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Activated nuclear transcription factor κB in patients with myocarditis and dilated cardiomyopathy—relation to inflammation and cardiac function

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Abstract

Objectives and background: Myocarditis is caused by various agents and autoimmune processes. It is unknown whether viral genome persistence represents inactive remnants of previous infections or whether it is attributed to ongoing adverse processes. The latter also applies to the course of autoimmune myocarditis. One principal candidate for an adverse remodeling is nuclear factor-κB (NFκB).

Methods: A total of 93 patients with suspected myocarditis/cardiomyopathy was examined. Hemodynamics were assessed by echocardiography as well as right and left heart catheterization. Endomyocardial biopsies were taken from the left ventricle. Biopsies were examined by immunohistochemistry and PCR for viral genomes. Selective immunostaining of activated NFκB was performed.

Results: NFκB was increased in patients with myocarditis when compared with controls ($11.1 \pm 7.1\%$ vs. $5.0 \pm 5.3\%$, $P < 0.005$) whereas dilated cardiomyopathy showed no significant increase. Patients with myocarditis and preserved left ventricular function exhibited increased activated NFκB when compared with reduced function ($r^2 = 0.72$, $P < 0.001$). In parallel, inverse correlation of NFκB and left ventricular enddiasstolic volume was found ($r^2 = 0.43$, $P < 0.02$). Increased activated NFκB was found in adenovirus persistence when compared with controls ($P = 0.001$). Only a trend of increased NFκB activation was seen in cytomegalovirus persistence. Parvovirus B19 persistence did not affect NFκB activation.

Conclusions: Increased activation of NFκB is related to inflammatory processes in myocarditis. Since activated NFκB correlates with left ventricular function, it could be assumed that NFκB activation occurs at early stages of inflammation. Potentially, NFκB could inhibit loss of cardiomyocytes by apoptosis and protect from cardiac dilation. Since NFκB is a crucial key transcription factor of inflammation, its prognostic and future therapeutic relevance should be addressed.

Keywords: NF-κB; Myocarditis; Dilated cardiomyopathy; Adenovirus; Cytomegalovirus; Enterovirus; Parvovirus B19; Heart failure; Hemodynamics

Diagnosis and treatment of myocarditis is still a challenging issue in cardiology. Myocarditis is induced by various agents, e.g., viral, bacterial, fungal, rickettsial, spirochetal, helminthic, and protozoal causes. In addition, autoimmune processes are crucial. Common viral agents are entero-, cytomegalo-, herpes-, and adenoviruses. Intramyocardial occurrence of parvovirus B19 genome has been reported more frequently within the last few years. Nevertheless, the etiological and prognostic significance of myocardial virus genome persistence remained unsettled [1–5]. It is unknown whether viral genome persistence in the heart represents inactive remnants of previous infections or whether it is attributed to ongoing adverse processes with poor prognosis. The latter also applies to the course of autoimmune myocarditis. One principal candidate for a remodeling of cardiomyocytes is nuclear factor-κB (NFκB), an intracellular transcription factor that exists nonactivated as latent cytoplasmic complex bound to its inhibitory protein κB (IκB) [6–10]. After activation by viruses, cytokines, oxidants, and protein kinases that

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results in IκB degradation [11], NFκB translocates into the nucleus and induces expression of various proinflammatory products, e.g., cytokines, intracellular adhesion molecules, and inducible nitric oxide, which are involved in the inflammatory response to viral myocarditis [12]. Therefore, we addressed the hypothesis that inflammatory processes could be assessed and quantified by selective measurement of activated NFκB in inflammatory heart disease.

Methods

A total of 93 patients with suspected myocarditis due to a history or new onset/progression of typical cardiac chest pain or dyspnea and ECG ST-segment alteration or T wave inversion, creatine kinase or troponin elevation was examined in this single-center study. Characteristics of the patients are summarized in Tables 1–3.

