts in early stages of myocardial tissue repair. Although this would increase contraction forces to prevent ventricular dilatation, TN-C also has been reported to inhibit cell contraction by suppressing focal adhesion kinase and Rho A activity in fibrinogen-fibronectin matrix.[43,44] Furthermore, TN-C may loosen adhesion of cardiomyocytes[8] and stimulate MMP expression in some types of cells.[56] Although these effects, as well as integrin shedding, should be beneficial for cells to modify cell-ECM interaction during tissue remodeling,[57] they also have potential to cause slippage of myocytes resulting in ventricular dilatation. Therefore, TN-C could be a key molecule in controlling the balance of beneficial and undesirable cellular responses in cardiac remodeling.

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References

1. Chiquet-Ehrismann R, Chiquet M: Tenascins: regulation and putative functions during pathological stress. J Pathol 2003, 200:488–499
2. Chiquet-Ehrismann R, Tucker RP: Connective tissues: signalling by tenascins. Int J Biochem Cell Biol 2004, 36:1085–1089
3. Jones FS, Jones PL: The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn 2000, 218:235–259
4. Jones PL, Jones FS: Tenascin-C in development and disease: gene regulation and cell function. Matrix Biol 2000, 19:581–596
5. Imanaka-Yoshida K, Matsumoto K, Hara M, Sakakura T, Yoshida T: The dynamic expression of tenascin-C and tenascin-X during early heart development. Differentiation 2003, 71:291–298
6. Tamura A, Kusachi S, Nogami K, Yamanishi A, Kajikawa Y, Hirohata S, Tsuji T: Tenascin expression in endomyocardial biopsy specimens in patients with dilated cardiomyopathy: distribution along margins of fibrotic lesions. Heart 1996, 75:291–294
7. Willems IE, Arends JW, Daemen MJ: Tenascin and fibronectin expression in healing human myocardial scars. J Pathol 1996, 179:321–325
8. Imanaka-Yoshida K, Hiroe M, Nishikawa T, Ishiyama S, Shimojo T, Ohta Y, Sakakura T, Yoshida T: Tenascin-C modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction. Lab Invest 2001, 81:1015–1024
9. Imanaka-Yoshida K, Hiroe M, Yasutomi Y, Toyozaki T, Tsuchiya T, Noda N, Maki S, Nishikawa T, Sakakura T, Yoshida T: Tenascin-C is a useful marker for disease activity in myocarditis. J Pathol 2002, 197:388–394
10. Imanaka-Yoshida K, Hiroe M, Yoshida T: Interaction between cell and

