

To mark the 60th birthday of *Circulation Research* (1953-2013), the editors have commissioned *Circulation Research Classics*, a series of commentaries highlighting seminal articles published in the journal over the past six decades that have importantly shaped cardiovascular research. Written by leading experts, *Circulation Research Classics* are intended to describe the impact of these articles on the field by putting them in a historical perspective. The concept of “classic” is inextricably linked to time - a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of *Circulation Research*, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

### Is Tumor Necrosis Factor- $\alpha$ Friend or Foe for Chronic Heart Failure?

Masatsugu Hori, Osamu Yamaguchi

**Dilated Cardiomyopathy in Transgenic Mice With Cardiac-Specific Overexpression of Tumor Necrosis Factor- $\alpha$**

**Kubota et al**

*Circ Res.* 1997;81:627-635.

**Although detrimental effects of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) have been reported in failing myocardium, clinical trials using TNF- $\alpha$  antagonists did not show the benefit of TNF- $\alpha$  inhibition in patients with chronic heart failure (CHF). The double-edged effects of TNF- $\alpha$ /Toll-like receptors (TLRs)-related proinflammatory cytokines and downstream signal transduction, nuclear factor (NF)- $\kappa$ B activation on failing myocardium are discussed.**

It is well known that neurohormonal activation in heart failure plays a key role in deterioration of myocardial failure, accelerating the vicious cycle in pathophysiology of heart failure. Proinflammatory cytokines are also increased in patients with heart failure. Since the first report by Levine et al<sup>1</sup> that serum TNF- $\alpha$  is increased in patients with severe heart failure, other cytokines, such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and their soluble receptors, have been reported to increase in heart failure.<sup>2</sup> Several reports indicated that plasma levels of these proinflammatory cytokines are correlated with severity of heart failure, New York Heart Association functional class.<sup>3</sup> The question was whether these proinflammatory cytokines deteriorate the failing myocardium as a cause of detrimental mechanism or merely secondary phenomenon in heart failure.

To answer this question, Kubota et al<sup>4</sup> established a murine transgenic line of TNF- $\alpha$  in which expression was driven by

the murine  $\alpha$ -myosin heavy chain promoter. The transgenic heart with chronic overexpression of TNF- $\alpha$  showed (1) ventricular hypertrophy, (2) ventricular dilatation, (3) interstitial infiltrates, (4) interstitial fibrosis, (5) rare myocyte apoptosis, (6) a diminished ejection fraction, (7) attenuation of  $\beta$ 1-adrenergic responsiveness, and (8) expression of atrial natriuretic factor in the ventricle. Moreover, mice overexpressing the TNF- $\alpha$  transgene had a marked increase in mortality, suggesting that they died of congestive heart failure because most of the mice presented an increase in lung weight and pleural effusion. In their earlier study, they demonstrated that robust overexpression of TNF- $\alpha$  in mice resulted in the development of lethal myocarditis,<sup>5</sup> whereas in this study, they obtained expression of TNF- $\alpha$ , demonstrating a phenotype of CHF. Because 5 founders overexpressing TNF- $\alpha$  constructs demonstrated similar cardiac phenotypes, it was unlikely that identical insertion sites could have been a causative factor for the development of myocarditis and CHF in these animals, but transgenic progeny may represent phenotypic heterogeneity.

If TNF- $\alpha$  is a detrimental factor or mediator for myocardial failure, inhibition of TNF- $\alpha$  either in blood or in TNF- $\alpha$  receptors may be an effective treatment for CHF. For this purpose, TNF- $\alpha$  antagonists were developed; one is a recombinant human TNF receptor, etanercept, which binds the circulating TNF and functionally inactivates TNF by preventing from binding to its receptors on cell surface membranes. The other is a chimeric (mouse/human) IgG monoclonal antibody (infliximab), which binds with the soluble and transmembrane TNF. Clinical trial named Randomized Etanercept North American Strategy of Study Antagonism of Cytokines (RENAISSANCE) was conducted using etanercept in the North America, whereas Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER) trial was conducted in Europe. Combined RENAISSANCE and RECOVER trials, a total 1500 patients' data were analyzed as Randomized Etanercept Worldwide Evaluation (RENEWAL) study.<sup>6</sup> Unfortunately, both trials were terminated prematurely because of lack of benefit. Consequently, in RENEWAL analysis, etanercept was shown to be ineffective for primary end point (ie, death and hospitalization for chronic heart failure). Although left ventricular ejection fraction was improved

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in the small pilot study, a long-term efficacy of TNF- $\alpha$  inhibiting therapy was not observed both in primary and in secondary outcomes.<sup>6</sup>

Chimeric IgG monoclonal antibody, infliximab, was tested as a pilot study in 150 patients with CHF (anti-TNF Therapy Against Congestive Heart Failure [ATTACH] trial).<sup>7</sup> In this trial also, the positive results were not obtained with infliximab of 5 mg/kg and even worse with a larger dose, 10 mg/kg, than the placebo arm. In ATTACH trial, during the initial 14 weeks, inflammatory response was attenuated with infliximab as evidenced with decreases in inflammatory markers, C-reactive protein, and IL-6; however, these beneficial responses were lost during the long-term treatment. Although the mechanisms for antagonizing the biological effects of TNF- $\alpha$  are different between etanercept and infliximab, negative results in RENEWAL and ATTACH trials may indicate that the inhibition of TNF- $\alpha$  could not improve the long-term outcome in patients with CHF, raising a question whether TNF- $\alpha$  may not be a detrimental factor for failing hearts.

