Department of Microbiology and Immunology, Faculty of Pharmacy, Belgrade, Serbia

Fusidin ameliorates experimental autoimmune myocarditis in rats by inhibiting TNF-α production

M. Milenković, N. Arsenović-Ranin, D. Vučićević, B. Bufan, Z. Stojić-Vukanić

Received October 20, 2006, accepted November 27, 2006

Marina Milenković, M.D., Ph.D., Department of Microbiology and Immunology, Faculty of Pharmacy, 450 Vojvode Stepe, 11221 Belgrade (Kumodraž), Serbia
marinama@pharmacy.bg.ac.yu

Pharmazie 62: 445–448 (2007) *doi: 10.1691/ph.2007.6.6739*

Experimental autoimmune myocarditis (EAM) represents a model for human autoimmune myocarditis, a condition for which no optimal treatment is currently available. It has been reported that tumor necrosis factor-α (TNF-α) plays a crucial role in pathogenesis of EAM. The immunomodulating antibiotic fusidic acid and its sodium salt (sodium fusidate-fusidin) were previously shown to reduce TNF-α production and its end-organ cytotoxicity, thus proving beneficial in several animal models of organ-specific autoimmune diseases. To investigate the effects of fusidin on EAM the drug was given at dose 80 mg/kg i.m. to EAM rats. Fusidin was administered as an early, from day 0 to 10, or late treatment, from day 10 to 21, after induction of disease. Both early and late treatment with fusidin markedly ameliorated the clinical and histological signs of the disease. Fusidin-treated rats had significantly decreased blood levels of TNF-α compared with vehicle-treated animals. Similarly, TNF-α production by *in vitro* sensitized lymph node cells in both fusidin treated groups was significantly lower than that in EAM rats. The present findings suggest that fusidin ameliorated EAM, at least partly, through an inhibitory action on the secretion of TNF-α.

1. Introduction

Myocarditis, inflammation of the heart, is characterized by cardiomyocyte necrosis and degeneration with mononuclear cell infiltration in the presence or absence of fibrosis (Pisani et al. 1997). The pathogenesis of myocarditis is not fully understood, but there is substantial evidence suggesting that autoimmune responses to heart antigens, particularly cardiac myosin, following viral infection may contribute to the disease process (Lauer et al. 1994).

Experimental autoimmune myocarditis (EAM) in rodents may be elicited by immunization with cardiac myosin. In rats, EAM is evoked 2 weeks after antigen injection, reaches its peak around day 19, gradually subsides between days 25 and 40, and is followed by dilated cardiomyopathy (Kodama et al. 1994; Dimitrijević et al. 1998). EAM is histologically similar to human fulminant myocarditis in the acute phase and dilated cardiomyopathy (DCM) in the chronic phase. We chose EAM as an animal model because of the single pathogenic process (autoimmune) of the disease compared with viral myocarditis, where primary direct infectious and subsequent autoimmune processes might be involved in the disease development, and, therefore, the pathogenic process might be complex. According to several studies, the inflammatory process of EAM is induced by a cellular immune rather than by a humoral immune reaction, because the disease is adoptively transferable into naive rats by T cells, not by antibodies (Kodama et al. 1992; Okura et al. 1998; Smith and Allen 1991).

Proinflammatory cytokine tumor necrosis factor-α (TNF-α is exclusively detected only during the maximum inflammatory phase (Okura et al. 1997). This cytokine probably plays an important role in the induction of EAM (Smith and Allen 1992).

Fusidic acid and its sodium salt (fusidin) have been shown to have immunomodulatory properties *in vitro* and *in vivo* (Christiansen 1999). In many inflammatory diseases fusidin exhibits powerful inhibitory effects on TNF-α production/release (Bendtzen et al. 1990; Chen et al. 1994; Nicoletti et al. 1995; Genovese et al. 1996; Nicoletti et al. 1997).