Measurement of hemodynamic parameters. Echocardiographic studies were performed in all patients using standard parasternal long-axis and apical views. Beside global and regional wall motion left ventricular endsystolic (LVESD) and enddiasstolic diameters (LVEDD) were assessed. In addition, the LVEDD index (LVEDDI) was defined as LVEDD normalized to body surface area (m^2), which was calculated as square route of body length * weight/3600 [13]. All patients underwent right and left heart catheterization using Judkins technique in local anesthesia. Coronary artery disease was excluded by angiography in every patient. Left ventricular functional parameters including ejection fraction (EF), enddiasstolic volume (EDV), and endsystolic volume (ESV) were assessed by ventriculography using 7-F standard Pigtail catheters after measurement of enddiasstolic pressure (LVEDP). In addition, presence of heart valve disease was excluded. Right heart catheterization was performed with Swan-Ganz catheters to assess right ventricular enddiasstolic pressure (RVEDP), pulmonary artery pressure, and pulmonary capillary wedge pressure (PC). Cardiac output (CO) was measured by thermodilution and Fick technique (differences were <12%); the mean value of both (cardiac output: CO) was normalized to body surface area and expressed as cardiac index ($CI = CO/m^2$). Pulmonary and systemic vascular resistance were calculated in standard manner and values are given as dyne (=80 Woods).

Assessment, preparation, and examination of left ventricular endomyocardial biopsies. Using the arterial approach, 7–9 endomyocardial biopsies were taken from the left ventricle [14–17]. Biopsies of each patient were snap-frozen in liquid nitrogen and stored at −80 °C. Serial sections were examined by immunohistochemistry with monoclonal antibodies (DAKO, Hamburg, Germany) for CD3, CD45, and CD45RO leukocytes as well as CD11C and CD14 macrophages. The presence of at least 14 infiltrating leukocytes (including a maximum of up to 4 macrophages of CD11C or CD14) per mm^2 of endomyocardial biopsy was necessary for the diagnosis of myocarditis in accordance with the World Heart Federation Task force criteria [18]. Since there is no general consensus on criteria involving leukocyte count for defining myocarditis, i.e., also lower counts of infiltrating leukocytes were judged to qualify for myocarditis [19–24], we used the highest usual cut-off value to enhance specificity [25,26]. The vast majority of infiltrating leukocytes were of type CD45RO. Characteristics are shown in Tables 1 and 2. Patients with fulminant myocarditis were not enrolled [27]. Persistence of viral genome was determined by polymerase

Table 1
Characteristics of patients with myocarditis and dilated cardiomyopathy

	Controls ($n = 26$)	Myocarditis ($n = 13$)	Dilated cardiomyopathy ($n = 13$)	P
Characteristic				
Age (years)	45.1 ± 14.7	53.9 ± 17.8	48.2 ± 10.5	ns
Body surface (m^2)	2.0 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	ns
Body mass index (kg/m^2)	26.8 ± 3.4	26.3 ± 3.9	25.5 ± 2.0	ns
Left ventricular parameters				
Ejection fraction (%)	72.3 ± 12.7	59.8 ± 22.9	39.0 ± 15.0	<0.001
EDV (ml)	208.8 ± 71.5	253.3 ± 174.5	327.9 ± 104.6	<0.02
ESV (ml)	57.7 ± 35.2	119.5 ± 127.3	203.5 ± 90.1	<0.001
EDP (mmHg)	12.6 ± 10.5	12.9 ± 8.8	21.7 ± 22.7	ns
CI ($1/min/m^2$)	2.6 ± 0.6	2.3 ± 0.7	2.2 ± 1.4	ns
LVEDD (mm)	49.8 ± 7.5	55.3 ± 11.3	65.8 ± 4.2	<0.001
LVEDDI (mm/m^2)	24.8 ± 2.2	27.6 ± 3.4	34.7 ± 4.8	<0.001
Right ventricular parameter				
EDP (mmHg)	6.0 ± 5.5	7.3 ± 5.2	8.7 ± 4.2	ns
Other hemodynamic parameters				
PC wedge mean (mmHg)	8.7 ± 7.6	11.0 ± 6.6	15.1 ± 8.2	ns
Pulmonary resist. (dyne)	128.3 ± 67.2	173.1 ± 126.0	189.6 ± 138.7	ns
Systemic resist. (dyne)	1550.2 ± 450.3	1679.0 ± 747.8	1790.6 ± 654.6	ns
Myocardial leukocytes				
CD45RO	2.3 ± 2.2	10.6 ± 5.1	2.9 ± 1.7	<0.001
CD11C	0.5 ± 1.1	4.0 ± 2.2	1.4 ± 0.5	<0.001
CD14	0.8 ± 1.3	2.8 ± 2.3	2.2 ± 1.9	<0.05
Pro- and antiinflammatory cytokines				
TNF-α (pg/ml)	10.6 ± 9.9	13.2 ± 10.0	15.6 ± 10.0	ns
IFN-γ (pg/ml)	1.3 ± 3.4	0.7 ± 1.4	5.5 ± 8.6	ns
IL-6 (pg/ml)	3.7 ± 9.5	2.4 ± 2.6	3.3 ± 7.1	ns
IL-10 (pg/ml)	3.8 ± 4.1	8.7 ± 16.4	19.2 ± 52.2	ns
NF-κB (%)	5.0 ± 5.3	11.1 ± 7.1	8.4 ± 2.6	0.005

