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Circulating cytokine levels in mice with heart failure are etiology dependent

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Vistnes M, Wæhre A, Nygård S, Sjaastad I, Andersson KB, Husberg C, Christensen G. Circulating cytokine levels in mice with heart failure are etiology dependent. *J Appl Physiol* 108: 1357–1364, 2010. First published March 11, 2010; doi:10.1152/japplphysiol.01084.2009.—*Objectives*: The aim of this study was to examine whether alterations in circulating cytokine levels are dependent on the etiology of myocardial hypertrophy and heart failure (HF). *Background*: Several heart diseases are associated with altered levels of circulating cytokines. Cytokines are regarded as possible therapeutic targets or biomarkers, but such approaches are currently not in clinical use. If alterations in circulating cytokines are etiology dependent, this should be taken into consideration when using cytokines as disease markers and therapeutic targets. *Methods*: The serum levels of 25 cytokines were quantified with Luminex and/or ELISA in four murine models of heart disease: banding of the ascending aorta (AB) or the pulmonary artery (PB), myocardial infarction (MI), and a cardiomyopathy model with inducible cardiomyocyte-specific knockout of the sarco(endo)plasmatic reticulum Ca^{2+}-ATPase (SERCA2KO). *Results*: No increase in circulating cytokine levels were found in mice 1 wk after AB, although substantial myocardial hypertrophy was present. After 1 wk of MI, only interleukin (IL)-18 was increased. In the SERCA2KO mice with HF, circulating levels of IL-1α, IL-2, IL-3, IL-6, IL-9, IL-10, IL-12p40, eotaxin, granulocyte-colony stimulating factor (G-CSF), interferon-γ, monocyte chemoattractant protein-1, macrophage inflammatory protein-1β were increased, and in mice with PB, IL-1α, IL-6, G-CSF, and monokine induced by gamma-interferon showed elevated levels. *Conclusions*: Serum levels of cytokines in mice with HF vary depending on the etiology. Increased serum levels of several cytokines were found in models with increased right ventricular afterload, suggesting that the cytokine responses result primarily from systemic congestion.

inflammation; infarction; interleukins

AN ASSOCIATION BETWEEN heart disease and alterations in serum cytokine levels has been known for almost two decades (14). High expectations have been placed on cytokines as both biomarkers and therapeutic targets in heart failure (HF). However, although some recent studies indicate positive effects of anti-inflammatory treatment in subgroups of HF patients (12, 22), the use of cytokines as therapeutic targets or as markers of cardiac disease is not a part of current clinical practice.

The lack of success in this field of research may be related to the fact that many studies have been performed in heterogenous patient populations with HF of different etiologies (4, 15). If cytokine activation differs according to etiology and form of HF, such approach might not identify the right targets or markers. This view is in accordance with a recent report from an expert workshop of The Heart Failure Association of the European Society of Cardiology, stating that the idea of a common inflammatory pathway that characterizes all types of HF appears unrealistic and that determination of the specific inflammatory pathways in different forms of HF may be essential (13).

Thus the aim of the present study was to examine alterations in circulating cytokine levels in cardiac disease with different etiologies and to relate cytokine activation to differences in pathophysiology. Analyses of 25 serum cytokines were carried out in four mouse models: banding of the ascending aorta (AB) or the pulmonary artery (PB), myocardial infarction (MI), and a cardiomyopathy model with inducible cardiomyocyte-specific knockout of the sarco(endo)plasmatic reticulum Ca^{2+}-ATPase (SERCA2KO). The mice were examined with echocardiography, necropsy, and histological measurements to characterize pathophysiological changes, and the AB, PB, and MI mice were killed at *day 6–7* after the primary operation. This time point was selected to obtain mice with new-onset HF, facilitating comparisons between left-sided HF with pulmonary congestion and right-sided HF with systemic congestion, before the other ventricle is affected.

As demonstrated by our findings, cytokine responses in cardiac disease are highly dependent on the primary etiology and the ensuing pathophysiological alterations. Interestingly, we found increased levels of several circulating cytokines primarily in mice with evidence of right-sided HF, indicating that the inflammatory response in cardiac disease relates to the presence of systemic congestion.

METHODS

The experimental procedures conformed to the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* and the protocols were approved by the Norwegian Council for Animal Research. The AB, PB, and MI mice were killed 6 or 7 days after the primary surgical procedure. See supplemental materials online for details regarding the surgical procedures.

Aortic banding. Animals with a maximum velocity of blood flow (V_{max}) over the stenosis of >3 m/s and a left ventricular weight (LVW)/tibial length (TL) of >4/3 of the mean value of the sham group ($sham_{mean}$) were included. Animals with a lung weight (LW)/TL of >5/3 of $sham_{mean}$ and an increased left atrial (LA) diameter >2 mm, a reliable criteria for HF with pulmonary congestion (10), were included in the HF group, while animals with LW/TL of <4/3 of $sham_{mean}$ were included in the non-HF group.

Pulmonary artery banding. Animals with a V_{max} over the stenosis of >1.8 m/s and a right ventricular weight (RVW)/TL of >1.8 mg/mm were included. No further subclassification was made in this model.

Myocardial infarction. Animals with an infarcted area covering more than 1/3 of the total endocardial area were included. Criteria for inclusion into the HF and non-HF groups were as described above (AB).

