J. Vet. Sci. (2008), **9**(1), 1−8

JOURNAL OF
Veterinary
Science

Review

Tactics of *Mycobacterium avium subsp. paratuberculosis* for intracellular survival in mononuclear phagocytes

Seng-Ryong Woo, Charles J. Czuprynski*

Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA

Johne's disease is a condition that refers to chronic granulomatous enteritis in ruminants. It is believed that survival and replication of *Mycobacterium* (*M.*) *paratuberculosis* in mononuclear phagocytes plays an important role in the pathogenesis of Johne's disease. However, it is not clear how *M. paratuberculosis* survives for long time periods in mononuclear phagocytes, nor is it clear which factors trigger multiplication of these bacilli and result in the development of Johne's disease. Investigating the intracellular fate of *M. paratuberculosis* is challenging because of its very slow growth (more than two months to form visible colonies on media). Existing animal models also have limitations. Despite those obstacles, there has been progress in understanding the intracellular survival tactics of *M. paratuberculosis* and the host response against them. In this review, we compare known aspects of the intracellular survival tactics of *M. paratuberculosis* with those of other mycobacterial species, and consider possible mycobactericidal mechanisms of mononuclear phagocytes.

Keywords: intracellular, Johne's disease, *Mycobacterium avium, Mycobacterium paratuberculosis*

Introduction

Mycobacterium (*M.*) *paratuberculosis* is the etiologic agent of chronic enteritis of ruminants, known as paratuberculosis or Johne's disease [14]. *M. paratuberculosis* is a Gram-positive, acid-fast bacillus that belongs to the *M. avium* complex [10]. It grows very slowly and requires mycobactin J, an iron-chelating cell wall component produced by most other mycobacteria, for growth *in vitro*. As a result, visible colony formation takes 8 to 12 weeks or longer. Identification of *M. paratuberculosis* depends on mycobactin-dependent growth and detection of the species-specific IS 900 insertion sequence by polymerase chain reaction (PCR) [20]. Like other mycobacteria, the cell wall of *M. paratuberculosis* is lipid-rich and consists of several layers. The main components of the cell wall are lipoarabinomannan (LAM) and arabinomannan (AM) [90]. It has been reported that LAM is highly immunogenic and reacts with sera from infected cattle [47,89]. Several proteins that induce a humoral immune response in infected cows are produced by *M. paratuberculosis*. These include 17 KDa, 34 KDa, and 400 KDa proteins, and the antigen 85 complex, which consists of four proteins [71]. These protein antigens have been investigated in an effort to find antigens that react specifically with sera from animals infected with *M. paratuberculosis*, and not those infected with other mycobacterial species. However, these antigens share epitopes with *M. avium* [71].

It is believed that young calves are infected by *M. paratuberculosis* via the oral route, through contaminated feces, colostrum, or milk. Most infected animals do not develop clinical symptoms. Only 10-15% of infected cows develop clinical disease, usually after two or more years of infection [72]. However, subclinically infected animals may shed bacilli intermittently in their feces, spreading infection in the herd. Cattle with clinical Johne's disease exhibit decreased production, diarrhea, and weight loss [19,71,90]. These animals usually shed bacilli in their feces and have detectable antibodies in their serum. The host response to *M. paratuberculosis* infection results in granulomatous lesions in the small intestine. The intestinal wall subsequently undergoes progressive thickening, which is caused by hypoproteinemia and edema due to decreased intravascular osmotic pressure [19]. Pathological changes in the small intestine result in malabsorption of nutrients and weight loss. It is not clear which bacterial factors trigger multiplication of *M. paratuberculosis*, or which host responses control *M. paratuberculosis* infection [14,18,21, 22,71,83,94].

*Corresponding author
Tel: +1-608-262-8102; Fax: +1-608-262-8102
E-mail: czuprync@svm.vetmed.wisc.edu

Entry of *M. paratuberculosis* into mononuclear phagocytes

After ingestion by young calves, it is thought that the bacilli enter intestinal tissue through M cells in the Peyer's patches of the small intestine [67]. *M. paratuberculosis* expresses fibronectin attachment proteins (FAPs) [77]. Fibronectin bound to these receptors can, in turn, bind to integrins on M cells and mediate the uptake of *M. paratuberculosis* [78,79,95]. After crossing the intestinal epithelial layer, subepithelial macrophages phagocytose bacilli, presumably using several different surface receptors.

Mononuclear phagocytes serve as the intracellular niche for *M. paratuberculosis* survival and multiplication. Macrophages are known to have several receptors that are involved in the uptake of mycobacteria [7,73,75,85-87]. These receptors include complement receptors (CR1, CR3, and CR4), immunoglobulin receptors (FcR), the mannose receptor, and scavenger receptors [2,29,30,39, 92]. Human macrophages exhibit decreased uptake of *M. avium* following the addition of anti-CR3 antibodies [11]. Serum opsonization of *M. tuberculosis* increases the uptake of bacilli by human monocytes, while blocking CR3 with specific antibody decreased the uptake of bacilli by about 87% [76]. Likewise, the uptake of *M. paratuberculosis* by murine macrophages was inhibited by preincubation with anti-CR3 monoclonal antibody [17]. Similarly, opsonization of *M. paratuberculosis* with serum from normal adult cows or from cows with clinical paratuberculosis enhanced the uptake of bacilli by bovine mononuclear phagocytes [43,106,112]. These observations suggest that complement opsonization is important to the uptake of *M. paratuberculosis* by bovine mononuclear phagocytes. It has also been reported that mononuclear phagocytes can synthesize and secrete complement proteins that opsonize particles for phagocytosis [62].

