ELSEVIER

European Journal of Pharmacology 415 (2001) 51–60

ejp

www.elsevier.nl/locate/ejphar

# Growth hormone interferes with the progression of myocarditis in rats

Zhi-ping Zong [a,*], Shinobu Matsui [b], Ai-li Li [c], Nobuo Yamaguchi [c], Shogo Katsuda [d], Mitsuru Hayase [e], Michael LX Fu [f]

[a] *Division of Basic Science, Medical Research Institute, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*
[b] *Department of Cardiology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*
[c] *Department of Serology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*
[d] *Department of Pathology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*
[e] *Department of Clinical Pathology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan*
[f] *Wallenberg Laboratory, Sahlgren's Hospital, Goteborg University, Gothenburg, Sweden*

Received 30 November 2000; received in revised form 29 January 2001; accepted 2 February 2001

**Abstract**

In this study, we investigated whether recombinant human growth hormone (rhGH) influences the progression of myocarditis. We induced experimental autoimmune myocarditis in F344 rats by subcutaneous injection of cardiac myosin, and divided the rats into three groups: (1) control group, saline injection; (2) pre-treated group, subcutaneous injection of rhGH (100 mIU/rat/day for 10 days) before induction of experimental autoimmune myocarditis; and (3) post-treated group, subcutaneous injection of rhGH (100 mIU/rat/day for 10 days) after induction of experimental autoimmune myocarditis. On the 35th day after induction of experimental autoimmune myocarditis, all rats were sacrificed and the hearts were examined. The increase in body weight was smaller in the control group than the pre-treated group and the rate of heart weight/body weight was larger in the control group than in the two treated groups. Histopathologically, rats in the control group showed multifocal infiltration by inflammatory cells, mainly neutrophils, lymphocytes and macrophages, extensive fibrosis, and a higher proportion of mast cells in the inflammed region. In contrast, rats in the two treated groups showed only minor changes. We found that rhGH did not influence the distribution of lymphocytes in peripheral blood in the three groups, and that rhGH induced G1 checkpoint dysfunction, thereby arresting the cell cycle in G1 and inhibiting the proliferation of mast cells in vitro. These findings suggest a possible role for mast cells in the progression of myocarditis and the rhGH may be a candidate for use as a new tool to treat myocarditis. 

*Keywords:* Growth hormone; Myocarditis; Mast cell

## 1. Introduction

Growth hormone (GH) is a neuroendocrine hormone secreted by acidophilic cells in the anterior pituitary. It exerts a wide range of growth-promoting effects in the body (Rudman et al., 1990). Insulin-like growth factor I (IGF-I, also known as somatomedin C) is produced and released by the liver and other tissues, and is believed to mediate most of the biological effects of GH, such as cellular proliferation and regulation of cellular metabolism (Isgaard et al., 1988, 1989; Duerr et al., 1995). Evidence is emerging that GH helps to control heart morphology and function by activating cardiac cell growth without changing the collagen content of the myocardium or the capillary density, and by inducing physiologic ventricular remodeling (Timsit et al.,1990; Merola et al., 1993). Numerous reports have indicated that GH exerts a wide variety of significant effects on the lymphohematopoietic system, for example, promoting the growth of the thymus (Kelley et al., 1986; Monroe et al., 1987) and T cell development (Mercola et al., 1981; Murphy et al., 1992; Postel-Vinay et al., 1997), and improving T cell function (Snow et al., 1981; Murphy et al., 1993) in animals using both in vitro and in vivo assays.

Myocarditis is an incompletely understood syndrome of multiple etiologies, including cardiotropic virus infection and host autoimmune reactions. It is proposed that the

* Corresponding author. Tel.: +81-76-286-2211 ext. 7211; fax: +81-76-286-2346.
*E-mail address:* zong3961@kanazawa-med.ac.jp (Z. Zong).

0014-2999/01/$ - see front matter 
PII: S0014-2999(01)00818-4

phenotypic characteristics of autoimmune myocarditis are different from those of the viral disease (Kodama et al., 1995). Some cases of myocarditis are progressive, resulting in chronic myocarditis. Accumulating evidence links chronic myocarditis with the eventual development of dilated cardiomyopathy; that is, the persistent myocardial cell damage involved in myocarditis may cause continuous destruction of contractile proteins and facilitate fibrosis, which may finally lead to dilated cardiomyopathy (Seko et al., 1995). Dilated cardiomyopathy is a multifaceted disease resulting from an insult to the ventricular myocardium and is characterized by progressive enlargement of cardiac chambers, thinning of ventricular walls, and reduced contractility. Dilated cardiomyopathy is also a prevalent cause of heart failure and sudden death, and there is no specific therapy except for cardiac transplantation.

Injection of porcine whole or rod cardiac myosin into susceptible strains of rats and mice has been shown to cause experimental autoimmune myocarditis (Inomata et al., 1995). Experimental autoimmune myocarditis, which resembles myocarditis in humans, was shown to occur in recurrent episodes, leading to dilated cardiomyopathy (Wilson et al., 1985; Kodama et al., 1994). Exogenous growth hormone (recombinant human growth hormone, rhGH) has been used in experimental animals (rats and mice), and shown to act as effectively as GH in those animals. Recently, the administration of rhGH in experimental animal models has been shown to result in a clear improvement of cardiac performance in experimental heart failure (Tajima et al., 1999). Moreover, it was reported that the administration of rhGH to patients with ischemic cardiomyopathy results in a significant improvement in hemodynamics and clinical function (Genth-Zotz et al., 1999). Using the model of experimental autoimmune myocarditis, the present study was designed to characterize the effect of rhGH on myocarditis. We show here that rhGH treatment markedly interferes with the progression of myocarditis.

## 2. Materials and methods

### 2.1. Reagents

The rhGH used in this study was kindly donated by Pharmacia-Upjohn (Stockholm, Sweden). The following monoclonal antibodies used for cytofluorometric analysis were purchased from Fujisawa Pharmaceutical: R-phycoerythrin-conjugated mouse anti-rat CD45R (for a pan B cell marker) and CD8a (for a suppressor T cell marker) monoclonal antibodies, and fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat CD4 (for a helper T cell marker) monoclonal antibody. The antibodies against rat p53, cyclin E, cdk2 and IGF-I used in this study were purchased from Wako, Tokyo. All other chemicals were of reagent grade and purchased from Sigma.

### 2.2. Experimental animals

Specific-pathogen-free male F344/NSIc rats weighing 150 g were purchased from Sankyo Laboratory, Japan. They were housed in animal quarters with controlled temperature (22–26°C), humidity (50–60%), and lighting (12-h cycle), and given free access to standard feed and water. Twenty animals were used per experimental condition. Body weights were measured at regular intervals.