extracellular matrix in heart disease: multiple roles of tenascin-C in tissue remodeling. Histol Histopathol 2004, 19:517–525
11. Sato M, Toyozaki T, Odaka K, Uehara T, Arano Y, Hasegawa H, Yoshida K, Imanaka-Yoshida K, Yoshida T, Hiroe M, Tadokoro H, Irie T, Tanada S, Komuro I: Detection of experimental autoimmune myocarditis in rats by 111In monoclonal antibody specific for tenascin-C. Circulation 2002, 106:1397–1402
12. Frangogiannis N, Shimoni S, Chang SM, Ren G, Dewald O, Gersch C, Espada R, Mahesh R, Entman ML, Zoghbi WA: Active interstitial remodeling: an important process in the hibernating human myocardium. J Am Coll Cardiol 2002, 39:1468–1474
13. Morimoto S, Imanaka-Yoshida K, Hiramitsu S, Kato S, Ohtsuki M, Uemura A, Kato Y, Nishikawa T, Toyozaki T, Hishida H, Yoshida T, Hiroe M: The diagnostic utility of tenascin-C for evaluation of activity of human acute myocarditis. J Pathol 2005, 205:460–467
14. Gabbiani G: Evolution and clinical implications of the myofibroblast concept. Cardiovasc Res 1998, 38:545–548
15. Gabbiani G: The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 2003, 200:500–503
16. Serini G, Gabbiani G: Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res 1999, 250:273–283
17. Powell DW: Myofibroblasts: paracrine cells important in health and disease. Trans Am Clin Climatol Assoc 2000, 111:271–292
18. Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S: Mice develop normally without tenascin. Gene Dev 1992, 6:1821–1831
19. Tsunoda T, Inada H, Kalembeyi I, Imanaka-Yoshida K, Sakakibara M, Okada R, Katsuta K, Sakakura T, Majima Y, Yoshida T: Involvement of large tenascin-C splice variants in breast cancer progression. Am J Pathol 2003, 162:1857–1867
20. Yoshimura E, Majima A, Sakakura Y, Sakakura T, Yoshida T: Expression of tenascin-C and the integrin alpha 9 subunit in regeneration of rat nasal mucosa after chemical injury: involvement in migration and proliferation of epithelial cells. Histochem Cell Biol 1999, 111:259–264
21. Yoshida T, Yoshimura E, Numata H, Sakakura Y, Sakakura T: Involvement of tenascin-C in proliferation and migration of laryngeal carcinoma cells. Virchows Arch 1999, 435:496–500
22. Mackie EJ, Halfter W, Liverani D: Induction of tenascin in healing wounds. J Cell Biol 1988, 107:2757–2767
23. Matsuda A, Yoshiki A, Tagawa Y, Matsuda H, Kusakabe M: Corneal wound healing in tenascin knockout mouse. Invest Ophthalmol Vis Sci 1999, 40:1071–1080
24. Koyama Y, Kusubata M, Yoshiki A, Hiraiwa N, Ohashi T, Irie S, Kusakabe M: Effect of tenascin-C deficiency on chemically induced dermatitis in the mouse. J Invest Dermatol 1998, 111:930–935
25. Nakao N, Hiraiwa N, Yoshiki A, Ike F, Kusakabe M: Tenascin-C promotes healing of Habu-snake venom-induced glomerulonephritis: studies in knockout congenic mice and in culture. Am J Pathol 1998, 152:1237–1245
26. Matsumoto K, Hiraiwa N, Yoshiki A, Ohnishi M, Kusakabe M: PDGF receptor-a deficiency in glomerular mesangial cells of tenascin-C knockout mice. Biochem Biophys Res Commun 2002, 290:1220–1227
27. Forsberg E, Hirsch E, Frohlich L, Meyer M, Ekblom P, Aszodi A, Werner S, Fassler R: Skin wounds and severed nerves heal normally in mice lacking tenascin-C. Proc Natl Acad Sci USA 1996, 93:6594–6599
28. Iglesia DD, Gaia PH, Qiu T, Stepp MA: Integrin expression during epithelial migration and restratification in the tenascin-C-deficient mouse cornea. J Histochem Cytochem 2000, 48:363–376
29. Camelliti P, Borg TK, Kohl P: Structural and functional characterisation of cardiac fibroblasts. Cardiovasc Res 2005, 65:40–51
30. Cleutjens JPM, Blankesteijn WM, Daemen MJAP, Smits JFM: The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. Cardiovasc Res 1999, 44:232–241
31. Heeneman S, Cleutjens JP, Faber BC, Creemers EE, van Suylen RJ, Lutgens E, Cleutjens KB, Daemen MJ: The dynamic extracellular matrix: intervention strategies during heart failure and atherosclerosis. J Pathol 2003, 200:516–525
32. Virag JI, Murry CE: Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. Am J Pathol 2003, 163:2433–2440
33. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA: Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002, 3:349–363
34. Grinnell F: Fibroblast biology in three-dimensional collagen matrices. Trends Cell Biol 2003, 13:264–269
35. Ramadori G, Schwogler S, Veit T, Rieder H, Chiquet-Ehrismann R, Mackie EJ, Meyer zum Buschenfelde KH: Tenascin gene expression in rat liver and in rat liver cells. In vivo and in vitro studies. Virchows Arch B Cell Pathol Incl Mol Pathol 1991, 60:145–153
36. Kalembeyi I, Yoshida T, Iriyama K, Sakakura T: Analysis of tenascin mRNA expression in the murine mammary gland from embryogenesis to carcinogenesis: an in situ hybridization study. Int J Dev Biol 1997, 41:569–573
37. Yoshida T, Matsumoto E, Hanamura N, Kalembeyi I, Katsuta K, Ishihara A, Sakakura T: Co-expression of tenascin and fibronectin in epithelial and stromal cells of benign lesions and ductal carcinomas in the human breast. J Pathol 1997, 182:421–428
38. Hanamura N, Yoshida T, Matsumoto E, Kawarada Y, Sakakura T: Expression of fibronectin and tenascin-C mRNA by myofibroblasts, vascular cells and epithelial cells in human colon adenomas and carcinomas. Int J Cancer 1997, 73:10–15
39. De Wever O, Nguyen QD, Van Hoorde L, Bracke M, Bruyneel E, Gespach C, Mareel M: Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. FASEB J 2004, 18:1016–1018
40. Hinz B, Dugina V, Ballestrem C, Wehrle-Haller B, Chaponnier C: {Alpha}-smooth muscle actin is crucial for focal adhesion maturation in myofibroblasts. Mol Biol Cell 2003, 14:2508–2519
41. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C: Alpha-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell 2001, 12:2730–2741
42. Kanekar S, Borg TK, Terracio L, Carver W: Modulation of heart fibroblast migration and collagen gel contraction by IGF-I. Cell Adhes Commun 2000, 7:513–523
43. Midwood KS, Schwarzbauer JE: Tenascin-C modulates matrix contraction via focal adhesion kinase- and Rho-mediated signaling pathways. Mol Biol Cell 2002, 13:3601–3613
44. Midwood KS, Valenick LV, Hsia HC, Schwarzbauer JE: Coregulation of fibronectin signaling and matrix contraction by tenascin-C and syndecan-4. Mol Biol Cell 2004, 15:5670–5677
45. Wirl G, Hermann M, Ekblom P, Fassler R: Mammary epithelial cell differentiation in vitro is regulated by an interplay of EGF action and tenascin-C downregulation. J Cell Sci 1995, 108:2445–2456
46. Dandachi N, Hauser-Kronberger C, More E, Wiesener B, Hacker GW, Dietze O, Wirl G: Co-expression of tenascin-C and vimentin in human breast cancer cells indicates phenotypic transdifferentiation during tumour progression: correlation with histopathological parameters, hormone receptors, and oncoproteins. J Pathol 2001, 193:181–189
47. Garcion E, Halilagic A, Faissner A, French-Constant C: Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. Development 2004, 131:3423–3432
48. Phillips GR, Krushel LA, Crossin KL: Domains of tenascin involved in glioma migration. J Cell Sci 1998, 111:1095–1104
49. LaFleur DW, Chiang J, Fagin JA, Schwartz SM, Shah PK, Wallner K, Forrester JS, Sharifi BG: Aortic smooth muscle cells interact with tenascin-C through its fibrinogen-like domain. J Biol Chem 1997, 272:32798–32803
50. Chung CY, Murphy-Ullrich JE, Erickson HP: Mitogenesis, cell migration, and loss of focal adhesions induced by tenascin-C interacting with its cell surface receptor, annexin II. Mol Biol Cell 1996, 7:883–892
51. Huang W, Chiquet-Ehrismann R, Moyano JV, Garcia-Pardo A, Orend G: Interference of tenascin-C with syndecan-4 binding to fibronectin blocks cell adhesion and stimulates tumor cell proliferation. Cancer Res 2001, 61:8586–8594
52. Orend G, Huang W, Olayioye MA, Hynes NE, Chiquet-Ehrismann R: Tenascin-C blocks cell-cycle progression of anchorage-dependent fibroblasts on fibronectin through inhibition of syndecan-4. Oncogene 2003, 22:3917–3926
53. Serini G, Bochaton-Piallat M-L, Ropraz P, Geinoz A, Borsi L, Zardi L, Gabbiani G: The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta 1. J Cell Biol 1998, 142:873–881

54. Dugina V, Fontao L, Chaponnier C, Vasiliev J, Gabbiani G: Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors. J Cell Sci 2001, 114:3285–3296
55. Matsumoto E, Yoshida T, Kawarada Y, Sakakura T: Expression of fibronectin isoforms in human breast tissue: production of extra domain A+/extra domain B+ by cancer cells and extra domain A+ by stromal cells. Jpn J Cancer Res 1999, 90:320–325
56. Kalembeyi I, Inada H, Nishiura R, Imanaka-Yoshida K, Sakakura T, Yoshida T: Tenascin-C upregulates matrix metalloproteinase-9 in breast cancer cells: direct and synergistic effects with transforming growth factor. Int J Cancer 2003, 105:53–60
57. Goldsmith EC, Carver W, McFadden A, Goldsmith JG, Price RL, Sussman M, Lorell BH, Cooper G, Borg TK: Integrin shedding as a mechanism of cellular adaptation during cardiac growth. Am J Physiol 2003, 284:H2227–H2234