Possible mechanisms of intracellular survival or death of *M. paratuberculosis*

It is very important to understand the survival mechanisms of *M. paratuberculosis* in bovine mononuclear phagocytes if we are to develop more effective ways to control Johne's disease. Because infected animals develop clinical symptoms of Johne's disease relatively slowly, infected cows presumably can suppress multiplication of *M. paratuberculosis* and delay or prevent the development of Johne's disease.

Different routes of entry can alter the intracellular fate of ingested bacilli [6,7,23,28,45]. For example, complement receptor CR1-mediated uptake of particles does not stimulate the production of superoxide anion [23,45]. Mannose receptor-mediated uptake of pathogenic or nonpathogenic mycobacteria does not activate NADPH oxidase in human macrophages [7], and selective receptor blockade did not alter the intracellular survival of *M. tuberculosis* in human macrophages [111]. CR3-mediated binding and uptake of *M. tuberculosis* by macrophages does not seem to affect the intracellular fate of bacilli [96]. There was no difference in bacterial burden or granulomatous response between wild-type and complement component C3-deficient mice following *M. avium* infection [12]. Opsonization of *M. paratuberculosis* with normal serum from adult cows, or serum containing specific antibodies against *M. paratuberculosis*, increased the uptake of bacilli by bovine mononuclear phagocytes. However, intracellular survival of *M. paratuberculosis* in the bovine mononuclear phagocytes was not affected [43].

Toll-like receptors (TLR) are pattern-recognition receptors that detect microbes or microbial components and initiate inflammatory responses [63,93]. Antigen- presenting cells (APCs), which include dendritic cells and macrophages, express TLR receptors and initiate an immune response, and then bind to pathogen-associated molecular patterns (PAMPs) of microbes [109]. The 19 kDa lipoprotein of *M. tuberculosis* activates murine and human macrophages through TLR2, and this, in turn, activates a signaling pathway that kills intracellular bacilli [84,91]. This TLR2-mediated mycobactericidal effect is dependent on the production of nitric oxide in murine macrophages and enhanced expression of vitamin D receptor in human macrophages [58,91]. Bovine monocytes and macrophages express mRNA for TLR2 and TLR4 [105]. TLR ligands such as lipopolysaccharide (LPS), *Salmonella dublin*, and *Listeria monocytogenes* activate bovine mononuclear phagocytes and induce the production of reactive oxygen intermediates (ROIs) [104]. Although there have been no reports of how TLR activation alters the intracellular survival of *M. paratuberculosis*, pretreatment of J774 cells and bovine monocytes with LPS and IFN-γ slightly decreased the number of viable intracellular *M. paratuberculosis* bacteria [44,110].

During the process of phagocytosis by macrophages, the NADPH oxidase complex on the plasma membrane produces a series of ROIs, including superoxide anion, hydrogen peroxide, and hydroxyl radical [27,40,80]. These ROIs have been implicated in killing mycobacteria [46,50]. However, there have been confilicting reports on the mycobactericidal effect of ROIs against *M. tuberculosis* [16,34, 55,56,61]. Mycobacteria have some ability to evade being killed by ROIs [41,61]. Bovine macrophages produce superoxide anion following stimulation with phorbol 12-myristate 13-acetate (PMA), zymosan, or LPS [104]. However, bovine monocytes and macrophages do not stimulate much ROI production after *M. paratuberculosis* infection, and IFN-γ activation of bovine monocytes and macrophages did little to increase the release of ROIs [103,113]. *M. paratuberculosis* secretes superoxide dismutase,

which is a possible protective mechanism for intracellular bacilli [59]. However, we need more evidence to clarify the role of ROIs on the intracellular fate of *M. paratuberculosis* in bovine mononuclear phagocytes.

Nitric oxide and other reactive nitrogen intermediates (RNIs) are known to be major mycobactericidal molecules, especially in mice [15,16,32]. After activation with IFN-γ and TNF-α, murine macrophages produce significantly increased amounts of RNI [60]. Mycobacteria have the ability to inhibit recruitment of nitric oxide synthase to mycobacteria-containing phagosomes [65]. IFN-γ activation of bovine macrophages did not result in increased nitric oxide production, whereas IFN-γ-activated murine macrophages produced significant amounts of nitric oxide (45-83 μM) at 72 and 96 h after treatment [3]. Bovine macrophages can produce increased nitric oxide in response to other stimuli. LPS, *Listeria monocytogenes*, and *Salmonella dublin* all enhanced the production of nitric oxide (20 to 70 μM) by bovine macrophages [3,104]. In contrast, *M. paratuberculosis*-infected bovine macrophages and monocytes produced small amounts of nitric oxide (2-3 μM), and IFN-γ treatment increased nitric oxide production only to 6-8 μM [103]. Stimulation of monocytes with IFN-γ or IFN-γ and LPS increased the production of nitric oxide, but the amount produced was inadequate to kill intracellular *M. paratuberculosis* [110]. In the granulomatous lesions of bovine Johne's disease, the immunoreactivity of inducible nitric oxide synthase (iNOS) is weak and localized at or near the intestinal crypts [42]. Chemically-generated nitric oxide kills extracellular *M. paratuberculosis*, but bovine mononuclear phagocytes do not produce enough nitric oxide to kill intracellular *M. paratuberculosis* [110]. Although the inhibition of nitric oxide production resulting from the addition of N^G- monomethyl-L-arginine (NMMA) increases the intracellular survival of *M. tuberculosis* in IFN-γ -pretreated murine macrophages [4], NMMA treatment does not promote intracellular survival of *M. paratuberculosis* in bovine monocytes [109]. These data suggest that nitric oxide might not be a major mycobactericidal mechanism with which to control *M. paratuberculosis* in infected cattle.

