Effect of interleukin-15 on the course of myocarditis in Coxsackievirus B3-infected BALB/c mice

Boris Bigalke MD[1], Peter L Schwimmbeck MD[2], Christian S Haas MD[1], Stephan Lindemann MD[1]

B Bigalke, PL Schwimmbeck, CS Haas, S Lindemann. Effect of interleukin-15 on the course of myocarditis in Coxsackievirus B3-infected BALB/c mice. Can J Cardiol 2009;25(7):e248-e254.

OBJECTIVE: Cytokines have an important role in both the initiation and perpetuation of viral myocarditis. Because a causative therapy of myocarditis is not yet well established and immunomodulation is a promising approach, the influence of interleukin (IL)-15, a proinflammatory cytokine, on the course of experimental myocarditis in Coxsackievirus B3 (CVB3)-infected mice was examined.
METHODS: Hearts from CVB3-infected (n=14), sham-infected (n=14) and CVB3-infected BALB/c mice treated with IL-15 (n=6) or a competitive IL-15 fusion protein (n=6) were analyzed for hemodynamic function, cellular infiltrates and myocardial collagen content.
RESULTS: Induction of myocarditis was associated with significant loss of body and heart weight, decreased left ventricular function, and increased collagen content and cellular infiltrates in the myocardium. Treatment of infected animals with IL-15 resulted in normalization of body and heart weight, and significantly improved systolic and diastolic left ventricular function, comparable with that of uninfected animals. This was paralleled by a significant reduction of myocardial collagen content to levels observed in animals without disease and by markedly reduced cellular infiltration of lymphocytes and macrophages in the myocardium. Inhibition of intrinsic IL-15 with IL-15 fusion protein tended to aggravate the disease.
CONCLUSIONS: Treatment with IL-15 has a positive effect on CVB3-induced murine myocarditis and seems to be a promising approach to modifying clinical course, hemodynamics and histopathology of virus-induced myocarditis. Further studies are needed to identify the underlying mechanisms.

Key Words: *Collagen; Coxsackievirus B3; Cytokines; Inflammation; Interleukin-15; Myocarditis*

Effet de l'interleukine-15 sur l'évolution de la myocardite chez des souris BALB/c infectées par le virus Coxsackie B3.

OBJECTIF : Les cytokines jouent un rôle important dans l'instauration et la perpétuation de la myocardite virale. Étant donné que le traitement axé sur la cause de la myocardite n'est pas encore bien établi et que l'immunomodulation est une approche prometteuse, les auteurs ont examiné l'influence de l'interleukine (IL)-15, une cytokine pro-inflammatoire, sur l'évolution d'une myocardite expérimentale chez des souris infectées par le virus Coxsackie B3 (CVB3).
MÉTHODES : Les auteurs ont analysé la fonction hémodynamique, les infiltrats cellulaires et le contenu myocardique en collagène de cœurs de souris BALB/c infectées par le CVB3 (n = 14), infectées facticement (n = 14) et infectées par le CVB3, mais traitées par IL-15 (n = 6) ou une protéine de fusion compétitive à base d'IL-15 (n = 6).
RÉSULTATS : L'induction de la myocardite a été associée à des baisses significatives des poids corporel et cardiaque, à une diminution de la fonction ventriculaire gauche et une augmentation du contenu myocardique en collagène et en infiltrats cellulaires. Traiter les animaux infectés au moyen d'IL-15 a permis une normalisation des poids corporel et cardiaque et une amélioration significative de la fonction ventriculaire gauche systolique et diastolique, comparables à celles qui s'observaient chez les animaux non infectés. Ces phénomènes se sont accompagnés d'une réduction significative du contenu myocardique en collagène, jusqu'au degré observé chez les animaux indemnes, et d'une réduction marquée de l'infiltration du myocarde par les lymphocytes et les macrophages. L'inhibition de l'IL-15 intrinsèque par la protéine de fusion à base d'IL-15 a eu tendance à aggraver la maladie.
CONCLUSION : Le traitement par IL-15 a exercé un effet positif sur la myocardite murine induite par le CVB3 et semble être une approche prometteuse pour modifier l'évolution clinique, l'hémodynamie et l'histopathologie des myocardites d'origine virale. D'autres études devront être réalisées pour comprendre les mécanismes sous-jacents.

Enteroviruses, particularly Coxsackie B viruses, are considered to have a crucial role in the etiology of viral myocarditis. Virus-induced damage to the heart tissue has been suggested as the main mechanism underlying myocarditis in the murine model (1,2). Previous studies have shown that T cell-derived cytokines, including some interleukins (ILs), have an important role in the development of Coxsackievirus B3 (CVB3) myocarditis (3-5).

ILs exert their effects through specific high-affinity receptors. These effects are pleiotropic, proinflammatory and even anti-inflammatory (6), which may explain damage to myocytes, cardiac autoimmunity and changes in the cardiac extracellular matrix (ECM).

IL-15 is a proinflammatory cytokine that is present in a broad variety of tissues and cells such as activated macrophages, keratinocytes, muscle cells, endothelial cells and neural cells. In addition, IL-15 has been shown to be expressed as a functionally active, membrane-bound form in monocytes (7). IL-15 binds to the alpha-chain of its own high-affinity receptor, but it also shares the beta-gamma c subunits with IL-2. Thus, similar biological activities of IL-15 and IL-2 can be explained (8). Both ILs cause a stimulation of T cell and B cell proliferation and activity, whereas IL-15 particularly promotes the proliferation and survival of natural killer cells (9,10). Natural killer cells are bone marrow-derived granular lymphocytes that, without previous sensitization and restriction by major histocompatibility proteins, are cytotoxic against malignant and virally infected cells (10-12).