Recently we have reported beneficial effects of fusidin on EAM in rats but the mechanism of the improvement in both clinical and histological parameters remains unclear (Milenković et al. 2005). Having in mind the crucial role of TNF-α in pathogenesis of EAM in the present study we investigated whether fusidin ameliorates EAM by affecting TNF-α production.

2. Investigations and results

2.1. Clinical and histological analysis

Dark Agouti rats were divided into four groups: group A, EAM rats treated with fusidin during the first 10 days; group B, EAM rats treated with fusidin from day 10 to 21; group C, EAM rats treated with vehicle; and group D, healthy control rats. No rats died prior to day 21. On day 21 all rats of group C showed typical features of myocarditis: the hearts were enlarged and contained large grayish

Table: Effects of fusidine on EAM in DA rats

	Group A (n = 8)	Group B (n = 8)	Group C (n = 8)	Group D (n = 8)
Disease incidence (%)	25.0	37.5	100.0	0.0
Heart weight/body weight	$2.95 \pm 0.44^{***}$	$3.47 \pm 0.19^{**}$	3.75 ± 0.14	2.75 ± 0.20
Macroscopic score	$0.2 \pm 0.4^{***}$	$1.0 \pm 0.7^{*}$	2.0 ± 0.6	0.0
Microscopic score	$0.6 \pm 0.5^{***}$	$1.5 \pm 0.5^{**}$	3.0 ± 0.7	0.0

Values are expressed as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to group C

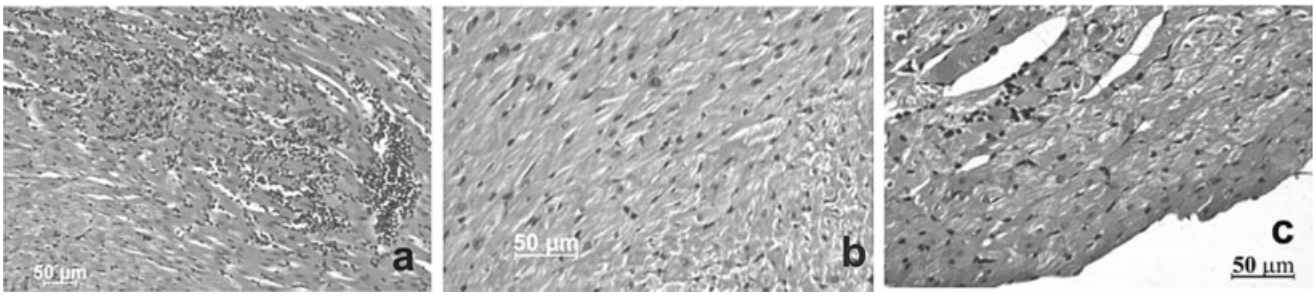

Fig. 1: Histopathology and the effects of fusidin treatment in rats with EAM. a) Representative histopathology of a rat with myocarditis treated with vehicle. Inflammatory infiltrate is shown; b) Representative histopathology of a rat with myocarditis treated with fusidin from day 0 to 10 (group A); c) A small focus of perivascular cellular infiltration in heart of a rat with myocarditis treated with fusidin from day 10 to 21 (group B). Hematoxylin and eosin staining, original magnification: x160 (a), x250 (b and c)

areas and massive pericardial effusion. The hearts of group B were slightly enlarged and showed small grayish areas on the surface. Only few grayish areas in the hearts of group A were detected. The heart weight/body weight ratio of groups A and B were significantly lower than that of group C (Table).