拡張型心筋症を呈する心臓サルコイドーシス —左室縮小形成術（バチスタ手術）症例を中心に—

寺崎文生，北浦　泰

【要旨】

心臓サルコイドーシス（心サ症）はサルコイドーシスによる死因の多くを占め，治療開始時期が予後を左右するため早期診断が極めて重要である．本研究は循環器内科の立場から観た心サ症患者の診断・治療・予後に関する現状と問題点を検討することを目的として行われた．臨床的に拡張型心筋症と診断され，心筋組織所見より心サ症が明らかになった患者10例（左室縮小形成術切除心筋6例，生検心筋2例（うち1例は手術中の生検），剖検心筋2例）を対照の特発性拡張型心筋症患者10例（左室縮小形成術施行例）と比較検討した．結果は心サ症患者において，1）心臓以外の臓器病変が臨床的に明らかでないため拡張型心筋症と誤診されている症例が多くみられた．2）拡張型心筋症と診断された心サ症患者は，中高年発症で非虚血性心筋症の病態を呈すること，高度房室ブロックや局所的心室壁運動異常（心室瘤，心室中隔菲薄化など）が先行するなどの特徴が認められた．3）経過は慢性進行性のものが多いが，一部に若年発症し急性激症の経過をとるものがみられた．4）分子生物学的および免疫組織学的検索で特異的な炎症性サイトカインの発現亢進が認められ，多くがI型ヘルパーT細胞（Th1）関連サイトカインであった．また，これらについて代表症例を挙げて問題点を指摘した．以上より，心臓以外の臓器病変が明らかでない場合は心サ症を拡張型心筋症または原因不明の心筋症と誤診することが多い．心サ症の疫学，病因，病態（活動性），治療，予後などに関して明らかにされていない点が多く，心サ症に対する感度および特異性が高い診断法，的確な活動性の評価法の確立のみならず病因・病態の解明が待たれる．

〔日サ会誌　2004;24:21-30〕

キーワード：心臓サルコイドーシス，拡張型心筋症，左室縮小形成術（バチスタ手術），完全房室ブロック，サイトカイン

Current Status of Cardiac Sarcoidosis with Dilated Cardiomyopathy — with Special Reference to Patients who Underwent Left Ventriculoplasty (Batista Operation)—

Fumio Terasaki, Yasushi Kitaura

【ABSTRACT】

Cardiac involvement is a crucial factor of death in sarcoidosis. Thus, the early diagnosis of cardiac sarcoidosis is quite important. This study was performed to assess the current status and problems involved in the diagnosis, treatment, and prognosis of cardiac sarcoidosis, from the viewpoint of cardiologists. We analyzed 10 patients with histologically proven cardiac sarcoidosis (6 cases by myocardial specimens obtained at the left ventriculoplasty, 2 at biopsy, and 2 at autopsy). Ten patients with idiopathic dilated cardiomyopathy (DCM) served as controls. We discovered the following: 1) We frequently found cardiac sarcoidosis patients who had been diagnosed as having DCM with lack of other organ involvements, 2) These patients had some characteristic clinical features, such as non-ischemic cardiomyopathy that developed in middle-age or older, and association with or precedence by advanced atrioventricular block, local ventricular wall motion abnormalities (ventricular aneurysm formation or thinning of the interventricular septum), 3) Though the clinical course of most patients was chronic and slowly progressive, fulminant progressive cases with poor prognosis were also observed, 4) From molecular biological and immunohistochemical studies of inflammatory cytokines, the expression of helper T cell type 1 (Th1) cytokines was enhanced specifically in the myocardium of cardiac sarcoidosis patients. In conclusion, the diagnosis of cardiac sarcoidosis was difficult, particularly in cases without involvement of any other organs. These cases are frequently misdiagnosed as idiopathic DCM or unclassified cardiomyopathy. Many questions and problems still remain unresolved concerning epidemiology, etiology, pathogenesis (activity), therapy, and prognosis of cardiac sarcoidosis. Further elucidation of the etiology and the development of specific tools are anticipated for the improvement of the diagnosis and the estimation of disease activity of cardiac sarcoidosis.

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keywords; Cardiac sarcoidosis, Dilated cardiomyopathy, Left ventriculoplasty (Batista operation), Complete atrioventricular block, Cytokines

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はじめに

サルコイドーシス（サ症）は原因不明の全身性多臓器疾患で非乾酪性類上皮細胞肉芽腫を特徴とする．我が国では心臓サルコイドーシス（心サ症）の頻度が高く，重症心不全や致死的不整脈により死因として極めて重要である[1-4]．従って，心サ症の治療と予後においてはとくに早期診断が重要であるが，心臓以外の臓器病変が明らかでない場合，症例呈示のごとくその早期診断は困難で，心臓手術の後や死後剖検で明らかになることも多い．また，心サ症の活動性や治療効果の判定も確立しているとは言えない．筆者らは，以前より重症拡張型心筋症と診断され左室縮小形成術（バチスタ手術）が施行された症例の切除心筋を用いて病因論的および病理組織学的検索を行ってきた[5-8]．その結果，2004年2月の時点で，左室縮小形成術等を受けた非虚血性拡張型心筋症119例中7例（6%）が心サ症と判明した．この頻度は看過できないものである．本研究報告では，先ず，拡張型心筋症の病態を呈した心サ症の1症例を呈示する．次に，自験例に基づいて心サ症患者の診断・病態・治療・予後に関する現状と問題点を呈示する．さらに，心サ症の診断や活動性の評価，治療効果の判定に役立つ新たな指標・検査の可能性を考察する．

症例呈示

62歳，男性．［主訴］起座呼吸．［家族歴］兄，胃潰瘍．弟，感染性心内膜炎．［既往歴］53歳，うっ血性心不全．59歳，完全房室ブロックのため恒久的ペースメーカー植え込み手術．61歳，心房細動．喫煙歴，20年前に禁煙．飲酒歴，無し．［現病歴］53歳時よりうっ血性心不全の診断で某病院に通院していた．59歳時より心不全症状が増悪し同病院で心臓カテーテル検査をうけた．冠動脈に異常なく，左室のびまん性壁運動低下が認められたため拡張型心筋症と診断された．心筋生検は施行されなかった．同時期に完全房室ブロックが出現し恒久的ペースメーカー植え込み手術をうけた．その後，ジギタリス，利尿薬，ACE阻害薬，β遮断薬による内科的治療が行われたが，左室拡大，左室収縮能低下および僧帽弁逆流が次第に進行した．61歳時よりうっ血性心不全症状が著明に増悪し内科的治療の限界と考えられ62歳時左室縮小形成術が施行された．［左室縮小形成術前検査所見］胸部X線検査（Figure 1）：心拡大と肺うっ血を認める．肺門部リンパ節腫脹（BHL）は認めない．心電図（Figures 2A, 2B）：57歳時の心電図では第1度房室ブロック，Ⅱ，Ⅲ，aVF誘導におけるprominent QおよびST-T変化，心室性期外収縮を認める（Figure 2A）．62歳時（手術前）の心電図はペースメーカーリズムで心室性期外収縮の頻発を認める（Figure 2B）．心臓超音波検査（Figures 3A, 3B）：左室拡大とびまん性の壁運動低下（Figure 3A），心室中隔上部（心基部）の菲薄化（Figure 3B）が認められる．胸部CT検査（Figure 4）：縦隔リンパ節（気管前リンパ節，主気管支周囲リンパ節）の腫大を認める．心筋シンチグラムおよび全身ガリウム（^{67}Ga）シンチグラムは施行されていない．［術後経過］左室縮小形成術時に切除した心筋の組織学的検査にて類上皮細胞性肉芽腫と高度の線維化が認められ，術後初めて心臓サルコイドーシスと診断された（Figure 5）．ステロイド治療を含む内科的治療の継続目的にて大阪医科大学附属病院第三内科に転院したが，うっ血性心不全と多臓器不全のため2.5ヶ月後に永眠された．

