

Macrophage apoptosis in mycobacterial infections

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Abstract: Mycobacterial diseases are a major public health concern. In the case of tuberculosis, the problem has been exacerbated due to the emergence of drug-resistant strains of *Mycobacterium tuberculosis*, and *Mycobacterium avium* is the major opportunistic pathogen in HIV-1 infection in the United States. *M. tuberculosis* and *M. avium* replicate in human macrophages and induce apoptosis. Incubation of freshly added uninfected autologous macrophages with apoptotic *M. avium*-infected macrophages results in 90% inhibition of bacterial growth. Apoptosis also prevents the release of intracellular components and the spread of mycobacterial infection by sequestering the pathogens within apoptotic bodies. Consistent with the model that host cell apoptosis is a defense mechanism against mycobacteria is the finding that the virulent *M. tuberculosis* strain H37Rv induces substantially less macrophage apoptosis than the attenuated strain H37Ra. Evasion of apoptosis by this pathogen is achieved by enhanced release of sTNFR2 by H37Rv-infected macrophages and subsequent formation of inactive TNF- α -TNFR2 complexes. These observations contribute to the hypothesis that apoptosis of the host macrophage is an important defense mechanism in mycobacterial infections, which prevents the spread of the infection. *J. Leukoc. Biol.* 66: 763–764; 1999.

Key Words: *Mycobacterium tuberculosis* · *Mycobacterium avium* · tumor necrosis factor · HIV-1

INTRODUCTION

Mycobacterium avium and *Mycobacterium tuberculosis* are pathogens with a worldwide distribution. *M. avium* is ubiquitous in the environment and is the prevalent cause for opportunistic infections among HIV-1-infected individuals. In these people *M. avium* infection results in disseminated disease with involvement of visceral organs and is a major cause for morbidity and mortality [1]. *M. tuberculosis* is the predominant cause for mortality from infectious agents and the severity of the global problem of tuberculosis has been exacerbated due to the emergence of drug-resistant strains [2].

The defense of healthy individuals against mycobacteria is thought to be mostly based on cellular immune mechanisms but

a detailed understanding of these events is missing. The principal host cell of mycobacteria is the macrophage (M ϕ) and these cells respond to inoculation with *M. avium* by undergoing apoptosis, an innate program by which cells undergo death [3]. Apoptosis, which is characterized by DNA fragmentation, nuclear chromatin condensation, compacting of cellular organelles, and membrane blebbing, seems to be important in the regulation of cell numbers and in the removal of unwanted and potentially dangerous cells [4]. *M. avium*-induced M ϕ apoptosis is dependent on the function of tumor necrosis factor α (TNF- α) because it is inhibited by the presence of anti-TNF- α antibodies [5].

We present evidence that the apoptotic response of M ϕ to mycobacterial infections plays a role in the defense against these microorganisms.

We first demonstrate that apoptosis prevents spreading of the mycobacterial infection by sequestering the mycobacteria within apoptotic bodies. These experiments were performed with human monocyte-derived M ϕ and *M. avium*. To understand this approach it is important to know that tissue transglutaminase, an enzyme that cross-links proteins through γ -glutamyl residues, seems to be important for the stabilization of apoptotic cell membranes necessary to retain intracellular material [6]. Thus, by blocking transglutaminase activity with dansyl-cadaverine, a specific transglutaminase inhibitor, cross-linking of membrane proteins is prevented, and the apoptotic cells undergo necrosis and lysis at an earlier time point. When M ϕ were inoculated with *M. avium* in the presence of 0.3 μ M dansyl-cadaverine, necrosis of 50% of the M ϕ was already visible on day 4 in comparison to only 20% necrotic cells in the absence of dansyl-cadaverine. This finding corresponds with a 100-fold increased release of bacteria into the cell-free supernatant in the presence of dansyl-cadaverine. Similarly, the release of intracellular lactate dehydrogenase into M ϕ culture supernatants after 3 days in culture was increased in *M. avium*-infected M ϕ cultures incubated with dansyl-cadaverine to 40% of the total amount of lactate dehydrogenase present in the cells compared with the release of this enzyme in cultures incubated without dansyl-cadaverine (0%) [7].

Second, addition of uninfected autologous M ϕ to apoptotic infected M ϕ resulted in significant growth inhibition of the bacteria: when an equal number of fresh, uninfected autologous

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M ϕ was added for 6 h to apoptotic M ϕ infected with *M. avium* 3 days earlier, a more than 10-fold reduction of the mycobacterial load from 10⁴ to 10³ bacteria/mL in the culture ($n = 6$; $P = 0.002$) was seen. To test whether the apoptotic condition is necessary for optimal bacterial growth inhibition, uninfected autologous M ϕ were also added to (1) M ϕ infected for 3 days with *M. avium* and made necrotic by ultrasound treatment and (2) M ϕ infected with *M. avium* whose apoptosis was inhibited by treatment with interleukin-10 (IL-10) and anti-TNF- α antibodies. Clearly, only when new M ϕ were added to apoptotic infected M ϕ was there a 10-fold reduction of bacterial burden in the culture. There was no effect by newly added M ϕ on the growth of *M. avium* in necrotic M ϕ and in non-apoptotic M ϕ . Thus, the apoptotic condition is essential for mycobacterial growth inhibition [7].