Various histological, immunohistological and biochemical studies have shown that cytokines change the cardiac ECM. Characteristic changes have been described in dilated cardiomyopathy, myocarditis, ischemic cardiomyopathy and hypertensive heart disease (13). Among cardiac collagens (COLs), fibrillar and interstitial COL types I, III and V are most abundant (14), with mainly COL I (80%) and COL III (20%) providing functional integrity for the cardiac ECM (13). An increase of COL I, which provides stiffness and tensile strength, leads to systolic and diastolic dysfunction in dilated cardiomyopathy (15). Mechanical forces and various polypeptide factors are stimulators of collagen synthesis and subsequent fibrosis, although their mechanisms are still unknown (14). Collagen is degraded via intracellular and extracellular pathways. The intracellular pathway degrades lysosomal procollagens immediately after their synthesis to prevent the secretion

[1]*Medizinische Klinik III, Klinik für Kardiologie und Kreislauferkrankungen, Eberhard Karls Universität Tübingen;* [2]*Medizinische Klinik I, Klinikum Leverkusen, Germany*
Correspondence: Dr Stephan Lindemann, Medizinische Klinik III, Eberhard Karls Universität Tübingen, Otfried-Müller-Str. 10, D-72076 Tübingen, Germany. Telephone 49-7071-29-83688, fax 49-7071-29-5749, e-mail s.lindemann@st-petri-hospital.de
Received for publication May 16, 2007. Accepted November 26, 2007

 ©2009 Pulsus Group Inc. All rights reserved

TABLE 1
Body weight, heart rate and left ventricular pressure (LVP) analysis

Groups	Body weight, g	Heart weight, g	Heart rate, beats/min	LVP, mmHg
Control (n=14)	26.8±2.5	0.153±0.02	290±47	85.3±8.5
CVB3 (n=14)	20.1±2.4*	0.112±0.01*	241±61*	72.9±6*
CVB3 + IL-15 (n=6)	24.5±3.3†	0.144±0.01†	320±27†	75.9±4.6*
CVB3 + IL-15fp (n=6)	19.4±0.8*	0.105±0.01*	198±29*	71.4±8.8*

*Data presented as mean ± SD. *P<0.05 versus controls; †P<0.05 versus Coxsackievirus B3 (CVB3)-infected mice. IL-15 Interleukin-15; IL-15fp Interleukin-15 fusion protein*

of defective molecules and regulate the level of collagen production in response to extracellular stimuli (14). The extracellular pathway is regulated by matrix metalloproteinases (MMPs) that, acting as zymogens, need extracellular activation by proteinases and are deactivated by tissue inhibitors of metalloproteinases (TIMPs) (16-18). Synthesis and degradation pathways constitute a complex regulatory system modulated by various factors such as ILs.

In the present study, we investigated the effect of IL-15 on the course of myocarditis in BALB/c mice after infection with CVB3.

METHODS

Infection of animals

Eight-week-old male BALB/c (H-2^d) mice, originally obtained from The Jackson Laboratory (Bar Harbor, Maine, USA) and housed in the animal facilities at the Charité – Medical School, Campus Benjamin Franklin (Berlin, Germany), were used. The animals were intraperitoneally infected with 500,000 plaque-forming units of CVB3, strain Nancy (Professor Zeichhardt, Department of Virology, Charité – Medical School, Campus Benjamin Franklin, Berlin, Germany). Groups of six mice were subsequently treated with daily injections of recombinant murine IL-15 (250 ng) (PeproTech, USA) or an equivalent dose (250 ng) of IL-15 fusion protein (IL-15fp) (Professor Bulfone-Paus, Department of Immunology, Borstel Research Institute, Borstel, Germany), which inhibits the physiological effects of IL-15. Groups of 14 mice, with or without infection, served as controls. No animals died during the administration of IL-15 or IL-15fp. The investigation was performed in accordance with the German law on animal protection.

Determination of body weight and heart weight, and hemodynamic evaluation

After 12 days, the animals were weighed, anesthetized using intraperitoneal administration of thiopental (125 µg/g body weight), intubated and ventilated. The chest was surgically opened, and an SPR-407 1.4 Fr Millar-tip catheter with a 1.4 Fr SPR-40M Ultraminiature Pressure Transducer (Millar Instruments Inc, USA) was advanced into the left ventricle as described previously (19). Hemodynamic parameters, such as heart rate, left ventricular pressure (LVP) and slope of the LVP pulse (dP/dt max in mmHg/s), were assessed using a heart rate module type 669 (Hugo Sachs Electronic, Germany), a DC bridge amplifier type 660 (Hugo Sachs Electronic) and a slope quotient-coupler type 575 (Hugo Sachs Electronic), and were recorded on an oscillograph (WR3101 Mark VII Linearcorder; Graphtec Corporation, Japan). Following hemodynamic evaluation, the heart of each animal was harvested and weighed before further processing for immunohistology.

Immunohistochemistry

Harvested hearts and spleens were embedded in a tissue medium and frozen in liquid nitrogen. Cryosections (5 µm) were prepared using a cryotome. According to the staining method described by Kühl et al (20), specific monoclonal rat antimouse primary antibodies directed against the leukocyte surface antigens CD4, CD8a, CD11a and CD11b (Becton Dickinson PharMingen, USA), and CD3 and CD54/ICAM-1 (Dianova, Germany) were embedded for 60 min. Unbound antibodies were eluted twice with phosphate-buffered saline (PBS) for 5 min each. A peroxidase-conjugated rabbit antirat secondary antibody (Dianova), diluted to 1:200 in PBS containing 10% fetal calf serum, was added and incubated for 60 min. After another two washing steps using PBS, a 12 min stain reaction was performed using 3-amino-9-ethylcarbazole; hematoxylin served as a counterstain. Cellular infiltrates were detected by light microscopy at a final magnification of ×450. At least 10 high-power fields in five different sections were evaluated for each single antibody.

Picrosirius red staining

For quantitative assessment of total collagen content in the myocardium, the picrosirius red staining method was used. Myocardial tissue, which had previously been formalin-fixed, was embedded in paraffin. Using a microtome, sections (5 µm) were prepared and stained with picrosirius red (Polysciences Inc, USA). According to the method of Whittacker et al (21), the sections were examined under circularly polarized light microscopy, which was assisted by a computer-based digital image analysis software program (LUCIA G MV-1500 version 4.6, Nikon Inc, USA). As previously described (21), the software program was calibrated to exclude interstitial space and to give a collagen percentage output using the following equation:

$$\text{Collagen (\% output)} = \frac{\text{Collagen area fraction in total tissue}}{\text{Total tissue without gaps in tissue}} \times 100\%$$

Each sample was evaluated with a total of 10 fields at a final magnification of ×400.