Histological analysis was performed to evaluate inflammatory infiltration in the heart on day 21 after immunization. Severe inflammatory lesions were observed in the hearts of group C rats (mean microscopic score 3.0). These lesions were composed of extensive myocardial necrosis and showed infiltration by mononuclear cells (Fig. 1a). In contrast, hearts of group B rats showed little perivascular infiltration by inflammatory cells (Fig. 1c), while in the hearts of group A cardiac myocytes were well preserved (Fig. 1b) and only few inflammatory cells were detected. In both fusidin treated groups microscopic scores were significantly decreased in comparison with group C. Taken together, 25% and 37.5% of animals developed myocarditis when they were treated from day 0 to 10 and from day 10 to day 21 after induction of the disease, respectively (Table). Fusidin was well tolerated and the only side-effect noted was a mild inflammatory reaction occuring at the site of injection.

2.2. The effects of fusidin on lymphocyte proliferation

Lymphocytes obtained from the early fusidin (group A) and late the fusidin (group B) and the vehicle (group C) animals displayed similar proliferation following stimulation with the non-specific mitogen Con A (mean OD of 0.18 ± 0.03, 0.18 ± 0.01 and 0.25 ± 0.02, respectively). Similarly, proliferation of lymphocytes upon *in vitro* myosin stimulation did not differ significantly between group A (mean OD of 0.17 ± 0.02), group B (0.18 ± 0.02) or group C (0.19 ± 0.01).

2.3. The effects of fusidin on TNF-α production

Since fusidin has previously been demonstrated to influence the secretion of TNF-α (Bendtzen et al. 1990; Chen et al. 1994; Nicoletti et al. 1995; Genovese et al. 1996; Nicoletti et al. 1997), we next studied the effects of fusidin-treatment on the TNF-α production *in vivo* and *in vitro*. Because the blood level of TNF-α was below the limit of sensitivity of the assay (5 pg/ml) we injected rats with Con A. By doing so an *in vivo* activation of T cells and macrophages was elicited which caused the release of TNF-α into the circulation. In rats this occurs within 2–6 h (Di Marco et al. 1999). As shown in Fig. 2, serum levels of TNF-α in both the fusidin treated groups were significantly lower than that in vehicle treated animals. In culture supernatants TNF-α levels were also significantly decreased in both fusidin treated groups compared with the vehicle-treated group regardless which stimulators were used: Con A or myosin (Fig. 3).

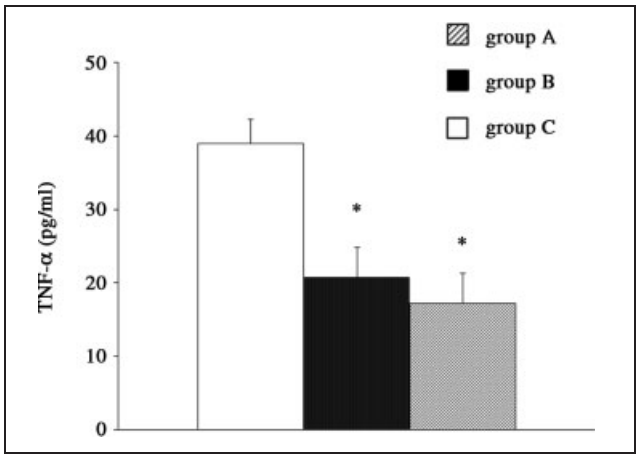

Fig. 2: The effect of fusidin on blood levels of TNF-α. On day 21 after immunization, fusidin and vehicle-treated rats were challenged with 20 mg/kg of ConA and serum levels of TNF-α were measured 6 h after ConA-challenge. Results are expressed as mean values ± SD. For statistical analysis each group was compared to vehicle-treated rats by Mann-Whitney U-test. * $p < 0.05$

3. Discussion

In this study we demonstrated that fusidin markedly ameliorated autoimmune myocarditis, as evidenced by reduc-