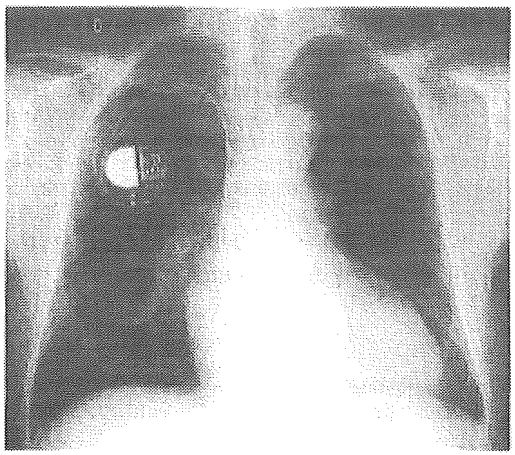


Figure 1. Chest X-ray film before left ventriculoplasty (Batista operation), showing cardiomegaly and pulmonary congestion. Bilateral hilar lymphadenopathy is not apparent.

対象と方法

拡張型心筋症の病態を呈し，心筋組織でサルコイド肉芽腫が証明された心サ症患者10例（左室縮小形成術切除心筋6例，生検心筋2例（うち1例は手術中の生検），剖検心筋2例）および年齢を一致させた特発性拡張型心筋症患者10例（左室縮小形成術施行例）を対象とした（Table 1A）．臨床的には，患者背景として，術前診断，既往歴，初発症状，病悩期間，術前NYHA心機能分類，心臓外他臓器病変の有無，心臓部ガリウムシンチグラフィ所見，胸部CT所見，心筋生検，剖検時心筋所見，術後経過（治療と予後）等の因子を検討した．また，血清中の炎症性サイトカイン濃度を測定した．基礎研究的には，定量的PCR法を用いて心筋組織における諸種サイトカインmRNAの発現解析を行った．さらに，サイトカインに対する抗体を用いた免疫組織化学的検索を行い心筋組織におけるサイトカイン蛋白の局在を検討した．本研究施行に際して，対象患者に研究目的と方法につき説明を行い承諾（インフォームドコンセント）を得た．

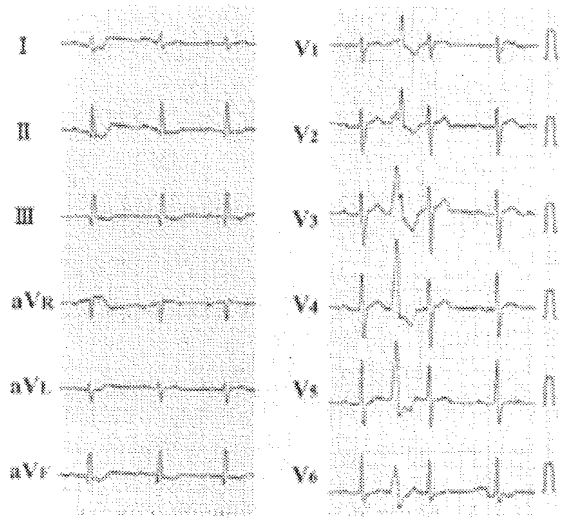


Figure 2A. Electrocardiogram at the age of 57, showing prominent Q wave and ST-T alterations in II, III, aVF, and a premature ventricular contraction.

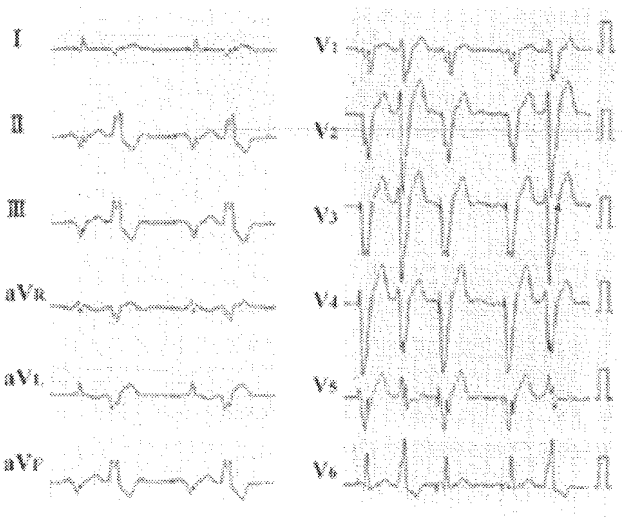
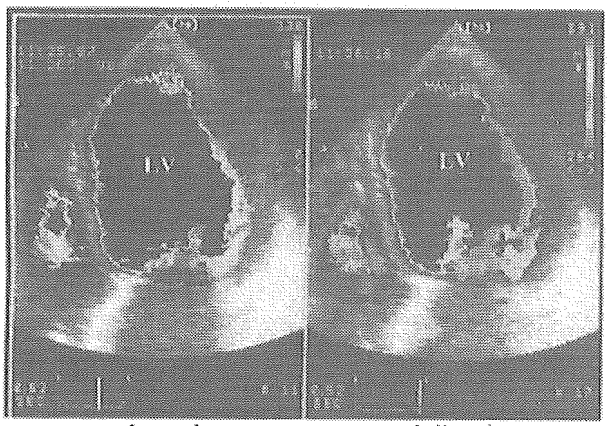
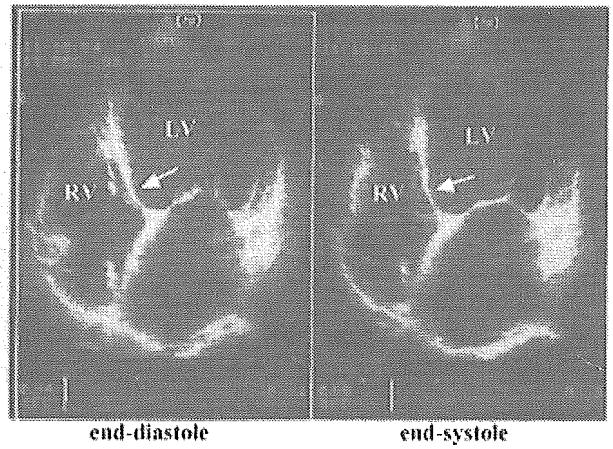


Figure 2B. Electrocardiogram at the age of 62 before left ventriculoplasty, showing basic artificial pacemaker rhythm, and frequent premature ventricular contractions.



