Letters to the Editor


Nitric oxide synthase in CHF

We read with great interest the paper by Comini et al., which demonstrates the induction of inducible nitric oxide synthase (iNOS) expression in circulating monocytes of patients with chronic heart failure. As we believe this paper reports upon a very important aspect of current research, we would like to comment on two aspects which we think are of special interest.

Firstly, the activation of the nitric oxide (NO)/cGMP pathway in the heart can be induced experimentally by relatively high concentrations of TNFα and the results by Comini et al. strongly support such a potential pathophysiological link in a clinical setting. There is, however, evidence from in vitro studies of NO-independent cardiodepression at lower, pathophysiologically more relevant concentrations. In this respect, the effects of TNFα on the heart vary with regard to the kinetics of the process. Immediate negative inotropic effects of TNFα were mediated by sphingosine and included suppression of the calcium transient. Chronic administration of TNFα caused a reduction in the synthesis of the precursors of the phosphoinositide pathway and inhibited pyruvate dehydrogenase activity and mitochondrial function. Secondary cytokines induced by TNFα or endotoxin in cardiomyocytes through the CD14 pathway, as mentioned by the authors, could contribute to cardiodepression independent of NO. Whether the direct activation of apoptotic signals by TNFα is involved in cardiodepression remains unclear.

Cyto-kine mediated chronotropic effects on cardiomyocytes were both NO-dependent and NO-independent. Finally, TNFα also impairs and abolishes, in an NO-independent manner, inotropic responses of rat cardiomyocytes to the cardiac glycoside ouabain and to β1-adrenoreceptor agonists. In summary, a complex picture of the contribution of TNFα, either by NO-dependent or NO-independent mechanisms, is evolving and more data are required to complete our understanding.

Comini et al. did not find an association between cytokotoxic TNFα and iNOS expression. This may be partly due to methodological difficulties using a bioactive cytokotoxic assay. This method requires the culture of cells in fetal calf serum, which is a fundamental point of criticism as all sera are potentially contaminated with cytokines, leading to either a potentiation or reduction of TNFα-mediated cytotoxicity. To confirm specific TNFα-mediated cytotoxicity, the authors tested the bioassay with a recombinant TNFα antibody, but unfortunately the results have not been reported. The addition of actomyosin D, a transcription inhibitor, usually increases sensitivity and reduces the incubation time of the bioassay. However, failed to increase further the highly sensitive cell line WEHI 164 by treating the cells with actomyosin D. This in turn, may suggest, that the incubation time of 24 h, as used by Comini et al. might have been too short. As the bioassay only measures bioactive, trimeric TNFα, this procedure has the potential to exclude the usual ELISA to recognize both trimeric (bioactive) and recombinant monomeric TNFα (personal communication with the manufacturers). We are not aware of any ELISA test kit that only recognizes trimeric, i.e. bioactive TNFα.

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References
and the recovery of mechanical for CHF points out the correlation on patients with mechanical assistance in particular re
tumour necrosis factor alpha (TNF)
Indeed, transgenic mice for TNF
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Because both of these mechanisms
marked cardiodepression, we
suggest they may represent comple-
mental role in congestive heart failure (CHF)\[1,2\].
In this complex scenario, tumour necrosis factor alpha (TNF\(\alpha\)) in
r reflects a primary role as a
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ated to induce NO induction) or
 NO-independent mechanisms.

Because both of these mechanisms
exert marked cardiodepression, we
suggest they may represent comp-
mental aspects of the same issue.

Indeed, transgenic mice for TNF\(\alpha\) pro-
duction in cardiac myocytes suggest that
cytokine production is sufficient to
duce severe CHF, supporting the
aul role of TNF\(\alpha\) in the pathogen-
osis of the disease\[3\].
A recent paper on patients with mechanical assistance for
points out the correlation between intracardiac levels of TNF\(\alpha\) and
the recovery of mechanical function\[4\].

However, our paper entitled ‘Induc-
duction of functional nitric oxide synthase
monocytes of patients with congestive
heart failure’\[5\] evaluated the role
of TNF\(\alpha\) in iNOS induction in periph-
eral monocytes, and we can only
peculate on the control mechanisms
of cardiac function (infiltrated macro-
ages and iNOS induction). Alterna-
tively, we can hypothesize that iNOS
duced in monocytes can modulate
vascular reactivity and blood cells/endothelial interaction.

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

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

To confirm specific TNF\(\alpha\)-mediated
cytotoxicity, we tested the bioassay with a recombinant TNF\(\alpha\) antibody
and indicated in the legend of Table 2 the values obtained by subtracting
aspecific from total TNF\(\alpha\) cytotoxic
activity (specific TNF\(\alpha\) activity). The
TNF\(\alpha\)-independent cytotoxicity was
relevant and highly variable, ranging
from 40 to 100% of the total
TNF\(\alpha\) 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 propor-
tion of these cells die after 24 h
incubation with TNF\(\alpha\) standard solu-
tions. Thus, longer incubation may
not be necessary to kill the cells. How-
ever, 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 TNF\(\alpha\), as the
latter appears to be biologically in-
active\[6\]. It would be desirable to have
an assay on hand that could detect
trimeric TNF\(\alpha\) only. The availability of
an assay specific for trimeric TNF\(\alpha\)
has been reported in the literature\[7\].
However, although this assay was
proven to be suitable for biochemical
studies on TNF\(\alpha\) trimmer–monomer
transitions, could not measure the
trimeric TNF\(\alpha\) in serum samples.

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References

receptors in patients with various de-
grees of congestive heart failure. Circu-
lation 1995; 92: 1479–86.

[2] Agnoletti L, Curello S, Bachetti T et al. Serum from patients with severe heart
failure downregulates eNOS and is
proapoptotic-Role of tumor necrosis

[3] Bryant D, Becker L, Richardson J et al. Cardiac failure in transgenic mice with
myocardial expression of tumor necrosis

KA, Durand JB, Radavancevic B,
Delgado RM, Frazier OH, Entman
ML, Noon GP. Decreased expression of
tumor necrosis factor-alpha in failing
human myocardium after mechanical
circulatory support: A potential mech-
nism for cardiac recovery. Circulation
1999; 100: 1189–93.

Induction of functional nitric oxide syn-
thenase in monocytes of patients with
congestive heart failure. Eur Heart J

[6] Smith RA, Baglioni C. The active form
of tumor necrosis factor is a trimer.

tumor necrosis factor alpha slowly
converts into inactive forms at bioactive

online at http://www.idealibrary.com

Classification of acute MI

Porela et al. proposed that the defi-
nition for epidemiological classifica-
tion of acute myocardial infarction
could be based solely on a ‘specific
cardiac marker’ such as creatine
kinase MB mass. These suggestions
exhibit a touching faith in the abilities
of clinical biochemistry by the
authors but betray a certain lack of
understanding.

1. Reference ranges and diagnostic
cut offs are set by reference to

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