Statistical analysis

Statistical significance was evaluated by a nonparametric Mann-Whitney U test for two independent samples. P<0.05 was defined as statistically significant. For quantitative analysis, all data were expressed as mean ± SD. Statistical calculation was performed with Microsoft Excel 2007 (Microsoft Corporation, USA) and SPSS for Windows version 15.0 (SPSS Inc, USA).

RESULTS

Treatment with IL-15 reverses myocarditis-associated loss of body and heart weight

CVB3-infected animals had a significantly reduced body weight compared with the uninfected group (–25%, P<0.05; Table 1). Interestingly, treatment with IL-15 significantly increased the mean body weight in CVB3-infected mice (+22%, P<0.05), virtually reversing the substantial myocarditis-associated loss. In contrast, IL-15fp did not alter the mean body weight in the myocarditis group. The changes in mean body weights were paralleled by similar changes in the heart weights – the significant, myocarditis-associated reduction of the mean heart weight was reversed by IL-15 but not IL-15fp.

IL-15 improves hemodynamics in CVB3-infected mice

Table 1 demontrates that heart rates in CVB3-infected mice were significantly lower than in the control group (241±61 beats/min versus 290±47 beats/min; P<0.05). CVB3-infected mice treated with IL-15fp also had lower heart rates than control animals (198±29 beats/min versus 290±47 beats/min; P<0.05). However, treatment with IL-15 in CVB3-infected mice resulted in a significant increase in heart rate (+33%) compared with the results in CVB3-infected animals that did not receive IL-15 therapy (320±27 beats/min versus 241±61 beats/min; P<0.05). Thus, IL-15 therapy in CVB3-infected mice led to normalization of heart rate, which did not significantly differ from that in the control group (320±27 beats/min versus 290±47 beats/min).

In addition, LVP was significantly decreased in the CVB3-infected mice (P<0.05; Table 1). While LVP did not change in IL-15fp-treated

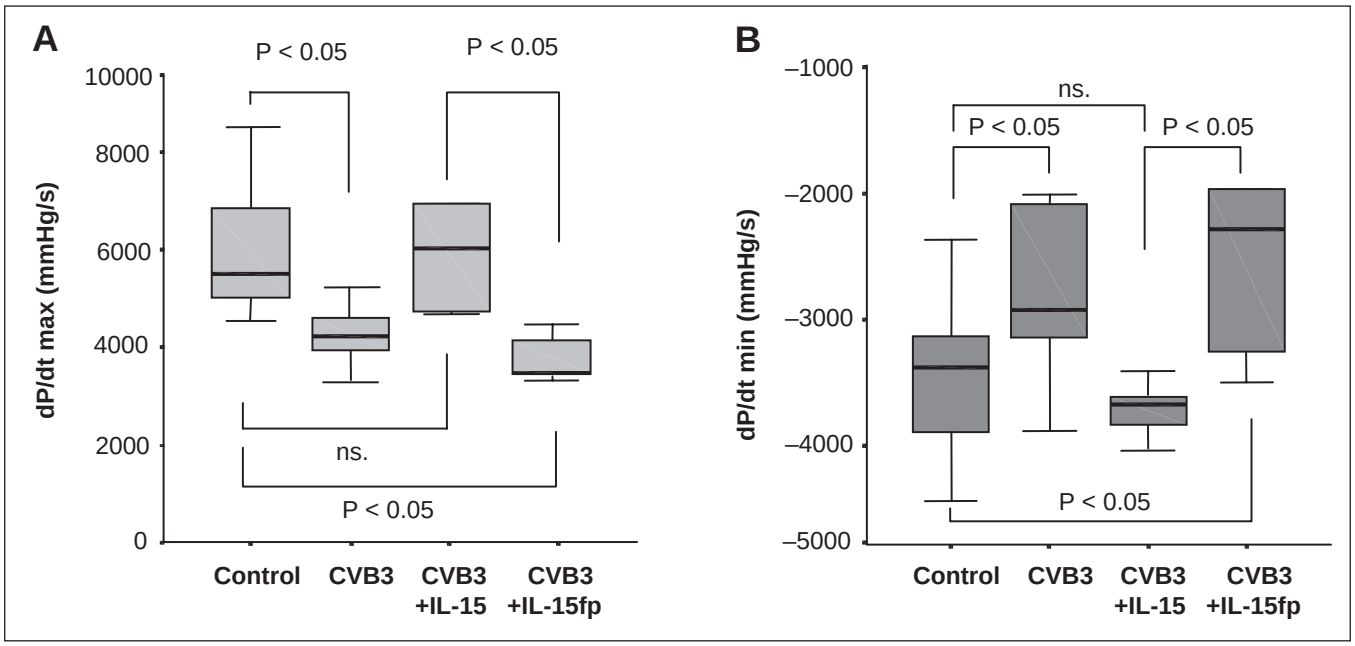

Figure 1) *Systolic (***A***) and diastolic (***B***) slope of the left ventricular pressure pulse (dP/dt max).* **A** *Coxsackievirus B3 (CVB3)-infected mice treated with interleukin (IL)-15 fusion protein (IL-15fp) showed no significant improvement, but there was a trend toward deterioration compared with the infected group. In contrast, CVB3-infected IL-15-treated animals demonstrated a significantly improved left ventricular function comparable with that in uninfected animals. Similar results were found in the diastolic slope (***B***) of left ventricular pressure pulse. max Maximum; min Minimum; ns Nonsignificant*

CVB3-infected animals, administration of IL-15 tended to improve LVP, although this effect was not statistically significant.

Figure 1A demonstrates the results of the systolic slopes of the LVP pulse. CVB3-infected mice treated with IL-15fp showed no significant improvement but rather a trend toward deterioration (–12%) compared with the infected group. In contrast, treatment with IL-15 resulted in significantly improved systolic left ventricular function (+34%, P<0.05), with values comparable with healthy subjects. Assessment of the diastolic slope of LVP pulse produced similar results (Figure 1B).

