*Biochemical Pharmacology*, Vol. 42, No. 7, pp. 1323–1327, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00


# COMMENTARY

# MECHANISMS OF THE PROTECTIVE EFFECTS OF TRANSFORMING GROWTH FACTOR-β IN REPERFUSION INJURY

ALLAN M. LEFER*

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A.

## *Biochemistry and cell biology of transforming growth factor-β*

Transforming growth factor-β (TGF-β) is a homodimeric peptide having a molecular size of 25,000 daltons. It was initially characterized and purified in 1983 [1–3]. Shortly thereafter, TGF-$\beta_1$ was cloned and its precursor structure elucidated [4]. TGF-β is now known to exist in three mammalian isoforms (i.e. TGF-$\beta_1$, TGF-$\beta_2$, and TGF-$\beta_3$), all of which exist as latent forms before activation. Although it was first discovered for its ability to stimulate the growth of fibroblasts in culture, it is now clear that TGF-β may be an important regulator of cellular processes. Thus, its nomenclature is outdated and gives a misimpression of the true range of actions of this regulatory peptide.

TGF-β is an essentially ubiquitous molecule and is found in almost every cell type in the cardiovascular system, including cardiac myocytes, arterial and venous smooth muscle cells, endothelial cells, and fibroblasts, as well as in macrophages, neutrophils, and platelets [5]. Thus, TGF-β appears to be a widely distributed peptide.

TGF-β exerts its biological effects following its binding to specific membrane receptors on a variety of cell types. At present, over 150 cell types bind TGF-β, mostly in the picomolar range [6]. Some of the important biological effects of this multifunctional peptide include: (a) enhancement of the synthesis of matrix proteins, (b) suppression of release or secretion of proteases, as well as enhancement of synthesis of protease inhibitors, (c) stimulation of the expression for cell adhesion proteins (e.g. integrins), (d) regulation of myogenesis, (e) stimulation of osteoblast growth, (f) suppression of monocyte function, (g) antagonism of the effects of certain cytokines, and (h) promotion of angiogenesis [5]. Recently, TGF-β has been characterized as a "switch" [7] in that it may mediate the interaction of a cell with its environment or with other cells. It thus serves as an adaptive mechanism allowing for enhanced plasticity of cells. In this regard, TGF-β can either activate certain processes which are inactive, or it can brake other processes which are very active. However, additional work remains to clarify the precise molecular role of TGF-β in cellular processes.

* Correspondence: Dr. Allan M. Lefer, Department of Physiology, Jefferson Medical College, Thomas Jefferson University, 1020 Locust St., Philadelphia, PA 19107, U.S.A.

## *Pharmacology of TGF-β*

Although TGF-β has been studied intensively in isolated cell preparations and in a variety of cultured cell systems, very little is known about the acute pharmacological effects of this family of peptides. Only scattered reports exist on the effects of TGF-β on components of the circulatory system *in vivo*, although there is a large literature on the effects of TGF-β on endothelial cells in culture [8].

The heart is a rich source of TGF-β [9, 10]; however, TGF-β did not exert any inotropic effects in cat papillary muscles over a 30-min period (Table 1). Furthermore, TGF-β did not exert any apparent vasoactive effect in isolated cat coronary artery rings even in the presence of an intact endothelium (Fig. 1). Nevertheless, TGF-β did exert vasculogenic and angiogenic effects indicating chronic effects on the

Table 1. Lack of inotropic activity of TGF-β in isolated cat papillary muscles

| Substance | Concentration | Δ Developed force (mg) | Significance |
|---|---|---|---|
| TGF-$\beta_1$ | 0.2 nM | +3 ± 6 | NS |
| TGF-$\beta_1$ | 2 nM | −4 ± 8 | NS |
| TGF-$\beta_1$ | 20 nM | +8 ± 5 | NS |
| Pentobarbital | 200 μM | −521 ± 63 | $P < 0.001$ |

All values are means ± SEM for six muscles. NS = not significant.
Control resting force = 1.85 ± 0.16 g.