One of the potential microbicidal mechanisms of phagocytes is phagosome-lysosome fusion [24,25,98]. The lysosomal vacuoles contain potent hydrolytic enzymes that kill and degrade ingested microbes [8]. These enzymes require an acidic environment for their optimal activity. This acidic condition is maintained by a membrane ATP-dependent proton pump, the vacuolar H+-ATPase [64]. Mycobacterial phagosomes inhibit the recruitment of vacuolar H+- ATPase and phagosomal acidification [88]. Phagosomal maturation is also inhibited by retention of the small GTP- binding protein, Rab5, and by reduced recruitment of early endosomal autoantigen 1 (EEA1) to mycobacterial phagosomes [36,99]. *M. tuberculosis* LAM inhibits phagosome maturation [37,38,97]. Live *M. paratuberculosis* cells inhibit phagosome- lysosome fusion and phagosomal acidification in the J774 murine macrophage cell line, but killed *M. paratuberculosis* cells do not have this effect [51]. Similar results were seen in bovine mononuclear phagocytes, in which killed *M. paratuberculosis* cells were associated with greater phagosome-lysosome fusion than live *M. paratuberculosis* cells [107]. Pretreatment with IFN-γ and LPS enhanced phagosome-lysosome fusion in murine macrophages infected with *M. avium* or *M. bovis* BCG [74,100]. Treatment of *M. paratuberculosis*-infected J774 cells with IFN-γ and LPS also enhanced phagosome-lysosome fusion and the killing of intracellular bacilli [44]. However, the effect of IFN-γ and LPS treatment on the maturation of phagosomes containing *M. paratuberculosis* in bovine mononuclear phagocytes is unknown. There have been few investigations of the molecular mechanisms of phagosomal maturation and of the mycobacterial molecules that inhibit phagosome maturation in bovine mononuclear phagocytes.

After infection with *M. paratuberculosis*, bovine monocytes produce TNF-α [1]. Although gene expression of TNF-α was identified in ileal tissues of cattle infected with *M. paratuberculosis*, no difference was seen between uninfected and infected cattle [57]. TNF-α treatment of murine macrophages infected with *M. paratuberculosis* resulted in either enhanced or decreased viability of intracellular bacilli, depending on the TNF-α concentrations and the lengths of incubation [82]. No report has yet been published on the effect of TNF-α on intracellular survival of *M. paratuberculosis* in bovine mononuclear phagocytes.

Although *M. avium* is antigenically and genetically very similar to *M. paratuberculosis*, it is generally considered to be relatively nonpathogenic in cattle. Bovine macrophages expressed greater amounts of IL-10 mRNA following infection with *M. paratuberculosis* than with *M. avium* [103]. IL-10 is an anti-inflammatory cytokine that suppresses the activation of macrophages [33]. The IL-10 gene is expressed to a greater extent in intestinal tissues and lymph nodes from cows clinically infected with *M. paratuberculosis* than in subclinically infected or healthy cows [49]. Bovine macrophages infected with *M. paratuberculosis* produce IL-10, and neutralization of IL-10 enhances the killing of intracellular bacilli [101]. Another report showed greater production of IL-10 from peripheral blood mononuclear cells (PBMC) isolated from cows with clinical Johne's disease than from healthy cows [49]. Addition of IL-10 increased intracellular survival of *M. paratuberculosis* in PBMCs isolated from healthy cows [48]. Neutralization of IL-10 also increased the production of IFN-γ by bovine peripheral blood mononuclear cells after infection with *M. paratuberculosis*, and enhanced phagosome acidification and apoptosis of bovine macrophages [13,101]. However, the general role of IL-10 in resistance to mycobacterial infection is not clear. IL-10/ mice did not show greater resistance to acute *M. tuberculosis*

infection than did wild-type mice [69].

Apoptosis is a process of programmed cell death that is characterized by DNA fragmentation, nuclear chromatin condensation, compacting of cellular organelles, and membrane blebbing [35]. It has been suggested that apoptosis of mycobacteria-infected macrophages induces the intracellular killing of bacilli, but that necrotic macrophage death does not induce the killing of mycobacteria [66]. There have been reports that apoptotic stimuli like Fas ligand, TNF-α, picolinic acid, ATP, and the mycobacterial 19 kDa lipoprotein can kill intracellular mycobacteria in mononuclear phagocytes [66,70,72,91]. However, it is not clear whether apoptosis of infected macrophages is required for intracellular killing of mycobacteria, nor is it clear whether stimuli that induce intracellular killing of bacilli are distinct from those that trigger apoptosis. *M. tuberculosis* induced 30-50% apoptosis of infected human alveolar macrophages at 2 and 4 days after infection, and this occurred via a TNF-α-dependent mechanism. Interestingly, pathogenic *M. tuberculosis* evades apoptosis of macrophages by inducing the secretion of TNF-R2 from infected macrophages that, in turn, is dependent on the production of IL-10 [9]. *M. paratuberculosis* induced 18 to 27% apoptosis of infected bovine monocytes at 6 and 48 h after infection, with live bacilli causing greater apoptosis than heat-killed *M. paratuberculosis* [5]. In that paper, however, the authors did not quantify changes in the intracellular survival of *M. paratuberculosis*. Interestingly, *M. paratuberculosis* caused less apoptosis in bovine macrophages than did *M. avium* [102]. More recently, evidence has indicated that the longer survival of *M. paratuberculosis* within bovine monocytes *in vitro* (4 to 8 days) results in morphological changes that may reflect reduced differentiation or survival of the infected monocytes [107].