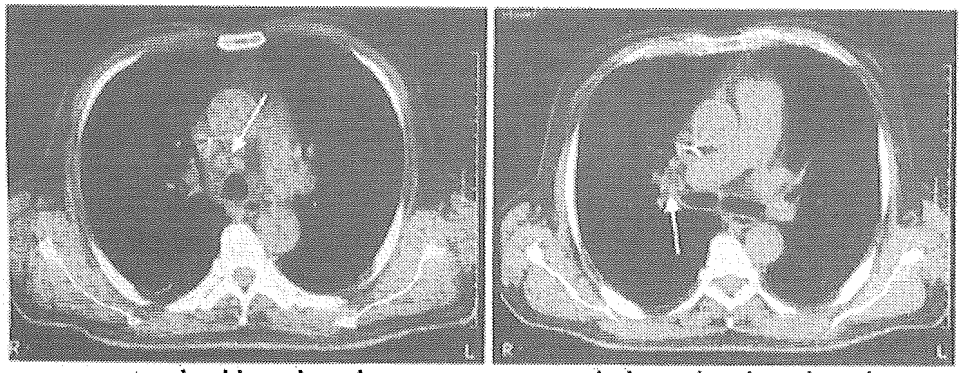
end-systole end-diastole

Figure 3A. Ultrasonic echocardiogram before left ventriculoplasty from four-chamber view under color-kinesis mode, showing severely and diffusely reduced left ventricular wall motion. (LV: left ventricle)



end-diastole end-systole

Figure 3B. Ultrasonic echocardiogram before left ventriculoplasty from four-chamber view, showing thinning of basal portion of interventricular septum (arrows). (LV: left ventricle, RV: right ventricle)



pretracheal lymph nodes main bronchus lymph nodes

Figure 4.
Computed tomography at the time of left ventriculoplasty reveals the swelling of mediastinal lymphnodes, including pretracheal and main bronchus lymphadenopathy (arrows).

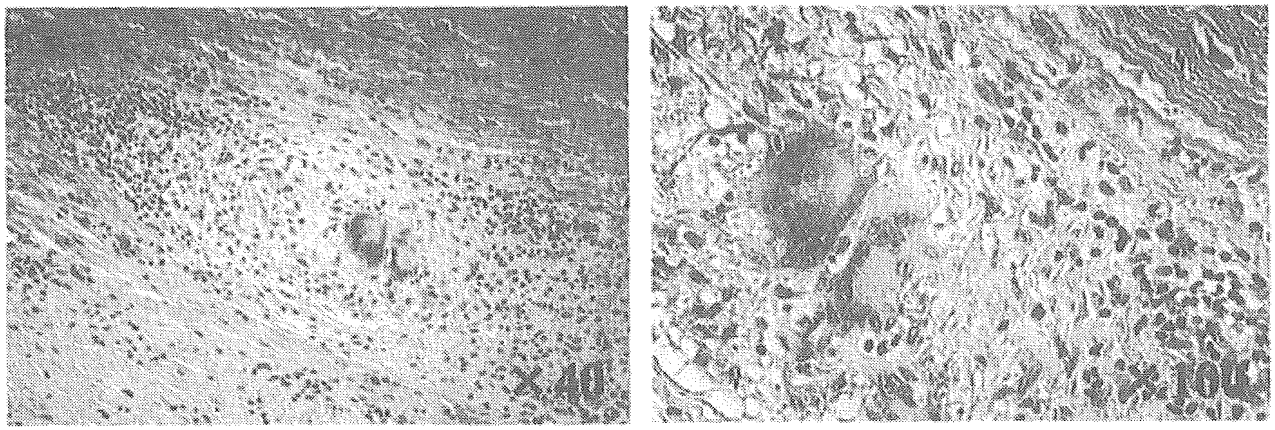


Figure 5. Histological findings of resected myocardium at left ventriculoplasty reveal noncaseous epithelioid cell granulomas including multinuclear giant cells associated with relatively severe fibrosis.

Table 1A. Clinical features of patients with cardiac sarcoidosis. Preoperative (premortem) diagnosis, duration and complications.

Case	Age	Gender	Mode of detection	Preoperative (premortem) diagnosis	Symptoms at onset	Duration of illness (years)	History of cardiac problem	
1	60	M	LVP	DCM	DOE	9	MVP (MR)	・at the time of onset
2	63	M	LVP	DCM	DOE	9	PMI (III° AVB)	・6 years after onset
3	51	F	LVP	UCM	III° AVB	8	PMI (III° AVB)	・at the time of onset
4	59	F	LVP	DCM	DOE	5	PMI (II° AVB)	・1 year after onset
5	51	F	LVP	DCM	SOB	10	LV aneurysm	・at the time of onset
6	36	M	LVP	DCM	DOE	0.2	(−)	
7	49	F	MVPL	UCM	DOE	0.3	(−)	
8	51	F	cardiac biopsy	UCM	palpitation	3	LV aneurysm	・at the time of onset
9	31	M	autopsy	cardiac sarcoidosis	DOE	0.7	(−)	
10	43	F	autopsy	DCM	DOE	1.3	(−)	

AVB: Atrioventricular block, DCM: Dilated cardiomyopathy, DOE: Dyspnea on exertion, LV: left ventricular
LVP: Left ventriculoplasty, MR: Mitral regurgitation, MVP: Mitral valve prolapse
MVPL: Mitral valvuloplasty, PMI: Permanent pacemaker implantation, SOB: Shortness of breath
UCM: Unclassified cardiomyopathy

[定量的PCR法のプロトコール]

左室縮小形成術時切除心筋の一部は直ちに凍結し，-80℃に保存，グアネジウムチオサイアネート法によるmRNAの抽出を行った．Random hexamersをプライマーとして逆転写反応を行い，ABI社提供のコントロール用 Human total RNAと切除心筋のRNAからcDNAを作成した．これらのcDNAを用いてABI社PRISM7700により11種のサイトカイン（interleukin-1α（IL-1α），IL-1β，IL-2，IL-4，IL-8，IL-10，IL-12p35，IL-12p40，IL-15，interferon-γ（IFN-γ）およびTNF-α）のmRNAについて定量的解析を行った．すなわち各々のcDNAに対して，ribosomal RNAとターゲット遺伝子に対するプライマー，プローブの組を用い，ABI社PRISM7700にて同一チューブでリアルタイムの定量的PCRを行った．心筋におけるターゲット遺伝子の発現量はコントロールRNA内のターゲット遺伝子の量に対する相対的な値として求めた[8]．