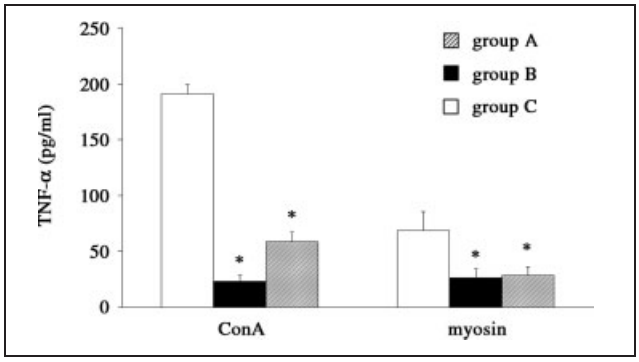

Fig. 3: The effect of fusidin on TNF-α in culture supernatants of Con A- and myosin-primed lymph node cells. Results are expressed as mean values ± SD. For statistical analysis each group was compared to vehicle-treated rats by Mann-Whitney U-test. * $p < 0.05$

tion in cardiac hypertrophy and the incidence and severity of inflammation and necrosis. The effect was associated with inhibition of TNF-α secretion.

Tumor necrosis factor-α is a proinflammatory cytokine produced principally by monocyte/macrophages and T lymphocytes (Beutler and Cerami 1989). Several clinical studies have described the participation of TNF-α in the pathogenesis of cardiac diseases. Increased plasma concentration and myocardial expression of TNF-α have been reported in patients with myocarditis and cardiomyopathy (Hegewisch et al. 1990; Toore-Amione et al. 1996), and TNF-α has been found to contribute to myocardial injury and dysfunction (Bozkurt et al. 1998; Bryant et al. 1998). In EAM, TNF-α is also expressed in myocarditic hearts, and its functional blockade or production results in attenuation of cardiac inflammation (Okura et al. 1997; Smith and Allen 1999; Bachmaier et al. 1997; Ishiyama et al. 1999). Administration of TNF-α promoted the aggravation of coxackievirus B3-induced myocarditis in the virus resistant mice (Lane et al. 1992). It was therefore demonstrated that the suppression of TNF-α has beneficial effects in ameliorating acute myocarditis (Furukawa et al. 2001; Shioji et al. 2001; Kishimoto et al. 2001).

In recent years, the potential use of fusidin as an antiinflammatory drug has gained increasing interest because *in vivo* studies have shown that fusidin reduces the production of TNF-α, and beneficial effects have been reported in TNF-α-mediated disorders such as septic shock, insulin-dependent diabetes mellitus, experimental allergic encephalomyelitis, experimental allergic neuritis, and dinitrobenzenesulfonic acid-induced colitis in rats (Nicoletti et al. 1995; Genovese et al. 1996; Di Marco et al. 1999, 2001, 2003). These results are consistent with our data which show that treatment of EAM rats with fusidin, early as well as late, led to a significant reduction of TNF-α levels in blood in comparison to EAM rats treated with vehicle. Also, both Con A- and myosin-induced *in vitro* TNF-α productions were diminished in both fusidin-treated groups. Accordingly, the beneficial effects of fusidin in EAM may be partly due to the suppression of TNF-α.

The molecular mechanisms of suppression of TNF-α are not fully understood. However, an inhibition at the post-transcriptional level has been postulated (Di Marco et al. 1999).

Although we have proposed a mechanism of the effect of fusidin based on the findings of the present study, there may be other explanations. For example, in other inflammatory diseases fusidin has been found to downregulate Th1 cytokine production and upregulate some Th2 type anti-inflammatory cytokines (Di Marco et al. 1999, 2001, 2003). Th1 cytokines are believed to play a pivotal role in EAM pathogenesis, and the transition of Th1/Th2 cytokine balance to Th2 cytokines could reduce myocardial inflammation. Thus, another possibility for suppression of EAM by fusidin could be its ability to induce prevalence of Th2 over Th1 cytokines. This assumption warrants further investigation.

Effective strategies for treating myocarditis have not yet been established (Mason et al. 1995). A principal disadvantage of the current therapeutic management of human acute myocarditis is general immune suppression by non-selective agents such as steroids and cytotoxic drugs (Feldman et al. 2000). Since we have found that fusidin had no effect on the proliferative capacity of lymphocytes stimulated by Con A, it is likely that cellular immunity was not altered.