TNF- $\alpha$  is a key molecule among the proinflammatory cytokines, which activates a transcriptional factor (NF- $\kappa$ B) in the nucleus.<sup>8</sup> Activation of NF- $\kappa$ B triggers and mediates the inflammatory response: activation of neutrophil immigration, production of proinflammatory cytokines, and activation of metalloproteinase. It is reported that TNF- $\alpha$  induces cell apoptosis and enhances cell necrosis during ischemia, and NF- $\kappa$ B elicits these responses as one of the key effectors of TNF- $\alpha$ . In contrast to the detrimental effects of NF- $\kappa$ B on surviving myocardium, other reports support the beneficial effect of this molecule in surviving the stressed/injured myocardium. Activation of NF- $\kappa$ B inhibits BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3)-dependent permeability transition pore through silencing BNIP3 in mitochondria and inhibits the cell death.<sup>9</sup> Cell survival by NF- $\kappa$ B is also supported by the mechanism of an increase in antioxidant proteins (eg, manganese superoxide dismutase [MnSOD] in the stressed hearts).<sup>10</sup> Protective effect of NF- $\kappa$ B was also reported in reovirus-infected myocarditis.<sup>11</sup> These findings strongly suggest that complete inhibition of TNF- $\alpha$  may lose the benefit of NF- $\kappa$ B activation. In ATTACH trial, apparently worse results in the arm of larger dose (10 mg/kg) of infliximab may be attributable to this double-edged effect of TNF- $\alpha$ , although an increased tissue injury secondary to infliximab-induced complement activation may be an alternative explanation of the negative results in this trial.

The role of proinflammatory cytokines may not be simple but multifaced depending on the cause, stage, and pathological state of heart failure. In severe heart failure, pulmonary infection, mechanical stress to the heart or myocardial ischemic insult may often cause precipitation to decompensated heart failure. In this clinical setting, increased proinflammatory cytokines activate metalloproteinase and protease to eliminate the damaged cells and repair the injured extracellular matrix.<sup>12</sup> This biological response is inevitable for tissue survival and repairment. Although the precise mechanisms are not clear, cytokines are regulators and mediators of cellular injury, as well as cellular repair in the injured tissues. However, these cellular responses are initiated and activated with some causing events (eg, ischemia, infection, and metabolic/mechanical stress). In myocarditis in which the infection of bacteria or

viruses is a trigger of inflammation, proinflammatory cytokines are activated to eliminate the infected bacteria/viruses and the damaged/dead cells.

However, even without infections of microbial organism, damaged-associated molecular pattern (DAMP) could be a cause of inflammatory response because DAMPs are recognized by TLRs through which immunologic mechanisms are activated.<sup>13</sup> TLRs are a family of transmembrane receptors, which recognize microbial molecular patterns or pathogen-associated molecular patterns and activate the innate immune system to mount inflammatory responses against pathogens. It has been clarified that different microbial moieties signal through different TLRs. In addition to pathogen-associated molecular patterns, TLR2 and TLR4 also recognize endogenous danger signals through DAMPs.<sup>14</sup> DAMPs signal the threat of either infection or injury to the organism, independently of either nonself or self identity. Several endogenous molecules are generated on tissue injury, some are intracellular molecules that are released into the extracellular milieu as a result of cell necrosis or activation after injury and others are extracellular matrix molecule fragments that are also released on tissue injury or upregulated in response to tissue damage. Signaling pathways activated by DAMP ligation of the TLRs result in activation of several signaling molecules among which NF- $\kappa$ B pathway is the most distinctive. Activation of NF- $\kappa$ B further promotes the expression of proinflammatory cytokines, angiogenic factors, adhesion molecules, nitric oxide synthase, matrix metalloproteinases, and antiapoptotic genes. Chronic NF- $\kappa$ B activation by DAMPs induces subsequent inflammation, angiogenesis, tissue repair, and regeneration. It is of note that low tissue levels of DAMPs are beneficial during tissue repair to include physiological immune response, whereas high levels of DAMPs are generated during chronic inflammation.