These experiments indicate that M ϕ apoptosis might be part of an anti-mycobacterial defense mechanism involving sequestering of the pathogens within apoptotic bodies and subsequent elimination of the mycobacteria packaged in the apoptotic bodies by phagocytes.

Thus, it was not surprising that some mycobacteria have developed mechanisms to block M ϕ apoptosis. We describe here a mechanism by which *M. tuberculosis* prevent M ϕ apoptosis by an IL-10-dependent mechanism. This mechanism is based on the release of soluble TNF- α receptor class 2 (TNFR2), resulting in the inactivation of TNF- α . This study was performed using human alveolar M ϕ , the virulent *M. tuberculosis* strain H37Rv, and the attenuated *M. tuberculosis* strain H37Ra.

We observed previously that the attenuated *M. tuberculosis* strain H37Ra induces more death of alveolar M ϕ than the virulent H37Rv strain (29 \pm 7 vs. 13 \pm 3% apoptotic M ϕ after 5 days in culture, $n = 3$; $P < 0.001$) [5]. This H37Ra-induced M ϕ apoptosis was inhibited by anti TNF- α antibodies (7 \pm 3 vs. 29 \pm 7% apoptotic M ϕ , $n = 3$; $P = 0.0001$). Differential induction of apoptosis by H37Ra and H37Rv was not due to different TNF- α production because both H37Ra and H37Rv induced the production of comparable amounts of TNF- α by the M ϕ (at 24 h in both cultures 1.7 \pm 0.3 ng TNF- α /mL). Instead the supernatants from H37Rv-infected cell cultures contained less TNF- α bioactivity than the supernatants from M ϕ cultures infected with the H37Ra strain. Although the amounts of TNF- α antigen as measured by enzyme-linked immunosorbent assay was not different in H37Ra- and H37Rv-infected cultures, the supernatants from H37Rv-infected cultures contained less cytotoxic activity attributable to TNF- α than culture supernatants from H37Ra-infected cells (at 24 h 0.3 \pm 0.1 vs. 1.4 \pm 0.4 ng TNF- α /mL as measured by a cytotoxicity assay, $n = 3$; $P < 0.05$), correlating with less apoptosis in M ϕ cultures infected with H37Rv (at 24 h 11 \pm 4 vs. 38 \pm 3% apoptotic cells, $n = 3$; $P < 0.05$) [8].

Because TNF- α receptors are also released from the cells in soluble form [9], we measured the release of soluble TNF receptors in culture supernatants from infected alveolar M ϕ . No increase in TNFR1 release from the cells was observed when the cells were infected with H37Ra or with H37Rv. However, infection with H37Rv resulted in release of significantly more TNFR2 than infection with H37Ra (at 48 h, 450 \pm 10 vs. 110 \pm 4 pg/mL soluble TNFR2, $n = 5$; $P < 0.05$).

Next we investigated whether soluble TNFR2 acts by

neutralizing TNF- α bioactivity. When soluble TNFR2 (50 ng/mL) was added to H37Ra-infected cultures TNF- α cytotoxic activity as measured by a mouse fibroblast cytotoxicity assay, TNF- α activity was significantly diminished from 1.4 \pm 0.2 to 0.7 \pm 0.3 ng TNF- α /mL ($n = 3$; $P < 0.05$). The release of soluble TNFR2 from the alveolar M ϕ depended on the action of IL-10 because neutralizing anti-IL-10 antibodies significantly reduced the release of TNFR2 induced by H37Rv from 160 \pm 5 in the absence to 80 \pm 4 pg/mL soluble TNFR2 in the presence of anti-IL-10 antibodies ($n = 4$; $P < 0.05$). In contrast, addition of IL-10 to H37Ra-infected alveolar M ϕ cultures resulted in abatement of H37Ra-induced apoptosis (27 \pm 3% apoptotic M ϕ in the presence of 20 ng/mL anti-IL-10 antibodies vs. 50 \pm 4% apoptotic M ϕ in the absence of antibodies, $n = 3$; $P < 0.05$). These data were corroborated by our finding that in alveolar M ϕ from seven of nine donors there was increased induction of IL-10 production by the virulent H37Rv in comparison to the attenuated H37Ra strain (58 \pm 11 pg/mL induced by H37Ra and 103 \pm 18 pg/mL IL-10 induced by H37Rv, $n = 12$; $P = 0.009$), which correlates directly with the decreased induction of alveolar M ϕ apoptosis in H37Rv-inoculated alveolar M ϕ cultures [8].

Thus, M ϕ apoptosis appears to be an important defense mechanism against mycobacteria, preventing the spread of infection by sequestering the pathogens and contributing to their elimination by activation of newly recruited uninfected M ϕ . The release of soluble TNFR2 from the M ϕ , which is mediated by IL-10, seems to be an important down-regulatory mechanism for TNF- α -dependent apoptosis, which is specifically induced by virulent strains of mycobacteria.

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