Abbreviations: EDV, enddiastolic volume; ESV, endsystolic volume; EDP, enddiastolic pressure; LVEDD, left ventricular enddiastolic diameter; LVEDDI, LVEDD normalized to body surface area (m^2).

Table 2
Characteristics of patients with myocarditis stratified by LVEDDI

	LVEDDI (<28 mm/m^2) $n = 7$ (54%)	LVEDDI (>28 mm/m^2) $n = 6$ (46%)	P
Characteristic			
Age (years)	59.0 ± 14.9	47.8 ± 20.3	ns
Body surface (m^2)	1.9 ± 0.2	2.1 ± 0.2	ns
Body mass index (kg/m^2)	26.3 ± 4.2	26.4 ± 4.0	ns
Left ventricular parameters			
Ejection fraction (%)	74.7 ± 6.7	42.3 ± 23.1	<0.005
EDV (ml)	150.6 ± 48.7	373.2 ± 195.6	<0.02
ESV (ml)	36.7 ± 13.3	216.0 ± 134.0	0.005
EDP (mmHg)	10.9 ± 2.7	15.8 ± 13.6	ns
CI (1/min/m^2)	2.3 ± 0.5	2.3 ± 0.9	ns
LVEDD (mm)	47.1 ± 3.3	66.8 ± 7.3	<0.001
LVEDDI (mm/m^2)	25.3 ± 1.9	30.8 ± 2.2	0.001
Right ventricular parameter			
EDP (mmHg)	6.1 ± 4.3	8.8 ± 6.3	ns
Other hemodynamic parameters			
PC wedge mean (mmHg)	10.6 ± 4.6	11.6 ± 9.5	ns
Pulmonary resist. (dyne)	209.3 ± 134.7	122.4 ± 104.6	ns
Systemic resist. (dyne)	1941.7 ± 826.1	1311.2 ± 473.6	ns
Myocardial leukocytes			
CD45RO	13.3 ± 4.8	7.4 ± 3.7	<0.05
CD11C	4.5 ± 2.6	3.6 ± 2.1	ns
CD14	3.3 ± 3.2	2.3 ± 1.5	ns
Pro- and antiinflammatory cytokines			
TNF-α (pg/ml)	13.6 ± 11.6	12.8 ± 8.8	ns
IFN-γ (pg/ml)	0.0 ± 0.0	1.4 ± 1.7	0.05
IL-6 (pg/ml)	3.3 ± 2.0	1.5 ± 2.9	ns
IL-10 (pg/ml)	8.2 ± 10.8	9.2 ± 22.5	ns
NF-κB (%)	16.8 ± 3.6	4.5 ± 3.2	<0.001

Abbreviations: as in Table 1.

chain reaction and Southern blot of adenovirus (ADV), cytomegalovirus (CMV), enterovirus (EV), and parvovirus B19 (PB19) [28]. Patient characteristics with virus genome persistence are summarized in Table 3. A total of 26 patients who presented neither pathohistological and -immunological findings nor viral genome persistence were judged as controls as shown in Tables 1 and 3; their complaints could not be attributed to standard cardiac disorders. Dilated cardiomyopathy (DCM) was diagnosed in patients who presented neither viral persistence nor inflammation, but had increased left ventricular diameters with LVEDDI > 28 mm/m^2 [29].