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Mice with cardiomyocyte-specific disruption of the Serca2 gene (SERCA2KO). SERCA2KO and SERCA2FF (control) were bred and genotyped as described (2). Both genotypes are backcrossed onto the BL6/J background, matching the genetic background of the other mouse models used in this study. The mice were killed 4 (SERCA2KO4w) and 7 wk (SERCA2KO7w) after the first day of tamoxifen treatment. At 4 wk, in vivo cardiac function is moderately affected in SERCA2KO mice. At 7 wk, these animals develop severe left ventricular dysfunction and congestive HF with right ventricular overload and hypertrophy, whereas control SERCA2FF animals are unaffected (2).

Histology of liver. Livers were fixed in 4% buffered formalin, embedded in paraffin wax, sectioned in 3.5 μm slices and stained with hematoxylin and eosin, Van Gieson and Gomori stain, and immunostained with CD68 antibody. To assess liver congestion, the following morphological criteria were used: degeneration of hepatocytes and fibrosis around the central vein, erythrocyte phagocytosing macrophages, irregular cord architecture, and single hepatocyte necrosis.

Statistical analysis. All values are presented as means ± SE. For comparisons, unpaired two-sided Student's *t*-test was used. Differences between groups were considered significant at a P value of <0.05.

Power analysis predicted that a sample size of eight per group was sufficient to detect a difference of 33% between groups with a power greater than 0.85, assuming a standard deviation of 20% of the mean and a level of significance of 0.05. See supplemental material online for details.

