Nitric oxide and the resolution of inflammation: implications for atherosclerosis

Catherine A Shaw, Emma L Taylor*, Ian L Megson, Adriano G Rossi*/+

Centre for Cardiovascular Science, Hugh Robson Building, George Square *MRC Centre for Inflammation Research, University of Edinburgh, Teviot Place, Edinburgh, Scotland, UK

The ubiquitous free radical, nitric oxide (NO), plays an important role in many biological processes including the regulation of the inflammatory response. Alterations in NO synthesis by endogenous systems likely influence inflammatory processes occurring in a wide range of diseases including many in the cardiovascular system (e.g. atherosclerosis). Progression of inflammatory conditions depends not only upon the recruitment and activation of inflammatory cells but also upon their subsequent removal from the inflammatory milieu. Apoptosis, or programmed cell death, is a fundamental process regulating inflammatory cell survival and is critically involved in ensuring the successful resolution of an inflammatory response. Apoptosis results in shutdown of secretory pathways and renders effete, but potentially highly histotoxic, cells instantly recognisable for non-inflammatory clearance by phagocytes (e.g., macrophages). However, dysregulation of apoptosis and phagocytic clearance mechanisms can have drastic consequences for development and resolution of inflammatory processes. In this review we highlight the complexities of NO-mediated regulation of inflammatory cell apoptosis and clearance by phagocytes and discuss the molecular mechanisms controlling these NO mediated effects. We believe that manipulation of pathways involving NO may have previously unrecognised therapeutic potential for limiting or resolving inflammatory and cardiovascular disease.

Key words: nitric oxide - apoptosis - inflammation - resolution

The inorganic free radical, nitric oxide (NO), was first identified as an endothelium-derived endogenous messenger responsible for the regulation of vascular tone (Furchgott & Zawadzki 1980, Palmer et al. 1987). However, since then it has become clear that NO is the signalling molecule responsible for several diverse physiological and pathophysiological processes. Synthesised from L-arginine by three isoforms of the enzyme nitric oxide synthase (NOS), NO is now known to control vascular smooth muscle tone, inhibit platelet and inflammatory cell adhesion and activation, and to be a transmitter at non-adrenergic non-cholinergic (NANC) synapses (Moncada et al. 1991, Quinn et al. 1995). Recent studies have revealed that NO can also modulate apoptosis, or programmed cell death, in a variety of cell types, including human inflammatory cells (Taylor et al. 2003). Apoptosis of inflammatory cells is a highly regulated process whereby cellular death occurs without the disruption of the cell membrane and subsequent release of the pro-inflammatory and histotoxic contents of the dying cell (Haslett 1997, Rossi et al. 2003). Apoptotic cells are instantly recognised and ingested by phagocytes, such as macrophages, using mechanisms that down-regulate pro-inflammatory mediator release and increase the release of agents with anti-inflammatory potential from the ingesting cell (Meagher et al. 1992, Fadok et al. 1998, Liu et al. 1999). Hence, apoptosis represents a non-inflammatory mechanism to remove potentially damaging pro-inflammatory cells from the site of inflammation and is therefore critical to the successful resolution of the inflammatory response. Pharmacological manipulation of the rate of apoptosis in inflammatory cells, such as granulocytes and macrophages, may represent a potential therapeutic strategy for the treatment of chronic inflammatory disorders (Ward et al. 1999, Gilroy et al. 2004).

NO can be both pro- and anti-apoptotic, depending on local concentrations and the specific cell type in question (Quinn et al. 1995, Kim et al. 1999, Taylor et al. 2003). Current evidence suggests that lower concentrations of NO produced by the constitutive endothelial and neuronal isoforms of NOS (eNOS and nNOS) are cytoprotective, whilst supraphysiological concentrations produced by the inducible NOS isoform (iNOS) trigger cell death (Nicotera et al. 1997). This paradox may be explained, at least in part, by the free radical nature of NO and hence the ease with which it will react with other radicals, particularly reactive oxygen species, present in the milieu to form various NO-related species in vivo. For example, NO combines rapidly with inflammatory cell derived superoxide anions (O_2^-) to form highly cytotoxic peroxynitrite ($ONOO^-$) (Maxwell & Lip 1997).