Extracellular ATP can kill intracellular mycobacteria, presumably through the activation of purinergic receptors on the surface of infected mononuclear phagocytes. ATP is released by nonlytic and lytic mechanisms from both resting cells, as well as by cells undergoing apoptotic or necrotic cell death. ATP released from lymphocytes or mononuclear phagocytes in foci of mycobacterial infection might activate purinergic receptors on infected mononuclear phagocytes. Extracellular ATP is known to be cytotoxic to macrophages, and triggers the killing of mycobacteria in murine and human macrophages [26,31, 52-54,68]. This response reflects increased intracellular calcium and phospholipase D (PLD) activity following ATP activation of $P2X_7$ receptors. This subsequently results in increased phagosome-lysosome fusion of mycobacterium-containing phagosomes [52,53]. It is not clear whether macrophage cytotoxicity is required for the mycobactericidal effect of ATP to occur. Bovine macrophages express mRNA for the $P2X_7$ receptor, and the addition of ATP to bovine macrophages infected with *M. bovis* BCG induced the killing of intracellular bacilli [81]. Elimination of extracellular ATP by the addition of apyrase increased the survival of *M. paratuberculosis*-infected bovine monocytes, but unexpectedly reduced the survival of bacilli. Similarly, the addition of ATP or benzyl-ATP reduced the survival of *M. paratuberculosis*-infected monocytes, but not the survival of the bacilli themselves [108]. Thus, *M. paratuberculosis* may differ from other mycobacterial species in terms of how the presence of ATP affects the intracellular survival of bacilli within bovine mononuclear phagocytes.

In this review, we have described possible intracellular survival mechanisms of *M. paratuberculosis* in infected macrophages, mainly in comparison to *M. tuberculosis* and *M. avium*. *M. paratuberculosis* seemed to have shared strategies with other closely-related mycobacteria for intracellular survival, but it also has unique mechanisms. It is important to clarify the intracellular survival tactics of *M. paratuberculosis* in order to understand the pathogenesis of Johne's disease and develop means by which to prevent this disease. However, it is difficult to investigate the survival mechanisms of *M. paratuberculosis* in infected macrophages because of the long incubation period required to count visible colonies in media and the limited availability of bovine reagents to use for this purpose. With the development of new technologies and vigorous efforts, these survival mechanisms will be better elucidated in the future, and will allow us to understand the pathogenesis of this, one of the most challenging bacteria to study.

References

1. **Adams JL, Czuprynski CJ.** Mycobacterial cell wall components induce the production of TNF-alpha, IL-1, and IL-6 by bovine monocytes and the murine macrophage cell line RAW 264.7. Microb Pathog 1994, **16**, 401-411.
2. **Aderem A, Underhill DM.** Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 1999, **17**, 593-623.
3. **Adler H, Frech B, Thöny M, Pfister H, Peterhans E, Jungi TW.** Inducible nitric oxide synthase in cattle. Differential cytokine regulation of nitric oxide synthase in bovine and murine macrophages. J Immunol 1995, 154, 4710-4718.
4. **Akaki T, Tomioka H, Shimizu T, Dekio S, Sato K.** Comparative roles of free fatty acids with reactive nitrogen intermediates and reactive oxygen intermediates in expression of the anti-microbial activity of macrophages against *Mycobacterium tuberculosis*. Clin Exp Immunol 2000, **121**, 302-310.
5. **Allen S, Sotos J, Sylte MJ, Czuprynski CJ.** Use of Hoechst 33342 staining to detect apoptotic changes in bovine mononuclear phagocytes infected with *Mycobacterium avium subsp. paratuberculosis*. Clin Diagn Lab Immunol 2001, **8**, 460-464.
6. **Armstrong JA, Hart PD.** Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and

observations on bacterial survival. J Exp Med 1975, **142**, 1-16.

