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Nitric oxide and cell viability in inflammatory cells: a role for NO in macrophage function and fate

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Abstract

Macrophages participate actively in the inflammatory response by releasing cytokines, chemokines and factors that recruit additional cells to sites of infection or tissue injury or alteration. In addition to this, activated macrophages rapidly activate the expression of genes responsible for the high-output synthesis of reactive oxygen and nitrogen species (NO, O_2^-, H_2O_2 and peroxynitrite, among others) and bioactive lipids derived from arachidonic acid. All of these agents contribute to the regulation of the inflammatory response. Most of these molecules, when synthesized at these high concentrations, exert pro-apoptotic effects in many cell types. Macrophages themselves are a notable and important exception, being resistant to apoptotic death upon activation. This resistance is necessary to enable these cells to perform their functional role during the early phases of an inflammatory response. However, after cumulative damage, or when the synthesis of inflammatory mediators decreases, macrophages undergo the characteristic mitochondrial-dependent cell death program, contributing in this way to the resolution of the inflammatory reaction. In the case of infectious diseases, this also helps to prevent the development of parasitic strategies by phagocytosed pathogens.

Keywords: Nitric oxide; Inflammatory cells; Macrophage function

1. The fine balance between survival and apoptosis

The fate of a particular cell ultimately depends on the balance between the activities of classes of gene. (1) Regulators of survival. These are typically represented by oncogenes and anti-apoptotic genes of the Bcl-2-family. (2) Promoters of apoptosis. These include most genes that encode for the expression pro-inflammatory cytokines (such as TNF-α, IL-1β, IFN-γ) and enzymes that catalyze the high throughput synthesis of inflammatory mediators such as nitric oxide and prostaglandins. (3) A series of 'gene modulators' that directly or indirectly exert a control over both preceding groups. Tumor suppressor genes are a good example of this type of regulator of the balance between

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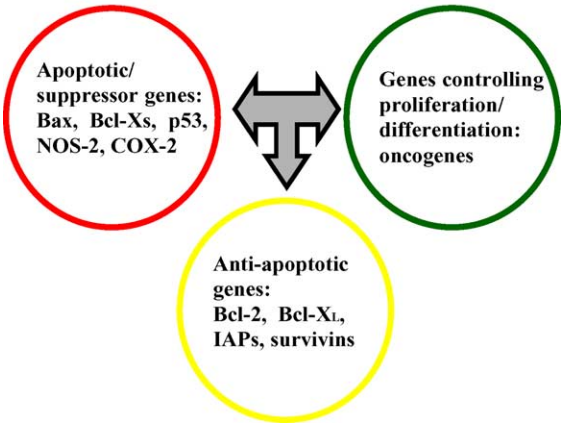

Fig. 1. The balance between three gene groups governs cell survival vs. apoptosis. (1) Genes that promote apoptosis of damaged or aged cells (tumor suppressor, pro-apoptotic genes, genes encoding the high output of reactive oxygen and nitrogen species). (2) Genes controlling proliferation/differentiation and/or survival. (3) Anti-apoptotic genes such as Bcl-2, survivins, usurpins, and IAPs. The decision of whether a cell lives or dies depends on the contribution of each gene group to preventing the cell reaching the executioner phase of the apoptotic pathway.

cell death and survival or proliferation/differentiation (Fig. 1) (DuBois et al., 1998; Tendler et al., 2001; Hinz et al., 1999; Liu et al., 2001; Williams et al., 1999; Chipuk et al., 2003). The interaction between these groups of genes has important consequences for the regulation of many pathophysiological processes; including the extension and resolution of inflammation, host defense, cancer, and neurodegenerative diseases.

2. Nitric oxide-dependent regulation of cell death and survival

Nitric oxide (NO) has long been recognized as an important molecule involved simultaneously in the regulation of apoptotic death and cell viability (Brockhaus and Brune, 1999; Mojena et al., 2001; Bosca and Hortelano, 1999; Chen et al., 2002; Hinz et al., 1999; Karin and Lin, 2002; Wang et al., 1998; Nicholson and Thornberry, 2003; Yang et al., 2000; Castrillo et al., 2000b; Chipuk et al., 2003; Hortelano et al., 2000). NO is a short-lived gas that can diffuse freely through cells, and whose effects can be propagated via the interaction with thiol groups on cysteines and glutathione, or protein heme groups (Eu et al., 2000; Jaffrey et al., 2001). This diverse protein targeting by NO includes freely reversible reactions such as the formation of adducts with thiols, and also covalent modifications of aminoacids (such as tyrosine) and nucleotides from nucleic acids. These aspects have been reviewed in depth by various authors (Manderscheid et al., 2001; Matsumoto et al., 2003; Foster et al., 2003; Stamler and Toone, 2002).

An important aspect of the activity of NO as a regulator of cell survival and death is its influence on mitochondrial function. Nitric oxide competes with oxygen for substrate binding sites in several enzyme components of the bioenergetic pathways, and also affects catalytic activity by forming complexes with heme and iron-sulfur clusters present in many mitochondrial proteins. In addition to this, NO has the ability to directly diminish the mitochondrial inner membrane potential ($\Delta\psi_m$) and to induce 'swelling' in isolated mitochondria. Both these phenomena are early steps in the mitochondrial-dependent apoptotic pathway (Hortelano et al., 1997, 1999; Bossy-Wetzel et al., 1998; Green et al., 2004; Costa et al., 2003; Radi et al., 2002; Brown and Borutaite, 2002; Boveris et al., 2002; Brookes et al., 2002; Kuwana et al., 2002). Releases of cytochrome *c* and Apoptosis Inducing Factor (AIF) have been detected after treatment of isolated mitochondria or intact cells with NO donors (at the concentrations prevailing under inflammatory conditions), or expressing the high-output NOS synthase (NOS-2) (Fig. 2).

However, the impact of NO on cell viability is quite variable. Indeed, it appears that NO plays a dual role, favoring cell viability or inducing apoptotic death depending on the cell type and the concentration of NO produced (Hortelano et al., 2000; Bosca and Hortelano, 1999; Arnett et al., 2002; Barsacchi et al., 2002; D'Acquisto et al., 2001; Edwards et al., 2000; Mohr et al., 1998; Mojena et al., 2001; Genaro et al., 1995; Mannick et al., 1994; Castrillo et al., 2000a; Brune and von Knethen, 2002; Edwards et al., 2000; von Knethen et al., 1999a, 1999b). For example, moderate NO concentrations inhibit basal apoptosis in B lymphocytes (Mannick et al., 1994), apoptosis induced by the potent cytokine TGF-β in hepatocytes, and Fas-dependent apoptosis in several cellular models of inflammation (Table 1). Several modes of action have been identified for these protective effects of NO. These include the 'desensitization' of the initial pro-apoptotic signaling pathway (although the mechanisms of this have been