Measurement of pro- and antiinflammatory cytokines. Increased concentrations of proinflammatory cytokines had been reported to be related to myocarditis [30]. Therefore, blood was taken from all patients directly before heart catheterization. Samples were centrifuged and stored at −80 °C. Serum concentrations of TNF-α, IFN-γ, IL-6, and antiinflammatory IL-10 were measured using ELISA technique with specific antibodies each (R&D Systems, Minneapolis, USA) [31,32].

Measurements of activated NFκB in left ventricular endomyocardial biopsies. A selective antibody for the released activated p65 subunit of NFκB (cat. no. 1697838, Roche Diagnostics, Mannheim, Germany) from mouse–mouse hybrid cells (clone 12H11) was used for immunostaining of left ventricular endomyocardial biopsies [33]. Subsequently, the tissue sections were incubated in a microwave oven in 0.1 mol/L citrate buffer, pH 6.0, for 10 min. After washing, the sections were covered with antibody solution (1:500 dilution) and incubated in a humid chamber for 12 h. The sections were wiped dry, covered with a peroxidase conjugated secondary antibody, and incubated again. After hematoxylin counterstaining, the slides were mounted for microscopy. Typical brown colored areas of immunostained activated NFκB in cytoplasm and nuclei were observed (Fig. 1) and quantified by the "Quantimed 600" system (Leica, Wetzlar, Germany). This observation is in accordance with the fact that translocation of NFκB into the nucleus occurs after its activation in the cytoplasm [34]. Using previously defined color ranges, the stained areas were determined and their percentage values referred to the entire section. This procedure was performed for three different areas of each section and the mean value was calculated. The determinations were carried out in a double-blind manner by two independent investigators.

Statistical analysis. Comparisons between groups were made by one-way analysis of variance (ANOVA). Tuckey post test was used for multiple comparisons. All probability values are 2-sided. In addition, patients with myocarditis were stratified into two groups using a standardized cut-off value for LVEDDI of 28 mm/m^2 [13]. All values were expressed as means ± standard deviation unless specified otherwise. Statistical significance was assumed at $P < 0.05$.

Results

The clinical characteristics of 93 patients are summarized in Tables 1–3. Each 13 patients exhibited myocarditis and DCM, respectively. ADV was present in 13, CMV in 9, EV in 10, and PB19 in 9 patients. Applying the criteria mentioned above, 26 individuals were judged as controls.

Patients with myocarditis and DCM

Patients with DCM showed impaired left ventricular hemodynamics when compared with controls (EF: $P < 0.001$, EDV: $P = 0.01$, ESV: $P < 0.001$, and LVEDD and LVEDDI < 0.001, Table 1). Right ventricular functional parameters as well as systemic and pulmonary vascular resistance were not affected. Infiltrating leukocytes were elevated in patients with myocarditis when compared with controls and DCM (CD45RO and CD11C: $P < 0.01$, Table 1). Systemic pro- and antiinflammatory cytokines did not differ between the groups. Immunostained activated NFκB was increased in patients with myocarditis when compared with controls ($P < 0.005$) whereas patients with DCM exhibited no significant increase (Figs. 1 and 2).

Relation of left ventricular function and activated NFκB in myocarditis

Patients with myocarditis had been stratified into two groups using a LVEDDI of 28 mm/m^2 as cut-off value. The characteristics are summarized in Table 2. As expected, hemodynamic parameters of patients with increased left ventricular diameters were negatively affected (EF, $P < 0.005$; EDV, $P < 0.02$; ESV, $P = 0.005$). Infiltrating leukocytes of type CD45RO and immunostained activated NFκB were elevated in patients with LVEDDI < 28 mm/m^2 when compared to LVEDDI > 28 mm/m^2 ($P < 0.05$, $P < 0.001$, Fig. 3). Patients with myocarditis and preserved left ventricular function exhibited increased activated NFκB when compared to patients with reduced function ($r^2 = 0.72$, $P < 0.001$, Fig. 4A). In parallel, inverse correlation of NFκB and LVEDV was found ($r^2 = 0.43$, $P < 0.02$, Fig. 4B). No relation was found between NFκB and