[心筋におけるサイトカインの局在]

左室縮小形成術時切除心筋を10%緩衝ホルマリンで固定した後，パラフィンに包埋した．心外膜側から心内膜側まで全層を含む切片を作成，HE染色でサルコイド肉芽腫の観察を行った．続いて，白血球表面マーカー（LCA［リンパ球］，CD45Ro［ヘルパーT細胞］，CD8［サプレッサーT細胞］，CD68［マクロファージ］；DAKO JAPAN，Tokyo，Japan）を用いて心筋組織の免疫染色を行いサルコイド肉芽腫における炎症性浸潤細胞の種類を検討した．さらに炎症性サイトカイン（IL-1β，IL-8，IL-12，IL-15，IFN-γ，TNF-α；DAKO JAPAN，Tokyo，Japan）に対する抗体を用いて免疫染色を行い心筋サルコイド肉芽腫におけるこれらサイトカインの局在を検討した．

[血清中サイトカインの測定]

心筋のサルコイド肉芽腫病変が極めて高度な3症例についてステロイド治療前に検討を行った．患者からの採血を直ちに低温状態で遠心分離し血清を-80℃で凍結保存した．炎症性サイトカインおよび関連因子としてIL-2，IL-4，IL-6，IL-8，IL-10，IL-12，IL-18，TNF-α，IFN-γ，可溶性IL-2受容体（sIL-2R）を測定した．また，ACEおよびリゾチームの測定も併せて施行した．

Table 1B. Clinical features of patients with cardiac sarcoidosis. Extracardiac organ involvements and Gallium uptake of heart.

Case	Age	Gender	Myocardial biopsy	Extracardiac involvement	Mediastinal lymphadenopathy at chest CT	Cardiac uptake at Ga scintigraphy
1	60	M	ND	(−)	(+)	ND
2	63	M	ND	(−)	(+)	ND
3	51	F	negative	eye	(+)	ND
4	59	F	ND	(−)	(−)	ND
5	51	F	positive	(−)	(+)	ND
6	36	M	ND	uncertain	ND	ND
7	49	F	ND	(−)	(+)	(−)
8	51	F	positive	(−)	(+)	(+)
9	31	M	ND	BHL(+) eye	(+)	(−)
10	43	F	negative	(−)	ND	ND

BHL: Bilateral hilar lymphadenopathy, CT: computed tomography, ND: not done

Table 1C. Clinical features of patients with cardiac sarcoidosis. Treatment and prognosis.

Case	Age	Gender	Granulomatous lesions in myocardium	Steroid therapy	Prognosis · Postoperative period · Cause of death	NYHA (pre/post operative)
1	60	M	mild	(+)	alive · 5 years	3/2
2	63	M	marked	(+)	dead · 0.2 year · heart failure	4/4
3	51	F	marked	(+)	dead · 1 year · heart failure	4/4
4	59	F	moderate	(+)	alive · 3 years	4/2
5	51	F	marked	(+)	alive · 1 year	4/2
6	36	M	marked	(+)	dead · postoperative · heart failure	4/4
7	49	F	marked	(+)	alive · 0.2 year	4/2
8	51	F	marked	(+)	alive · 4 years	3/2
9	31	M	marked	(+)	dead · 0.7 year · sudden death	4/2
10	43	F	marked	(+)	dead · 1.3 years · cerebral emboli	4/2

NYHA: New York Heart Association functional classification of cardiovascular disability

Table 2. Expression of cytokine mRNA in myocardium from patients with cardiac sarcoidosis (S) and idiopathic dilated cardiomyopathy (D).

Case	Age	Gender	IL-1 α	IL-2	IL-12p40	IFN-γ
S1	60	M	n	0.883	n	0.0429
S3	51	F	53.4	22.5	22.2	128
S4	59	F	4.38	1.34	0.865	1.67
S5	51	F	4.72	0.35	0.868	45.3
S6	36	M	1.72	n	0.591	7.24
S10	43	F	n	3.01	2.53	9.51
D1	60	M	n	n	n	n
D2	56	M	n	n	n	n
D3	56	F	n	n	n	n
D4	55	M	n	n	n	n
D5	48	F	n	n	n	n
D6	65	M	n	n	n	0.0096
D7	61	M	n	n	n	n
D8	49	M	n	n	n	n
D9	51	F	17.9	n	n	n
D10	47	M	n	n	n	n

Relative Quantification = 2^(-Average ΔΔCT).

Because the values for the calibrator wells are always substracted from themselves, their relative quantification values will always be 1.

n: negative, S: Cardiac sarcoidosis, D: Idiopathic dilated cardiomyopathy

結果

[臨床像] (Table 1A, 1B, 1C)

非虚血性拡張型心筋症の診断のもとに左室縮小形成術が施行され，術中生検1例を含め，術後初めて組織学的に心サ症の確定診断がなされた症例が7例/119例 (6.0%) (Table 1A 症例1-7) であった．

今回対象とした心サ症患者10例において心臓以外の臓器病変が臨床的に明かでない症例が9例で，その術前（生前）診断は拡張型心筋症または分類不能の心筋症であった．初発症状は多くが労作時呼吸困難等の左心不全症状で，10例中1例（症例3）が完全房室ブロックであった．また，病悩期間は0.2-10年，中高年発症で慢性進行性のものが多いが，若年発症・急性激症型で予後不良の症例が10例中2例（症例6, 9）みられた．心臓に関する既往歴，合併症としては，高度房室ブロックによる恒久的ペースメーカー植え込み術が3例，左心室瘤が2例，僧帽弁逸脱症が1例認められた（Table 1A）．

術前または生前に心筋生検が行われた症例が10例中4例でその内，後ろ向きの検討を含めて巨細胞を含む典型的な類上皮細胞肉芽腫が認められた症例は2例であった．臨床的に明かな心臓以外の臓器病変がみられたのは10例中1例（症例9）のみであった．しかし，後ろ向きに胸部CT検査を検索した結果，10例中7例に縦隔リンパ節腫大が認められた．術前にガリウムシンチグラムが施行された症例は少なく（3例），陽性例は1例（症例8）であった（Table 1B）．

診断確定後全例にステロイド治療が行われた．予後については，10例中5例が比較的早期に死亡し，死因は心不全3例，突然死1例，脳塞栓1例であった．死亡例では心筋組織病変が高度であった．生存例では，左室縮小形成術後，ステロイド治療により病状が安定し心不全症状が改善した (Table 1C)．

[心筋におけるサイトカインmRNAの定量的PCR] (Table 2)

