We thank Dr Rauchhaus and his colleagues for their comments on our article.

We agree with the authors that the cytokine network plays an important role in congestive heart failure (CHF)[1-2]. In this complex scenario, tumour necrosis factor alpha (TNFα) in particular reflects a primary role as a modulator of myocardial contractility either via NO-dependent (usually ascribed to inducible NOS induction) or NO-independent mechanisms.

Because both of these mechanisms exert marked cardiodepression, we suggest they may represent complementary aspects of the same issue. Indeed, transgenic mice for TNFα production in cardiac myocytes suggest that cytokine production is sufficient to induce severe CHF, supporting the causal role of TNFα in the pathogenesis of the disease[3]. A recent paper on patients with mechanical assistance for CHF points out the correlation between intracardiac levels of TNFα and the recovery of mechanical function[4].

However, our paper entitled ‘Induction of functional nitric oxide synthase in monocytes of patients with congestive heart failure’[5] evaluated the role of TNFα in iNOS induction in peripheral monocytes, and we can only speculate on the control mechanisms of cardiac function (infiltrated macrophages and iNOS induction). Alternatively, we can hypothesize that iNOS induced in monocytes can modulate vascular reactivity and blood cells/endothelium interaction.

We entirely agree with the authors that the bioactive cytotoxic assay on WEHI 164 cells implies some technical difficulties due, for example, to the use of fetal calf serum, usually not tested for cytokine contamination. However, this potential negative impact is minimized by the fact that both standard curve and samples are prepared in the same culture medium (RPMI 1640) enriched with FCS, providing data affected by the same bias.

To overcome the limitation of WEHI bioassay, we set up a new bioassay using human umbilical vein endothelial cells (HUVEC,) instead of fibroblasts. As reported by Agnoletti et al.[2], TNFα from CHF patients (NYHA class IV) when administered directly to HUVECs, mimicking a physiopathological cytotoxic assay, exerts a significant apoptotic effect on these cells, underlying a primary role of TNFα in inducing cytotoxicity.

To confirm specific TNFα-mediated cytotoxicity, we tested the bioassay with a recombinant TNFα antibody and indicated in the legend of Table 2 the values obtained by subtracting aspecific from total TNFα cytotoxic activity (specific TNFα activity). The TNFα-independent cytotoxicity was relevant and highly variable, ranging from 40 to 100% of the total TNFα cytotoxic activity, and could not be correlated with the NYHA class.

Concerning the length of the WEHI 164 bioassay, we observed that, under our assay conditions, a high proportion of these cells die after 24 h incubation with TNFα standard solutions. Thus, longer incubation may not be necessary to kill the cells. However, we do not know whether the increase in the incubation time of the assay could ameliorate the assay detectability.

Finally, we agree that bioassays and ELISAs may differentially detect trimeric and monomeric TNFα, as the latter appears to be biologically inactive[6]. It would be desirable to have an assay on hand that could detect trimeric TNFα only. The availability of an assay specific for trimeric TNFα has been reported in the literature[7]. However, although this assay was proven to be suitable for biochemical studies on TNFα trimer–monomer transitions, could not measure the trimeric TNFα in serum samples.

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