Table 3
Characteristics of patients with myocardial virus persistence

	Controls ($n = 26$)	ADV ($n = 13$)	CMV ($n = 9$)	EV ($n = 10$)	PB19 ($n = 9$)	P
Characteristic						
Age (years)	45.1 ± 14.7	49.2 ± 12.2	53.7 ± 10.3	49.4 ± 14.0	37.2 ± 4.8	ns
Body surface (m^2)	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.3	ns
Body mass index (kg/m^2)	26.8 ± 3.4	28.8 ± 5.3	28.5 ± 4.7	24.8 ± 3.4	26.7 ± 6.3	ns
Left ventricular parameters						
Ejection fraction (%)	72.3 ± 12.7	59.9 ± 26.3	64.6 ± 12.7	64.6 ± 15.4	66.2 ± 20.0	ns
EDV (ml)	208.8 ± 71.5	197.2 ± 53.1	191.6 ± 49.1	266.9 ± 137.1	181.0 ± 11.6	ns
ESV (ml)	57.7 ± 35.2	87.2 ± 74.1	69.1 ± 41.2	95.2 ± 74.2	60.2 ± 34.5	ns
EDP (mmHg)	12.6 ± 10.5	17.4 ± 8.6	4.8 ± 13.6	9.0 ± 87.5	19.8 ± 8.1	ns
CI ($1/min/m^2$)	2.6 ± 0.6	2.8 ± 0.7	2.1 ± 0.4	2.2 ± 0.8	2.1 ± 0.3	ns
LVEDD (mm)	49.8 ± 7.5	55.0 ± 6.2	61.1 ± 4.8	55.8 ± 10.5	51.4 ± 8.4	0.005
LVEDDI (mm/m^2)	24.8 ± 2.2	26.8 ± 3.0	29.2 ± 2.6	28.5 ± 3.4	25.6 ± 1.6	<0.001
Right ventricular parameter						
EDP (mmHg)	6.0 ± 5.5	10.6 ± 6.5	6.8 ± 3.9	8.2 ± 3.6	9.4 ± 2.3	ns
Other hemodynamic parameters						
PC wedge mean (mmHg)	8.7 ± 7.6	17.6 ± 15.0	5.3 ± 3.9	10.0 ± 3.7	11.6 ± 6.0	ns
Pulmonary resist. (dyne)	128.3 ± 67.2	146.2 ± 172.9	158.9 ± 45.5	121.7 ± 125.7	198.6 ± 72.9	ns
Systemic resist. (dyne)	1550.2 ± 450.3	1302.9 ± 293.8	1810.1 ± 301.2	1713.8 ± 737.8	1593.6 ± 148.8	ns
Myocardial leukocytes						
CD45RO	2.3 ± 2.2	2.4 ± 1.7	1.9 ± 1.6	2.4 ± 2.1	2.4 ± 2.4	ns
CD11C	0.5 ± 1.1	0.6 ± 0.5	4.0 ± 2.2	0.7 ± 0.8	0.0 ± 0.0	ns
CD14	0.8 ± 1.3	0.4 ± 0.5	0.8 ± 0.8	0.0 ± 0.0	1.2 ± 2.6	ns
NF-κB (%)	5.0 ± 5.3	13.0 ± 7.6	10.6 ± 6.9	6.4 ± 4.3	2.0 ± 1.0	<0.001

Abbreviations: as in Table 1.

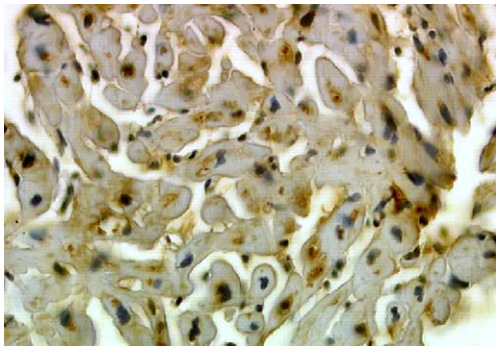

Fig. 1. Immunostaining of activated NFκB in a patient with myocarditis.

pulmonary capillary wedge pressure (not shown). In contrast, NFκB activation was not related to left ventricular EF or EDV in patients with DCM. However, NFκB activation was less in patients with myocarditis and left ventricular dilation (LVEDD > 28 mm/m^2) when compared with DCM ($P = 0.01$).