7. **Astarie-Dequeker C, N'Diaye EN, Le Cabec V, Rittig MG, Prandi J, Maridonneau-Parini I.** The mannose receptor mediates uptake of pathogenic and nonpathogenic mycobacteria and bypasses bactericidal responses in human macrophages. Infect Immun 1999, **67**, 469-477.
8. **Bainton DF.** The discovery of lysosomes. J Cell Biol 1981, **91**, 66s-76s.
9. **Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG.** Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-alpha. J Immunol 1998, **161**, 2636-2641.
10. **Bannantine JP, Zhang Q, Li LL, Kapur V.** Genomic homogeneity between *Mycobacterium avium subsp. avium* and *Mycobacterium avium subsp. paratuberculosis* belies their divergent growth rates. BMC Microbiol 2003, **3**, 10.
11. **Bermudez LE, Young LS, Enkel H.** Interaction of *Mycobacterium avium* complex with human macrophages: roles of membrane receptors and serum proteins. Infect Immun 1991, **59**, 1697-1702.
12. **Bohlson SS, Strasser JA, Bower JJ, Schorey JS.** Role of complement in *Mycobacterium avium* pathogenesis: *in vivo* and *in vitro* analyses of the host response to infection in the absence of complement component C3. Infect Immun 2001, **69**, 7729-7735.
13. **Buza JJ, Hikono H, Mori Y, Nagata R, Hirayama S, Aodon-geril, Bari AM, Shu Y, Tsuji NM, Momotani E.** Neutralization of interleukin-10 significantly enhances gamma interferon expression in peripheral blood by stimulation with Johnin purified protein derivative and by infection with *Mycobacterium avium subsp. paratuberculosis* in experimentally infected cattle with paratuberculosis. Infect Immun 2004, **72**, 2425-2428.
14. **Chacon O, Bermudez LE, Barletta RG.** Johne's disease, inflammatory bowel disease, and *Mycobacterium paratuberculosis*. Annu Rev Microbiol 2004, **58**, 329-363.
15. **Chakravortty D, Hensel M.** Inducible nitric oxide synthase and control of intracellular bacterial pathogens. Microbes Infect 2003, **5**, 621-627.
16. **Chan J, Xing Y, Magliozzo RS, Bloom BR.** Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 1992, **175**, 1111-1122.
17. **Cheville NF, Hostetter J, Thomsen BV, Simutis F, Vanloubbeeck Y, Steadham E.** Intracellular trafficking of *Mycobacterium avium ss. paratuberculosis* in macrophages. Dtsch Tierarztl Wochenschr 2001, **108**, 236-243.
18. **Chiodini RJ.** Immunology: resistance to paratuberculosis. Vet Clin North Am Food Anim Pract 1996, **12**, 313-343.
19. **Cocito C, Gilot P, Coene M, de Kesel M, Poupart P, Vannuffel P.** Paratuberculosis. Clin Microbiol Rev 1994, **7**, 328-345.
20. **Collins MT.** Paratuberculosis: review of present knowledge. Acta Vet Scand 2003, **44**, 217-221.
21. **Coussens PM.** Model for immune responses to *Mycobacterium avium subspecies paratuberculosis* in cattle. Infect Immun 2004, **72**, 3089-3096.
22. **Coussens PM.** *Mycobacterium paratuberculosis* and the bovine immune system. Anim Health Res Rev 2001, **2**, 141-161.
23. **Da Silva RP, Hall BF, Joiner KA, Sacks DL.** CR1, the C3b receptor, mediates binding of infective *Leishmania major* metacyclic promastigotes to human macrophages. J Immunol 1989, **143**, 617-622.
24. **Desjardins M.** Biogenesis of phagolysosomes: the 'kiss and run' hypothesis. Trends Cell Biol 1995, **5**, 183-186.
25. **Desjardins M, Huber LA, Parton RG, Griffiths G.** Biogenesis of phagolysosomes proceeds through a sequential series of interactions with the endocytic apparatus. J Cell Biol 1994, **124**, 677-688.
26. **Di Virgilio F, Chiozzi P, Falzoni S, Ferrari D, Sanz JM, Venketaraman V, Baricordi OR.** Cytolytic P2X purinoceptors. Cell Death Differ 1998, **5**, 191-199.
27. **Dinauer MC, Orkin SH.** Chronic granulomatous disease. Annu Rev Med 1992, **43**, 117-124.
28. **Drevets DA, Leenen PJ, Campbell PA.** Complement receptor type 3 (CD11b/CD18) involvement is essential for killing of *Listeria monocytogenes* by mouse macrophages. J Immunol 1993, **151**, 5431-5439.
29. **El-Etr SH, Cirillo JD.** Entry mechanisms of mycobacteria. Front Biosci 2001, **6**, D737-747.
30. **Ernst JD.** Macrophage receptors for *Mycobacterium tuberculosis*. Infect Immun 1998, **66**, 1277-1281.
31. **Fairbairn IP, Stober CB, Kumararatne DS, Lammas DA**. ATP-mediated killing of intracellular mycobacteria by macrophages is a P2X(7)-dependent process inducing bacterial death by phagosome-lysosome fusion. J Immunol 2001, **167**, 3300-3307.
32. **Fang FC.** Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. J Clin Invest 1997, **99**, 2818-2825.
33. **Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A.** IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991, **146**, 3444-3451.
34. **Flesch IE, Kaufmann SH.** Attempts to characterize the mechanisms involved in mycobacterial growth inhibition by gamma-interferon-activated bone marrow macrophages. Infect Immun 1988, **56**, 1464-1469.
35. **Fratazzi C, Arbeit RD, Carini C, Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG.** Macrophage apoptosis in mycobacterial infections. J Leukoc Biol 1999, **66**, 763-764.
36. **Fratti RA, Backer JM, Gruenberg J, Corvera S, Deretic V.** Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. J Cell Biol 2001, **154**, 631-644.
37. **Fratti RA, Chua J, Deretic V.** Induction of p38 mitogen-activated protein kinase reduces early endosome autoantigen 1 (EEA1) recruitment to phagosomal membranes. J Biol Chem 2003, **278**, 46961-46967.
38. **Fratti RA, Chua J, Vergne I, Deretic V.** *Mycobacterium tuberculosis* glycosylated phosphatidylinositol causes phagosome maturation arrest. Proc Natl Acad Sci U S A 2003, **100**, 5437-5442.
39. **Gatfield J, Pieters J.** Molecular mechanisms of host-patho-