### 2.3. Cell line and culture conditions

Mast cell line (RBL-2H3 cells, syngeneic to rats) (Turner et al., 1995) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories), 50 U/ml of penicillin and 50 μg/ml of streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% $CO_2$ and 95% air. The viability of the cells used in these experiments was consistently more than 95% when evaluated by the trypan blue exclusion method.

### 2.4. Induction of experimental autoimmune myocarditis

Purified cardiac myosin prepared from the muscles of porcine hearts and purchased from Sigma was used as an antigen. Experimental autoimmune myocarditis was induced as described by Inomata et al. (1995) with minor modifications. Briefly, the cardiac myosin was emulsified with complete Freund's adjuvant supplemented with Mycobacterium tuberculosis H37Ra at a concentration of 10 mg/ml. To induce myocarditis, F344 rats were injected twice subcutaneously with a 7-day interval in the rear footpads with 0.1 ml of the emulsified solution.

### 2.5. Administration of rhGH

F344 rats were randomly assigned to three groups receiving subcutaneous injection: (1) saline diluent (designated the control group), (2) rhGH at 100 mIU/rat/day for 10 days before induction of experimental autoimmune myocarditis (designated the pre-treated group), and (3) rhGH at 100 mIU/rat/day for 10 days after induction of experimental autoimmune myocarditis (designated the post-treated group).

### 2.6. Histopathologic and immunohistochemical analyses

For the histopathological study of cardiac tissue, the rats were sacrificed under ether anesthesia on day 35 after the cardiac myosin injection. The hearts were removed from the rats, weighed, sliced transversely (at least four biventricular cardiac cross-sections/heart), fixed in 10% formalin, embedded in paraffin, and stained. The extent of inflammatory cell infiltration was estimated using hema-

toxylin-eosin staining. The extent of fibrosis was estimated using Mallory Azan staining. Mast cells were visualized by toluidine blue staining and counted.

Microscopic findings were expressed in terms of myocardial infiltration and fibrosis scores. Microscopic findings of the severity of myocardial infiltration and the extent of fibrosis were graded as follows: 0 (no lesion), 1 (presence of a few small lesions, not exceeding 0.25 $mm^2$ in size), 2 (presence of multiple small lesions or a few moderately sized lesions, not exceeding 6.25 $mm^2$), and 3 (the presence of multiple moderately sized lesions or numerous, larger lesions) (Okura et al., 1998).

Microscopic scores represent the average and mast cell numbers represent the total number in the whole fields in all sections obtained from the same heart. Therefore, the microscopic scores and the mast cell numbers were defined as value/heart.

To verify the rhGH is effective in rats, rhGH was subcutaneously injected in normal rats at 100 mIU/animal/day for 10 days. The livers were removed from anesthetized rats, fixed in 10% formalin and embedded in paraffin. Immunostaining of IGF-I protein in hepatic tissues was performed according to the staining manual of the DAKO LSAB2 Kit (Dako, CA) using 40 μg/ml of anti-rat IGF-I goat immunoglobulin G1 (IgG1) monoclonal antibody. Fig. 1 shows representative photographs. Immunoreactive IGF-I protein was mainly localized round central veins of the liver (Fig. 1A). rhGH injection induced the upregulation of IGF-I protein expression (Fig. 1B), indicating that rhGH is effective in rats.

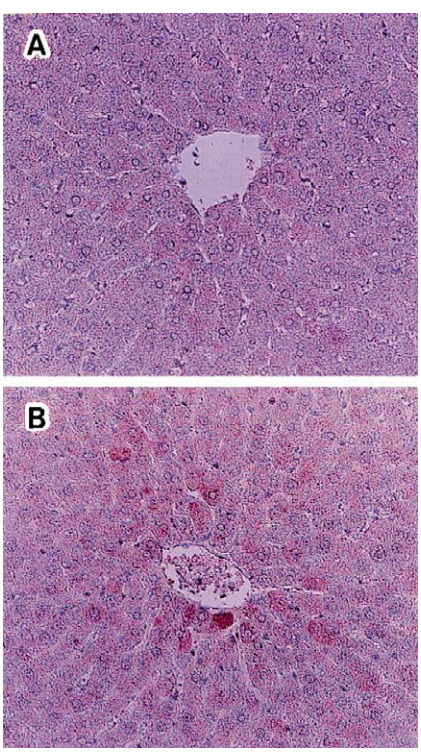


Fig. 1. Representative photographs of IGF-I expression in rat liver. rhGH was subcutaneously injected into normal rats at 100 mIU/animal/day for 10 days. The livers were removed from anesthetized rats treated without (A) or with (B) rhGH, and stained with monoclonal antibody against IGF-I protein as described in Section 2.

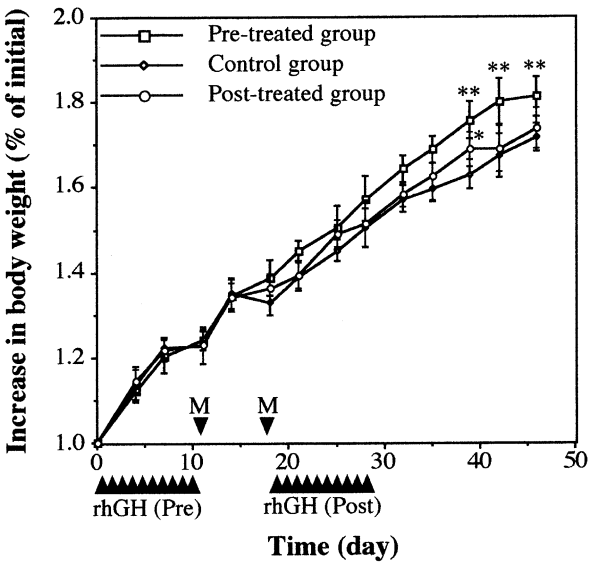


Fig. 2. Time course of rhGH effect on body weight. Pre, rhGH treatment before induction of experimental autoimmune myocarditis; M, induction of experimental autoimmune myocarditis; and Post, rhGH treatment after induction of experimental autoimmune myocarditis, as described in Section 2. Values are expressed as the mean ± S.D. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control group.

### 2.7. Immunofluorescence lymphocyte subtyping

Peripheral blood was obtained at regular intervals from anesthetized rats by cardiac puncture. The blood was placed in a 1.2% EDTA solution in phosphate buffered saline (PBS). The lymphocytes in 100 μl of blood were labeled with 20 μl of the fluorochrome-conjugated mouse anti-rat monoclonal antibodies specific for lymphocyte surface antigens (CD4, CD8a and CD45R). The lymphocytes were then incubated for 30 min and washed twice with PBS at 1500 rpm for 10 min. The tagged lymphocytes were fixed with 1% formaldehyde and stored at 4°C until they were analyzed.

