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David G. Russell, Sheila Sturgill Koszycki, Tambryn Vanheyningen, Helen Collins and Ulrich E. Schaible

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Why intracellular parasitism need not be a degrading experience for *Mycobacterium*

DAVID G. RUSSELL*, SHEILA STURGILL-KOSZYCKI, TAMBRYN VANHEYNINGEN, HELEN COLLINS† AND ULRICH E. SCHAIBLE†

Department of Molecular Microbiology, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110, USA (russell@borcim.wustl.edu)

SUMMARY

The success of mycobacteria as pathogens hinges on their ability to infect and persist within the macrophages of their host. However, activation of host macrophages by cytokines from a productive cellular immune response can stimulate the cells to kill their resident pathogens. This suggests that the interaction between host cell and microbe is in delicate balance, which can be tipped in favour of either organism. Biochemical analysis of mycobacterial vacuoles has shown them to be integral to the host cell's recycling endosomal system. As such they show limited acidification and hydrolytic activity despite possession of known lysosomal constituents such as cathepsins D, B and L, and LAMP 1. Even in established infections, they remain dynamic compartments accessible to several plasmalemma-derived constituents. Once the macrophage has been activated by IFN-γ and TNF-α the vacuoles coalesce and acidify. This marks a distinct alteration in vacuole physiology and leads to stasis and death of the mycobacteria. Mycobacteria have developed several strategies to avoid this outcome. Most notably, live bacilli induce sustained release of IL-6 from infected macrophages. IL-6 blocks the ability of both polyclonal primary T cells and T-cell hybridomas to respond to appropriate stimuli. Such an activity could render the centres of infection foci, such as granulomas, anergic and thus avoid release of macrophage-activating cytokines. This paper discusses both the mechanisms by which mycobacteria try to ensure their success as intracellular pathogens and the relevance of these strategies to the overall understanding of mycobacterial diseases.

1. INTRODUCTION

Pathogenic mycobacteria reside primarily within the macrophages of their host. Macrophages have an impressive battery of antimicrobial responses ranging from acidic lysosomes and their hydrolases, bactericidal peptides, the ability to generate reactive oxygen and nitrogen intermediates, to their role in antigen presentation and the induction of cellular immune responses (Britton 1993; Kaufmann 1995; Wallis & Ellner 1994). However, the frequency of interaction between phagocyte and pathogen will have placed strong evolutionary pressure on the pathogen to develop intramacrophage survival mechanisms. It is therefore not surprising that the shifting balance of this interplay yields an array of disparate results. Mycobacterial infections, most notably with *M. tuberculosis* and *M. leprae*, induce complex spectra of disease (Britton 1993; Hastings *et al.* 1988; Kaplan 1993). The immune responses to these pathogens are strongly influenced by the genetics of the host and can lead to presentations ranging from the more usual dormant infections and controlled, localized granulomas to diffuse, systemic infections. This heterogeneity is regulated primarily at the interface between the infected macrophage and the cellular immune system, suggesting that the major decision points that determine the course of infection hinge on the nature of the interplay between mycobacteria and their host cells.

The ability of certain mycobacterial species to enter and survive within macrophages provided one of the first intracellular infections studied through the application of cell biological approaches. The seminal work of D'Arcy Hart and his colleagues in the late 1960s and early 1970s, in which they demonstrated that vacuoles inhabited by viable *Mycobacterium tuberculosis* did not fuse with lysosomes (Armstrong & Hart 1971, 1975), whereas those containing dead bacilli, or nonpathogenic mycobacteria did, marked a new era in the field of microbial pathogenesis. It is only within the past few years that biochemical and immunocytological techniques have expanded our appreciation of this vacuole and, in consequence, the strategies behind the survival of pathogenic mycobacteria within their host phagocytes. This paper attempts to describe recent work on the compartments in which mycobacteria reside and

*Author for correspondence.
†Present address: MPI für Infektionsbiologie, Monbijoustr. 2, D-10117 Berlin, Germany.

how the bacilli modulate macrophage behaviour to maximize their chance of maintaining a productive infection.

2. TO FUSE OR NOT TO FUSE?

The original observation by D'Arcy Hart and colleagues regarding the restricted fusion capacity of mycobacterial vacuoles has been reproduced in numerous laboratories (Clemens & Horwitz 1995; Frehel *et al*. 1986; Sibley *et al*. 1987; Xu *et al*. 1994). However, a few reports suggested that these vacuoles might not be isolated or sequestered totally outwith the endosomal–lysosomal continuum of the host cell. Although vacuoles containing virulent *M. avium* showed limited fusion with preloaded lysosomes and did not accumulate fluid-phase markers delivered to infected cells, de Chastellier and colleagues reported that horseradish peroxidase, which is internalized via both the fluid phase and the mannose receptor, could be detected within mycobacterial vacuoles (de Chastellier *et al*. 1995). The authors suggested that although these vacuoles did not fuse with lysosomes they may be accessible to earlier vesicles of the endosomal network.