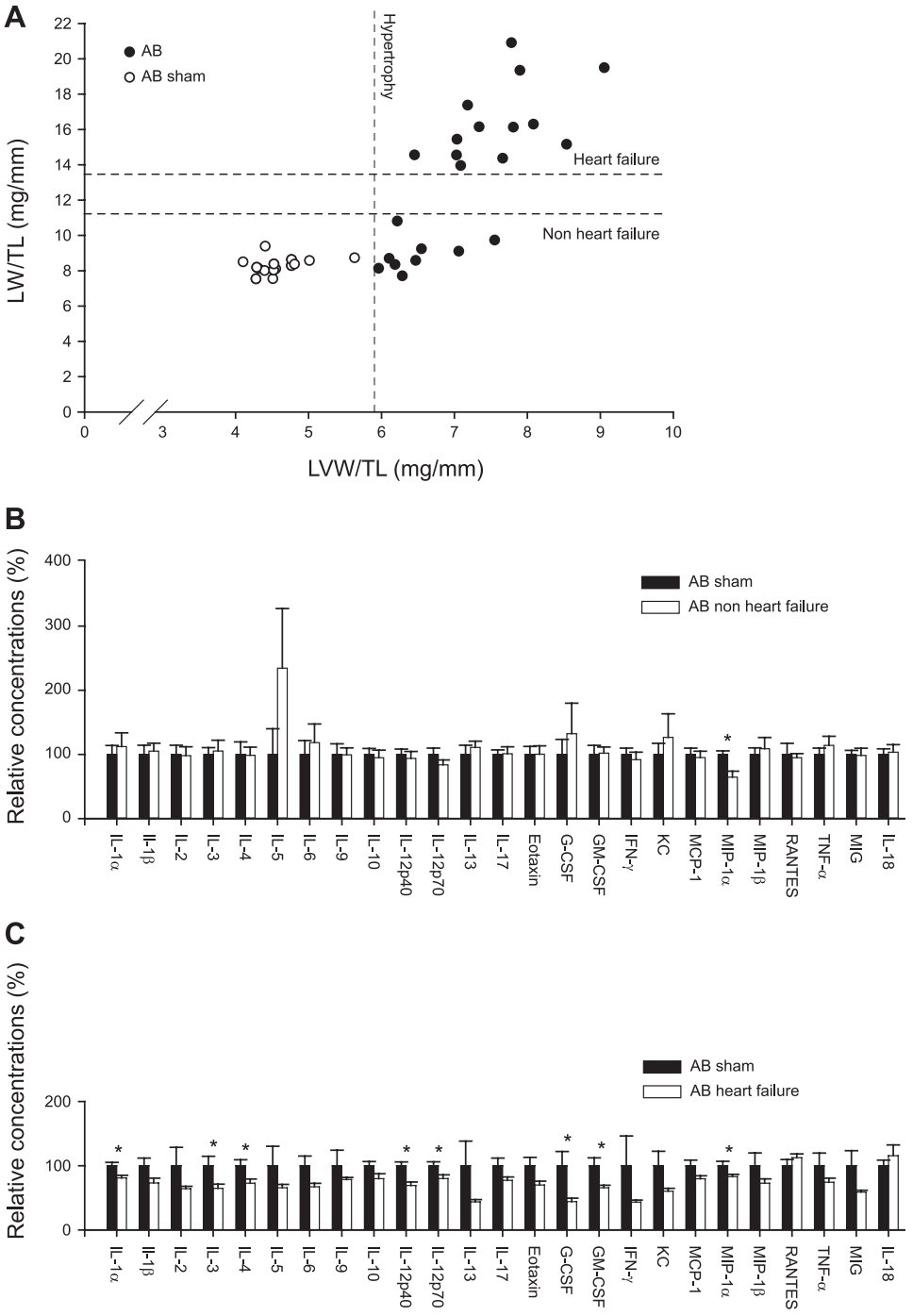

Fig. 1. Characteristics and cytokine concentrations—aortic banding mice. *A*: based on lung weight (LW)/tibial length (TL) and left ventricular weight (LVW)/TL after banding of aorta (AB) the mice were included in the heart failure and non-heart failure groups as described in METHODS. Relative concentrations of cytokines in serum of AB mice in the non-heart failure (*B*) and the heart failure (*C*) groups. Concentrations in AB sham are set to 100%. IL, interleukin; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN, interferon; KC, keratinocyte chemoattractant; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T-cell expressed, and secreted; TNF, tumor necrosis factor; MIG, monokine induced by gamma-interferon. *$P < 0.05$.

Table 1. *Echocardiographic measurements and organ weights in AB mice*

	AB Sham	AB Non-HF	AB Sham	AB HF
n	9	9	12	13
		Echocardiography		
Vmax, m/s	1.12 ± 0.07†	3.87 ± 0.16*	1.38 ± 0.12‡	3.66 ± 0.22*
LA diameter, mm	1.52 ± 0.04†	1.81 ± 0.06*	1.59 ± 0.09‡	2.41 ± 0.07*
		Organ weights		
LVW/TL, mg/mm	4.57 ± 0.10	6.49 ± 0.17*	4.59 ± 0.11	7.61 ± 0.19*
RVW/TL, mg/mm	1.24 ± 0.05	1.28 ± 0.06	1.25 ± 0.06	1.56 ± 0.06*
LW/TL, mg/mm	8.26 ± 0.11	8.90 ± 0.31	8.22 ± 0.15	16.42 ± 0.62*
Body wt, g	23.20 ± 0.57	22.61 ± 0.49	23.33 ± 0.42	20.53 ± 0.56*
Change in body wt, Δg	0.28 ± 0.50	−0.66 ± 0.49	−0.13 ± 0.40	−2.84 ± 0.37*

Values are mean ± SE. AB, aortic banding; Vmax, maximum velocity of blood flow over the stenosis; LA, left atrial; LVW, left ventricular weight; RVW, right ventricular weight; LW, lung weight; TL, tibial length. *$P < 0.05$ vs. AB sham; †measured in a subset of mice ($n = 4$); ‡measured in a subset of mice ($n = 3$).

Other methods. Anesthesia, echocardiographic examination, tissue and serum sampling, Luminex analysis of cytokines, and validation of the assay are detailed in online supplemental material.

RESULTS

Circulating cytokines in mice with AB. To determine the effect of left ventricular pressure overload on circulating cytokine levels, we analyzed serum obtained from mice with AB. Figure 1*A* shows development of myocardial hypertrophy confirmed by an elevated LVW/TL in the groups without (42%) and with HF (66%) compared with the sham group. In the HF group, mean increases were 100% and 52% in LW/TL and LA diameter, respectively, while RW/TL was increased by 25% compared with sham values (Table 1).

Despite substantial left ventricular hypertrophy, we found no increase in circulating levels of cytokines in the AB mice (Fig. 1, *B* and *C*). The level of MIP-1α was decreased in both groups, whereas the levels of seven additional cytokines, interleukin (IL)-1α, IL-3, IL-4, IL-12p40, IL-12p70, granulocyte colony stimulating factor (G-CSF), and granulocyte macrophage colony stimulating factor (GM-CSF), were significantly reduced only in the AB HF group. For verification, the concentration of IL-12p40 was measured by both Luminex and ELISA in two separate subsets of AB mice, giving similar results with 45% and 35% reduction, respectively. The levels of 14 of the measured cytokines, including IL-1β, IL-6, and tumor necrosis factor (TNF)-α, showed a significant positive correlation to RVW (Table 2, Fig. 2).

Circulating cytokines in mice with PB. The effect of right ventricular pressure overload on serum cytokine levels was evaluated in mice with PB. Hypertrophy of the right ventricle was confirmed by an 84% increase in RVW/TL compared with sham mice (Table 3). Histology of the liver showed presence of systemic congestion in PB mice (Fig. 3) and ascites was observed at necropsy.

A reduced level of IL-12p40 was found in PB mice, similar to the finding in the HF group after AB. In contrast, we found that the levels of four other cytokines, IL-1α, IL-6, G-CSF, and monocyte induced by gamma interferon (MIG), were increased after PB compared with sham-treated mice (Fig. 4).

Circulating cytokines in mice with MI. To assess the cytokine response after MI, cytokines in serum were analyzed. No difference in infarct size and LVW (noninfarcted)/TL between the two groups with MI were observed. The HF group was characterized by a 107% increase in LW/TL compared with sham (Fig. 5*A*) and a 16% increase in LA diameter compared with non-HF mice. RVW/TL was similar in the MI and the sham-treated mice (Table 4).

In the non-HF group, we found no alterations in circulating cytokine levels, except for an increased level of IL-18 (Fig. 5*B*), which was even more pronounced in the HF group (Fig. 5*C*). Four cytokines were decreased in the HF group compared with sham. As in the AB HF and PB group, a decreased level of IL-12p40 was found. In addition, IL-1β, IL-3, and keratinocyte chemoattractant (KC) showed decreased levels. Eight of the measured cytokines in the HF group showed a positive correlation to RVW/TL (Table 2).