NO as a mediator of inflammatory cell apoptosis

The pro- and anti-apoptotic actions of NO have been well documented in many cell systems. For example, high concentrations of either exogenous or endogenous iNOS-derived NO have been shown to induce apoptosis in murine macrophage cell lines (Albina et al. 1993, Sarih et al. 1993). However, pre-treatment with low concentrations of exogenous NO protects RAW 264 cells against cell death upon subsequent exposure to higher concentra-

+Corresponding author. E-mail: a.g.rossi@ed.ac.uk
Received 8 November 2004
Accepted 30 December 2004

tions of NO which would normally be cytotoxic (Yoshioka et al. 2003). However, despite the apparent reduced capacity of human macrophages in comparison to murine macrophages (Albina 1995, Schneemann & Schoedon 2002), to generate iNOS derived NO (Thomassen & Kavuru 2001), human macrophages do undergo apoptosis in response to exogenous NO. For example, the NO donors, S-nitrosoglutathione (GSNO), and spermine diazenium diolate (SPER/NO) induce apoptosis in primary human monocyte-derived macrophages (von Knethen et al. 1999). Exogenously delivered NO from NO donors (e.g., sodium nitroprusside; SNP and GSNO) induce apoptosis in human neutrophils (Fortenberry et al. 1999, Singhal et al. 1999). However, it has also been established that NO may have anti-apoptotic potential in neutrophils; low concentrations of NO generated from the spontaneous NO donors, SPER/NO and DEA/NO, reduce the rate of neutrophil apoptosis (Taylor et al. 2001). In contrast, the same study showed that the oxatriazole derivative, GEA-3162, at equivalent concentrations produced no such inhibition. However, it was demonstrated that GEA-3162 decomposes to co-generate both NO and O_2^-, which then react to form $ONOO^-$ (Taylor et al. 2004). This suggests that the pro- or anti-apoptotic effects of NO may be critically governed by the specific NO-related species generated.

Interestingly, the production of $ONOO^-$ may be of particular importance at sites of inflammation where the concentration of reactive oxygen species is likely to be elevated (Crow & Beckman 1995). However, the precise role of $ONOO^-$ in inflammatory cell apoptosis remains to be fully elucidated. There is some evidence to suggest that $ONOO^-$ at high concentrations increases apoptosis in murine RAW 264.7 cells (Sandoval et al. 1997), whilst at lower concentrations it may have a protective effect against lipopolysaccharide (LPS) and interferon (IFN)-γ-induced apoptosis in these cells (Scivittaro et al. 1997). A scavenger of $ONOO^-$, uric acid, had no effect on apoptosis induced by the NO donors GSNO or SPER/NO in RAW 264.7 macrophages, but abolished apoptosis induced by the $ONOO^-$ generator SIN-1, suggesting that $ONOO^-$ is a mediator of apoptosis, at least not in this cell type (Brockhaus & Brune 1999).

As is the case with macrophages, there are conflicting reports about the ability of $ONOO^-$ to induce or suppress apoptosis in neutrophils. Several investigators have demonstrated that SIN-1 and GEA-3162 increases the rate of apoptosis in human neutrophils (Blaylock et al. 1998, Ward et al. 2000, Taylor et al. 2004). Conversely, Blaylock et al. (1998) reported SIN-1 produced no significant increase in neutrophil apoptosis. However, this may be due to experimental differences and the exact amounts of $ONOO^-$ present rather than a true difference in the effect of $ONOO^-$ (Taylor et al. 2003).