3. LIMITED ACIDIFICATION AND THE ANOMALOUS DISTRIBUTION OF LYSOSOMAL MARKERS

One of the most obvious characteristics of the endosomal–lysosomal network is the decreasing pH encountered by material being transferred down the system to the lysosome. The early studies by D'Arcy Hart had suggested that mycobacterial vacuoles showed limited acidification (Armstrong & Hart 1975; Hart & Young 1991); however, it was not until Crowle employed a dinitrophenol (DNP) derivatized weak base to label the vacuoles in established *M. avium* infections that the relatively high pH of these compartments was demonstrated formally (Crowle *et al*. 1991). In 1994, these observations were extended by calibrating the pH of phagosomes containing *M. avium*, *Leishmania mexicana*, IgG-beads and zymosan after internalization by bone-marrow-derived murine macrophages. Whereas the vacuoles containing the last three particles acidified rapidly below pH 5.5, phagosomes containing live, virulent *M. avium* equilibrated to a pH between 6.3 and 6.5 (figure 1) (Sturgill-Koszycki *et al*. 1994). Biochemical analysis of the different phagosomes following isolation revealed the presence of proton-ATPase subunits in the IgG-bead and *Leishmania* phagosomes, whereas those containing *M. avium* did not contain detectable concentrations of the vacuole-acidifying complex (Sturgill-Koszycki *et al*. 1994). One of the antibodies used recognized the 110 kDa accessory protein, which is a transmembrane subunit and indicated that the complete pump complex was absent from the vacuole. These data appeared to reinforce the concept that mycobacterial vacuoles were non-interactive with endosomes; however, characterization of the vacuoles by immunoelectron microscopy and immunoblot revealed the presence of lysosome-associated membrane protein 1 (LAMP 1) (Sturgill-Koszycki *et al*. 1994; Xu *et al*. 1994), a protein usually associated with late endosomal–lysosomal compartments. The presence of LAMP proteins in mycobacterial vacuoles was also noted by Clemens & Horwitz (1995), although these authors indicated that these proteins were less concentrated in mycobacterial vacuoles than in dense lysosomes.

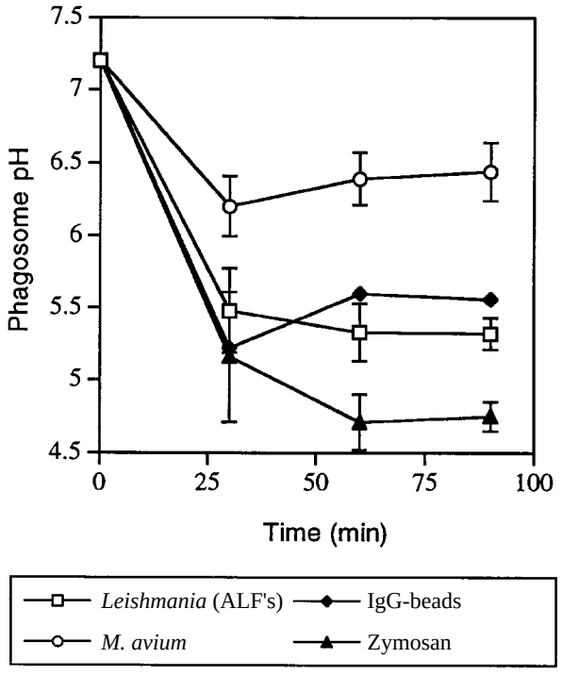

Figure 1. Measurement of phagosomal pH. This graph illustrates the pH of phagosomes formed around human-IgG-coated latex beads, *Leishmania mexicana* (MYNC/BZ/62/M379) amastigote-like forms (ALFs), zymosan, and *M. avium* after internalization by murine macrophages. The pH of IgG-bead, zymosan, and *Leishmania* phagosomes rapidly dropped below 5.5 within the first 30 min after uptake. In contrast, the *M. avium* phagosome fell to around pH 6.1, before equilibrating to pH 6.5. The pH of the phagosomes was measured by spectrofluorimetry following *n*-hydroxysuccinimide carboxyfluorescein labelling of the particles (Sturgill-Koszycki *et al*. 1994).

This distribution, the presence of LAMP 1 and absence of proton-ATPase, is difficult to rationalize within existing models for phagosome biogenesis based on fusion of phagosomes with 'preformed' endosomal compartments, all of which are postulated to possess proton-ATPases, discussed by Desjardins *et al*. (1994) and Mellman (1992).