Circulating cytokines in mice with cardiomyopathy due to SERCA2KO. The alterations in circulating cytokine levels in mice with cardiomyopathy were examined using the SERCA2KO model. Left ventricular hypertrophy was not evident in SERCA2KO mice at 4 or at 7 wk after *Serca2* gene disruption (Table 5). However, SERCA2KO animals had a 27% and 31% increase in RVW/TL after 4 and 7 wk, respectively. At 4 wk, a 39% increase in LW/TL and 248% increase in atrial weight/TL were found. After 7 wk, a 71% increase in LW/TL and a 729% increase in atrial weight/TL compared with control, indicated progression into HF (Table 5), comparable to previous findings

Table 2. *Correlation between cytokine levels and RVW/TL*

	AB HF		MI HF	
	P	*r*	*P*	*R*
IL-1α	0.003*	0.747	0.044*	0.719
Il-1β	<0.001*	0.811	0.364	0.372
IL-2	0.018*	0.641	0.339	0.390
IL-3	0.041*	0.572	0.049*	0.707
IL-6	0.012*	0.674	0.340	0.390
IL-12p70	0.046*	0.561	0.023*	0.777
IL-13	0.003*	0.751	0.361	0.374
IL-17	0.152	0.421	0.009*	0.842
Eotaxin	<0.001*	0.823	0.036	0.739
GM-CSF	0.010*	0.682	0.028*	0.762
IFN-γ	0.005*	0.729	0.013*	0.819
MCP-1	0.001*	0.794	0.023*	0.777
MIP-1α	0.042*	0.569	0.002*	0.900
MIP-1β	0.006*	0.715	0.381	0.360
TNF-α	0.011*	0.676	0.826	−0.093

r, Correlation coefficient; *P, P* value. Only cytokines with a significant correlation with RVW/TL are included in the table.

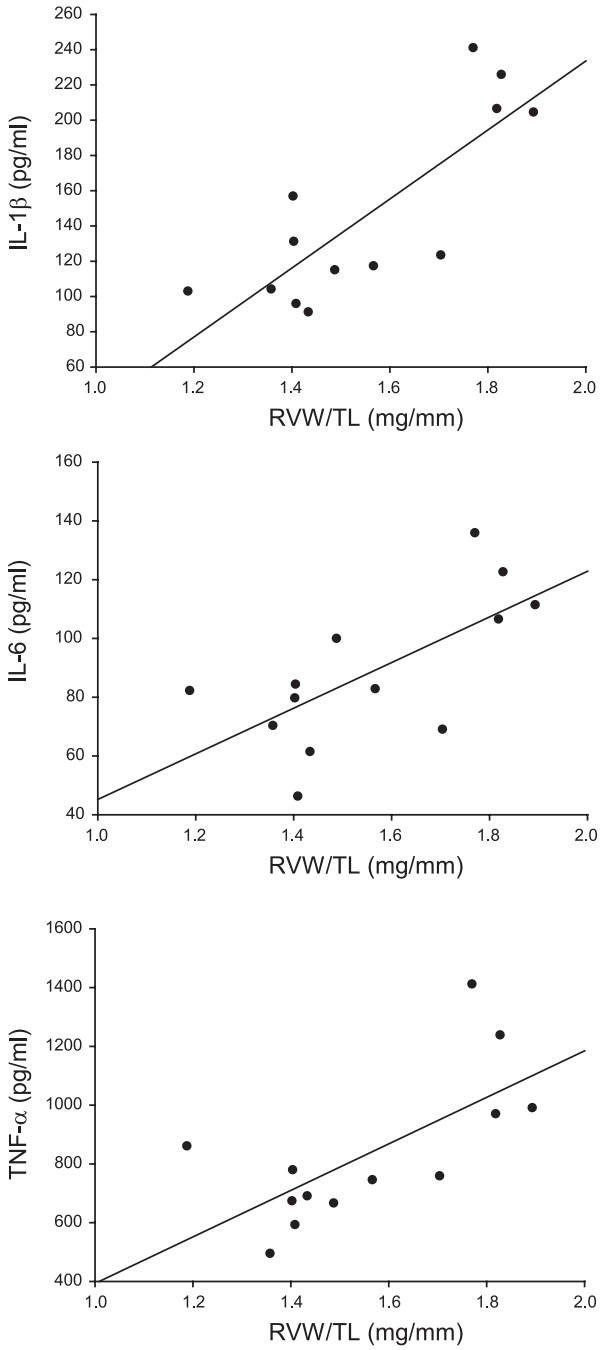

Fig. 2. Correlation between right ventricular weight (RVW)/TL and cytokine levels in mice after AB. Fourteen cytokines showed a positive correlation with RVW/TL, including IL-1β, IL-6, and TNF-α (shown as examples).

(2). Histology of liver showed presence of systemic congestion in SERCA2 KO7w mice (Fig. 3) and ascites was observed during necropsy.