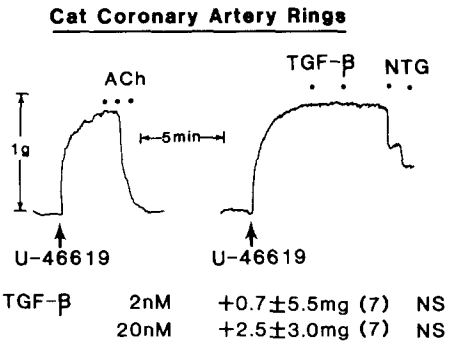


Fig. 1. Representative recordings of isolated cat coronary artery rings. Rings were contracted with 100 nM U-46619, a thromboxane $A_2$-mimetic, and tested with acetylcholine (ACh) at 1, 10, and 100 nM at each dot to demonstrate intactness of the endothelium. TGF-$\beta$ at 2 and 20 nM did not alter vascular tone, whereas nitroglycerin at 200 nM and 2000 $\mu$M relaxed the ring. At the bottom are tabulated results for seven coronary artery rings. NS = not significant.

vascular endothelium. Thus, TGF-$\beta$ blocks the mitogenic effect of fibroblast growth factor (FGF) on cultured endothelial cells [11]. TGF-$\beta$ also inhibits phorbol ester stimulated migration of endothelial cells into collagen gels [12], although in three-dimensional matrix structures TGF-$\beta$ promotes organization of endothelial cells into tubular arrangements [13]. TGF-$\beta$ also promotes endothelial cell formation of plasminogen activator inhibitor [14]. The half-life of the mature TGF-$\beta$ molecule *in vivo* is very short [15], although it can be extended significantly when complexed with its latency-associated peptide (LAP) [16]. LAP has been isolated and characterized recently [17, 18]. Clearly, the basic pharmacology of TGF-$\beta$ is in its early stage, and there is need for a more complete characterization of this important regulatory peptide.

## TGF-$\beta$ IN ISCHEMIA–REPERFUSION

### *Ischemia–reperfusion mechanisms*

Severe ischemia of a vascular bed for periods of 30–90 min followed by reperfusion of the ischemic region is known to lead to a severe tissue injury. This ischemia–reperfusion injury is will known in the heart (i.e. myocardial ischemia–reperfusion) [19, 20], but occurs in the splanchnic [21, 22], cerebral [23] and renal [24] vasculatures as well. Thus, ischemia–reperfusion is interruption of blood flow to a vascular bed for a significant period of time, followed by an abrupt restoration of blood flow sometime later. In the case of myocardial ischemia, the reperfusion injury is characterized by an extension of necrotic myocardial tissue (i.e. so-called infarct extension). In the case of splanchnic ischemia and reperfusion, this results in severe hypotension and acute circulatory shock of a highly lethal nature [22].

In general, soon after the start of reperfusion, endothelial dysfunction of the ischemic vascular bed occurs [21, 25] followed by a marked neutrophil adherence to the endothelium and eventual migration into the tissues perfused by that vasculature. The initial endothelial dysfunction appears to be due to superoxide radicals formed by endothelial cells [26–28]. The increased superoxide radicals oppose the actions of endothelium-derived relaxing factor (EDRF) [29–31], now thought to be nitric oxide (NO) [32] or a nitrosothiol compound which releases NO [33]. Since EDRF, in addition to acting as a vasodilator, also inhibits platelet aggregation as well as platelet and neutrophil adherence [34, 35], blockade of NO represents a significant pathological event sensitizing the endothelium to subsequent neutrophil invasion.

When activated neutrophils diapedese through the endothelium, they release their own mediators of cell injury including oxygen derived free radicals, cytokines [e.g. tumor necrosis factor (TNF), and interleukin-1 (IL-1)] and proteolytic enzymes (e.g. elastase). These mediators clearly aggravate the ischemic state and provoke additional reperfusion injury, the so-called amplification factor of Bulkley [21]. In addition, neutrophils can also plug microvessels, the so-called "no reflow phenomenon" [1, 2], further contributing to post-reperfusion ischemia or maldistribution of blood flow within the microcirculation.