Immunofluorescence from individual cells was measured with a flow cytometer (Cytofluorograf system 50H,

Table 1
Heart and body weights of experimental autoimmune myocarditis rats

| Group | BW (g) | HW (mg) | HW (mg)/BW (g) |
|---|---|---|---|
| Pre-treated group | 285 ± 7 | 847 ± 46 | 2.97 ± 0.17[a] |
| Control group | 262 ± 12 | 899 ± 74 | 3.44 ± 0.35 |
| Post-treated group | 275 ± 11 | 842 ± 57 | 3.07 ± 0.26[a] |

BW indicates body weight at the time of sacrifice; and HW, heart weight. Values are expressed as the mean ± S.D.

[a] $P < 0.01$ vs. control group.

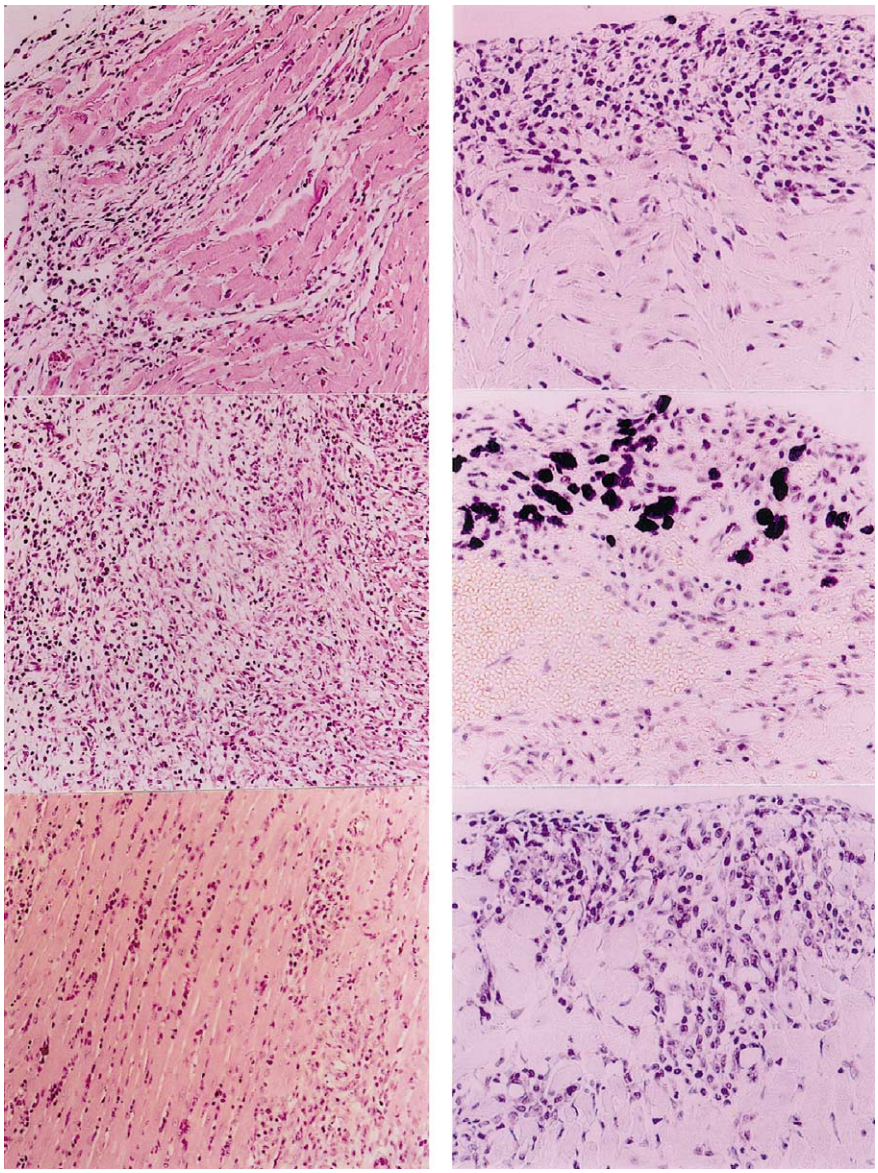

Fig. 3. Representative photomicrographs of inflammatory cell infiltration and mast cell accumulation. Upper panel, pre-treated group; middle panel, control group; and lower panel, post-treated group, as described in Section 2. Left panels are hematoxylin and eosin stained and right panels are toluidine blue stained; magnification ×250.

Ortho Instruments) and a FACSort (Becton Dickinson Immunocytometry System). At least $10^4$ lymphocytes were acquired in each run, and the results were analyzed using CELLQuest software.

### *2.8. Measurement of cellular proliferation and cell cycle analysis*

The number of cultured mast cells was determined using a Coulter counter model ZM and a Channelyzer model 256 (Coulter Electronics). The analyzer was calibrated using 9.61-μm styrene beads.

The DNA content of the mast cells was determined as described previously (Zong et al., 1996). Briefly, the cells were fixed in 70% ethanol, and then treated exhaustively with pancreatic RNase A and stained with propidium iodide (10 μg/ml in PBS). Fluorescence from individual cells was measured with a flow cytometer.

Table 2
Cardiac pathology of experimental autoimmune myocarditis rats

| Group | Mast cells/heart | Microscopic scores | |
|---|---|---|---|
| | | Infiltration | Fibrosis |
| Pre-treated group | 267 ± 91[a] | 1.85 ± 0.67[b] | 2.25 ± 0.64[a] |
| Control group | 361 ± 177 | 2.55 ± 0.69 | 2.65 ± 0.59 |
| Post-treated group | 176 ± 80[a] | 1.95 ± 0.76[a] | 2.15 ± 0.75[a] |

Values are expressed as the mean ± S.D.
[a] $P < 0.05$.
[b] $P < 0.01$ vs. control group.

The p53, cyclin E and cdk2 proteins of the mast cells were detected by indirect immunofluorescence using specific antibodies as described previously (Zong et al., 2000). Briefly, the mast cells were collected, washed in PBS, fixed with 1% paraformaldehyde for 30 min at room temperature and treated with 0.3% Triton X-100 for 10 min at 37°C. They were then incubated with the primary antibodies against p53, cyclin E or cdk2 for 24 h at 4°C. Next, they were washed in PBS containing 1% bovine serum albumin and stained with the secondary anti-IgG FITC-conjugated antibodies. Finally, the cells were washed again, resuspended in PBS containing RNase A and propidium iodide, and analyzed by flow cytometry.