gen interaction: entry and survival of mycobacteria in macrophages. Adv Immunol 2003, **81**, 45-96.
40. **Gordon AH, Hart PD.** Stimulation or inhibition of the respiratory burst in cultured macrophages in a mycobacterium model: initial stimulation is followed by inhibition after phagocytosis. Infect Immun 1994, **62**, 4650-4651.
41. **Heym B, Zhang Y, Poulet S, Young D, Cole ST.** Characterization of the katG gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. J Bacteriol 1993, **175**, 4255-4259.
42. **Hostetter J, Huffman E, Byl K, Steadham E.** Inducible nitric oxide synthase immunoreactivity in the granulomatous intestinal lesions of naturally occurring bovine Johne's disease. Vet Pathol 2005, **42**, 241-249.
43. **Hostetter J, Kagan R, Steadham E.** Opsonization effects on *Mycobacterium avium subsp. paratuberculosis*-macrophage interactions. Clin Diagn Lab Immunol 2005, **12**, 793-796.
44. **Hostetter JM, Steadham EM, Haynes JS, Bailey TB, Cheville NF.** Cytokine effects on maturation of the phagosomes containing *Mycobacteria avium subspecies paratuberculosis* in J774 cells. FEMS Immunol Med Microbiol 2002, **34**, 127-134.
45. **Ishibashi Y, Arai T**. Roles of the complement receptor type 1 (CR1) and type 3 (CR3) on phagocytosis and subsequent phagosome-lysosome fusion in Salmonella-infected murine macrophages. FEMS Microbiol Immunol 1990, **2**, 89-96.
46. **Jackett PS, Aber VR, Lowrie DB.** Virulence of *Mycobacterium tuberculosis* and susceptibility to peroxidative killing systems. J Gen Microbiol 1978, **107**, 273-278.
47. **Jark U, Ringena I, Franz B, Gerlach GF, Beyerbach M.** Development of an ELISA technique for serodiagnosis of bovine paratuberculosis. Vet Microbiol 1997, **57**, 189-198.
48. **Khalifeh MS, Stabel JR.** Effects of gamma interferon, interleukin-10, and transforming growth factor beta on the survival of *Mycobacterium avium subsp. paratuberculosis* in monocyte-derived macrophages from naturally infected cattle. Infect Immun 2004, **72**, 1974-1982.
49. **Khalifeh MS, Stabel JR.** Upregulation of transforming growth factor-beta and interleukin-10 in cows with clinical Johne's disease. Vet Immunol Immunopathol 2004, **99**, 39-46.
50. **Klebanoff SJ, Shepard CC.** Toxic effect of the peroxidase-hydrogen peroxide-halide antimicrobial system on *Mycobacterium leprae*. Infect Immun 1984, **44**, 534-536.
51. **Kuehnel MP, Goethe R, Habermann A, Mueller E, Rohde M, Griffiths G, Valentin-Weigand P.** Characterization of the intracellular survival of *Mycobacterium avium ssp. paratuberculosis*: phagosomal pH and fusogenicity in J774 macrophages compared with other mycobacteria. Cell Microbiol 2001, **3**, 551-566.
52. **Kusner DJ, Adams J.** ATP-induced killing of virulent *Mycobacterium tuberculosis* within human macrophages requires phospholipase D. J Immunol 2000, **164**, 379-388.
53. **Kusner DJ, Barton JA.** ATP stimulates human macrophages to kill intracellular virulent *Mycobacterium tuberculosis* via calcium-dependent phagosome-lysosome fusion. J Immunol 2001, **167**, 3308-3315.
54. **Lammas DA, Stober C, Harvey CJ, Kendrick N, Panchalingam S, Kumararatne DS.** ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z(P2X7) receptors. Immunity 1997, **7**, 433-444.
55. **Laochumroonvorapong P, Paul S, Elkon KB, Kaplan G.** H2O2 induces monocyte apoptosis and reduces viability of *Mycobacterium avium-M. intracellulare* within cultured human monocytes. Infect Immun 1996, **64**, 452-459.
56. **Laochumroonvorapong P, Paul S, Manca C, Freedman VH, Kaplan G.** Mycobacterial growth and sensitivity to H_2O_2 killing in human monocytes *in vitro*. Infect Immun 1997, **65**, 4850-4857.
57. **Lee H, Stabel JR, Kehrli ME Jr.** Cytokine gene expression in ileal tissues of cattle infected with *Mycobacterium paratuberculosis*. Vet Immunol Immunopathol 2001, **82**, 73-85.
58. **Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL.** Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006, **311**, 1770-1773.
59. **Liu X, Feng Z, Harris NB, Cirillo JD, Bercovier H, Barletta RG.** Identification of a secreted superoxide dismutase in *Mycobacterium avium ssp. paratuberculosis*. FEMS Microbiol Lett 2001, **202**, 233-238.
60. **MacMicking J, Xie QW, Nathan C.** Nitric oxide and macrophage function. Annu Rev Immunol 1997, **15**, 323-350.
61. **Manca C, Paul S, Barry CE, Freedman VH, Kaplan G.** *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes *in vitro*. Infect Immun 1999, **67**, 74-79.
62. **McPhaden AR, Whaley K.** Complement biosynthesis by mononuclear phagocytes. Immunol Res 1993, **12**, 213-232.
63. **Medzhitov R, Preston-Hurlburt P, Janeway CA Jr.** A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 1997, **388**, 394-397.
64. **Mellman I, Fuchs R, Helenius A.** Acidification of the endocytic and exocytic pathways. Annu Rev Biochem 1986, **55**, 663-700.
65. **Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, Marletta MA, Deretic V.** Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. Infect Immun 2004, **72**, 2872-2878.
66. **Molloy A, Laochumroonvorapong P, Kaplan G.** Apoptosis, but not necrosis, of infected monocytes is coupled with killing of intracellular bacillus Calmette-Guérin. J Exp Med 1994, **180**, 1499-1509.
67. **Momotani E, Whipple DL, Thiermann AB, Cheville NF.** Role of M cells and macrophages in the entrance of *Mycobacterium paratuberculosis* into domes of ileal Peyer's patches in calves. Vet Pathol 1988, **25**, 131-137.
68. **Murgia M, Pizzo P, Steinberg TH, Di Virgilio F.** Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. Biochem J 1992, **288**, 897-901.
69. **North RJ.** Mice incapable of making IL-4 or IL-10 display