### *TGF-$\beta$ in myocardial ischemia–reperfusion injury*

Against this setting, TGF-$\beta$ was tested in a rat model of myocardial ischemia and reperfusion [36]. When rats were subjected to 10 min of occlusion of the left coronary artery followed by reperfusion, they suffered a significant loss of left ventricular creatine kinase (CK) activity equivalent to 8.7 ± 0.9 I.U./mg protein, compared to a loss of only 2.3 ± 0.8 I.U./mg protein in sham-operated controls. However, when 10 $\mu$g of TGF-$\beta$ was given intravenously 24 hr prior to occlusion, cardiac CK loss was reduced to 3.1 ± 0.7 I.U./mg protein ($P < 0.02$ from untreated), indicating that post-treatment also was effective. When the TGF-$\beta$ was given intravenously at the time of reperfusion, the cardiac CK activity was 4.1 ± 0.9 I.U./mg protein ($P < 0.05$ from untreated). These cardioprotective effects of TGF-$\beta_1$ are quite significant, and indicate that post-ischemic treatment TGF-$\beta$ can be effective in myocardial ischemia. One of the interesting findings in this study suggesting a potential mechanism of protection by TGF-$\beta$ was a marked attenuation of thoracic cavity fluid and blood levels of TNF [36]. Since TNF is known to exert a number of pathophysiological effects [37] including impairment of endothelial-dependent vasodilation [38], this blunting of TNF suggests an important protective mechanism of TGF-$\beta$ corroborating the anti-TNF effects of TGF-$\beta$ [39, 40].

To ascertain the effects of ischemia and TGF-$\beta$ on endothelial dysfunction, further studies were conducted in isolated perfused rat hearts subjected to global ischemia for 30 min (i.e. reduction of flow to 15% of control) followed by 20 min of reperfusion (i.e. restoration of flow to pre-ischemic control levels). These rats hearts were perfused under a constant flow with oxygenated Krebs–Henseleit solution in the absence of plasma or blood cells. In this milieu, coronary vasodilator responses were

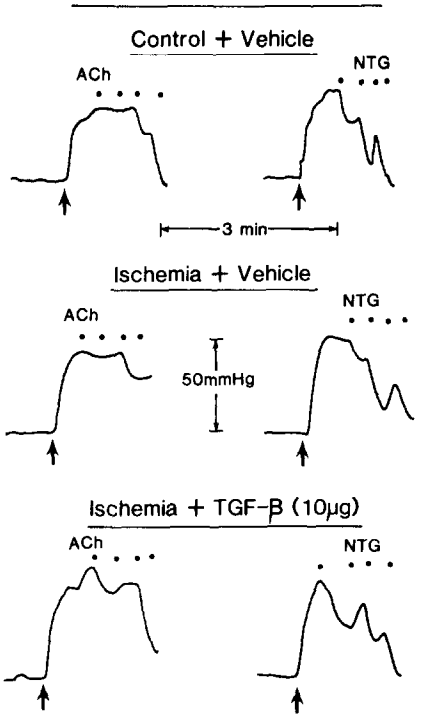


Fig. 2. Representative recordings of responses of the coronary vasculature in constant flow perfused rat hearts. Control + vehicle = non-ischemic, no TGF-β. Ischemia + vehicle = 30 min of global ischemia to 15% of control coronary flow + 20 min reperfusion, no TGF-β. Ischemia + TGF-β = 30 min ischemia + 20 min reperfusion + TGF-β (10 μg to rat i.v. 2 hr before ischemia). Arrows indicate addition of U-46619 (200 nM). ACh = acetylcholine, 1, 10, 100, 1000 nM; NTG = nitroglycerin.

determined to acetylcholine (ACh), an endothelial-dependent dilator requiring the release of EDRF to produce its dilation, and nitroglycerin (NTG), a direct vasodilator not requiring EDRF for its vasodilator response [36]. In the presence of an intact endothelium, both ACh and NTG fully dilated the coronary vasculature (Fig. 2, top panel). However, when the endothelium became dysfunctional or injured, the ACh response was attenuated, whereas the NTG response was normal (Fig. 2, middle panel). This indicates that myocardial ischemia depresses endothelial function specifically, and does not impair coronary vascular smooth muscle function. The degree of endothelial dysfunction appeared to be 65–70% in reperfused ischemic hearts. In contrast, 10 μg of TGF-β given intravenously 2 hr prior to ischemia virtually obliterated the ischemia–reperfusion-induced endothelial dysfunction (Fig. 2, bottom panel). Moreover, this endothelial protective effect was associated with a reduced formation of oxygen-derived free radicals (e.g. superoxide radicals) generated immediately upon reperfusion [36]. The source of these superoxide radicals is probably the coronary endothelium [27, 28].