### 2.9. Data analysis

All values were expressed as the mean ± S.D. Data were compared between the groups by using analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Effect of rhgh on body and heart weights

Body weight is shown in Fig. 2. The weights of all rats of the control, pre-treated and post-treated groups in-

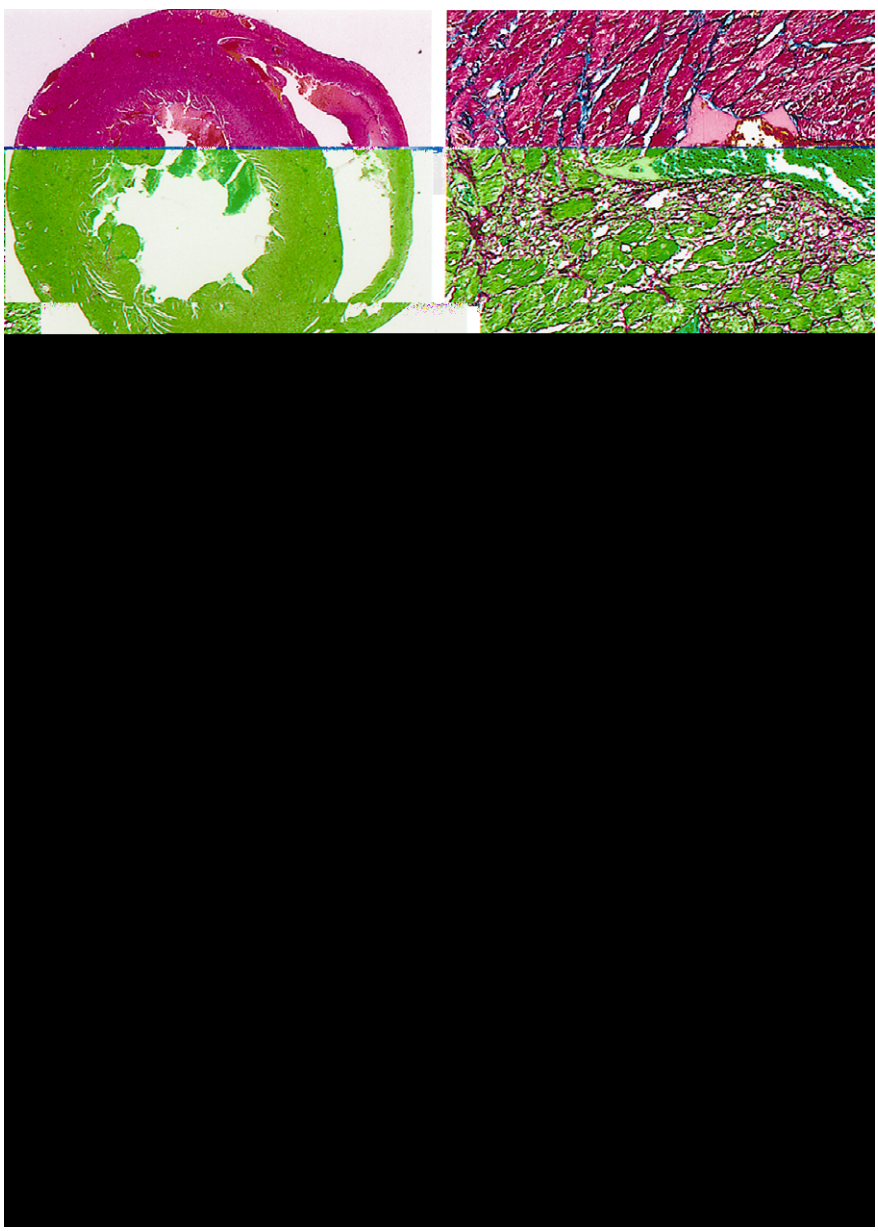

Fig. 4. Representative photomicrographs of myocardial fibrosis. Upper panel, pre-treated group; middle panel, control group; and lower panel, post-treated group, as described in Section 2. Mallory Azan staining; magnification of left panel is B ×30 and right panel is ×250.

creased until sacrifice with a mild delay appearing during the induction of experimental autoimmune myocarditis. After the induction, the rate of increase was more marked in rats of the pre-treated group than in the control and post-treated groups.

With regard to heart weight, there was a marked difference between the control group and the two treated groups (Table 1). The mean heart weight was heaviest in the control group ($899 \pm 74$ mg), and lighter in the pre-treated group ($842 \pm 57$ mg) and post-treated group ($847 \pm 46$ mg). When normalized to the body weight at the time of sacrifice, there was a marked increase in the control group ($3.44 \pm 0.35$) compared with the pre-treated group ($2.97 \pm 0.17$) and post-treated group ($3.07 \pm 0.26$). These results suggested that a more severe inflammation occurred in the hearts of the control group.

### 3.2. Histopathological findings of myocarditis

Histopathological changes in the myocardium were observed in the hearts of all rats from the three groups. Myocarditis with the characteristic features of an inflammatory cell infiltration, consisting of numerous neutrophils, lymphocytes and macrophages, was detected in all the hearts, but varied in distribution and intensity in the different groups. In the control group, severe inflammatory infiltration and edema together with myocardial necrosis were observed throughout the ventricular wall. However, in the two treated groups, the hearts showed microscopically detectable mild inflammation consisting of sporadic inflammatory foci around the vessels or scattered between the muscle fibers and small fragments of degenerated myocardial fibers. Moreover, in the area of inflammatory cell infiltration, we found that there was a more marked accumulation of mast cells, visualized by toluidine blue staining, in the control group than in the two treated groups (Fig. 3 and Table 2).

Corresponding with the intensity of inflammatory cell infiltration and mast cell accumulation, fibrosis was observed throughout the ventricular wall in the control group, but was mild in the two treated groups (Fig. 4 and Table 2). These results indicate that rhGH interferes with the progression of myocarditis and suggest that mast cells play an important role in the progression of myocarditis.

### 3.3. Characterization of the phenotypes of lymphocytes in peripheral blood

To investigate the effect of rhGH on the phenotypic distribution of lymphocytes in peripheral blood obtained on days 15, 25 and 35 (just before sacrifice) after the cardiac myosin injection, the phenotype was analyzed by flow cytometry. In the control group, CD4-positive T cells appeared as the major population and CD8-positive T cells appeared as a minor population similar to the results reported by Okura (Okura et al., 1998). We found no significant differences in the proportions of CD4-, CD8- and CD45R-positive cells in the control group compared with the two treated groups (Fig. 5). These results are not consistent with the notion that rhGH interferes with the progression of myocarditis through systemic disturbance of the immune system.