IL-15 but not IL-15fp ameliorates inflammation in CVB3-induced myocarditis

Myocardial tissue was analyzed for cellular infiltration of inflammatory cells using CD3, CD4, CD8a, CD11a and CD11b as specific markers. In addition, immunohistochemical staining for the adhesion molecule CD54/ICAM-1 was performed (Table 2). Spleen samples served as positive controls (data not shown).

The myocardium of CVB3-infected mice showed significantly more CD3-positive (+), CD4+ and CD8a+ lymphocytes than the myocardium of noninfected controls (P<0.05). Similarly, infiltration with CD11a+ and CD11b+ macrophages was significantly increased in the myocarditis group (P<0.05). While treatment with IL-15 resulted in significantly fewer CD3+, CD4+, CD8a+, CD11a+ and CD11b+ leukocytes (P<0.05), IL-15fp did not alter the influx of inflammatory cells. Interestingly, myocardial expression of CD54/ICAM-1 doubled in the CVB3 mice, an effect that was completely reversed by IL-15 administration (P<0.05). Compared with the uninfected animals, CVB3-infected animals and CVB3-infected animals treated with IL-15fp demonstrated a significantly increased number of cellular infiltrates per high-power field.

Figure 2 shows a representative staining for CD11b in the myocardium of uninfected and CVB3-infected mice with and without treatment. Infiltration of CD11b+ macrophages was clearly present in the myocarditis group (Figure 2B) compared with the controls (Figure 2A). Figure 2C depicts the myocardium of a CVB3-infected IL-15-treated mouse showing reduced cellular infiltrates. Figure 2D represents the myocardium of an infected, IL-15fp-treated mouse with persistent abundant CD11b+ cellular infiltration.

TABLE 2
Immunohistochemical evaluation

Groups	Control (n=14)	CVB3 (n=14)	CVB3 + IL-15 (n=6)	CVB3 + IL-15fp (n=6)
CD3	0.008±0.02	0.84±0.34*	0.43±0.07†	0.89±0.22*
CD4	0.006±0.02	0.88±0.30*	0.54±0.16†	0.70±0.33*
CD8a	0.003±0.01	0.78±0.41*	0.41±0.07†	0.58±0.22*
CD11a	0.022±0.39	0.96±0.35*	0.52±0.11†	0.69±0.16*
CD11b	0.018±0.04	0.76±0.22*	0.50±0.07†	1.07±0.28*
CD54/ ICAM-1	0.48±0.14	1.03±0.31*	0.48±0.11†	1.07±0.23*

*Data presented as mean (± SD) cells per high-power field. *P<0.05 versus controls; †P<0.05 versus Coxsackievirus B3 (CVB3). IL-15 Interleukin-15; IL-15fp IL-15 fusion protein*

Treatment with IL-15 prevents myocardial fibrosis in CVB3-infected mice

To determine myocardial fibrosis and ECM deposition, overall collagen content was assessed using picrosirius red staining. A significant increase in collagen (+36%, P<0.05) was demonstrated in the myocardium of CVB3-infected mice compared with the uninfected group (Figure 3). Treatment with IL-15 significantly decreased collagen content of CVB3-infected mice (–34%, P<0.05), whereas IL-15fp did not have an effect on myocardial fibrosis in CVB3 myocarditis.

DISCUSSION

Evaluation of body and heart weight

In the present study, we investigated the effect of IL-15 in a murine model of CVB3-induced myocarditis. It is still challenging to identify affected animals clinically (22). Describing the visible clinical aspects, such as smooth or rough fur and agility of the mice, provided an initial hint of the animals' state of health. While controls and CVB3-infected, IL-15 treated mice showed an unobtrusive habitus and smooth fur, suggesting a healthy state, nontreated and IL-15fp-treated CVB3-infected mice were clearly sick. Besides these subjective parameters, body and heart weight permitted a more objective judgement; viral infection and myocarditis were associated with a significant loss of body and heart weight compared with