Patients with virus genome persistence

Patients with CMV persistence showed increased LVEDD and LVEDDI when compared with controls ($P < 0.005$, $P = 0.001$). EV persistence was related to increased LVEDDI when compared with controls ($P < 0.005$). Other hemodynamic parameters and systemic

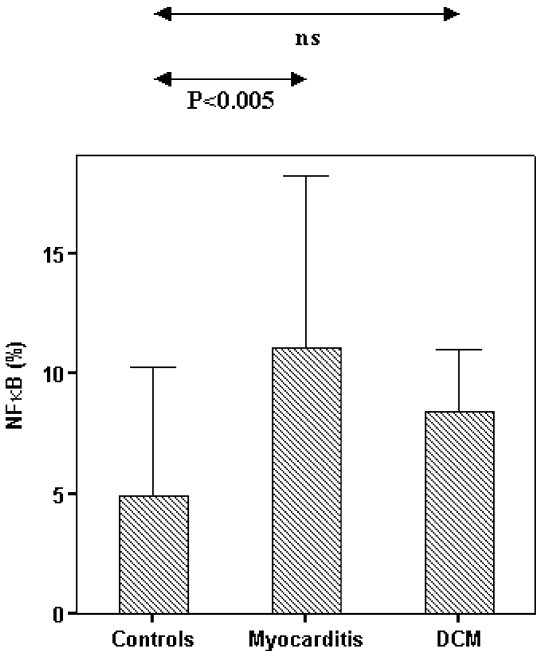

Fig. 2. Extent of immunostained activated NFκB in controls, myocarditis, and dilated cardiomyopathy (DCM).

concentrations of pro- and antiinflammatory cytokines did not differ between the groups with virus persistence (Table 3). Increased activated NFκB was found in patients with ADV persistence in comparison to controls

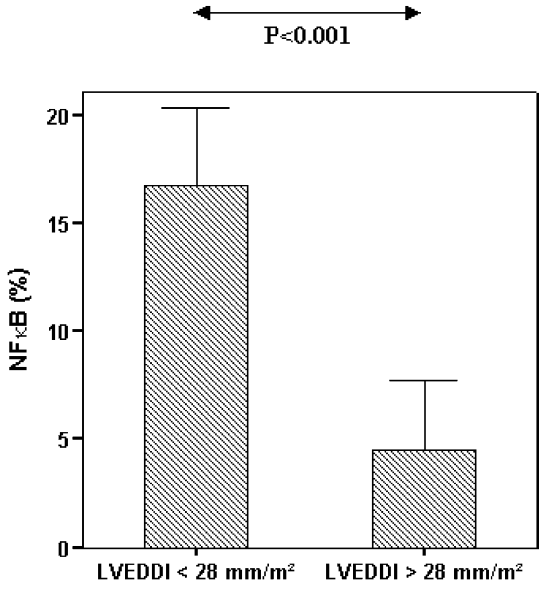

Fig. 3. Extent of immunostained activated NFκB in patients with myocarditis and normal left ventricular enddiastolic diameters (LVEDDI < 28 mm/m^2) compared with dilated hearts (LVEDDI > 28 mm/m^2).

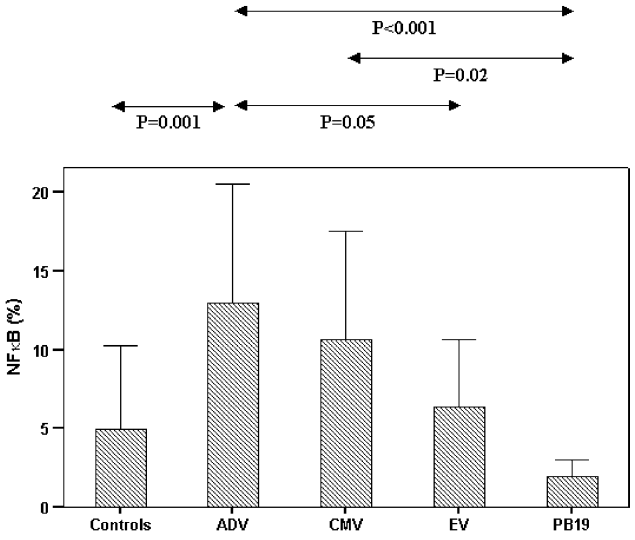

Fig. 5. Extent of immunostained activated NFκB in controls and patients with various viral genome persistence. All significant differences are shown.