### 3.4. Effects of rhgh on cell growth, cell cycle and g1 checkpoint

For cell growth and cell cycle studies, exponentially growing mast cells in non-synchronized cultures were treated with 0, $10^{-10}$, $10^{-9}$ or $10^{-8}$ M of rhGH and then

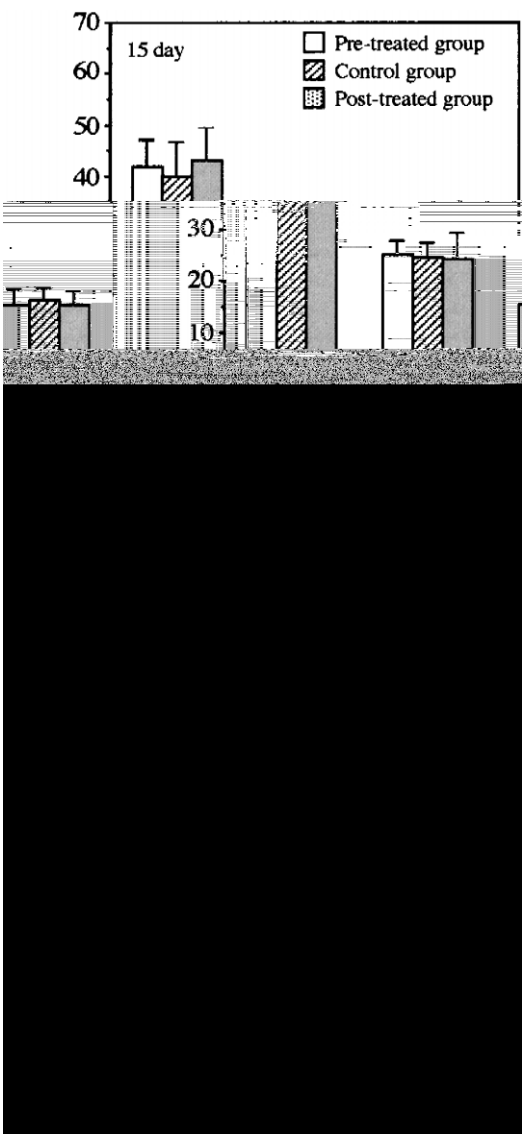


Fig. 5. Characterization of the phenotypes of lymphocytes in peripheral blood. The phenotypic distributions of lymphocytes in peripheral blood obtained respectively on days 15, 25 and 35 (just before sacrifice) after the cardiac myosin injection from the control group, pre-treated group or post-treated group were analyzed by flow cytometry as described in Section 2. Values are expressed as the mean ± S.D.

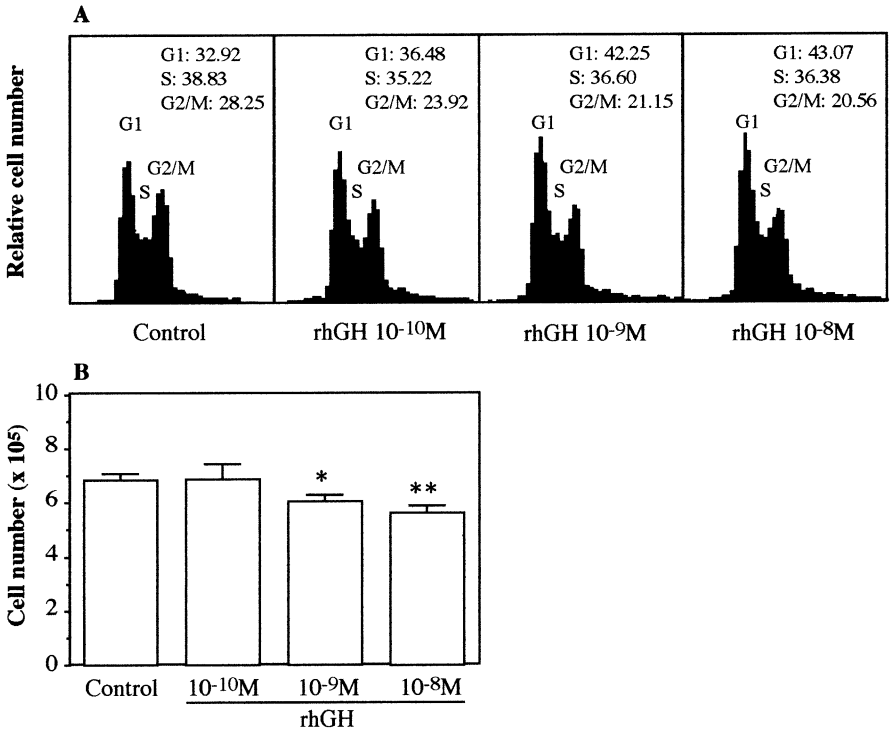


Fig. 6. Effects of rhGH on cell growth and cell cycle in mast cells. Mast cells were treated with 0, $10^{-10}$, $10^{-9}$ or $10^{-8}$ M of rhGH for 24 h, and then used to determine the distribution of DNA content by flow cytometry (A) and cell number by a Coulter counter (B) as described in Section 2. Values are expressed as the mean ± S.D. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control group.

cultivated for 24 h. As shown in Fig. 6A, treatment with rhGH increased the proportion of cells in G1 phase in a concentration-dependent manner. Cell counts show that the proliferation of mast cells was inhibited by rhGH in a concentration-dependent manner (Fig. 6B). These results indicate that rhGH inhibits the proliferation of mast cells in vitro through G1 phase arrest.

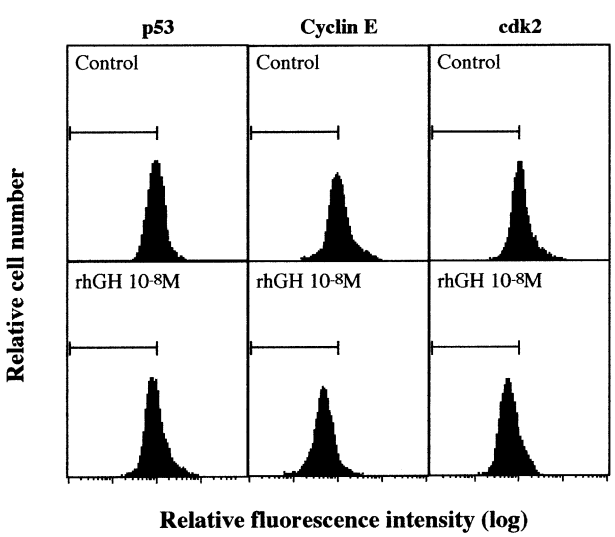


Fig. 7. Effect of rhGH on G1 checkpoint in mast cells. Mast cells were treated with 0 or $10^{-8}$ M of rhGH for 24 h, and then used to determine the expression of p53, cyclin E and cdk2 proteins in mast cells as described in Section 2.