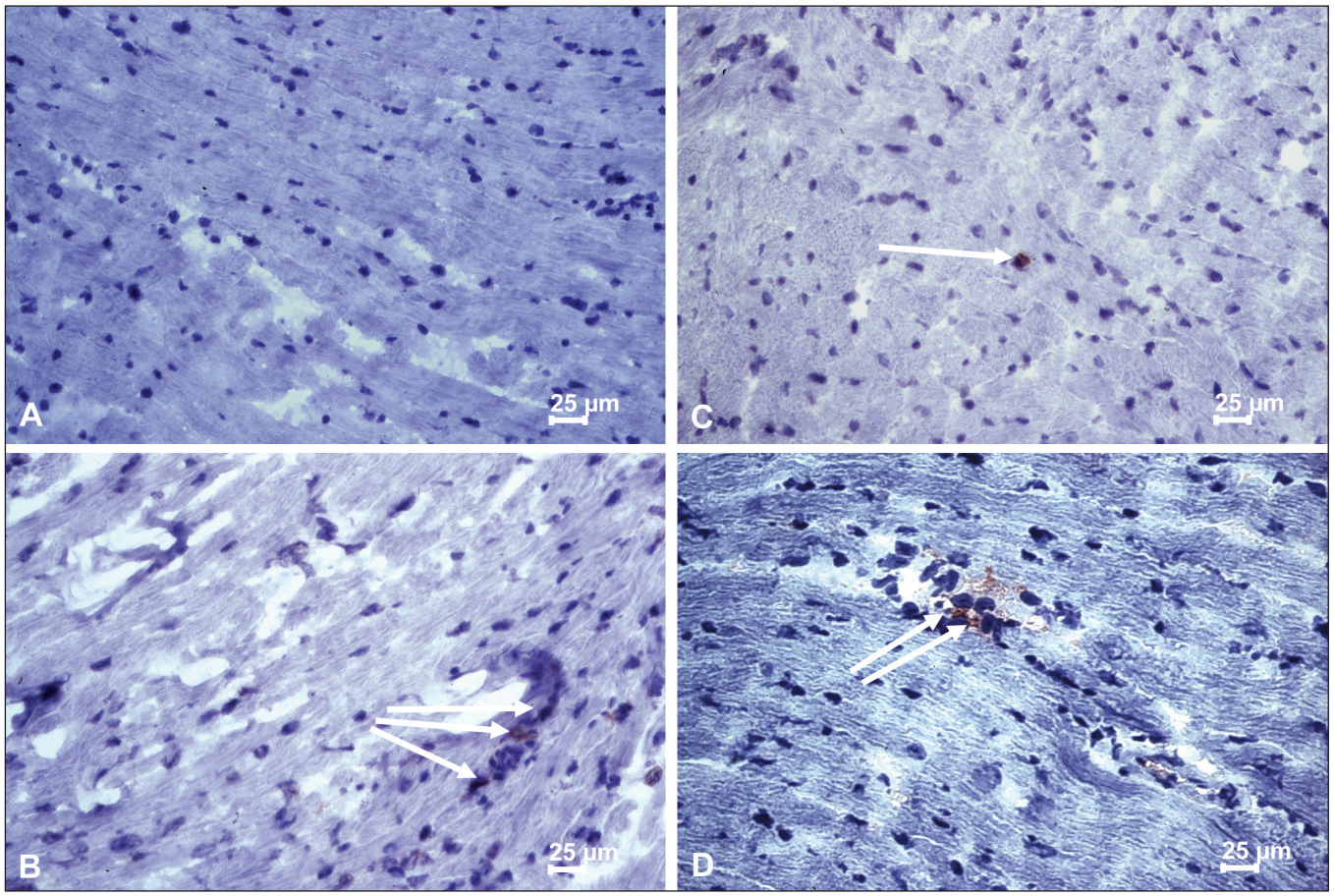

Figure 2) *CD11b staining reflecting infiltration of macrophages in the myocardium.* **A** *In the myocardium of a normal, sham-infected mouse, no significant infiltrating cells are found.* **B** *Infiltrating CD11b-positive cells (arrows) in the heart of a Coxsackivirus B3 (CVB3)-infected mouse.* **C** *After treatment with interleukin (IL)-15, cellular infiltrates are significantly reduced. The white arrow marks a single CD11b-positive cell.* **D** *Treatment with IL-15 fusion protein did not decrease the number of cellular infiltrates compared with CVB3-infected animals. The arrows indicate a cluster of CD11b cellular infiltrates*

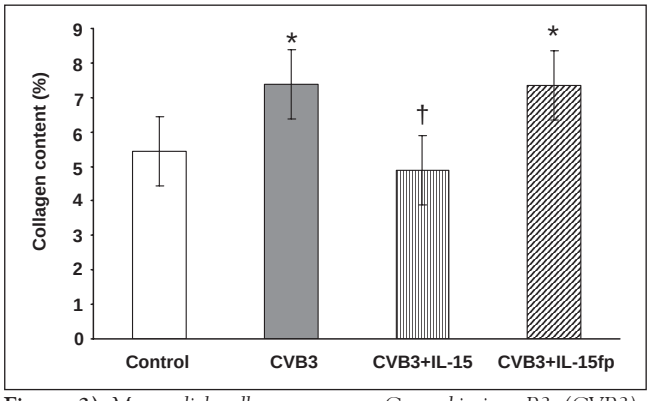

Figure 3) *Myocardial collagen content. Coxsackievirus B3 (CVB3)-infected interleukin (IL)-15-treated animals did not show a significant change in collagen compared with the uninfected group. However, they were significantly decreased compared with the infected group. CVB3-infected IL-15 fusion protein (IL-15fp)-treated mice, however, presented a significantly increased collagen content compared with the uninfected group. *P<0.05 compared with control group; †P<0.05 compared with CVB3-infected group*

healthy animals. Treatment with IL-15, but not IL-15fp, reversed that effect, pointing to a beneficial role of IL-15 on the clinical course of myocarditis. This correlated with our findings on hemodynamics, collagen content and infiltration of inflammatory cells. These results are consistent with previous studies that have described a correlation of hemodynamic changes to the heart weight to body weight ratio (23), as well as an association between left ventricular dysfunction, collagen degradation and cellular infiltrates in the myocardium, involving regulatory cytokines in CVB3-induced mice with myocarditis (24).

Hemodynamic parameters

The uninfected group had a significantly higher heart rate than the infected group, suggesting impaired myocardial performance in the CVB3-infected mice. Although narcotics were weight-adjusted, we cannot exclude that the lower heart rate is, in part, due to deeper anesthesia. However, we made an effort to minimize these influences by performing the hemodynamic measurements under steady-state conditions with constant ventilation, thus avoiding a significant influence of the heart rate on left ventricular function. Indeed, decreased LVP as well as deteriorated diastolic and systolic left ventricular function in CVB3-infected mice reflected impaired cardiac function, which was significantly improved by IL-15 treatment, albeit not by IL-15fp.

Cytokines, particularly proinflammatory cytokines such as tumour necrosis factor-alpha and IL-6 (25-27), have an important role as negatively inotropic factors leading to myocardial dysfunction. They have been shown to have an influence on the changes in the ECM, causing left ventricular remodelling and myocardial fibrosis. However, a recent study by Kuhl et al (28) demonstrated that treatment with interferon-beta eliminates cardiotropic viruses and improves left ventricular function, although virtually all patients reported flu-like side effects. Despite the known side effects, immunomodulation is a promising approach to the detrimental clinical outcome in patients with manifest dilated cardiomyopathy due to myocarditis. Intriguingly, in the present study, we observed a protective effect of proinflammatory

IL-15, resulting in amelioration of left ventricular dysfunction, whereas inhibition of endogenous IL-15 with IL-15fp did not improve, but rather, tended to impair cardiovascular hemodynamics.