Thus, reperfusion following myocardial ischemia results in superoxide formation leading to endothelial dysfunction as manifested by a reduced EDRF response to ACh and other endothelium-dependent vasodilators. TGF-β appears to markedly inhibit superoxide radical formation (it does not scavenge superoxide radicals), and in so doing preserves endothelial function. This may result in a delay or inhibition of neutrophil adherence to the endothelium, which would be of great significance in the intact animal, and could explain the reduced TNF concentration observed in TGF-β-treated ischemia-reperfused rats. In this connection, Gamble and Vadas [41] have shown that TGF-β inhibits neutrophil adherence to endothelial cells. This has been confirmed recently [42], and could relate to the deactivating effect of TGF-β on macrophages [43], particularly if a comparable effect were to occur in neutrophils.

The finding that TGF-β protected during myocardial ischemia is interesting for other reasons. First, Eghbali [10] has shown that immunofluorescence of TGF-β is intense in the rat heart, particularly around blood vessels. Second, Thompson *et al.* [9] have shown that an antibody to TGF-$\beta_1$ stains intensively in rat myocardium, and that this staining decreases markedly within 1 hr following coronary ligation. However, 24–48 hr following coronary occlusion, there is intense staining at the border between normal and infarcted tissue. These findings suggest a possible role for TGF-β in myocardial infarction. Coupled with the results of Lefer *et al.* [36], TGF-β would appear to be a potentially useful agent in myocardial ischemia.

*TGF-β in splanchnic ischemia–reperfusion injury*

Occlusion of the splanchnic arteries which supply the gastrointestinal tract (i.e. celiac, superior mesenteric, and inferior mesenteric arteries) for 2 hr in the cat followed by abrupt reperfusion results in a precipitous decline in arterial blood pressure and death within 2 hr [22, 44]. This circulatory shock state is characterized by lysosomal disruption in splanchnic viscera, particularly the ischemic pancreas and liver [44] resulting in enhanced plasma proteolysis and the formation of a myocardial depressant factor (MDF) [45, 46]. Superoxide radicals are also an important mediator of splanchnic ischemia–reperfusion [47]. Thus, reperfusion of the ischemic splanchnic viscera sets into motion the formation and release of powerful mediators which overwhelm circulatory homeostasis and promote the progression of a lethal shock state.

In a recent series of experiments, TGF-$\beta_1$ was tested in a feline model of splanchnic artery occlusion (SAO) and reperfusion. This model was uniformly lethal in cats (Fig. 3). However, intravenous administration of 50 μg/3 kg cat just 5 min prior to reperfusion (i.e. after 115 min of ischemia) resulted in a significant improvement in survival (Fig. 3). Accompanying this improvement in survival was a significant attenuation of plasma MDF activity. Plasma MDF activity increased from 11 ± 3 to 67 ± 8 MDF units/mL in untreated SAO shock cats, but increased only from 12 ± 3 to 31 ± 5 MDF units in TGF-β-treated cats. Thus, a marked attenuation in the circulating MDF activity was observed in response to administration of TGF-β. Moreover,

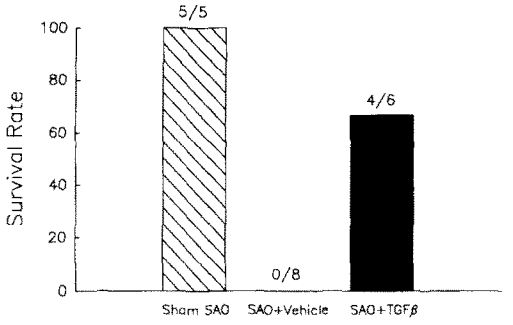


Fig. 3. Survival rate in cats subjected to sham splanchnic artery occlusion (SAO) and reperfusion or SAO + reperfusion and given either vehicle or 50 μg TGF-β intravenously.

when TGF-β was added to isolated cat papillary muscles incubated with high MDF activity, no amelioration of the negative inotropic effect of MDF was observed, so that the low plasma MDF activity in TGF-β-treated cats could not be attributed to a positive inotropic effect of TGF-β in the presence of an MDF-depressed myocardium. Therefore, TGF-β appeared to prevent the formation or release of MDF or to bind it so that it failed to exert its marked pathophysiologic effects.