To clarify the behaviour of the G1 checkpoint in the rhGH-induced G1 arrest, flow cytometry was used to quantify the expression of p53, cyclin E and cdk2 proteins for the G1 checkpoint. Fig. 7 shows the expression of p53, cyclin E and cdk2 proteins in mast cells subjected to flow cytometry. After exposure to $10^{-8}$ M of rhGH for 24 h, the expression of p53 protein was not significantly affected. However, the expressions of cyclin E and cdk2 proteins were significantly inhibited. These results indicate that G1 checkpoint dysfunction is a causal factor of rhGH-induced G1 phase arrest.

## 4. Discussion

As a new tool for future use in treating heart diseases, rhGH has been studied in clinical patients and laboratory animals. Several independent lines of evidence suggest that rhGH increases the amounts of IGF-I mRNA (Isgaard et al., 1988, 1989) and protein (Amato et al., 1993), increases left-ventricular-wall thickness in dilated cardiomyopathy (Fazio et al., 1996), and enhances contractile reserve in heart failure (Cuneo et al., 1989; Duerr et al., 1995; Yang et al., 1995; Beranek, 1999; Genth-Zotz et al., 1999; Ross, 1999; Tajima et al., 1999). However, effective strategies for treating myocarditis have not been established, and especially, little is known about the effect of rhGH on myocarditis. Because evidence is emerging that rhGH

interacts with the lymphohematopoietic system (Kelley, 1989) and experimental autoimmune myocarditis is a T cell-mediated autoimmune disease (Hanawa et al., 1996), we hypothesized that rhGH might influence the progression of experimental autoimmune myocarditis. To test this hypothesis, we used this model of experimental autoimmune myocarditis to observe the effects of rhGH in rats and found that rhGH markedly interfered with the progression of myocarditis. Our results showed that on day 35 after induction of experimental autoimmune myocarditis, histopathological findings of myocarditis, including inflammatory cell infiltration involving numerous neutrophils, lymphocytes and macrophages, edema, myocardial necrosis and fibrosis, were significantly more severe in the control group than in the two treated groups.

Although the specific mechanisms of actions of rhGH in myocarditis are not known, our data suggest several possible kinds of interactions. First, it is conceivable that a small population of adherent dendritic major histocompatibility complex class II-bearing resident cardiac antigen-presenting cells that constitutively express myosin peptide-bound major histocompatibility complex class II molecules play a role in the inhibitory effect of rhGH on the progression of myocarditis. In other words, it seemed possible that the prevention of myocarditis by rhGH is a direct effect on the heart, i.e., rhGH may stabilize the myosin-stimulated antigen-presenting cells and inhibit their expression of major histocompatibility complex class II antigen and antigen presentation. A series of studies has documented that major histocompatibility complex molecules normally are not expressed by human cardiac myocytes (Daar et al., 1984; Rose et al., 1986; Ahmed-Ansari et al., 1988), that human cardiac myocytes are incapable of inducing primary immune responses even when they express major histocompatibility complex molecules (Ansari et al., 1992, 1993a,b,c, 1994), and that these resident cells induce myosin-specific responses that lead to myocarditis upon immunization with myosin (Smith and Allen, 1992a,b; Suzuki, 1995; Pummerer et al., 1996). Our observations revealed that in rats of the three groups suffering from myocarditis, rhGH did not significantly influence the phenotypic distribution of helper T cells, suppressor T cells and B cells in the peripheral blood, which is compatible with the possibility described above, although the results presented here do not exclude the possibility that rhGH inhibits the progression of myocarditis via an immune response.

Second, it is assumed that the inhibition of the chemotaxis and growth of mast cells is responsible for the prevention of myocarditis by rhGH, because the present study showed that the mast cell infiltration is more marked in the control group, as is the severity of the inflammation. Recently, as master cells controlling inflammation, the role of mast cells in the effective operation of the immune response has been emphasized (Chung et al., 1986; Brown et al., 1987; Young et al., 1987; Burd et al., 1989; Plaut et al., 1989; Wodnar et al., 1989; Gordon and Galli, 1990, 1991). More recently, it has been found that mast cells are capable of producing tumor necrosis factor-a (TNF-δ), which modulates the phenotypic characteristics of fibroblasts and synthesis of extracellular matrix as well as participates in the fibrotic process (Frangogiannis et al., 1998a,b), and that TNF-δ is important for the pathogenesis of myocarditis and dilated cardiomyopathy (Shioi et al., 1996; Kubota et al., 1997; Okura et al., 1997). Hara et al. (1999) showed that mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells in vitro. In an in vitro experiment, with a short treatment time (24 h), we found that the proliferation of mast cells was significantly inhibited by rhGH and that G1 checkpoint dysfunction is a causal factor of the inhibition to arrest the cells in G1. These in vitro results indirectly support the hypothesis that the inhibitory effect of rhGH on the progression of myocarditis results from the effect of rhGH on mast cells, though it is necessary to investigate the rhGH-induced functional change of mast cells to confirm this.

This is the first study to evaluate the effects of rhGH on myocarditis. Although the mechanisms are largely unknown, it is a fact that rhGH treatment in rats suffering from experimental autoimmune myocarditis effectively interferes with the progression of myocarditis. We anticipate the administration of rhGH to be helpful in pharmacological therapy of autoimmune myocarditis and the prevention of postmyocarditis dilated cardiomyopathy.

## Acknowledgements

This study was supported in part by a Grant for Project Research from the High-Technology Center of Kanazawa Medical University (H98-1, H00-1) and Kanazawa Medical University Project Research Grants (P96-2, P98-6), Japan.

## References

Ahmed-Ansari, A., Tadros, T.S., Knopf, W.D., Murphy, D.A., Hertzler, G., Feighan, J., Leatherbury, A., Sell, K.W., 1988. Major histocompatibility complex class I and II expression by myocytes in cardiac biopsies post transplantation. Transplantation, 45, 972–978.

Amato, G., Carella, C., Fazio, S., Montagna, G.L., Cittadini, A., Sabatini, D., Marciano-Mone, C., Sacca, L., Bellastella, A., 1993. Body compositio μn, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J. Clin. Endocrinol. Metab., 77, 1671–1676.

Ansari, A.A., Wang, Y.C., Kanter, K., Naucke, N., Sell, K.W., 1992. Indirect presentation of donor histocompatibility antigens contributes to the allogeneic response against human cardiac myocytes. J. Heart Transplant., 11, 467–477.

Ansari, A.A., Neckelmann, N., Wang, Y.C., Gravanis, M.B., Sell, K.W., Herskowitz, A., 1993a. Immunologic dialogue between cardiac myocytes, endothelial cells, and mononuclear cells. Clin. Immunol. Immunopathol., 68, 208–214.

Ansari, A.A., Wang, Y.C., Kanter, K., Villinger, F., Mayne, A., Sell, K.W., Herskowitz, A., 1993b. Host T cell primary allosensitization of MHC class I- and II-expressing human cardiac myocytes requires the presence of a second signal. Hum. Immunol., 37, 108–118.