Immunohistochemical evaluation

CVB3 can infect cells of the immune system and it localizes to follicles in the spleen and lymph nodes of infected mice. It has been suggested that CVB3 directly alters the immune response, delaying viral clearance in affected organs (29) and leading to persistent inflammatory lesions in the heart with loss of cardiac myocytes (30). Thus, control of viral load in an early stage of myocarditis would improve histological and clinical features of myocarditis. Both CD4+ and CD8+ T cells have been shown to be affected by IL-15. Although IL-15 was considered not to be required for memory CD4+ cell proliferation (31), a study by Purton et al (32) demonstrated that antiviral CD4+ memory T cells are IL-15-dependent. In addition, IL-15 seems to be crucial for both survival and homeostatic proliferation of memory CD4+ cells (32), while a lack of IL-15 results in the suboptimal priming of CD4+ T cell response (33). In the present study, IL-15 treatment resulted in downregulation of CD4+ lymphocytes in the myocardium, suggesting that IL-15 may not exert its possible effects on viremia control and CVB3 viral load in this model. Of note, another study (34) demonstrated that IL-15 can induce viral replication in HIV-infected macaque monkeys.

Likewise, CD8+ cells are IL-15 dependent and appear to function in both innate and adaptive immunity (35). Prolonged in vivo survival and sustained biological action on target cells may account for the proposed persistent action of IL-15 that aids in the long-term survival of functional CD8 memory T cells in vivo (36). Other studies reported that IL-15 is not essential for generation but for proliferation of memory CD8+ cells (31,37). Recently, Fonseca et al (37) demonstrated that CD8+ T cells infiltrating the heart in Chagas disease showed higher expression of the alpha- and gamma c-chain of the IL-15 high-affinity receptor than CD4+ T cells, thereby favouring a predominance of CD8+ cells in the presence of IL-15. In our study, IL-15 resulted not only in a reduction of CD4+ T cells but also CD8+ T cells, thereby not changing the CD4+ to CD8+ ratio and implying that the beneficial effect of IL-15 is not likely via control of viremia and viral load, but instead, via other pathways. Besides its effect on T cell activation and proliferation, IL-15 has been shown to promote release of pro- and anti-inflammatory cytokines (38-40). However, it remains unclear if and how IL-15 ameliorates CVB3-induced myocarditis via those cytokines.

Downregulation of both CD4+ and CD8a+ cells, albeit not to levels observed in normal myocardial tissue, pointed to a reduced but still present inflammatory response. IL-15 treatment was also associated with a reduction of CD3+ lymphocytes in the myocardial tissue. Myocardial inflammation has been shown to be associated with an increased number of CD3+ lymphocytes and anti-CD3 antibodies have been used to treat myocarditis (41,42), suggesting that IL-15 may exert its anti-inflammatory effects via inhibition of CD3+ cells.

Myocardial upregulation of the adhesion molecule CD54/ICAM-1, which is predominantly expressed in endothelial cells, has been previously demonstrated in experimental autoimmune myocarditis in rats (43). Increased levels of soluble CD54/ICAM-1 have been observed in various cardiovascular diseases, eg, myocarditis (44). Intriguingly, the extent of CD54/ICAM-1 expression on endothelial cells correlated with the severity of endothelial dysfunction in patients with myocardial inflammation (29). In the present study, we showed that IL-15-mediated downregulation of myocardial CD54/ICAM-1 expression is associated with less infiltration of CD11b+ macrophages and lymphocytes, suggesting that IL-15 inhibits leukocyte adhesion and migration.

While IL-15 has proinflammatory effects in experimental and human arthritis, and has a potential pathogenetic role in inflammatory bowel disease and T cell leukemia (30,45-47), administration of IL-15 seems to be a promising approach to modify the inflammatory process in viral myocarditis.

Evaluation of myocardial collagen content

In a study by Pauschinger et al (15), patients with dilated cardiomyopathy had increased COL I and COL III messenger RNA in the myocardium, causing systolic and partially diastolic dysfunction. Of interest, immunomodulation using IL-4 suppresses MMPs and improves cardiac function in murine myocarditis (48). In our experiments, a significant increase of myocardial collagen content was demonstrated in the BALB/c mouse model for myocarditis. The high percentage of collagen was not altered by treatment with IL-15fp, whereas collagen staining in IL-15-treated animals was indistinguishable from that in uninfected animals. Comparing these results with the hemodynamic parameters supports the beneficial use of IL-15 that might have an indirect impact on collagen content via stimulation of MMP and TIMP production (49). Li et al (50,51) reported the effect of the proinflammatory cytokines tumour necrosis factor-alpha and IL-1-beta in downregulating TIMPs and enhancing the activity of MMPs, respectively. As a consequence, the fibrillar collagens are denatured and new fibrous tissue is produced (52). Furthermore, reduced activity of MMPs due to inhibition of these enzymes and enhanced activity of TIMPs may result in reduced collagen expression, an increase in insoluble collagen and a higher nondenatured to total soluble collagen ratio. These mechanisms may prevent myocardial hypertrophy and diastolic dysfunction. However, further experiments are needed to examine the mechanisms by which cytokines interact with the synthesis and degradation pathways of collagen during viral myocarditis.

BALB/c mice infected with CVB3 as an in vivo model for human myocarditis

In the present study, we exclusively used male mice because Coxsackievirus concentrations are consistently lower in the heart of female animals, resulting in minimal to nonexistent myocarditis, an observation that is explained on the basis of differences in sex-associated hormones (53,54). The BALB/c mouse model introduced by Woodruff et al (55,56) became an established murine model for CVB3-induced myocarditis, featuring a quite extensive interstitial and focal inflammatory cell infiltration similar to the lesions observed in humans (54). Interestingly, Leipner et al (57) showed a marked age-related susceptibility to myocardial CVB3 infections. Experimental reproducibility and exact characterization of the genetic and immunological factors allow the study of the pathogenic mechanisms in this animal model (57), and can be used to test further approaches for an immunomodulatory therapy of viral myocarditis.