In conclusion, this study confirmed our previous finding of fusidin as ameliorating agent of EAM in rats, and furthermore showed that beneficial effects were associated with a reduction of TNF-α. Although additional mechanisms could mediate the favorable effects of fusidin, it may be worth to further investigate this drug as a potential treatment modality in autoimmune myocarditis.

4. Experimental

4.1. Animals

Male, 8 weeks of age Dark Agouti (DA) rats were purchased from the Military Medical Academy Animal House, Belgrade, and were maintained in a local animal house under conventional conditions. Throughout the studies, the protocol for these experiments was approved by the Institutional Animal Care and Use Commitee.

4.2. Induction of experimental autoimmune myocarditis

Rats were immunized twice (on days 0 and 7), subcutaneously with 1 mg of porcine cardiac myosin (Sigma) mixed with an equal volume of Freund's complete adjuvant (FCA) supplemented with 10.0 mg/ml of heat-killed *Mycobacterium tuberculosis* R37a (Difco) (Okura et al. 1997; Kodama et al. 1990).

4.3. Treatment with sodium fusidate (fusidin)

Rats immunized with cardiac myosin were divided into three groups. Group A ($n = 8$) was treated (i.m.) with 80 mg of sodium fusidate (Sigma, St. Louis MO, USA) per kilogram of body weight, starting from day 0 to day 10 (early treatment group) while group B ($n = 8$) was treated from day 10 to day 21 (late treatment group) in relation to cardiac myosin injection. The dose of fusidin was chosen on the base of our previous study (Milenković et al. 2005). The third group C, ($n = 8$) i.m. received vehicle. The other six DA rats (group D), received neither immunization nor sodium fusidate therapy, were used as normal controls. All rats were sacrificed under ether anesthesia on day 21 after immunization.

4.4. Histopathology

Macroscopic findings were graded according to the following criteria: 0 (normal appearance), 1 (a focal discolored area), 2 (multiple or diffuse discolored areas not exceeding 1/3 of the heart), 3 (diffuse discolored areas not exceeding 2/3 of the heart) and 4 (diffuse discolored areas exceeding 2/3 of the heart). After the heart weight was measured, the ratio of heart weight/body weight (Hw/Bw) was evaluated. Then the heart was fixed in 10% buffered formalin, embedded in paraffin, sectioned into 4 μm slices, and stained with hematoxylin-eosin staining for histological examination. Microscopic findings were graded: 0 (normal), 1 (lesion extent not exceeding 1% of a transverse section), 2 (not exceeding 10%), 3 (not exceeding 50%), and 4 (exceeding 50%). We measured the lesion area using a square lattice scale in an eye lens of a microscope. Three sections were obtained from one heart, and the mean score of the 3 sections was recorded as the microscopic score. Two observers scored the histopathological scores blindly.

4.5. TNF-α assay

To evaluate the effects of the fusidin treatment on TNF-α secretion *in vivo*, on day 21 after immunization four animals from each groups were injected with 20 mg/kg b.w. of concanavalin A (Con A) (Sigma-Aldrich Chemie

Gmb H, Taufkirchen, Germany) and 6 h later blood samples were collected. The serum levels of TNF-α were measured by Quantikine ELISA (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

4.6. Lymphocyte proliferation assay

Lymph node (LN) cells were harvested and single-cell suspensions were obtained by passing through a stainless steel mesh screen. Cells were suspended in RPMI-1640 (Sigma-Aldrich Chemie Gmb) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco, Grand Island, N.Y. USA), 2 mM L-glutamine (Serva, Heidelberg, Germany), 1 mM sodium pyruvate (Serva), 100 units/ml penicillin (ICN, Costa Mesa, CA, USA) and 100 μg/ml streptomycin (ICN).