($P = 0.001$, Fig. 5). Only a trend of increased NFκB activation was seen in patients with CMV persistence (not significant). Patients with PB19 persistence showed no increase of NFκB activation.

Discussion

The present study shows substantial increase of activated NFκB in left ventricular endomyocardial biopsy specimen of patients with myocarditis. The extent of activated NFκB was related to preserved left ventricular function in patients with myocarditis. In turn, NFκB was not increased in myocarditis when left ventricular dilation had occurred. An increased NFκB activation was observed in patients with intramyocardial ADV genome persistence. In addition, a trend of elevated NFκB was found in patients with CMV genome persistence. EV and PB19 virus persistence did not affect the extent of activated NFκB. Myocardial prevalence of PB19 viral genome in patients with DCM has been reported to occur in up to 50% [22,35]. Nonetheless, its pathophysiological and clinical significance is still unsettled. It remains to be shown whether enteroviruses induce mechanisms counteracting NFκB activation or whether their shorter life cycle has any influence. Also, recruitment of other proinflammatory mediators had to be taken into account. Since pro- and antiinflammatory serum cytokine concentrations did not differ between the observed groups, they cannot be responsible for differences of activated NFκB. It remains to be shown whether local effects of cytokines or viral replication are involved in NFκB activation [36].

Although the electrophoretic mobility shift assay is appropriate for measuring released activated NFκB [37], it requires amounts of tissue which cannot be provided

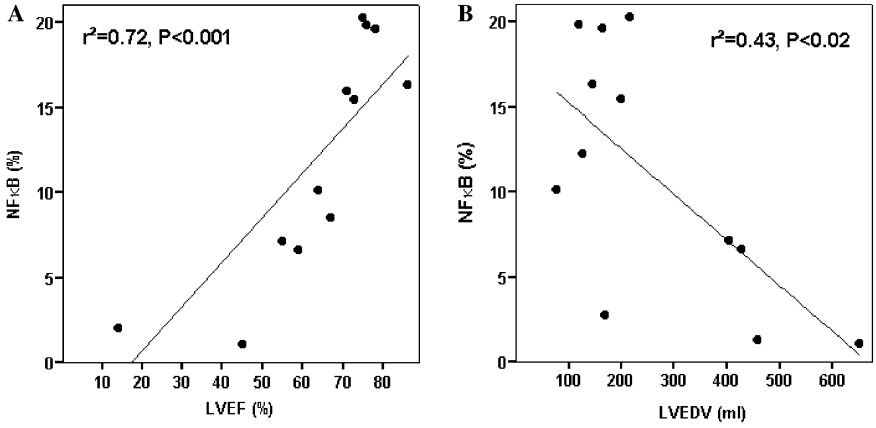

Fig. 4. Correlation of the extent of activated NFκB with (A) left ventricular ejection fraction (LVEF) and (B) left ventricular enddiastolic volume (LVEDV).

by endomyocardial biopsies. Therefore, immunostaining of NFκB was performed with an antibody recognizing an epitope overlapping the nuclear location signal of the p65 subunit of the NFκB heterodimer. Thus, the activated form of NFκB was detected selectively [9].

NFκB is a principal intracellular transcription factor and usually exists as latent cytoplasmic complex bound to its inhibitory protein IκB [38]. Activation by viruses, cytokines, oxidants, and protein kinase results in IκB degradation. Subsequently, NFκB translocates into the nucleus and induces expression of various proinflammatory products, e.g., cytokines, intracellular adhesion molecules, and inducible nitric oxide, which could be cardiodepressive [6,7,10,12,39–44].

Activation of NFκB was shown in experimental murine myocarditis [45]. NFκB is a key regulator in the progression of autoimmune myocarditis and in vivo transfection of NFκB decoy reduced the severity of disease [46]. Particularly, TNF-α has been shown to depress heart function and to favor dilatation of the heart [47,48]. In accordance, when high levels of IκB were induced by dexamethasone, the myocardial TNF-α production was reduced and cardiac contractility improved [49,50]. Overexpression of cardiomyocyte IκB was also sufficient to block TNF-α production and prevented cardiac dysfunction after a challenge with lipopolysaccharide [51]. Peroxisome proliferator-activated receptors (PPAR) are judged to regulate cardiomyocyte hypertrophy at least partially through the NFκB pathway [52,53]. It was shown that use of left ventricular assist devices decreases the extent of NFκB activation in failing human hearts, suggesting that NFκB may be involved in the process of reverse remodeling in patients with severe heart failure [54].