normal resistance to infection with *Mycobacterium tuberculosis*. Clin Exp Immunol 1998, **113**, 55-58.

70. **Oddo M, Renno T, Attinger A, Bakker T, MacDonald HR, Meylan PR.** Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. J Immunol 1998, **160**, 5448-5454.
71. **Olsen I, Sigurgardottir G, Djonne B.** Paratuberculosis with special reference to cattle. A review. Vet Q 2002, **24**, 12-28.
72. **Pais TF, Appelberg R.** Macrophage control of mycobacterial growth induced by picolinic acid is dependent on host cell apoptosis. J Immunol 2000, **164**, 389-397.
73. **Roecklein JA, Swartz RP, Yeager H Jr.** Nonopsonic uptake of *Mycobacterium avium* complex by human monocytes and alveolar macrophages. J Lab Clin Med 1992, **119**, 772-781.
74. **Schaible UE, Sturgill-Koszycki S, Schlesinger PH, Russell DG.** Cytokine activation leads to acidification and increases maturation of *Mycobacterium avium*-containing phagosomes in murine macrophages. J Immunol 1998, **160**, 1290-1296.
75. **Schlesinger LS.** Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. J Immunol 1993, **150**, 2920-2930.
76. **Schlesinger LS, Bellinger-Kawahara CG, Payne NR, Horwitz MA.** Phagocytosis of *Mycobacterium tuberculosis* is mediated by human monocyte complement receptors and complement component C3. J Immunol 1990, **144**, 2771-2780.
77. **Secott TE, Lin TL, Wu CC.** Fibronectin attachment protein homologue mediates fibronectin binding by *Mycobacterium avium subsp. paratuberculosis.* Infect Immun 2001, **69**, 2075-2082.
78. **Secott TE, Lin TL, Wu CC.** Fibronectin attachment protein is necessary for efficient attachment and invasion of epithelial cells by *Mycobacterium avium subsp. paratuberculosis.* Infect Immun 2002, **70**, 2670-2675.
79. **Secott TE, Lin TL, Wu CC.** *Mycobacterium avium subsp. paratuberculosis* fibronectin attachment protein facilitates M-cell targeting and invasion through a fibronectin bridge with host integrins. Infect Immun 2004, **72**, 3724-3732.
80. **Segal AW, Abo A.** The biochemical basis of the NADPH oxidase of phagocytes. Trends Biochem Sci 1993, **18**, 43-47.
81. **Smith RA, Alvarez AJ, Estes DM.** The P2X7 purinergic receptor on bovine macrophages mediates mycobacterial death. Vet Immunol Immunopathol 2001, **78**, 249-262.
82. **Stabel JR.** Temporal effects of tumor necrosis factor-alpha on intracellular survival of *Mycobacterium paratuberculosis.* Vet Immunol Immunopathol 1995, **45**, 321-332.
83. **Stabel JR.** Transitions in immune responses to *Mycobacterium paratuberculosis*. Vet Microbiol 2000, **77**, 465-473.
84. **Stenger S, Modlin RL.** Control of *Mycobacterium tuberculosis* through mammalian Toll-like receptors. Curr Opin Immunol 2002, **14**, 452-457.
85. **Stokes RW, Doxsee D.** The receptor-mediated uptake, survival, replication, and drug sensitivity of *Mycobacterium tuberculosis* within the macrophage-like cell line THP-1: a comparison with human monocyte-derived macrophages. Cell Immunol 1999, **197**, 1-9.
86. **Stokes RW, Haidl ID, Jefferies WA, Speert DP.** Mycobacteria-macrophage interactions. Macrophage phenotype determines the nonopsonic binding of *Mycobacterium tuberculosis* to murine macrophages. J Immunol 1993, **151**, 7067-7076.
87. **Stokes RW, Thorson LM, Speert DP.** Nonopsonic and opsonic association of *Mycobacterium tuberculosis* with resident alveolar macrophages is inefficient. J Immunol 1998, **160**, 5514-5521.
88. **Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG.** Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. Science 1994, **263**, 678-681.
89. **Sugden EA, Corner AH, Samagh BS, Brooks BW, Turcotte C, Nielsen KH, Stewart RB, Duncan JR.** Serodiagnosis of ovine paratuberculosis, using lipoarabinomannan in an enzyme-linked immunosorbent assay. Am J Vet Res 1989, **50**, 850-854.
90. **Tessema MZ, Koets AP, Rutten VP, Gruys E.** How does *Mycobacterium avium subsp. paratuberculosis* resist intracellular degradation? Vet Q 2001, **23**, 153-162.
91. **Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, Barnes PF, Rollinghoff M, Bolcskei PL, Wagner M, Akira S, Norgard MV, Belisle JT, Godowski PJ, Bloom BR, Modlin RL.** Induction of direct antimicrobial activity through mammalian toll-like receptors. Science 2001, **291**, 1544-1547.
92. **Underhill DM, Ozinsky A.** Phagocytosis of microbes: complexity in action. Annu Rev Immunol 2002, **20**, 825-852.
93. **Underhill DM, Ozinsky A.** Toll-like receptors: key mediators of microbe detection. Curr Opin Immunol 2002, **14**, 103-110.
94. **Valentin-Weigand P, Goethe R.** Pathogenesis of *Mycobacterium avium subspecies paratuberculosis* infections in ruminants: still more questions than answers. Microbes Infect 1999, **1**, 1121-1127.
95. **Valentin-Weigand P, Moriarty KM.** *Mycobacterium paratuberculosis* binds fibronectin. Res Microbiol 1992, **143**, 75-79.
96. **Velasco-Velázquez MA, Barrera D, González-Arenas A, Rosales C, Agramonte-Hevia J.** Macrophage--*Mycobacterium tuberculosis* interactions: role of complement receptor 3. Microb Pathog 2003, **35**, 125-131.
97. **Vergne I, Chua J, Deretic V.** Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca2+/calmodulin-PI3K hVPS34 cascade. J Exp Med 2003, **198**, 653- 659.
98. **Vergne I, Chua J, Singh SB, Deretic V.** Cell biology of *mycobacterium tuberculosis* phagosome. Annu Rev Cell Dev Biol 2004, **20**, 367-394.
99. **Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V.** Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. J Biol Chem 1997, **272**, 13326-13331.
100. **Via LE, Fratti RA, McFalone M, Pagan-Ramos E, Deretic D, Deretic V.** Effects of cytokines on mycobacte-