We did not find altered cytokine levels in SERCA2KO4w compared with SERCA2FF4w mice (Fig. 6*A*). However, in SERCA2KO7w mice, the level of 12 cytokines were increased (Fig. 6*B*). IL-1α, IL-6, and G-CSF were increased, similar to the findings in the PB HF mice. Interestingly, the level of IL-12p40 was increased in this group, in contrast to the decreased values seen in all the other HF groups examined. IL-2, IL-3, IL-9, IL-10, eotaxin, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1, and macrophage inflammatory protein (MIP)-1β also showed increased levels.

DISCUSSION

In this study, we show that the levels of circulating cytokines in mice with heart disease are dependent on etiology and that the alterations are related to pathophysiological features. No increase in circulating cytokine levels was found in AB mice, although the animals had substantial myocardial hypertrophy and pulmonary congestion. In mice with MI, only the level of IL-18 was increased. In contrast, several cytokines were increased in the circulation in both mouse models with right ventricular overload (PB and SERCA2KO7w).

Increased levels of cytokines in mice with systemic congestion. Common for PB and SERCA2KO7w was the presence of systemic congestion, suggesting that this is a stimulus for cytokine release into the circulation. The presence of systemic congestion in PB and SERCA2KO mice was demonstrated by observation of ascites and congested livers, with typical morphological characteristics such as centrilobular necrosis, showed by histology. The liver weight was maintained or slightly decreased, but to a much lesser degree than in the left-sided HF (data not shown). A decrease in liver weight in chronic HF in rats has previously been described (7). Moreover, in a rat model of right-sided HF (17), liver weight remained unchanged despite macroscopic evidence of hepatic congestion supporting that the liver weight does not increase in rodent models of right-sided HF. In AB and MI mice, mean RVW/TLs were only moderately affected. However, a positive correlation was shown between the levels of several cytokines and RVW/TL in these models, also supporting a role for increased right ventricular afterload in enhancing circulating cytokine levels.

Cytokine levels in mice with pulmonary congestion. Surprisingly, in mice with pulmonary congestion (AB and MI), several cytokines showed unaltered or even decreased levels. Previous studies have shown increased levels of cytokines in heart disease of these etiologies. However, the majority has focused on severe HF and symptomatic disease (9), where congestion in the systemic circulation may also be the cause of increased serum levels. Few studies have examined circulating cytokines in rodents, but an increase in the plasma levels of IL-10 and IL-1β 6 mo after aortic constriction has been found

Table 3. *Echocardiographic measurements and organ weights in PB mice*

	PB Sham	PB
n	9	9
	Echocardiography	
Vmax, m/s	not measured	2.02 ± 0.07
	Organ weights	
RVW/TL, mg/mm,	1.16 ± 0.06	2.14 ± 0.08*
LVW/TL, mg/mm	5.01 ± 0.13	4.26 ± 0.09*
LW/TL, mg/mm	8.45 ± 0.29	7.49 ± 0.29*
Body wt, g	26.69 ± 0.69	23.15 ± 0.43*†
Change in body wt, Δg	0.83 ± 0.21	−1.21 ± 0.46*

Values are mean ± SE. *$P < 0.05$ vs. PB sham; †measured in a subset of mice ($n = 5$).