Additionally, TGF-β preserved endothelial integrity in isolated superior mesenteric artery (SMA) rings taken from cats in SAO shock. Thus, the normal vasodilator response of control SMA rings to 1 μM ACh was 90 ± 5%; in untreated SAO shock this declined to 18 ± 6% ($P < 0.001$). However, addition of 50 μg TGF-β just before reperfusion maintained the ACh response at 58 ± 6% ($P < 0.01$ from untreated SAO cat SMA rings).

Maintenance of endothelial-dependent vasorelaxation by TGF-β occurred in the splanchnic as well as the coronary vasculature subjected to ischemia and reperfusion, and probably represents a fundamentally important mechanism of vascular protection by TGF-β. Further work is necessary to elucidate the full mechanism of this endothelial protective effect of TGF-β, and to determine whether this represents a fundamental cytoprotective mechanism representative of other cellular preserving effects, or whether it is a unique action of TGF-β occurring only in ischemia–reperfusion states.

## SUMMARY

TGF-β appears to be an important regulatory peptide in cellular physiology. Although all of its actions are not presently known, TGF-β functions as a cell-switching molecule. In the case of ischemia–reperfusion states, TGF-β has been shown to exert remarkably effective protective effects. These effects appear to pertain to preservation of endothelial function, particularly to maintenance of EDRF formation by the endothelium. The endothelial protection may be related to actions of TGF-β opposing the endothelial-destabilizing actions of both TNF and superoxide radicals. However, other important mechanisms will undoubtedly be brought to light with further study of TGF-β in these situations.

*Acknowledgements*—This work was supported in part by Research Grant HL-25575 from the National Heart, Lung and Blood Institute of the NIH.

## REFERENCES

1. Frolik CA, Dart LL, Meyers CA, Smith DM and Sporn MB, Purification and initial characterization of a type β transforming growth factor from human placenta. *Proc Natl Acad Sci USA* **80**: 3676–3680, 1983.
2. Assoian RK, Komoriya A, Meyers CA, Miller DM and Sporn MB, Transforming growth factor-β in human platelets. Identification of a major storage site, purification, and characterization. *J Biol Chem* **258**: 7155–7160, 1983.
3. Roberts AB, Anzano MA, Meyers CA, Wideman J, Blacher R, Pan Y-Ce, Stein S, Lehrman SR, Smith JM, Lamb LC and Sporn MB, Purification and properties of a type β transforming growth factor from bovine kidney. *Biochemistry* **22**: 5692–5698, 1983.
4. Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB and Goeddel DV, Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. *Nature* **316**: 701–705, 1985.
5. Sporn MB and Roberts AB, The transforming growth factor-βs. In: *Peptide Growth Factors and Their Receptors I* (Eds. Sporn MB and Roberts AB), pp. 419–472. Springer, New York, 1990.
6. Massagué J and Like B, Cellular receptors for type β transforming growth factor. Ligand binding and affinity labeling in human and rodent cell lines. *J Biol Chem* **260**: 2636–2645, 1985.
7. Sporn MB and Roberts AB, TGF-β: Problems and prospects. *Cell Regulation* **1**: 875–882, 1990.
8. Madri JA, Kocher O, Merwin JR, Bell L, Tucker A and Basson CT, Interactions of vascular cells with transforming growth factors-β's. *Ann NY Acad Sci* **593**: 243–258, 1990.
9. Thompson NL, Bazoberry F, Speir EH, Casscells W, Ferrans VJ, Flanders KC, Kondaiah P, Geiser AG and Sporn MB, Transforming growth factor beta-1 in acute myocardial infarction in rats. *Growth Factors* **1**: 91–99, 1988.
10. Eghbali M, Cellular origin and distribution of transforming growth factor-$\beta_1$ in the normal rat myocardium. *Cell Tissue Res* **256**: 553–558, 1989.
11. Baird A and Durkin T, Inhibition of endothelial cell proliferation by type β-transforming growth factor: Interactions with acidic and basic fibroblast growth factors. *Biochem Biophys Res Commun* **138**: 476–482, 1986.
12. Müller G, Behrens J, Nussbaumer U, Böhlen P and Birchmeier W, Inhibitory action of transforming growth factor β on endothelial cells. *Proc Natl Acad Sci USA* **84**: 5600–5604, 1987.
13. Madri JA, Pratt BM and Tucker AM, Phenotypic modulation of endothelial cells by transforming growth factor-β depends upon the composition and organization of the extracellular matrix. *J Cell Biol* **106**: 1375–1384, 1988.
14. Saksela O, Moscatelli D and Rifkin DB, The opposing effects of basic fibroblast growth factor and transforming growth factor beta on the regulation of plasminogen activator activity in capillary endothelial cells. *J Cell Biol* **105**: 957–963, 1987.
15. Coffey RJ Jr, Kost LJ, Lyons RM, Moses HL and