NO and apoptosis in the resolution of inflammation

The ability of NO to induce apoptosis is particularly relevant during the resolution phase of inflammation. In a mouse model of kidney inflammation, activated macrophages have been shown to induce apoptosis in neighbouring mesangial cells prior to their ingestion by phagocytes (Duffield et al. 2000). The ability of these activated macrophages to induce apoptosis is greatly reduced in the presence of the NOS inhibitor N-É-monomethyl-L-arginine (L-NMMA), suggesting that macrophage-directed apoptosis of mesangial cell apoptosis occurs via a NO-dependent mechanism (Duffield et al. 2001). Similarly, several studies have demonstrated that activated macrophages infiltrating murine tumours induce apoptosis via a NO-dependent pathway in both activated anti-tumour T cells and in the tumour cells themselves (Saio et al. 2001, Chattopadhyay et al. 2002). Thus, it appears that macrophages have the capacity to induce apoptosis of nearby cells by the liberation of NO to enhance the clearance of apoptotic cells and thereby promote the resolution phase of inflammation (Figure).

Mechanism of action of NO

The classical pathway by which NO exerts many of its actions is via activation of the enzyme soluble guanylate cyclase (sGC) (Moncada et al. 1991) and resultant conversion of guanosine 5'-triphosphate (GTP) to the second messenger 3', 5'-cyclic guanosine monophosphate (cGMP) (Ignarro et al. 1999). However, recent studies have established that NO can also act via cGMP-independent pathways in various systems, particularly during the inhibition of platelet aggregation and regulation of inflammatory cell apoptosis (Gordge et al. 1998, Sogo et al. 2000, Ward et al. 2000, Crane et al. 2002).

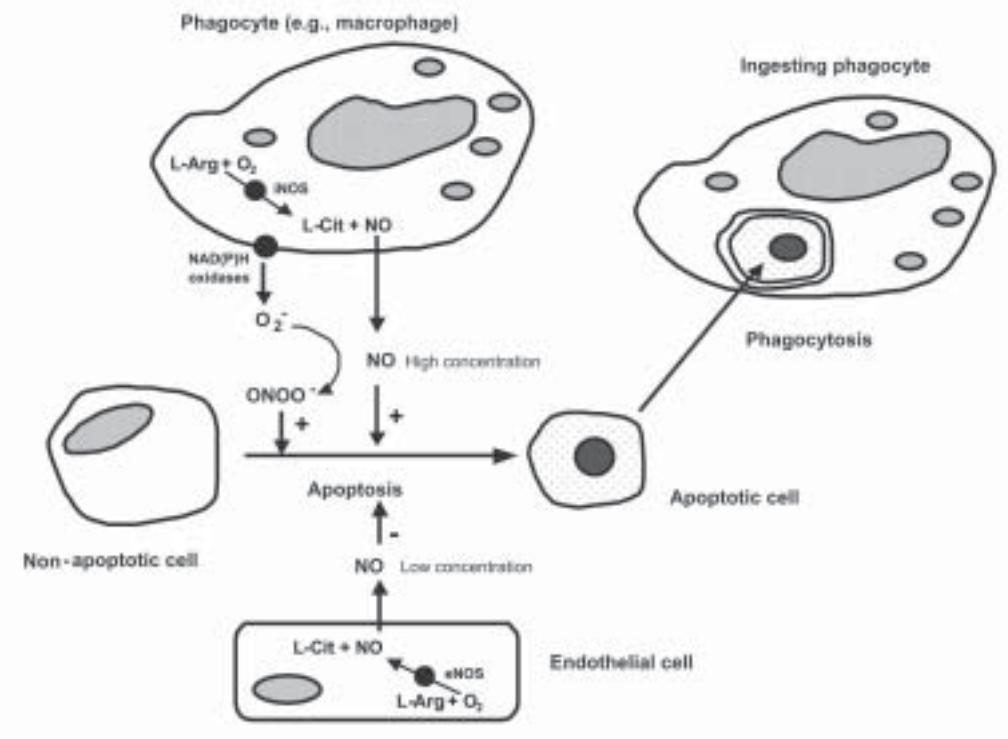
It is generally thought that lower concentrations of NO inhibit apoptosis via cGMP-dependent mechanisms, whilst higher concentrations are cytotoxic on account of cGMP-independent signalling. For example, Yoshoka et al. (2003) demonstrated that pre-treatment of RAW 264 cells with a low concentration of the NO donor SNP, inhibited cell death upon subsequent exposure to higher concentrations of NO. This protection was negated in the presence of sGC inhibitors and could be mimicked by cGMP analogues, suggesting that the cellular protection was conferred by cGMP.