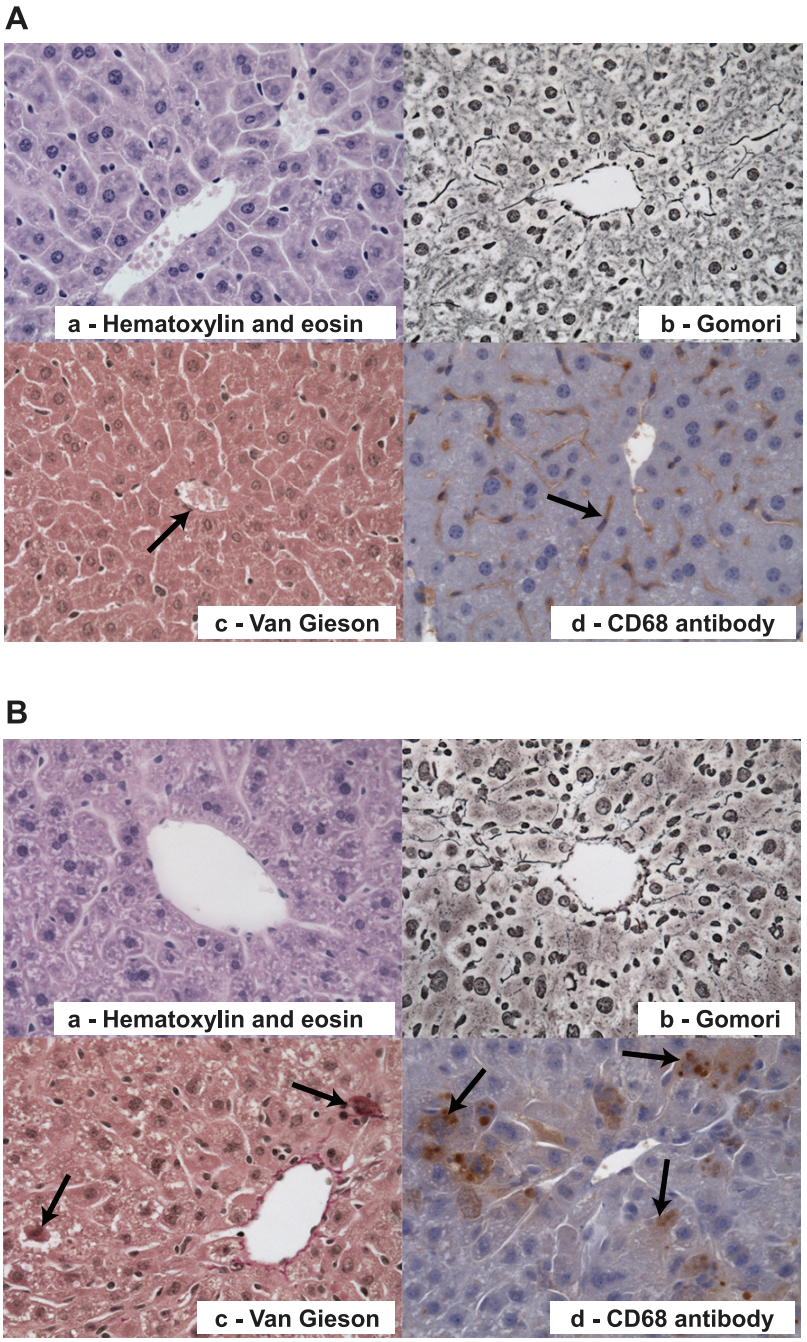

Fig. 3. Liver histology. *A*: morphology of livers without congestion (×40). a, No centrilobular necrosis; b, regular cord architecture; c, no fibrosis around the central vein (arrow); d, normal Kupffer cells along the sinusoids (arrow). *B*: morphology of congested livers (×40). Representative sections from mice with banding of the pulmonary artery and SERCA2KO mice. a, Centrilobular necrosis; b, irregular cord architecture; c, fibrosis around the central vein and single hepatocyte necrosis (arrows); d, erythrocyte phagocytosing macrophages (arrows).

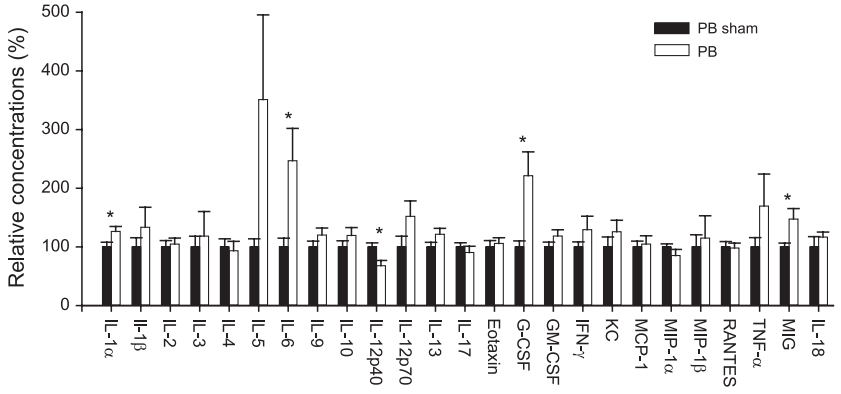

Fig. 4. Cytokine concentrations–pulmonary artery banding (PB) mice. Relative concentrations of cytokines in serum of mice with PB. Concentrations in PB sham are set to 100%. *$P < 0.05$.