Viral infection of the heart exhibits a standardized progression of illness following three stages: The acute phase (days 0–3) is characterized by systemic viremia, associated vascular response, and direct virus-induced cardiotoxicity including myofiber necrosis and myocytolysis in the absence of inflammatory cells [55,56]. NFκB can initiate and amplify the synthesis of proinflammatory cytokines involving not only IL-1, IL-2, IL-6, IL-8, and IFN-γ but also adhesion molecules, protein kinases, inducible nitric oxide synthase, and cyclooxygenase-2. The subacute stage (days 4–14) begins with invading infiltrating cells. Dendritic cells and macrophages ingest viral agents for subsequent processing. Synthesis of various cytokines is increased; ongoing involvement of NFκB has to be assumed [57]. Cytokines such as TNF-α and IL-6 are known to be released from cardiomyocytes possibly leading to an autocrine or paracrine action on neighboring cells. Particularly, TNF-α has been shown to depress heart function and to favor dilatation of the heart. The third stage (day 15 and beyond) could lead to dilated cardiomyopathy with increased fibrosis and potentially ongoing virus replication [55,56]. Viral persistence even without the ability of multiplication can induce DCM [32,58]. Loss of contractile cardiomyocytes and associated depression of functional performance could arise from necrosis and apoptosis.

Apoptosis is a central host defense mechanism to eliminate virus-infected cells. It is known that marked increase of apoptosis occurs in chronic myocarditis [15,16] and activation of NFκB suppresses apoptosis in vitro [59]. Thus, one of the two potential mechanisms or a combination of both could be hypothesized to explain the observed positive correlation between preserved left ventricular function and increased activation of NFκB:

1. Depressed cardiac function and left ventricular dilation could be an end-stage result of long-lasting inflammatory processes. In turn, a preserved cardiac function is observed at earlier stages.
2. Activation of NFκB per se occurs to a variable extent whereby the causes remain unknown. A pronounced increase of activated NFκB could preserve ventricular function by protection from contractile cardiomyocyte loss due to apoptosis [59]. In turn, insufficient activation of NFκB could lead to cell loss and consecutive ventricular dilatation.

Both hypotheses would be in accordance with the fact that, usually, initiation of myocarditis cannot be determined exactly in patients. It also has to be assumed that the extent of inflammatory response decreases at later stages. Although fibrosis could contribute to the decrease in leukocytosis and activated NFκB in dilated hearts, it appears unlikely that it can solely account for the observed reduction. Data from explanted hearts of patients with idiopathic dilated cardiomyopathy demonstrated that enterovirus RNA persists in myocardium of a significant proportion of patients with end-stage dilated cardiomyopathy in the absence of a continuing cell-mediated or humoral immune response [2]. Myocardial virus persistence and myocardial inflammation/endothelial activation are reported to be associated with endothelial dysfunction of the coronary microcirculation, and endothelial dysfunction could occur independently of myocardial inflammation in patients with virus persistence [60]. The hypotheses are concordant with experimental findings showing that sustained hemodynamic overloading in mice by transverse constriction of the aorta provoked a transient increase in proinflammatory cytokine and cytokine receptor gene expression. Decrease in proinflammatory cytokine gene expression occurred in the absence of changes in loading conditions, suggesting that the expression of proinflammatory cytokines in the heart is regulated, at least in part, by load-dependent and load-independent mechanisms [61].

In conclusion, the determination of activated NFκB in endomyocardial biopsies provides a novel diagnostic tool for detecting proinflammatory processes in cardiomyocytes of patients with myocarditis and myocardial viral genome persistence. Since NFκB is a crucial key transcription factor of inflammation, its prognostic relevance remains

intriguing. Potentially, NFκB could be a future target for treatment of inflammatory heart disease [5,36].

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