Lymphocyte proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay (Mosmann 1983). Initial experiments showed equivalent results using the MTT colorimetric assay or [^{3}H] thymidine incorporation in this system. For the proliferation assay, 2×10^5 cells/well (100 μl) were dispersed into plastic 96-well plates (Nunc A/S) and cultured for 72 h at 37 °C in a 5% CO_2 humified air atmosphere without or with 2.5 μg/ ml Con A, myosin (1 μg/ml) and fusidin (30 μg/ml) in a total volume of 200 μl complete RPMI 1640 culture medium. All cultures were run in triplicate. After 72 h, 20 μl MTT solution were added to each well. Cells were incubated for a further 4 h at 37 °C in 5% CO_2, then the medium was removed and the reaction was stopped with 100 μl 0.1 N HCl in phosphate-buffered saline (PBS) with 10% SDS. Optical densities (OD) were read at 580 nm.

4.7. Statistical analysis

Data are presented as mean ± standard deviation (SD). To assess the significance of differences between means, the Mann-Whitney U test was applied using the program SPSS 10.0 for Windows. $P < 0.05$ was considered significant.

References

Bachmaier K, Pummerer C, Kozieradzki I, Pfeffer K, Mak TW, Neu N, Penninger JM (1997) Low-molecular-weight tumor necrosis factor receptor p55 controls induction of autoimmune heart disease. Circulation 95: 655–661.

Bendtzen K, Diamant M, Faber V (1990) Fusidic acid, an immunosuppressive drug with functions similar to cyclosporine A. Cytokine 2: 423–429.

Beutler B, Cerami A (1989) The biology of cachectin/TNF-α primary mediator of the host response. Ann Rev Immunol 7: 625–655.

Bozkurt B, Kribbs B, Clubb FJ Jr, Michael LD, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL (1998) Pathophysiologically relevant concentrations of tumor necrosis factor-α promote progressive left ventricular dysfunction and remodeling in rats. Circulation 97: 1382–1391.

Bryant D, Becker L, Richardson J, Shelton J, Franco F, Peshock R, Thompson M, Giroir B (1998) Cardiac failure in transgenic mice with myocardial expression of tumor necrosis factor alpha. Circulation 97: 1375–1381.

Chen YL, Le Vraux V, Giroud JP, Chauvelot-Moachon L (1994) Anti-tumour necrosis factor properties of non-peptide drugs in acute-phase responses. Eur J Pharmacol 71: 319–327.

Christiansen K (1999) Fusidic acid non-antibacterial activity. Int J Antimicrob Agents 12: S73–78.

Di Marco R, Khademi M, Wallstrom E, Muhallab S, Nicoletti F, Olsson T (1999) Amelioration of experimental allergic neuritis by sodium fusidate (fusidin): supression of INF-γ and TNF-α and enhancement of IL-10. J Autoimmun 13: 187–195.

Di Marco R, Mangano K, Quattrocchi C, Musumeci R, Speciale AM, Papaccio G, Buschard K, Bendtzen K, Nicoletti F (2003) Curative effects of sodium fusidate on the development of dinitrobenzenesulfonic acid-induced colitis in rats. Clin Immunol 109: 266–271.

Di Marco R, Puglisi G, Papaccio G, Nicoletti A, Patti F, Reggio A, Bendtzen K, Nicoletti F (2001) Sodium fusidate (fusidin) ameliorates the course of monophasic experimental allergic encephalomyelitis in the Lewis rat. Mult Scler 7: 101–104.

Dimitrijević M, Milenković M, Milosavljević P, Stojić Vukanić Z, Čolić M, Bartlett R (1998) Beneficial effects of leflunomide on cardiac myosin-induced experimental autoimmune myocarditis in rats. Int J Immunotherapy 14: 9–21.

Feldman AM, McNamara D (2000) Myocarditis. N Engl J Med 343: 1388–1398.

Furukawa T, Kobuke K, Matsumori A (2001) Role of cytokines in autoimmune myocarditis and cardiomyopathy. Autoimmunity 34: 165–168.