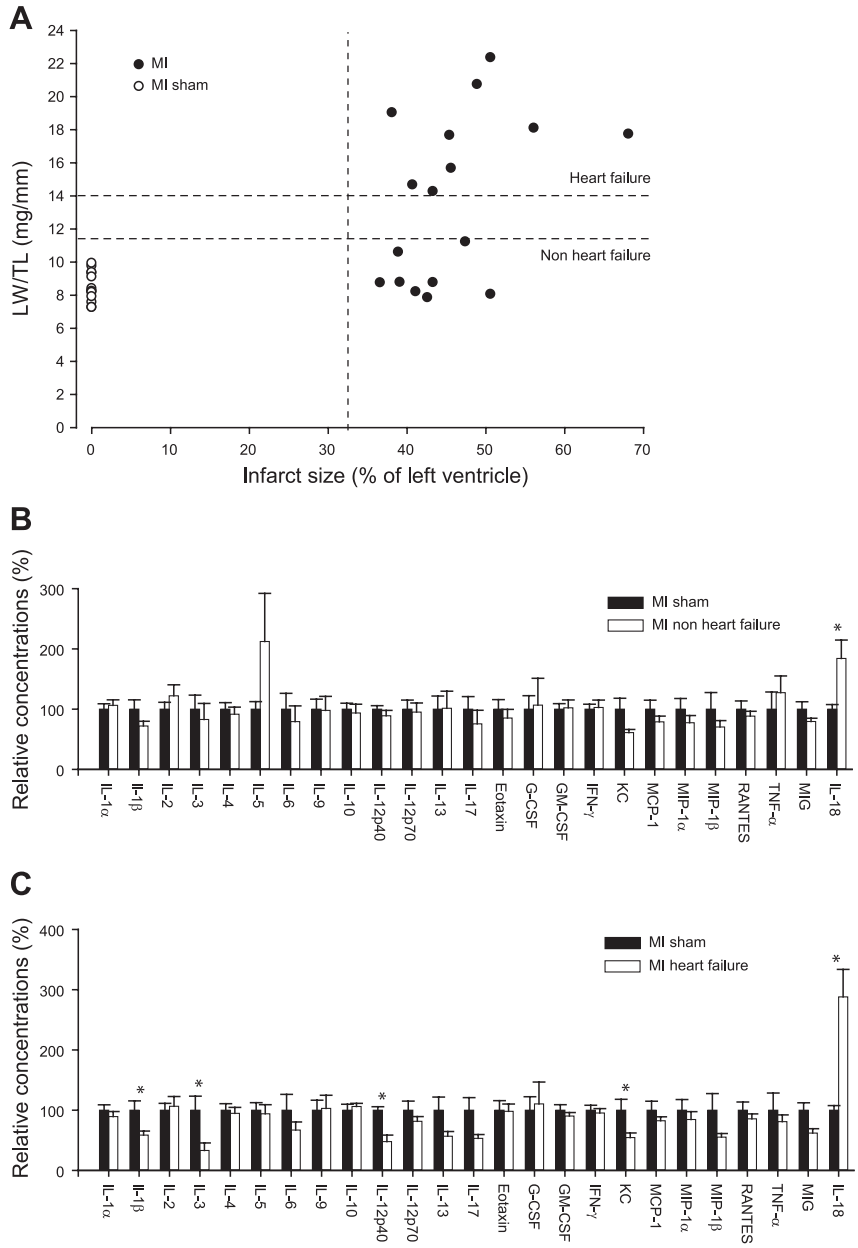

Fig. 5. Characteristics and cytokine concentrations—myocardial infarction (MI) mice. *A*: all mice included had MI size of >1/3 of the left ventricular wall. Based on LW/TL after induction of MI the mice were included in the heart failure and non-heart failure groups as described in METHODS. Relative concentrations of cytokines in serum of MI mice in the nonheart failure (*B*) and the heart failure (*C*) groups. Concentrations in the sham group are set to 100%. *$P < 0.05$.

Table 4. *Echocardiographic measurements and organ weights in MI mice*

	Sham	MI Non-HF	MI HF
n	12	8	9
	Echocardiography		
LA diam, mm	not measured	2.27 ± 0.06	2.63 ± 0.07*
	Organ weights		
LVW infarct/TL, mg/mm	–	1.64 ± 0.11	1.85 ± 0.17
LVW viable/TL, mg/mm	–	3.84 ± 0.14	3.62 ± 0.23
LVW/TL, mg/mm	4.97 ± 0.14	5.49 ± 0.17†	5.47 ± 0.32
RVW/TL, mg/mm	1.33 ± 0.06	1.18 ± 0.02	1.34 ± 0.05
LW/TL, mg/mm	8.61 ± 0.25	9.03 ± 0.43	17.80 ± 0.90†
Body wt, g	25.84 ± 0.54	24.49 ± 0.39	19.22 ± 0.67†
Change in body wt, Δg	0.04 ± 0.15	−0.91 ± 0.53	−5.53 ± 0.48†
Infarct size, %	–	43.4 ± 1.9	49.2 ± 3.4

Values are mean ± SE. *$P < 0.05$ vs. MI non-HF; †$P < 0.05$ vs. sham.

(6), a time point where systemic congestion may be present. Similarly, in patients, TNF-α, IL-6, IL-18, GM-CSF, MCP-1, MIP-1α, and regulated upon activation, normal T-cell expressed, and secreted (RANTES) have primarily been found in severe HF when the degree of peripheral edema is considerable (5, 8, 16, 18, 19). After MI, a transient increase in circulating cytokines has been shown, but a sustained long-term increase was found primarily in those patients who developed HF manifestations (11, 20). Thus the increase in cytokine levels found in these studies in animals and humans might be caused by systemic congestion following right ventricular failure.

Sources of cytokine release. Both the failing myocardium itself (25) and congested extracardiac organs, such as the liver (1, 15) and gut (3), have been suggested sources for the increased cytokine levels in the circulation during HF. Interestingly, in our study, no increased levels of cytokines were found in mice with hypertrophic left ventricle after AB. How-

Table 5. *Echocardiographic measurements and organ weights in SERCA2KO*

	SERCA2FF4w	SERCA2KO4w	SERCA2FF7w	SERCA2KO7w
n	10	12	10	11
		Echocardiography‡		
LA diam, mm	1.84 ± 0.04	2.1 ± 0.08*	1.9 ± 0.07	2.68 ± 0.19*
		Organ weights		
LVW/TL, mg/mm	4.74 ± 0.16	5.31 ± 0.23	4.39 ± 0.18	4.25 ± 0.18
RVW/TL, mg/mm	1.27 ± 0.05	1.61 ± 0.10*	1.08 ± 0.07	1.41 ± 0.08*
LW/TL, mg/mm	8.58 ± 0.16	11.95 ± 0.69*	7.85 ± 0.17	13.46 ± 0.69*
Atria/TL, mg/mm	0.27 ± 0.04†	0.94 ± 0.17*	0.24 ± 0.04†	1.99 ± 0.33*
Body wt, g	27.55 ± 0.88	29.55 ± 1.45	25.5 ± 1.06	25.08 ± 1.32

Values are mean ± SE. *$P < 0.05$ vs. SERCA2FF; ‡measured in representative groups of SERCA2KO and FF mice ($n = 5$); †measured in a subset of mice ($n = 9$).

ever, increased cytokine levels were associated with systemic congestion, indicating an extracardiac source of cytokine release. This finding may be in concordance with the endotoxin hypothesis, which suggests that edema of the intestines in right-sided HF induces translocation of endotoxins into the systemic circulation (3). In the blood stream, the endotoxins may activate monocytes to release cytokines, resulting in increased cytokine concentrations (21).