LaRusso NF, Hepatic processing of transforming growth factor β in the rat. Uptake, metabolism and biliary excretion. *J Clin Invest* **80**: 750–757, 1987.
16. Wakefield LM, Winokur TS, Hollands RS, Christopherson K, Levinson AD and Sporn MB, Recombinant latent transforming growth factor β1 has a longer plasma half-life in rats than active transforming growth factor β1, and a different tissue distribution. *J Clin Invest* **86**: 1976–1984, 1990.
17. Miyazono K, Hellman U, Wernstedt C and Heldin C-H, Latent high molecular weight complex of transforming growth factor β1. Purification from human platelets and structural characterization. *J Biol Chem* **263**: 6407–6415, 1988.
18. Wakefield LM, Smith DM, Flanders KC and Sporn MB, Latent transforming growth factor-β from human platelets. *J Biol Chem* **263**: 7646–7654, 1988.
19. Braunwald E and Kloner RA, Myocardial reperfusion: A double-edged sword? *J Clin Invest* **76**: 1713–1719, 1985.
20. Lucchesi BR, Modulation of leukocyte-mediated myocardial reperfusion injury. *Annu Rev Physiol* **52**: 561–576, 1990.
21. Bulkley GB, Mediators of splanchnic organ injury: Overview and perspective. In: *Splanchnic Ischemia and Multiple Organ Failure* (Eds. Marston A, Bulkley GB, Fiddian-Green RG and Haglund UH), pp. 183–191. Edward Arnold Publishers, London, 1989.
22. Glenn TM and Lefer AM, Role of lysosomes in the pathogenesis of splanchnic ischemic shock in cats. *Circ Res* **27**: 783–797, 1970.
23. Nishijima MK, Koehler RC, Hurn PD, Eleff SM, Norris S, Jacobus WE and Traystman RJ, Postischemic recovery rate of cerebral ATP, phosphocreatine, pH, and evoked potentials. *Am J Physiol* **257**: H1860–H1870, 1989.
24. Lieberthal W, Wold EF, Rennke HG, Valeri CR and Levinsky NG, Renal ischemia and reperfusion impair endothelium-dependent vascular relaxation. *Am J Physiol* **256**: F894–F900, 1989.
25. Lefer AM, Tsao PS, Lefer DJ and Ma X-l, Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J* **5**: 2029–2034, 1991.
26. Lefer AM and Ma X-l, Endothelial dysfunction in the splanchnic circulation following ischemia and reperfusion. *J Cardiovasc Pharmacol* **17** (Suppl 3): S186–S190, 1991.
27. Tsao PS and Lefer AM, Time course and mechanism of endothelial dysfunction in isolated ischemic- and hypoxic-perfused rat hearts. *Am J Physiol* **259**: H1660–H1666, 1990.
28. Zweier JL, Kuppasamy P and Lutty GA, Measurement of endothelial cell free radical generation: Evidence for a central mechanism of free radical injury in post-ischemic tissue. *Proc Natl Acad Sci USA* **85**: 4046–4050, 1988.
29. Gryglewski RJ, Palmer RMJ and Moncada S, Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* **320**: 454–456, 1986.
30. Rubanyi GM and Vanhoutte PM, Superoxide anions and hyperoxia in inactivate endothelium-derived relaxing factor. *Am J Physiol* **250**: H822–H827, 1986.
31. Stewart DJ, Pohl U and Bassenge E, Free radicals inhibit endothelium-dependent dilation in the coronary resistance bed. *Am J Physiol* **255**: H765–769, 1988.