Conversely, at higher concentrations, NO has been shown to induce apoptosis in rabbit macrophages – an effect which was unaffected by antagonism of cGMP-dependent kinases and not mimicked by cGMP analogues, suggesting that the pro-apoptotic action of NO is cGMP-independent (Wang et al. 1999). The peroxynitrite generators, SIN-1 and GEA-3162, have also been shown to produce a marked concentration-dependent induction of apoptosis in isolated human neutrophils (Ward et al. 2000). Again, this induction was unaffected by inhibitors of sGC, and cGMP analogues failed to elicit a pro-apoptotic response suggesting that a mechanism independent of cGMP signalling also featured in neutrophils. Interestingly, superoxide dismutase (SOD), the enzyme responsible for converting O_2^- to hydrogen peroxide (H_2O_2), antagonised the actions of SIN-1 and GEA-3162, whilst "authentic" peroxynitrite mimicked their effects. This result may, therefore, highlight the critical importance of NO-related species in determining an anti- or pro-apoptotic response, with the final outcome depending on the balance between reactive oxygen and nitrogen species.

Atherosclerosis

Atherosclerosis is a multi-factorial condition with a complicated aetiology, and, in combination with the associated cardiovascular syndromes, such as myocardial infarction and stroke, is a major cause of morbidity and mortality. However, it is now widely recognised that there is an inflammatory component to the disease pathogenesis and progression (Ross 1999a, b, Ludewig et al. 2002).

Atherosclerosis is characterised by the development of lipid-rich atherosclerotic plaques in the subendothelial space of conduit vessels, such as the coronary artery and aorta (Badimon et al. 1993). These plaques are usually eccentric, with the lipid rich core encapsulated by a fibrous, collagen-rich cap of smooth muscle cells and extracellular matrix (Davies 1997). The underlying causes of atherogenesis remain largely unknown, although a critical early stage is thought to be an insult to the endothelium, either physical or through oxidative stress. The consequences of this insult are multiple; firstly, in contrast to the situation in healthy endothelium, the injured endothelium becomes dysfunctional and production of NO by eNOS decreases, promoting vasoconstriction and platelet and inflammatory cell adhesion. Secondly, a protective inflammatory response is triggered. However, depending on the nature and duration of the insult, this protective response becomes excessive and over a period of years, comes to constitute the disease process itself (Ross 1999a, b). The inflammatory process begins with the expression of chemotactic and adhesion molecules for monocytes and lymphocytes, such as vascular cell adhesion molecule 1 (VCAM-1), on dysfunctional endothelial cells. Circulating monocytes adhere to the site of endothelial damage and translocate to the sub-endothelial space (Vogel 1997). Colony stimulating factors secreted from areas of endothelial damage induce monocytes to differentiate into macrophages, which then express scavenger receptors on their membranes, facilitating the internalisation of oxidised low density lipoprotein (ox-LDL). The accumulation of ox-LDL continues unchecked as, unlike LDL receptors, scavenger receptors are not down-regulated by cells in the cholesterol-replete state (Maxwell & Lip 1997). In this lipid-laden state, macrophages are known as foam cells and it is an aggregation of these foam cells in the vessel intima which form the earliest recognisable lesion of atherosclerosis – the fatty streak (Ross 1993). The plaque continues to grow via the accumulation of further macrophage foam cells and eventually becomes overlaid with a layer of smooth muscle cells forming a fibrous, collagen-rich cap. The cap serves to keep the highly thrombogenic contents of the plaque separate from the circulation. However, if the plaque cap is compromised and the contents exposed to the circulation, platelets are rapidly recruited and activated resulting in thrombus formation, leading to the more serious acute cardiovascular syndromes (Badimon et al. 1993).