CONCLUSION

The influence of recombinant murine IL-15 and inhibiting intrinsic IL-15, respectively, on the course of myocarditis was tested in BALB/c mice after infection with CVB3. The results of the present study show that treatment with IL-15 had a positive effect on the clinical course of CVB3-induced murine myocarditis, while inhibition of intrinsic IL-15fb tended to have a detrimental influence. IL-15 also resulted in a significantly improved systolic and diastolic left ventricular function comparable with that of uninfected animals. IL-15 treatment was paralleled by a normalization of body and heart weight, whereas animals infected and consecutively injected with IL-15fp showed the lowest body and heart weights. Finally, IL-15 resulted in markedly reduced cellular infiltrates in the myocardium. In summary, IL-15 seems to have a beneficial effect on viral-induced myocarditis. The underlying mechanisms, however, need to be determined.

ACKNOWLEDGEMENTS: The authors thank Dr Carsten Tschöpe and Dr Dirk Westermann (Charité – Medical School, Campus Benjamin Franklin, Berlin, Germany) for their expert technical assistance. The study was supported by the Deutsche Forschungsgemeinschaft (DFG) Sonderforschungsbereich/Transregio 19 'Inflammatorische Kardiomyopathie-Molekulare Pathogenese und Therapie' (Tübingen, Berlin, Greifswald) to SL and by the DFG 849/3/1 to SL.

REFERENCES

1. Seong IW, Choe SC, Jeon ES. Fulminant Coxsackieviral myocarditis. N Engl J Med 2001;345:379.
2. Padalko E, Verbeken E, DeClercq E, Neyts J. Inhibition of Coxsackie B3 virus induced myocarditis in mice by 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile. J Med Virol 2004;72:263-7.
3. Kishimoto C, Kuroki Y, Hiraoka Y, Ochiai H, Kurokawa M, Sasayama S. Cytokine and murine Coxsackievirus B3 myocarditis. Interleukin-2 suppressed myocarditis in the acute stage but enhanced the condition in the subsequent stage. Circulation 1994;89:2836-42.
4. Huber SA, Polgar J, Schultheiss P, Schwimmbeck P. Augmentation of pathogenesis of Coxsackievirus B3 infections in mice by exogenous administration of interleukin-1 and interleukin-2. J Virol 1994;68:195-206.
5. Fairweather D, Rose NR. Inflammatory heart disease: A role for cytokines. Lupus 2005;14:646-51.
6. Kunzendorf U, Tran TH, Bulfone-Paus S. The Th1-Th2-paradigm in 1998: Law and nature or rule with exceptions. Nephrol Dial Transplant 1998;13:2445-8.
7. Bulanova E, Budagian V, Pohl T, et al. The IL-15Rα chain signals through association with Syk in human B cells. J Immunol 2001;167:6292-302.
8. Rückert R, Herz U, Paus R, et al. IL-15- IgG2b fusion protein accelerates and enhances a Th2 but not a Th1 immune response in vivo, while IL-2-IgG2b fusion protein inhibits both. Eur J Immunol 1998;28:3312-20.
9. Dunne J, Lynch S, O'Farrelly C, et al. Selective expansion and partial activation of human NK cells and NK receptor-positive T cells by IL-2 and IL-15. J Immunol 2001;167:3129-38.
10. Carson WE, Fehniger TA, Haldar S, et al. A potential role for interleukin-15 in the regulation of human natural killer cell survival. J Clin Invest 1997;99:937-43.
11. Carson WE, Giri JG, Lindemann MJ, et al. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. J Exp Med 1994;180:1395-403.
12. Robertson MJ, Ritz J. Biology and clinical relevance of human natural killer cells. Blood 1990;76:2421-38.
13. Pauschinger M, Doerner A, Remppis A, Tannhäuser R, Kühl U, Schultheiss HP. Differential myocardial abundance of collagen type I and type III mRNA in dilated cardiomyopathy: Effects of myocardial inflammation. Cardiovasc Res 1998;37:123-9.
14. Bishop JE, Laurent GJ. Collagen turnover and its regulation in the normal and hypertrophying heart. Eur Heart J 1995;16(Suppl C):38-44.
15. Pauschinger M, Knopf D, Petschauer S, et al. Dilated cardiomyopathy is associated with significant changes in collagen type I/III ratio. Circulation 1999;99:2750-6.
16. D'Armiento J. Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. Trends Cardiovasc Med 2002;12:97-101.
17. Gomez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. Tissue inhibitors of metalloproteinases: Structure, regulation and biological functions. Eur J Cell Biol 1997;74:111-22.
18. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: Evolution, structure and function. Bioch Biophys Acta 2000;1477:267-83.
19. Tschöpe C, Heringer-Walther S, Koch M, et al. Myocardial bradykinin B2-receptor expression at different time points after induction of myocardial infarction. J Hypertens 2000;18:223-8.
20. Kühl U, Noutsias M, Seeberg B, Schultheiss HP. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. Heart 1996;75:295-300.
21. Whittacker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic Res Cardiol 1994;89:397-410.
22. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: An American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation 2006;113:1807-16.
23. Tschope C, Spillmann F, Altmann C, et al. The bradykinin B1 receptor contributes to the cardio-protective effects of AT1 blockade after experimental myocardial infarction. Cardiovasc Res 2004;61:559-69.
24. Li J, Schwimmbeck PL, Tschope C, et al. Collagen degradation in a murine myocarditis model: Relevance of matrix metalloproteinase in association with inflammatory induction. Cardiovasc Res 2002;56:235-47.
25. Nishimura M, Hashimoto T, Kobayashi H, et al. Possible involvement of TNF-alpha in left ventricular remodeling in hemodialysis patients. J Nephrol 2003;16:641-9.
26. Pathan N, Hemingway CA, Alizadeh AA, et al. Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock. Lancet 2004;363:203-9.
27. Sun M, Chen M, Dawood F, et al. Tumor necrosis factor-alpha mediates cardiac remodeling and ventricular dysfunction after pressure overload state. Circulation 2007;115:1398-407.
28. Kuhl U, Pauschinger M, Schwimmbeck PL, et al. Interferon-beta treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. Circulation 2003;107:2793-8.
29. Vallbracht KB, Schwimmbeck PL, Seeberg B, Kuehl U, Schultheiss HP. Endothelial dysfunction of peripheral arteries in patients with immunohistologically confirmed myocardial inflammation correlates with endothelial expression of human leukocyte antigens and adhesion molecules in myocardial biopsies. Am J Coll Cardiol 2002;40:515-20.
30. Yashihara K, Yamada H, Hori A, Yajima T, Kubo C, Yoshikai Y. IL-15 exacerbates collagen-induced arthritis with an enhanced CD4(+) T cell response to produce IL-17. Eur J Immunol 2007;37:2744-52.
31. Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. J Exp Med 2002;195:1523-32.
32. Purton JF, Tan JT, Rubinstein MP, Kim DM, Sprent J, Surh CD. Antiviral CD4+ memory T cells are IL-15 dependent. J Exp Med 2007;204:951-61.
33. Combe CL, Moretto MM, Schwartzman JD, Gigley JP, Bzik DJ, Khan IA. Lack of IL-15 results in the suboptimal priming of CD4+ T cell response against an intracellular parasite. Proc Natl Acad Sci U S A 2006;103:6635-40.
34. Hryniewicz A, Price DA, Moniuszko M, et al. Interleukin-15 but not interleukin-7 abrogates vaccine-induced decrease in virus level in simian immunodeficiency virus mac251-infected macaques. J Immunol 2007;178:3492-504.
35. Dubois S, Waldmann TA, Muller JR. ITK and IL-15 support two distinct subsets of CD8+ T cells. Proc Natl Acad Sci U S A 2006;103:12075-80.
36. Sato N, Patel HJ, Waldmann TA, Tagaya Y. The IL-15/IL-15Ralpha on cell surfaces enables sustained IL-15 activity and contributes to the long survival of CD8 memory T cells. Proc Natl Acad Sci U S A 2007;104:588-93.
37. Fonseca SG, Reis MM, Coelho V, et al. Locally produced survival cytokines IL-15 and IL-7 may be associated to the predominance of CD8+ T cells at heart lesions of human chronic chagas disease cardiomyopathy. Scand J Immunol 2007;66:362-71.
38. Mori A, Suko M, Kaminuma O, et al. IL-15 promotes cytokine production of human T helper cells. J Immunol 1996;156:2400-48.
39. Rueckert R, Brandt K, Braun A, et al. Blocking IL-15 prevents the induction of allergen-specific T cells and allergic inflammation in vivo. J Immunol 2005;174:5507-15.
40. Carson WE, Ross ME, Baiocchi RA, et al. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro. J Clin Invest 1995;96:2578-82.
41. Abbate A, Bussani R, Liuzzo G, et al. Sudden coronary death, fatal acute myocardial infarction and widespread coronary and myocardial inflammation. Heart 2008;94:737-42.
42. Perens G, Levi DS, Alejos JC, Wetzel GT. Muronab-CD3 for pediatric acute myocarditis. Pediatric Cardiol 2007;28:21-6.
43. Suzuki J, Ogawa M, Futamatsu H, Kosuge H, Sagseka YM, Isobe M. Tea catechins improve left ventricular dysfunction, suppress myocardial inflammation and fibrosis, and alter cytokine expression in rat autoimmune myocarditis. Eur J Heart Fail 2007;9:152-9.