32. Palmer RMJ, Ferrige AG and Moncada S, Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524–526, 1987.
33. Myers PR, Guerra R Jr and Harrison DG, Release of NO and EDRF from cultured bovine aortic endothelial cells. *Am J Physiol* **256**: H1030–H1037, 1989.
34. Furlong B, Henderson AH, Lewis MJ and Smith HA, Endothelium-derived relaxing factor inhibits *in vitro* platelet aggregation. *Br J Pharmacol* **90**: 687–692, 1987.
35. McCall T, Whittle BJR, Boughton-Smith NK and Moncada S, Inhibition of FMLP-induced aggregation of rabbit neutrophils by nitric oxide. *Br J Pharmacol* **95**: 517P, 1988.
36. Lefer AM, Tsao P, Aoki N and Palladino MA Jr, Mediation of cardioprotection by transforming growth factor-β. *Science* **249**: 61–64, 1990.
37. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ III, Zentella A, Albert JD, Shires GT and Cerami A, Shock and tissue injury induced by recombinant human cachectin. *Science* **234**: 470–474, 1986.
38. Aoki N, Siegfried M and Lefer AM, Anti-EDRF effect of tumor necrosis factor in isolated perfused cat carotid arteries. *Am J Physiol* **256**: H1509–H1512, 1989.
39. Ranges GE, Figari IS, Espevik T and Palladino MA Jr, Inhibition of cytotoxic T cell development by transforming growth factor β and reversal by recombinant tumor necrosis factor α. *J Exp Med* **166**: 991–998, 1987.
40. Espevik T, Figari I, Ranges GE and Palladino MA Jr, Transforming growth factor-$\beta_1$ (TGF-$\beta_1$) and recombinant human tumor necrosis factor-α reciprocally regulate the generation of lymphokine-activated killer cell activity. Comparison between natural porcine platelet-derived TGF-$\beta_1$ and TGF-$\beta_1$, and recombinant human TGF-$\beta_1$. *J Immunol* **140**: 2312–2316, 1988.
41. Gamble JR and Vadas MA, Endothelial adhesiveness for blood neutrophils is inhibited by transforming growth factor-β. *Science* **242**: 97–99, 1988.
42. Bereta J, Bereta M, Bauer D, Cohen S and Cohen MC, Tumor necrosis factor-α (TNF-α) enhancement of tumor cell binding to endothelium is reversed by transforming growth factor-β (TGF-β). *FASEB J* **4**: A1791, 1990.
43. Tsunawaki S, Sporn M, Ding A and Nathan C, Deactivation of macrophages by transforming growth factor-β. *Nature* **334**: 260–262, 1988.
44. Lefer AM and Barenholz Y, Pancreatic hydrolases and the formation of a myocardial depressant factor in shock. *Am J Physiol* **223**: 1103–1109, 1972.
45. Lefer AM, Role of a myocardial depressant factor in the pathogenesis of circulatory shock. *Fed Proc* **29**: 1836–1847, 1970.
46. Haglund UH, Myocardial depressant factors. In *Splanchnic Ischemia and Multiple Organ Failure* (Eds. Marston A, Bulkley GB, Fiddian-Green RG and Haglund UH), pp. 229–236. Edward Arnold Publishers, London, 1989.
47. Granger DN, Rutili G and McCord JM, Superoxide radicals in feline intestinal ischemia. *Gastroenterology* **81**: 22–29, 1981.