High concentrations of nitric oxide (NO) synthesised by iNOS in phagocytes, such as macrophages, induce apoptosis in neighbouring cells. In addition, apoptosis can also be induced by $ONOO^-$ generated as O_2^- produced by phagocytes reacts with NO. Apoptotic cells are subsequently recognised and ingested by phagocytes, thus aiding the resolution of inflammation. Conversely, low concentrations of NO produced constitutively by eNOS in endothelial cells can inhibit apoptosis.

Inflammatory cell apoptosis in atherosclerosis

Recruitment of inflammatory cells, particularly monocytes and macrophages, is the major driving force behind plaque growth and development. However, the plaque is dynamic and inflammatory cells are constantly turning over within the core. It is well established that apoptotic cells, particularly macrophages, are present in atherosclerotic plaques in both human and animal models of the disease. Apoptotic macrophages and smooth muscle cells have been identified by TUNEL staining in sections from human plaques by various authors (Bjorkerud & Bjorkerud 1996, Haunstetter & Izumo 1998). Because apoptotic cells are ingested by phagocytes without initiating any further proinflammatory response, it has been suggested that apoptosis may represent a mechanism to regress the plaque. NO is a particularly promising candidate for this strategy because, as well as the pro-apoptotic actions discussed above, it has several other powerful anti-atherogenic characteristics including a powerful inhibitory effect on platelet and inflammatory cell activation (Moncada et al. 1991, Armstrong 2001). Evidence is emerging in support of this hypothesis. For example, administration of L-arginine (the substrate for NOS) to hypercholesterolemic rabbits increases the number of apoptotic macrophages in intimal lesions by three fold. This increase in apoptosis was associated with a regression of the plaque, suggesting that manipulation of the NO synthase pathway may well represent a therapeutic approach to resolving the inflammatory response in the vessel wall (Wang et al. 1999). However, care must by exercised when considering this approach because NO is also known to induce apoptosis in smooth muscle cells (Labelle et al. 2004). Loss of cells from the fibrous cap during the latter stages of atherosclerosis may well be detrimental, destabilising the plaque and promoting rupture (Kockx & Knaapen 2000)

Conclusion

Apoptosis of inflammatory cells is a tightly regulated process whereby cells are removed from the site of inflammation without triggering a subsequent pro-inflammatory response that would instigate further tissue injury. Pharmacological manipulation of apoptosis during chronic inflammatory conditions, such as atherosclerosis, may aid the resolution of inflammation and hence halt, or delay, disease progression. The ubiquitous signalling molecule and inducer of apoptosis, NO, is a likely candidate for such manipulation and may represent a novel therapeutic target for the treatment of such conditions.

REFERENCES

Albina JE 1995. On the expression of nitric oxide synthase by human macrophages. Why no NO? *J Leukoc Biol 58*: 643-649.

Albina JE, Cui S et al. 1993. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *J Immunol 150*: 5080-5085.

Armstrong R 2001. The physiological role and pharmacological potential of nitric oxide in neutrophil activation. *Int Immunopharmacol 1*: 1501-1512.

Badimon JJ, Fuster V et al. 1993. Coronary atherosclerosis. A multifactorial disease. *Circulation 87*: II3-16.

Bjorkerud S, Bjorkerud B 1996. Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol 149*: 367-380.

Blaylock MG, Cuthbertson BH et al. 1998. The effect of nitric oxide and peroxynitrite on apoptosis in human polymorphonuclear leukocytes. *Free Radic Biol Med 25*: 748-752.