Increased level of IL-18 in myocardial ischemia. In mice that underwent MI, we found an increased level of IL-18, regardless of HF phenotype. This finding is supported by a previous study in mice (23), and the association between increased circulating levels of IL-18 and ischemic myocardial damage has also been reported in patients (24).

Clinical relevance. In the present study, differences in circulating cytokine concentrations were observed between four mouse models of HF. These models share phenotypic similarities with different human HF etiologies. It is therefore tempting to speculate that differences in circulating cytokine levels between murine HF models also occur among the heterogenous population of HF patients. If so, a further subclassification of HF patients according to cytokine levels could be advantageous when evaluating the clinical use of cytokines as biomarkers or therapeutic targets. However, more studies are needed to further explore the association between cytokine levels and specific pathophysiological features, such as systemic congestion.

Limitations of the study. The present study assessed cytokine levels in mouse models 6–7 days after induction of cardiac

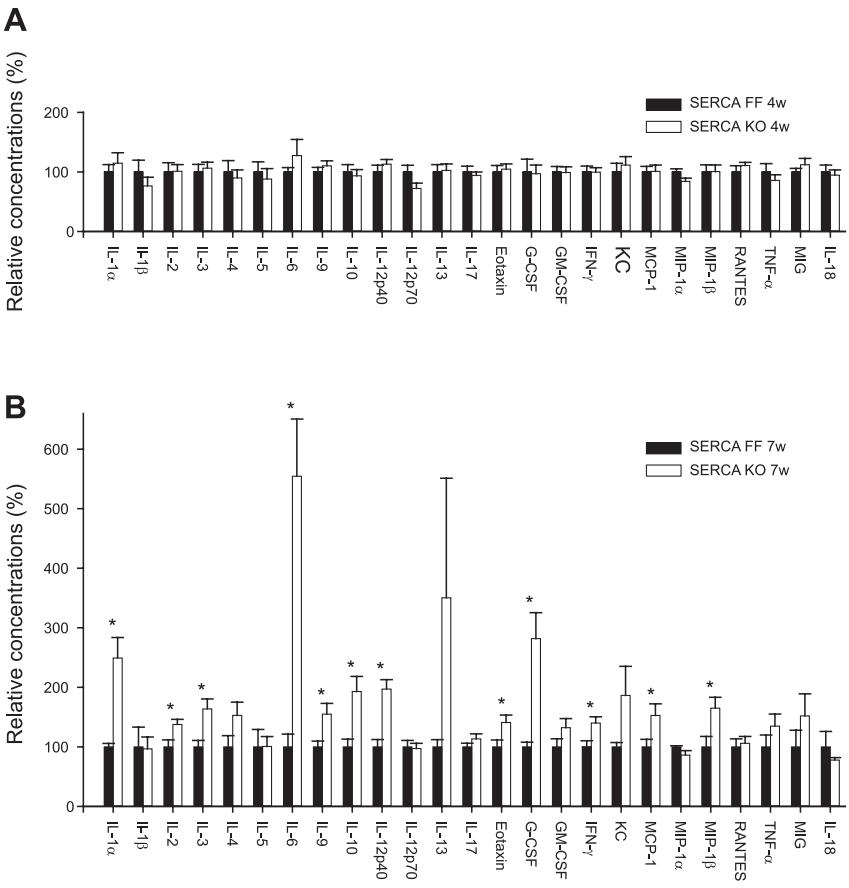

Fig. 6. Cytokine concentrations—SERCA2KO mice. Relative concentrations of circulating cytokines in SERCA2KO mice at 4 (*A*) and 7 wk (w) (*B*). Concentrations in SERCA2FF are set to 100%. *$P < 0.05$.

overload, except in the SERCA2 KO model where investigations were performed 4 and 7 wk after induction of KO. At the selected time points, mice in the HF groups developed congestion in the pulmonary or systemic circulation, thus reflecting congestive new-onset HF. However, our models do not reflect end-stage HF. It is possible that different alterations in circulating cytokines may occur in later stages of HF than those observed in our study, due to variations in pathophysiological characteristics such as development of cor pulmonale in left-sided HF. Thus further studies are needed to gain more insight into temporal fluctuations of circulating cytokine levels in various types of HF.

Conclusions. In conclusion, serum levels of cytokines in mice with heart disease are highly dependent on the etiology and the pathophysiological alterations in the heart and circulation. Increased serum levels of several cytokines were found in models with increased right ventricular afterload, suggesting that systemic congestion is an important stimulus for cytokine release into the circulation in HF.

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DISCLOSURES

G. Christensen and K. B. Andersson are partners in a patent filed by Oslo University Hospital Ullevål regarding the use of SERCA2KO as a cardiac disease model [European patent EP 2004808891 (Serca2 Mouse PCT/NO2004/000397)].

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