

Streptococcal-Induced Apoptosis of Macrophages

with

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What is known about group B *Streptococcus agalactiae*?

Elisabeth E. Adderson: Group B *Streptococcus* (GBS) is a major pathogen of newborn infants, with respiratory colonization at birth leading to pneumonia and other serious infections (1). Opsonin-independent defenses are critical to the early resistance to GBS infection, since sub-optimal levels of specific antibody and serum complement concentrations are typical of newborns. Following non-opsonic phagocytosis of GBS by macrophages,

however, bacteria persist within macrophages and induce apoptosis (2-5). GBS-induced macrophage apoptosis may limit inflammation during infection and facilitate bacterial evasion of normal host immune defenses. Moreover, since therapy of GBS infection in neonates generally involves antibiotics with little intra-phagocytic penetration, persistence within an intracellular niche could allow GBS to evade killing (1).

How do they induce apoptosis in macrophages?

Elisabeth E. Adderson: Critical regulators of GBS-induced macrophage apoptosis include the caspase family of cysteine-directed proteases and members of the Bcl-2 family of apoptotic regulators (7). GBS infection of murine macrophages provokes increases in cytosolic and mitochondrial Bad expression and dephosphorylation, and the translocation of Bax to mitochondria. Bad activity is inhibited by phosphorylation, which promotes its association with the scaffold protein 14-3-3. Decreased levels of phosphorylated Bad (phospho-Bad Ser112) are also observed following GBS infection of macrophages. These

events, in turn, lead to the activation of Bax which, together with Bak, activates caspase-3 and other downstream effectors of cell death. GBS induces significant increases in both caspase-3 and caspase-9, but not caspase-8, activity in macrophages following infection, and inhibition of caspase-3 activity reduces cell death (7). Compared with control uninfected cells, GBS-infected macrophages have reduced mitochondrial cytochrome c levels. GBS also causes a marked depolarization of mitochondrial membrane potential, confirming activation of the intrinsic (mitochondrial) pathway of apoptosis.

Are apoptotic regulators involved?

Elisabeth E. Adderson: The amount of 14-3-3 associated with mitochondria of GBS-infected macrophages is markedly increased, and increased levels of cytosolic Bcl-xL are also induced by GBS (7). In contrast, levels of Omi/HtrA2, a mitochondrial proapoptotic serine protease that induces both

caspase-dependent and caspase-independent cell death, are reduced following infection of macrophages with GBS compared with control cells. This dichotomy might be best understood as an progressive shift in the balance of competing pro- and anti-apoptotic regulators over the course of

infection. Although 14-3-3 levels are increased initially, this is ultimately insufficient to compensate for elevations of Bad. Omi/HtrA2 normally suppresses the inhibitor of apoptosis pro-

teins, which are potent and direct inhibitors of caspase-3. The unexpected reduction in Omi/HtrA2 levels may reflect another attempt by the host to circumvent cell death.

What is the role of nitric oxide?

Elisabeth E. Adderson: In murine macrophages, increases in TNF- α , IL-1, and iNOS gene expression coincide with GBS-induced apoptosis (6). Inhibition of iNOS gene expression by N(G)-monomethyl-L-arginine (NMMA) reduces apoptosis, whereas inhibition of TNF- α and IL-1 biological activity do not. Peritoneal macrophages from congenic iNOS-deficient mice are less susceptible to apoptosis than those of iNOS sufficient C57BL/6 mice. The NO donor S-nitroso-N-acetylpenicillamine also induces apoptosis in the absence of GBS infection, confirming that NO has a proapoptotic effect in the murine system.

In human monocyte-derived macrophages, however, NO production is minimal, even after costimulation with IFN- γ and lipopolysaccharide (6). Despite this, GBS-infected human macrophages also undergo inoculum-dependent apoptosis.

Thus, NO appears to be an important mediator of GBS-induced murine, but not human, macrophage apoptosis. GBS-induced apoptosis of murine macrophages is not completely abolished in peritoneal macrophages from iNOS-deficient mice and apoptosis is not completely prevented by treatment with NMMA, suggesting that a second NO-independent pathway of GBS-induced apoptosis exists. It is not clear at this time if this second "alternative" apoptotic pathway is shared by murine and human macrophages. NO exerts antimicrobial effects against many bacteria. Somewhat surprisingly, NO is not required for killing of GBS by murine or human macrophage, and NO has no direct antimicrobial activity against GBS in *in vitro* assays. Thus, human and murine macrophages killed GBS by one or more NO-independent mechanisms.

Is there therapeutical outlook for anti-infective therapy?

Elisabeth E. Adderson: At present it is unclear whether the apoptosis that results from GBS-macrophage interactions reflects a pathogen-driven virulence mechanism or a host defense mechanism. Induction of apoptosis by GBS may promote bacterial survival and dissemination and may tilt the balance of the host-microbial interaction in the favor of bacterial persistence. Future studies of the host-cell processes manipulated by GBS to bring about apoptosis will be important for understanding the role

played by apoptosis in infection. If the induction of apoptosis is beneficial to GBS and other microbial pathogens, however, the ability to modulate the activity of effector caspases or regulators of apoptosis may represent an unexploited avenue for therapeutic intervention in these infections.

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