Brockhaus F, Brune B 1999. Overexpression of CuZn superoxide dismutase protects RAW 264.7 macrophages against nitric oxide cytotoxicity. *Biochem J 338 (Pt 2)*: 295-303.

Chattopadhyay S, Das T et al. 2002. Protein A-activated macrophages induce apoptosis in Ehrlich's ascites carcinoma through a nitric oxide-dependent pathway. *Apoptosis 7*: 49-57.

Crane MS, Ollosson R et al. 2002. Novel role for low molecular weight plasma thiols in nitric oxide-mediated control of platelet function. *J Biol Chem 277*: 46858-46863.

Crow JP, Beckman JS 1995. Reactions between nitric oxide, superoxide, and peroxynitrite: footprints of peroxynitrite in vivo. *Adv Pharmacol 34*: 17-43.

Davies MJ 1997. The composition of coronary-artery plaques. *N Engl J Med 336*: 1312-1314.

Duffield JS, Erwig LP et al. 2000. Activated macrophages direct apoptosis and suppress mitosis of mesangial cells. *J Immunol 164*: 2110-2119.

Duffield JS, Ware CF et al. 2001. Suppression by apoptotic cells defines tumor necrosis factor-mediated induction of glomerular mesangial cell apoptosis by activated macrophages. *Am J Pathol 159*: 1397-1404.

Fadok VA, Bratton DL et al. 1998. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest 101*: 890-898.

Fortenberry JD, Owens ML et al. 1999. S-nitrosoglutathione enhances neutrophil DNA fragmentation and cell death. *Am J Physiol 276*: L435-442.

Furchgott RF, Zawadzki JV 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature 288*: 373-376.

Gilroy DW, Lawrence T et al. 2004. Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov 3*: 401-416.

Gordge MP, Hothersall JS et al. 1998. Evidence for a cyclic GMP-independent mechanism in the anti-platelet action of S-nitrosoglutathione. *Br J Pharmacol 124*: 141-148.

Haslett C 1997. Granulocyte apoptosis and inflammatory disease. *Br Med Bull 53*: 669-683.

Haunstetter A, Izumo S 1998. Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res 82*: 1111-1129.

Ignarro LJ, Cirino G et al. 1999. Nitric oxide as a signaling molecule in the vascular system: an overview. *J Cardiovasc Pharmacol 34*: 879-886.

Kim YM, Bombeck CA et al. 1999. Nitric oxide as a bifunctional regulator of apoptosis. *Circ Res 84*: 253-256.

Kockx MM, Knaapen MW 2000. The role of apoptosis in vascular disease. *J Pathol 190*: 267-280.

Labelle M, Beaulieu M et al. 2004. Integrating clinical practice guidelines into daily practice: impact of an interactive workshop on drafting of a written action plan for asthma patients. *J Contin Educ Health Prof 24*: 39-49.

Liu Y, Cousin JM et al. 1999. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. *J Immunol 162*: 3639-3646.

Ludewig B, Zinkernagel RM et al. 2002. Arterial inflammation and atherosclerosis. *Trends Cardiovasc Med 12*: 154-159.

Maxwell SR, Lip GY 1997. Free radicals and antioxidants in cardiovascular disease. *Br J Clin Pharmacol 44*: 307-317.

Meagher LC, Savill JS et al. 1992. Phagocytosis of apoptotic neutrophils does not induce macrophage release of thromboxane B2. *J Leukoc Biol 52*: 269-273.

Moncada S, Palmer RM et al. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev 43*: 109-142.

Nicotera P, Brune B et al. 1997. Nitric oxide: inducer or suppressor of apoptosis? *Trends Pharmacol Sci 18*: 189-190.

Palmer RM, Ferrige AG et al. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature 327*: 524-526.

Quinn AC, Petros AJ et al. 1995. Nitric oxide: an endogenous gas. *Br J Anaesth 74*: 443-451.

Ross R 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature 362*: 801-809.