44. Wikowska AM. Soluble ICAM-1: A marker of vascular inflammation and lifestyle. Cytokine 2005;31:127-34.
45. Fehniger TA, Caligiuri MA. Interleukin 15: Biology and relevance to human disease. Blood 2001;97:14-32.
46. Baslund B, Tvede N, Danneskiold-Samsoe B, et al. Targeting interleukin-15 in patients with rheumatoid arthritis: A proof-of-concept study. Arthritis Rheum 2005;52:2686-92.
47. Yamada H, Kaibara N, Okano S, et al. Interleukin-15 selectively expands CD57(+) CD28(–)CD4(+) T cells, which are increased in active rheumatoid arthritis. Clin Immunol 2007;124:328-35.
48. Li J, Leschka S, Rutschow S, et al. Immunomodulation by interleukin-4 suppresses matrix metalloproteinases and improves cardiac function in murine myocarditis. Eur J Pharmacol 2007;554:60-8.
49. Canstantinescu CS, Grygar C, Kappos L, Leppert D. Interleukin 15 stimulates production of matrix metalloproteinase-0 and tissue inhibitor of metalloproteinase-1 by human peripheral blood mononuclear cells. Cytokine 2001;13:244-7.
50. Li YY, Feng YQ, Kadokami T, et al. Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor α can be modulated by anti-tumor necrosis factor α therapy. Proc Natl Acad Sci USA 2000;97:12746-51.
51. Li YY, McTiernan CF, Feldman AM. Proinflammatory cytokines regulate tissue inhibitors of metalloproteinases and disintegrin metalloproteinase in cardiac cells. Cardiovasc Res 1999;42:162-72.
52. Li YY, Kadokami T, Wang P, McTiernan CF, Feldman AM. MMP inhibition modulates TNF-α transgenic mouse phenotype early in the development of heart failure. Am J Physiol Heart Circ Physiol 2002;282:H983-9.
53. Huber SA, Lyden DC, Lodge PA. Myocarditis: Immunopathogenesis of experimental coxsackievirus induced myocarditis: Role of autoimmunity. Herz 1985;10:1-7.
54. Huber SA, Pfaeffle B. Differential Th1 and Th2 cell responses in male and female BALB/c mice infected with coxsackievirus group B type 3. J Virol 1994;68:5126-32.
55. Woodruff JF, Kilbourne ED. The influence of quantitated post-weaning undernutrition on coxsackievirus B3 infection of adult mice. Viral persistence and increased severity of lesions. J Infect Dis 1970;121:137-63.
56. Woodruff JF, Woodruff JJ. Involvement of T lymphocytes in the pathogenesis of coxsackie virus B3 heart disease. J Immunol 1974;113:1726-34.
57. Leipner C, Grün K, Borchers M, Stelzner A. The outcome of Coxsackie B3-(CVB3)-induced myocarditis is influenced by the cellular immune status. Herz 2000;25:245-8.