Ansari, A.A., Kanter, K., Wang, Y.C., Matne, A., Sell, K.W., Herskowitz, A., 1993c. Major histocompatibility complex-expressing human cardiac myocytes are not the direct target of host cardiac-infiltrating cells: evidence for a prominent role of the indirect pathway in human cardiac allograft rejection. Transplant. Proc., 25, 89–93.

Ansari, A.A., Sundstrom, J.B., Runnels, H., Jensen, P., Kanter, K., Mayne, A., Herskowitz, A., 1994. The absence of constitutive and induced expression of critical cell-adhesion molecules on human cardiac myocytes: its role in transplant rejection. Transplantation, 57, 942–949.

Beranek, J.T., 1999. Why growth hormone therapy is efficient in heart failure? Eur. Heart J., 20, 242–243.

Brown, M.A., Pierce, J.H., Watson, C.J., Falco, J., Ihle, J.N., Paul, W.E., 1987. B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. Cell, 50, 809–818.

Burd, P.R., Rogers, H.W., Gordon, J.R., Martin, C.A., Jayaraman, S., Wilson, S.D., 1989. Interleukin 3-dependent and-independent mast cells stimulated with IgE and antigen express multiple cytokines. J. Exp. Med., 170, 245–257.

Chung, S.W., Wong, P.M., Shen, O.G., Ruscetti, S., Ishizaka, T., Eaves, C.J., 1986. Production of granulocyte-macrophage colony-stimulating factor by Abelson virus-induced tumorigenic mast cell lines. Blood, 68, 1074–1081.

Cuneo, R.C., Wilmshurst, P., Lowy, C., McGauley, G., Sonksen, P.H., 1989. Cardiac failure responding to growth hormone. Lancet, 1, 838–839.

Daar, A.S., Fuggle, S.V., Fabre, J.W., Tung, A., Morris, P.J., 1984. The detailed distribution of HLA–ABC antigens in normal human organs. Transplantation, 38, 287–292.

Duerr, R.L., Huang, S., Miraliakbar, H.R., Clark, R., Chien, K.R., Ross, J., 1995. Insulin-like growth factor I enhances ventricular hypertrophy and function during the onset of experimental cardiac failure. J. Clin. Invest., 95, 619–627.

Fazio, S., Sabatini, D., Capaldo, B., Vigorito, C., Giordano, A., Guida, R., Pardo, F., Biondi, B., Sacca, L., 1996. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. N. Engl. J. Med., 334, 809–814.

Frangogiannis, N.G., Lindsey, M.L., Michael, L.H., Youker, K.A., Bressler, R.B., Mendoza, L.H., Spengler, R.N., Smith, W., Entman, M.L., 1998a. Resident cardiac mast cells degranulate and release preformed TNF-, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation, 98, 699–710.

Frangogiannis, N.G., Perrard, J.L., Mendoza, L.H., Burns, A.R., Lindsey, M.L., Ballantyne, C.M., Michael, L.H., Smith, W., Entman, M.L., 1998b. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation, 98, 687–698.

Genth-Zotz, S., Zotz, R., Geil, S., Voigtlander, T., Meyer, J., Darius, H., 1999. Recombinant growth hormone therapy in patients with ischemic cardiomyopathy: effects on hemodynamics, left ventricular function, and cardiopulmonary exercise capacity. Circulation, 99, 18–21.

Gordon, J.R., Galli, S.J., 1990. Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. Nature, 346, 274–276.

Gordon, J.R., Galli, S.J., 1991. Release of both preformed and newly synthesized tumor necrosis factor alpha (TNF-alpha)/cachectin by mouse mast cells stimulated via the Fc epsilon RI. A mechanism for the sustained action of mast cell-derived TNF-alpha during IgE-dependent biological responses. J. Exp. Med., 174, 103–107.

Hanawa, H., Inomata, T., Sekikawa, H., Abo, T., Kodama, M., Izumi, T., Shibata, A., 1996. Analysis of heart-infiltrating T-cell clonotypes in experimental autoimmune myocarditis in rats. Circ. Res., 78, 118–125.

Hara, M., Matsumori, A., Ono, K., Kido, H., Hwang, M.W., Miyamoto, T., Iwasaki, A., Okada, M., Nakatani, K., Sasayama, S., 1999. Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells in vitro. Circulation, 100, 1443–1449.

Inomata, T., Hanawa, H., Miyanishi, T., Yajima, E., Nakayama, S., Maita, T., Kodama, M., Izumi, T., Shibata, A., Abo, T., 1995. Localization of porcine cardiac myosin epitopes that induce experimental autoimmune myocarditis. Circ. Res., 76, 726–733.

Isgaard, J., Carlsson, L., Isaksson, O.G.P., Jansson, J.O., 1988. Pulsatile intravenous growth hormone (GH) infusion to hypophysectomized rats increases insulin-like growth factor I messenger ribonucleic acid in skeletal tissues more effectively than continuous GH infusion. Endocrinology, 123, 2605–2610.

Isgaard, J., Nilsson, A., Vikman, K., Isaksson, O.G.P., 1989. Growth hormone regulates the level of insulin-like growth factor-I mRNA in rat skeletal muscle. J. Endocrinol., 120, 107–112.

Kelley, K.W., Brief, S., Westly, H.J., Novakofski, J., Bechtel, P.J., Simon, J., Walker, E.B., 1986. GH3, pituitary adenoma ácells can reserve thymic aging in rats. Proc. Natl. Acad. Sci. U. S. A., 83, 5663–5667.

Kelley, K.W., 1989. Growth hormone, lymphocytes and macrophages. Biochem. Pharmacol., 35, 705–713.

Kodama, M., Hanawa, H., Saeki, M., Hosono, H., Inomata, T., Suzuki, K., Shibata, A., 1994. Rat dilated cardiomyopathy after autoimmune giant cell myocarditis. Circ. Res., 75, 278–284.

Kodama, M., Zhang, S.S., Hanawa, H., Saeki, M., Inomata, T., Suzuki, K., Koyama, S., Shibata, A., 1995. Effects of 15-deoxyspergualin on experimental autoimmune giant cell myocarditis of the rat. Circulation, 91, 1116–1122.

Kubota, T., McTiernan, C.F., Frye, C.S., Slawson, S.E., Lemster, B.H., Koretsky, A.P., Demetris, A.J., Feldman, A.M., 1997. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-a. Circ. Res., 81, 627–635.

Mercola, K.E., Cline, M.J., Golde, D.W., 1981. Growth hormone stimulation of normal and leukemic human T-lymphocyte proliferation in vitro. Blood, 58, 337–340.