It is known that TLRs are expressed not only in immunologically activated blood cells, but also in cardiomyocytes, vascular endothelial cells, and vascular smooth muscle cells.<sup>15</sup> Activation of TLRs in these tissues stimulates the transfer of NF- $\kappa$ B into the nucleus and thus, expressions of proinflammatory cytokines are increased in the myocardium and vessels. Proinflammatory cytokines also exert the role in elimination of the dead cells and degradate cellular components to initiate the process of tissue repair. However, in addition to this physiological response to tissue injury, there is evidence that indicates endogenous TLR activators also contribute to the pathogenesis of many inflammatory and autoimmune diseases. Recently, it is reported that as a sterile inflammatory cause, mitochondrial DNA plays an important role.<sup>16</sup> Mitochondria in the myocardial cells are denatured with metabolic/mechanical stress and degraded in autophagosomes fused with lysosomes in which the denatured mitochondria are trapped and degraded. Accumulation of undigested mitochondrial DNA induced in the mechanically overloaded hearts is recognized with TLR9 and initiates the inflammatory response with production of proinflammatory cytokines.

It should also be noted that proinflammatory cytokines exert their biological responses in concert with other cytokines families; TNF- $\alpha$  activation is always associated with other proinflammatory cytokines (eg, IL-1 $\beta$  and IL-6). This

cytokine cascade is working in orchestra with other proinflammatory cytokines.<sup>17</sup> Among these cytokine networks, targeting a single component, TNF- $\alpha$  may not be sufficient for total suppression of proinflammatory cytokine cascade.

In case of excessive tissue injury, inflammatory responses are further augmented and proinflammatory cytokines (eg, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) are markedly activated, accelerating a positive feedback through NF- $\kappa$ B activations. As such, there is a possibility that augmented proinflammatory cytokines may not be a major cause of tissue injury in pathophysiologically relevant conditions such as in CHF. In the article of Kubota,<sup>4</sup> TNF- $\alpha$  content in transgenic mice was 1500 pg/mg, whereas in human tissues obtained from the patients with heart failure it was 0.2 to 4 pg/mg. The extremely high contents of TNF- $\alpha$  in the transgenic mice may mislead the playing role of TNF- $\alpha$  in CHF. It should be noted that in Crohn disease, anti-TNF neutralizing antibody, infliximab is very effective presumably through induction of apoptosis of TNF- $\alpha$ -expressing target cells<sup>18</sup> secondary to infliximab-induced complement activation. Although the target of the treatment should be always directed to the primary pathogenetic cause of the disease, searching the primary cause of the disease may not be easy because the leading actor is often switched depending on the abnormalities.

### Disclosures

None.

### References

- Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med*. 1990;323:236–241.
- Ferrari R, Bachetti T, Confortini R, Opasich C, Febo O, Corti A, Cassani G, Visioli O. Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. *Circulation*. 1995;92:1479–1486.
- Testa M, Yeh M, Lee P, Fanelli R, Loperfido F, Berman JW, LeJemtel TH. Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. *J Am Coll Cardiol*. 1996;28:964–971.
- Kubota T, McTiernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM. Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- $\alpha$ . *Circ Res*. 1997;81:627–635.
- Kubota T, McTiernan CF, Frye CS, Demetris AJ, Feldman AM. Cardiac-specific overexpression of tumor necrosis factor- $\alpha$  causes lethal myocarditis in transgenic mice. *J Card Fail*. 1997;3:117–124.
- Mann DL, McMurray JJ, Packer M, et al. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation*. 2004;109:1594–1602.
- Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT; Anti-TNF Therapy Against Congestive Heart Failure Investigators. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor- $\alpha$ , in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. *Circulation*. 2003;107:3133–3140.
- Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF- $\kappa$ B in the heart: to be or not to NF- $\kappa$ B. *Circ Res*. 2011;108:1122–1132.
- Baetz D, Regula KM, Ens K, Shaw J, Kothari S, Yurkova N, Kirshenbaum LA. Nuclear factor-kappaB-mediated cell survival involves transcriptional silencing of the mitochondrial death gene BNIP3 in ventricular myocytes. *Circulation*. 2005;112:3777–3785.
- Hikoso S, Yamaguchi O, Nakano Y, et al. The I $\kappa$ B kinase  $\beta$ /nuclear factor  $\kappa$ B signaling pathway protects the heart from hemodynamic stress mediated by the regulation of manganese superoxide dismutase expression. *Circ Res*. 2009;105:70–79.
- O'Donnell SM, Hansberger MW, Connolly JL, et al. Organ-specific roles for transcription factor NF- $\kappa$ B in reovirus-induced apoptosis and disease. *J Clin Invest*. 2005;115:2341–2350.
- Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res*. 2002;91:988–998.
- Piccinini AM, Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm*. 2010;doi:10.1155/2010/672395.
- Marchant DJ, Boyd JH, Lin DC, Granville DJ, Garmaroudi FS, McManus BM. Inflammation in myocardial diseases. *Circ Res*. 2012;110:126–144.
- Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol*. 2001;1:135–145.
- Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature*. 2012;485:251–255.
- Aukrust P, Ueland T, Lien E, Bendtzen K, Müller F, Andreassen AK, Nordøy I, Aass H, Espevik T, Simonsen S, Frøland SS, Gullestad L. Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1999;83:376–382.
- Van Deventer SJH. Transmembrane TNF- $\alpha$ , induction of apoptosis, and the efficacy of TNF-targeting therapies in Crohn's Disease. *Gastroenterology*. 2001;121:1242–1245.