対象とした心サ症患者10例中6例で心筋組織におけるmRNAの測定が可能であった．検索した諸種炎症性サイトカイン中，IL-1α，IL-2，IL-12p40，IFN-γ mRNAが心サ症患者心筋において発現が著明に亢進していた．IL-1β，IL-8，IL-10，TNF-α のmRNAの発現は心サ症および特発性拡張型心筋症両者の心筋に認められた．IL-4およびIL-5の発現は両疾患ともに認められなかった[9, 10]．

[サイトカイン蛋白の局在]

心筋サルコイド肉芽腫における浸潤細胞の多くはCD45Ro陽性のヘルパーT細胞およびCD68陽性の大型単核球であった．多核巨細胞はCD68陽性であった．

サイトカインの免疫組織学的検索ではIL-12およびIFN-γが心サ症患者心筋において特異的に陽性でIL-12はサルコイド肉芽腫内の大型単核球および多核巨細胞に，IFN-γの染色性が一部のリンパ球や血管壁に強く認められた[9-11]．また，肉芽腫内の大型単核球や多核巨細胞はIL-15も陽性であった（Figure 6）．特発性拡張型心筋症患者心筋でも軽度の炎症性細胞浸潤が存在するが，これらにおいてはIL-12およびIFN-γの発現はみられなかった．

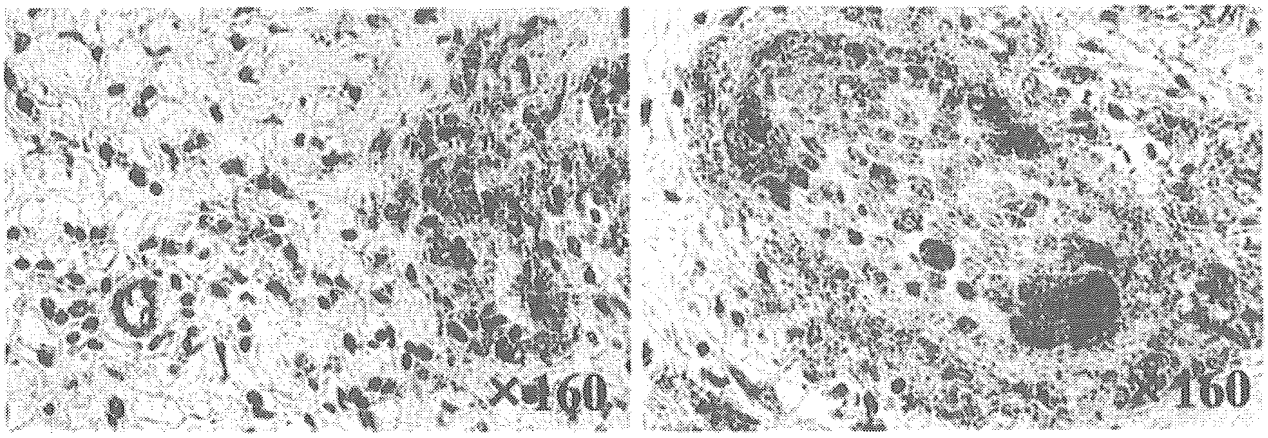


Figure 6. Immunohistochemical findings of resected myocardium at left ventriculoplasty using an anti-IL-15 antibody reveal specific positive staining in the cytosol of multinucleated giant cells and macrophages.

Table 3. Serum levels of cytokines in active cardiac sarcoidosis cases with marked granulomatous lesions in myocardium.

Cytokines	Case 5	Case 6	Case 7	Normal range
IFN-γ	<0.1	<0.1	<0.1	<0.1 IU/ml
IL-2	<0.8	<0.8	<0.8	<0.8 U/ml
IL-12	<7.8	<7.8	<7.8	<7.8 pg/ml
IL-18	162	591	295	36-258 pg/ml
S-IL-2R	869	6580	958	220-530 U/ml
IL-4	45.6	5.2	21.5	<6.0 pg/ml
IL-6	7.1	364	22.5	<4.0 pg/ml
IL-8	16.3	103	40.2	<20 pg/ml
IL-10	<2.0	16	<2.0	<5.0 pg/ml
TNF-α	<5.0	<5.0	<5.0	<5.0 pg/ml
ACE	12.9	9.3	25.2	8.3-21.4 IU/L
Lysozyme	7.4	9.7	18.9	5.0-10.2 μg/ml

[血清中サイトカインの測定] (Table 3)

心筋のサルコイド肉芽腫病変が高度な3症例（症例5-7）についてステロイド治療前の血清を用いて検討を行った．測定した血清サイトカイン中，IL-2, IL-12, IFN-γ, TNF-αは全例正常範囲であった．sIL-2RおよびIL-6は3例全例で，IL-4，IL-8，IL-18は3例中2例で，IL-10は1例で有意に増加していた．3例中1例（同一症例7）においてACEおよびリゾチームの上昇が認められた[12, 13]．

考察

[臨床像と診断]

本邦では心サ症は中高年女性に頻度が高いことが報告されている[1-3]．今回の対象症例についても同様の傾向がみられたが，比較的若年で発症し急性激症型で予後不良の症例も10例中2例（症例6，9）みられた．若年で急性発症の拡張型心筋症を診た場合にも心サ症を鑑別診断に挙げておく必要がある．

重症末期の拡張型心筋症と診断され，左室縮小形成術等の適応と考えられた患者の6%に心サ症が認められた事実は看過できないものである[14]．心サ症は頻度の高い疾患ではないが循環器内科医とくに心筋症を専門に扱う立場から心サ症に遭遇することがある．サ症患者の経過観察中に重篤な心サ症が出現する場合もあるが[15]，心臓以外の臓器病変の明らかでない場合も多いため[1, 12, 14]，確定診断に苦慮することがしばしばである．心サ症の確定診断は心筋生検で非乾酪性類上皮細胞肉芽腫を証明することにより行われるが，心病変が散在性でありサンプリングエラーが生じやすいため，巨細胞を含む典型的な肉芽腫が認められる機会は少なく心筋生検診断率は19%とする報告がある[16]．

今回，多くの症例では臨床的に明かな心臓外他臓器病変がみられなかった．胸部X線検査においても両側肺門リンパ節腫脹（BHL）が認められたのは10例中1例であった．しかし，後ろ向きに胸部CT検査を検索した結果，10例中7例に縦隔リンパ節腫大が認められた（Table 1B）．日本のサルコイドーシス剖検例の検討では，心病変を有した症例の80%以上に縦隔リンパ節腫脹が認められたとする報告があり[17]，心サ症を疑う症例では縦隔リンパ節病変の存在が診断の有力な手がかりになる可能性がある[18]．心臓のリンパ還流については未だ明かになっていない部分も多く，今後の研究課題として興味が持たれる分野である．