Genovese F, Mancuso G, Cuzzola M, Cusumano V, Nicoletti F, Bendtzen K, Teti G (1996) Improved survival and antagonistic effect of sodium fusidate on tumor necrosis factor alpha in a neonatal mouse model of endotoxin shock. Antimicrob Agents Chemother 40: 1733–1735.

Hegewisch S, Weh HJ, Hossfeld DK (1990) TNF-induced cardiomyopathy. Lancet 335: 294–295.

Ishiyama S, Hiroe M, Nishikawa T, Shimojo T, Hosokawa T, Ikeda I, Toyozaki T, Kasajima T, Marumo F (1999) Inhibitory effects of vesnarinone in the progression of myocardial damage in experimental autoimmune myocarditis in rats. Cardiovasc Res 43: 389–397.

Kishimoto C, Takada H, Kawamata H, Umatake M, Ochiai H (2001) Immunoglobulin treatment prevents congestive heart failure in murine encephalomyocarditis viral myocarditis associated with reduction of inflammatory cytokines. J Pharmacol Exp Ther 299: 645–651.

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

Kodama M, Matsumoto Y, Fujiwara M (1992) *In vivo* lymphocyte-mediated myocardial injuries demonstrated by adoptive transfer of experimental autoimmune myocarditis. Circulation 85: 1918–1926.

Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A (1990) A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. Clinical Immunol Immunopathol 57: 250–262.

Lane JR, Neumann DA, Lafond-Walker A, Herskowitz A, Rose NR (1992) Interleukin 1 or tumor necrosis factor can promote Coxsackie virus-induced myocarditis in resistant B10. A mice. J Exp Med 175: 1123–1129.

Lauer B, Padberg K, Schultheiss HP, Strauer BE (1994) Autoantibodies against human ventricular myosin in sera of patients with acute and chronic myocarditis. J Am Coll Cardiol 23: 146–153.

Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, Moon TE (1995) A clinical trial of immunosuppressive therapy for myocarditis. N Engl J Med 333: 269–275.

Milenković M, Vučićević D, Milosavljević P, Arsenović Ranin N, Stojić Vukanić Z, Čolić M (2005) Suppression of experimental autoimmune myocarditis by sodium fusidate (fusidin). Pharmacol Res 52: 491–496.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55–63.

Nicoletti F, Beltrami B, Raschi E, Di Marco R, Magro G, Grasso S, Bendtzen K, Fiorelli G, Meroni PL (1997) Protection from concanavalin A (Con-A)-induced T cell-dependent hepatic lesions and modulation of cytokine release in mice by sodium fusidate. Clin Exp Immunol 110: 479–484.

Nicoletti F, Zaccone P, Di Marco R, Magro G, Grasso S, Morrone S, Santoni A, Tempera G, Meroni PL, Bendtzen K (1995) Effects of sodium fusidate in animal models of insulin-dependent diabetes mellitus and septic shock. Immunology 85: 645–650.

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.

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

Pisani B, Taylor DO, Mason JW (1997) Inflammatory myocardial diseases and cardiomyopathies. Am J Med 102: 459–469.

Shioji K, Kishimoto C, Sasayma S (2001) Fc receptor-mediated inhibitory effect of immunoglobulin therapy on autoimmune giant cell myocarditis: Concomitant suppression of the expression of dendritic cells. Circ Res 89: 540–546.

Smith SC, Allen PM (1991) Myosin-induced acute myocarditis is a T cell-mediated disease. J Immunol 147: 2141–2147.

Smith SC, Allen PM (1992) Neutralization of endogenous tumor necrosis factor ameliorates the severity of myosin-induced myocarditis. Circ Res 70: 856–863.

Toore-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL (1996) Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: A report from the studies of left ventricular dysfunction (SOLVD). J Am Coll Cardiol 27: 1201–1206.