rial phagosome maturation. J Cell Sci 1998, **111**, 897-905.
101. **Weiss DJ, Evanson OA, de Souza C, Abrahamsen MS.** A critical role of interleukin-10 in the response of bovine macrophages to infection by *Mycobacterium avium subsp paratuberculosis*. Am J Vet Res 2005, **66**, 721-726.
102. **Weiss DJ, Evanson OA, Deng M, Abrahamsen MS.** Gene expression and antimicrobial activity of bovine macrophages in response to *Mycobacterium avium subsp. paratuberculosis*. Vet Pathol 2004, **41**, 326-337.
103. **Weiss DJ, Evanson OA, Moritz A, Deng MQ, Abrahamsen MS.** Differential Responses of Bovine Macrophages to *Mycobacterium avium subsp. paratuberculosis* and *Mycobacterium avium subsp. avium*. Infect Immun 2002, **70**, 5556-5561.
104. **Werling D, Hope JC, Howard CJ, Jungi TW.** Differential production of cytokines, reactive oxygen and nitrogen by bovine macrophages and dendritic cells stimulated with Toll-like receptor agonists. Immunology 2004, **111**, 41-52.
105. **Werling D, Jungi TW.** TOLL-like receptors linking innate and adaptive immune response. Vet Immunol Immunopathol 2003, **91**, 1-12.
106. **Woo SR, Sotos J, Hart AP, Barletta RG, Czuprynski CJ.** Bovine monocytes and a macrophage cell line differ in their ability to phagocytose and support the intracellular survival of *Mycobacterium avium subsp. paratuberculosis*. Vet Immunol Immunopathol 2006, **110**, 109-120.
107. **Woo SR, Heintz JA, Albrecht R, Barletta RG, Czuprynski CJ.** Life and death in bovine monocytes: The fate of *Mycobacterium avium subsp. paratuberculosis*. Microb Pathog 2007, **43**, 106-113.
108. **Woo SR, Barletta RG, Czuprynski CJ.** Extracellular ATP is cytotoxic to mononuclear phagocytes but does not induce killing of intracellular *Mycobacterium avium subsp. paratuberculosis*. Clin Vaccine Immunol 2007, **14**, 1078- 1083.
109. **Zarember KA, Godowski PJ.** Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol 2002, **168**, 554-561.
110. **Zhao B, Collins MT, Czuprynski CJ.** Effects of gamma interferon and nitric oxide on the interaction of *Mycobacterium avium subsp. paratuberculosis* with bovine monocytes. Infect Immun 1997, **65**, 1761-1766.
111. **Zimmerli S, Edwards S, Ernst JD.** Selective receptor blockade during phagocytosis does not alter the survival and growth of *Mycobacterium tuberculosis* in human macrophages. Am J Respir Cell Mol Biol 1996, **15**, 760-770.
112. **Zurbrick BG, Czuprynski CJ.** Ingestion and intracellular growth of *Mycobacterium paratuberculosis* within bovine blood monocytes and monocyte-derived macrophages. Infect Immun 1987, **55**, 1588-1593.
113. **Zurbrick BG, Follett DM, Czuprynski CJ.** Cytokine regulation of the intracellular growth of *Mycobacterium paratuberculosis* in bovine monocytes. Infect Immun 1988, **56**, 1692-1697.