本研究報告では心電図の詳細な検索は行われなかったが，心サ症の診断に際して心電図異常，とくにその経時的変化を見逃さないことが極めて大切である．本症の好発部位の一つに心室中隔心基部があり[19, 20]，そのため脚ブロックや房室ブロックが重要である．高度房室ブロック89例を後ろ向きに調査した結果，10例が心サ症であったとの報告もある[21]．今回の対象患者においても10例中3例が特発性高度房室ブロックと診断され恒久的ペースメーカー植え込み術が施行されていた（Table 1A）．拡張型心筋症で高度房室ブロックを合併した場合には心サ症を強く疑う必要がある．また，心室頻拍など重症心室性不整脈も高頻度に認められるため注意が必要である．一方，明かな心電図異常がみられない場合もあり注意を要する[22]．

心エコー図は心サ症を診断する上で重要である．本研究報告の対象患者においては10例中6例がびまん性の左室壁運動低下を認め拡張型心筋症と診断されていた．しかし，10例中3例が，左室壁の菲薄を認めない，局所的壁運動低下（心室瘤）があるなどの理由で典型的な拡張型心筋症とはいえず分類不能の心筋症と診断されていた（Table 1A）．重症末期的な心サ症は拡張型心筋症の病像を呈するが，病期や重症度により心エコー所見は多彩な像を示すと考えられる．心室壁が肥厚し肥大型心筋症に類似した症例も報告

されている[23]．診断的には局所的な心室壁厚異常や心室壁運動異常（心室瘤や心室中隔の非導化など）の存在が心サ症に特異度が高く[24, 25]，中高年発症で非虚血性心筋症の病態を呈する患者でこのような所見があれば心サ症を強く疑い検査を進める必要がある．なお，Yazakiらは心サ症例と拡張型心筋症症例との心電図，心エコー図所見などを比較検討した成績を報告している[26]．

今回の対象患者は当初より心サ症を疑っていなかった症例が多いため心臓核医学検査はあまり施行されていない．全身^{67}Gaシンチグラムが施行された3例中1例が心臓部に異常集積を認め，本症例ではプレドニゾロン40mg/日連日内服から漸減して2カ月後異常集積が消失した．^{67}Gaおよびテクネシウムピロリン酸（^{99m}Tc-PYP）シンチグラムはいずれも炎症性病変に特異性が高く，活動性の評価や治療効果判定に有用と考えられるが感度は高いとは言えない[24, 25]．タリウム（^{201}Tl）シンチグラムにおける集積低下像は炎症性病変に特異性が高いとは言えないが，心サ症において比較的高頻度にみられ感度が高い検査であり[24]，心電図等に異常がみられない早期から心サ症の診断に役立つ可能性がある[27]．今後期待される画像診断検査法としては核磁気共鳴（MRI）[28, 29]と陽電子放射断層法（positron emission tomography；PET）[30]が考えられる．MRIは壁厚異常の検出に優れており，ガドリニウム（Gd-DTPA）の造影により組織性状の変化も評価できる可能性があるが，恒久的ペースメーカー植え込み後の症例は本検査は施行できない．

心サ症の診断率を向上させるために，今直ぐに何ができるかという問いにたいしては，まず，心サ症の存在を疑うこと，「分類不能の（拡張型）心筋症，高度房室ブロック，心室瘤を観たら心サ症と思え」ということ，さらに注意深く経過を観察することが最も大切と言えるかも知れない．

[心臓サルコイドーシス診断基準]

心サ症の診断に際しては，厚生省特定疾患びまん性肺疾患調査研究班で作成された「心臓サルコイドーシス診断の手引き」[31]が参考になる．しかし，明かな心電図異常がみられない症例や診断の手引きを満たさない症例も経験されるため[22]，現在，日本サルコイドーシス/肉芽腫性疾患学会および日本心臓病学会の主導のもと心臓サルコイドーシス診断基準の見直しが進められている．

[サイトカインに関する検索]

諸種のサイトカインがサ症の病因・病態に関与することが報告されているが，心サ症におけるサイトカインの実態は明らかにされていなかった．本研究により心サ症患者の心筋組織内における炎症性サイトカインの発現とその特徴が明らかになった．すなわち，特異的な炎症性サイトカインの発現亢進が認められ，その多くが1型ヘルパーT細胞（Th1）関連サイトカインであった．これらは，他の臓器（主として肺，リンパ節）における過去の報告に類似するものである．しかし，本研究の対象患者においてはTh1サイトカインの末梢血レベルは必ずしも上昇しておらず，むしろ，一部の症例でTh2サイトカインの上昇が認められた．末梢血のサイトカインレベルは心筋組織内におけるTh1優位のサイトカイン発現を必ずしも反映しないと考えられる．すなわち，Th1サイトカインは心筋局所においてautocrineおよびparacrine的に働くことが示唆される．また，全身的レベルでTh1/Th2バランスを保つために末梢血のTh2サイトカインが上昇している可能性もある．さらに，sIL-2RおよびIL-6は測定した3例全例で有意に上昇しており，とくにsIL-2Rは心筋組織内でのサルコイド肉芽腫の活動性を反映している可能性が高いと考えられる．IL-6は重症うっ血性心不全の病態を反映している可能性がある[12, 13]．

[治療と予後]

今回の対象患者においては，診断確定後10例全例ステロイド治療が行われている．しかし，予後は不良で10例中5例が比較的早期に死亡し，死因は心不全3例，突然死1例，脳塞栓1例であった．その理由は，心不全に対する内科的治療の限界を超えたため左室縮小形成術を施行された以後にステロイド治療が開始された心サ症6例を含むためと考えられる．死亡例では心筋組織病変，とくに心筋線維化が高度であった．生存例では，左室縮小形成術後，ステロイド治療により病状が安定し心不全症状が改善した（Table 1C）．

原則的に心サ症はステロイド治療の適応となる[32]．ステロイド全身投与の適応は，1）高度房室ブロック，2）心室頻拍などの重症心室不整脈，および3）局所壁運動異常あるいはポンプ機能の低下とされている．最近，本邦における心サ症の治療と予後に関する調査研究により心不全の重症度（NYHA心機能分類，左室拡張終期径，持続性心室頻拍）が予後の規定因子であることが明かにされた[33]．さらに，ステロイド療法により心サ症の予後が改善され，とくに心収縮能が保たれている期間に投与した場合の方が，より有効であることが示唆されている．従って，心サ症をより早期に発見，診断して心機能が低下する前にステロイド治療を開始することが極めて重要と考えられる．同調査によるとステロイドの投与量については，投与開始量が30mg/日以下と40mg/日以上において予後に有意の差がなかった．従って，現在投与開始量は30mg/日または60mg/日隔日投