Merola, B., Cittadini, A., Colao, A., Longobardi, S., Fazio, S., Sabatini, D., Sacca, L., Lombardi, G., 1993. Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J. Clin. Endocrinol. Metab., 77, 1658–1661.

Monroe, W.E., Roth, J.A., Grier, R.L., Arp, L.H., Naylor, P.H., 1987. Effects of growth hormone on the adult canine thymus. Thymus, 9, 173–187.

Murphy, W.J., Durum, S.K., Longo, D.L., 1992. Role of neuroendocrine hormones in murine T cell development: growth hormone exerts thymopoietic effects in vivo. J. Immunol., 149, 3851–3857.

Murphy, W.J., Durum, S.K., Longo, D.L., 1993. Differential effects of growth hormone and prolactin on murine T cell development and function. J. Exp. Med., 178, 231–236.

Okura, Y., Yamamoto, T., Goto, S., Inomata, T., Hirono, S., Hanawa, H., Feng, L.L., Wilson, C.B., Kihara, I., Izumi, T., Shibata, A., Aizawa, Y., Seki, S., Abo, T., 1997. Characterization of cytokine and iNOS mRNA expression in situ during the course of experimental autoimmune myocarditis in rats. J. Mol. Cell. Cardiol., 29, 491–502.

Okura, Y., Takeda, K., Honda, S., Hanawa, H., Watanabe, H., Kodama, M., Izumi, T., Aizawa, Y., Seki, S., Abo, T., 1998. Recombinant murine interleukin-12 facilitates induction of cardiac myosin-specific type 1 helper T cells in rats. Circ. Res., 82, 1035–1042.

Plaut, M., Pierce, J.H., Watson, C.J., Hanley, H.J., Nordan, R.P., Paul, W.E., 1989. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. Nature, 339, 64–67.

Postel-Vinay, M.C., Coelho, V.M., Gagnerault, M.C., Dardenne, M., 1997. Growth hormone stimulates the proliferation of activated mouse T lymphocytes. Endocrinology, 138, 1816–1820.

Pummerer, C.L., Grassl, G., Sailer, M., Bachmaier, K.W., Penninger,

J.M., Neu, N., 1996. Cardiac myosin-induced myocarditis: target recognition by autoreactive T cells requires prior activation of cardiac interstitial cells. Lab. Invest., 74 845–852.

Rose, M.L., Coles, M.I., Griffin, R.J., Pomerance, A., Yacoub, M.H., 1986. Expression of class I and II major histocompatibility antigens in normal and transplanted human heart. Transplantation, 41, 776–780.

Ross, J., 1999. Growth hormone, cardiomyocyte contractile reserve, and heart failure. Circulation, 99, 15–17.

Rudman, D., Feller, A.G., Nagraj, H.S., Gergans, G.A., Lalitha, P.Y., Goldberg, A.F., Schlenker, R.A., Cohn, L., Rudman, I.W., Mattson, D.E., 1990. Effects of human growth hormone in men over 60 years old. N. Engl. J. Med., 323, 1–6.

Seko, Y., Ishiyama, S., Nishikawa, T., Kasajima, T., Hiroe, M., Kagawa, N., Osada, K., Suzuki, S., Yagita, H., Okumura, K., Yazaki, Y., 1995. Restricted usage of T cell receptor Va–Vb genes in infiltrating cells in the hearts of patients with acute myocarditis and dilated cardiomyopathy. J. Clin. Invest., 96, 1035–1041.

Shioi, T., Matsumori, A., Sasayama, S., 1996. Persistent expression of cytokine in the chronic stage of viral myocarditis in mice. Circulation, 94, 2930–2937.

Smith, S.C., Allen, P.M., 1992a. Expression of myosin-class II major histocompatibility complexes in the normal myocardium occurs before induction of autoimmune myocarditis. Proc. Natl. Acad. Sci. U. S. A., 89, 9131–9135.

Smith, S.C., Allen, P.M., 1992b. Neutralization of endogenous tumor necrosis factor ameliorates the severity of myosin-induced myocarditis. Circ. Res., 70, 856–863.

Snow, E.C., Feldbush, T.L., Oaks, J.A., 1981. The effect of growth hormone and insulin upon MLC responses and the generation of cytotoxic lymphocytes. J. Immunol., 126, 161–164.

Suzuki, K., 1995. A histological study on experimental autoimmune myocarditis with special reference to initiation of the disease and cardiac dendritic cells. Virchows Arch., 426, 493–500.

Tajima, M., Weinberg, E.O., Bartunek, J., Jin, H., Yang, R., Paoni, N.F., Lorell, B.H., 1999. Treatment with growth hormone enhances contractile reserve and intracellular calcium transients in myocytes from rats with postinfarction heart failure. Circulation, 99, 127–134.

Timsit, J., Riou, B., Bertherat, J., Wisnewsky, C., Kato, N.S., Weisberg, A.S., Lubetzki, J., Lecarpentier, Y., Winegrad, S., Mercadier, J.J., 1990. Effects of chronic growth hormone hypersecretion on intrinsic cotractility, energetics, isomyosin pattern, and myosin adenosine triphosphatase activity of rat left ventricle. J. Clin. Invest., 86, 507–515.

Turner, H., Reif, K., Rivera, J., Cantrell, D.A., 1995. Regulation of the adapter molecule Grb2 by the Fc epsilon R1 in the mast cell line RBL2H3. J. Biol. Chem., 270, 9500–9506.

Wilson, M.S., Barth, R.F., Baker, P.B., Unverferth, D.V., Kolibash, A.J., 1985. Giant cell myocarditis. Am. J. Med., 79, 647–652.

Wodnar, F.A., Heusser, C.H., Moroni, C., 1989. Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature, 339, 150–152.

Yang, R.H., Bunting, S., Gillett, N., Clark, R., Jin, H.K., 1995. Growth hormone improves cardiac performance in experimental heart failure. Circulation, 92, 262–267.

Young, J.D., Liu, C.C., Butler, G., Cohn, Z.A., Galli, S.J., 1987. Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. Proc. Natl. Acad. Sci. U. S. A., 84, 9175–9179.

Zong, Z.P., Fujikawa-Yamamoto, K., Tanino, M., Teraoka, K., Yamagishi, H., Odashima, S., 1996. Saikosaponin b2-induced apoptosis of cultured B16 melanoma cell line through down-regulation of PKC activity. Biochem. Biophys. Res. Commun., 219, 480–485.

Zong, Z.P., Fujikawa-Yamamoto, K., Li, A.L., Yamaguchi, N., Chang, Y.G., Murakami, M., Ishikawa, Y., 2000. Involvement of protein kinase C in taxol-induced polyploidization in a cultured sarcoma cell line. Eur. J. Pharmacol., 394, 181–187.