Ross R 1999a. Atherosclerosis is an inflammatory disease. *Am Heart J 138*: S419-420.

Ross R 1999b. Atherosclerosis is an inflammatory disease. *N Engl J Med 340*: 115-126.

Rossi AG, Ward C, Dransfield I, Haslett C 2003. Apoptosis of inflammatory cells. In NF Adkinson et al. (eds), *Middleton's Allergy: Principles and Practice*, Chapt 26, 6th ed., Mosby-Harcourt, p. 412-424.

Saio M, Radoja S et al. 2001. Tumor-infiltrating macrophages induce apoptosis in activated CD8(+) T cells by a mechanism requiring cell contact and mediated by both the cell-associated form of TNF and nitric oxide. *J Immunol 167*: 5583-5593.

Sandoval M, Ronzio RA et al. 1997. Peroxynitrite-induced apoptosis in epithelial (T84) and macrophage (RAW 264.7) cell lines: effect of legume-derived polyphenols (phytolens). *Nitric Oxide 1*: 476-483.

Sarih M, Souvannavong V et al. 1993. Nitric oxide synthase induces macrophage death by apoptosis. *Biochem Biophys Res Commun 191*: 503-508.

Schneemann M, Schoedon G 2002. Species differences in macrophage NO production are important. *Nat Immunol 3*: 102.

Scivittaro V, Boggs S et al. 1997. Peroxynitrite protects RAW 264.7 macrophage from Lipopolysaccharide/interferon-gamma-induced cell death. *Biochem Biophys Res Commun 241*: 37-42.

Singhal PC, Patel P et al. 1999. Ethanol-induced neutrophil apoptosis is mediated through nitric oxide. *J Leukoc Biol 66*: 930-936.

Sogo N, Magid KS et al. 2000. Inhibition of human platelet aggregation by nitric oxide donor drugs: relative contribution of cGMP-independent mechanisms. *Biochem Biophys Res Commun 279*: 412-419.

Taylor EL, Megson IL et al. 2001. Dissociation of DNA fragmentation from other hallmarks of apoptosis in nitric oxide-treated neutrophils: differences between individual nitric oxide donor drugs. *Biochem Biophys Res Commun 289*: 1229-1236.

Taylor EL, Megson IL et al. 2003. Nitric oxide: a key regulator of myeloid inflammatory cell apoptosis. *Cell Death Differ 10*: 418-430.

Taylor EL, Rossi AG et al. 2004. GEA 3162 decomposes to cogenerate nitric oxide and superoxide and induces apoptosis in human neutrophils via a peroxynitrite-dependent mechanism. *Br J Pharmacol 143*: 179-185.

Thomassen MJ, Kavuru MS 2001. Human alveolar macrophages and monocytes as a source and target for nitric oxide. *Int Immunopharmacol 1*: 1479-1490.

Vogel RA 1997. Coronary risk factors, endothelial function, and atherosclerosis: a review. *Clin Cardiol 20*: 426-432.

von Knethen A, Brockhaus F et al. 1999. NO-Evoked macrophage apoptosis is attenuated by cAMP-induced gene expression. *Mol Med 5*: 672-684.

Wang BY, Ho HK et al. 1999. Regression of atherosclerosis: role of nitric oxide and apoptosis. *Circulation 99*: 1236-1241.

Ward C, Dransfield I et al. 1999. Pharmacological manipulation of granulocyte apoptosis: potential therapeutic targets. *Trends Pharmacol Sci 20*: 503-509.

Ward C, Wong TH et al. 2000. Induction of human neutrophil apoptosis by nitric oxide donors: evidence for a caspase-dependent, cyclic-GMP-independent, mechanism. *Biochem Pharmacol 59*: 305-314.

Yoshioka Y, Yamamuro A et al. 2003. Nitric oxide at a low concentration protects murine macrophage RAW264 cells against nitric oxide-induced death via cGMP signaling pathway. *Br J Pharmacol 139*: 28-34.