4. MYCOBACTERIAL VACUOLES ARE DYNAMIC COMPARTMENTS THAT EXCHANGE WITH THE PLASMALEMMA

Previous studies revealing the limited communication of endosomes with mycobacterial vacuoles had employed ligands and markers that deliver preferentially to lysosomes (Clemens & Horwitz 1995; de Chastellier *et al*. 1995; Frehel *et al*. 1986; Xu *et al*. 1994). However, Russell *et al*. (1996) recently examined the partitioning of plasmalemma-derived gangliosides in

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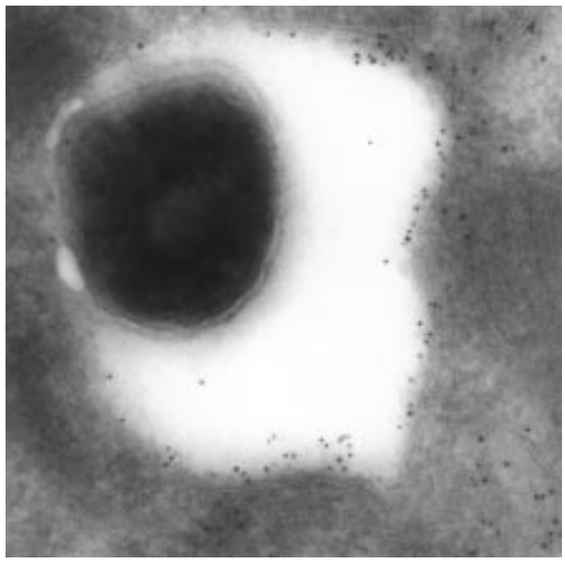

Figure 2. Transmission electron micrograph showing that biotinylated cholera toxin B-subunit (Ctx-B) enters vacuoles containing *M. avium*. Bone-marrow-derived macrophages, infected 72 h previously with *M. avium*, were incubated in 10 µg ml^{-1} biotinylated Ctx-B for 15 min, washed, and placed in prewarmed medium for a 45 min chase period. The section was probed with streptavidin–anti-streptavidin (anti-rabbit IgG–5 nm gold). The lumenal face of the *M. avium*-containing vacuole is decorated with 5 nm gold particles corresponding to the presence of Ctx-B, indicating that the GM1-binding subunit of the toxin has gained ready access to the mycobacterial vacuole (Russell *et al*. 1996).

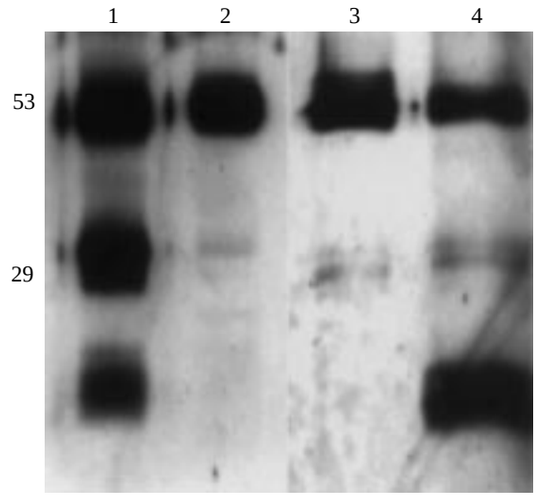

Figure 3. Immunoblot of *M. avium* and IgG-bead-containing phagosomes with anti-cathepsin D antibody, demonstrating the restricted processing of cathepsin D in the *M. avium*-containing vacuoles and the subsequent proteolysis of cathepsin D on acidification of isolated vacuoles. Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS–PAGE) (12%) gels were run with 60 min IgG-bead-containing phagosomes (lane 1), and *M. avium*-containing phagosomes isolated 9 d (lane 2) and 60 min post-infection (lanes 3 & 4). Lane 4 contains 60 min *M. avium*-containing phagosomes following incubation in 50 mM acetate buffer pH 4.5, with 0.05% Nonidet P40 at 37 °C for 10 min. Vacuole preparations shown in lanes 3 and 4 were isolated in the absence of proteinase inhibitors. The samples were normalized for protein content before electrophoresis. Source: Sturgill-Koszycki *et al*. 1996.

infected macrophages. Biotinylated cholera toxin B subunit was complexed with GM1 ganglioside on the surface of macrophages infected with *M. tuberculosis* and *M. avium*. The toxin–glycosphingolipid complex delivered to vacuoles containing both bacteria with remarkable speed and efficiency (figure 2). Kinetic analysis of the rate of delivery to mycobacterial vacuoles demonstrated that it reached steady state within 5–10 min of entry. The rate of delivery was much slower to the acidified IgG-bead phagolysosomes.

These data indicate that mycobacterial vacuoles are highly dynamic compartments that interact readily with certain plasmalemma-derived components. In addition, the kinetics of delivery and rapid attainment of steady state suggest that the vacuole presents some of the characteristics of a peripheral endosomal compartment. This was one of the possible explanations suggested by Clemens & Horwitz (1995) for their observation that *M. tuberculosis* vacuoles in human peripheral blood-derived monocytes remained positive for transferrin receptor even in established infections. Transferrin receptors are stable constituents of the early and recycling endosomal apparatus.

5. MYCOBACTERIAL VACUOLES ARE ENDOSOMAL COMPARTMENTS OF LIMITED HYDROLYTIC CAPACITY

Despite the data regarding delivery of plasmalemma constituents to mycobacterial vacuoles, it was still unclear whence the intracellular vacuolar components originated. The elucidation of this exploited the fact that certain lysosomal hydrolases, most notably the cathepsins, are synthesized as proenzymes that are processed to their active and mature forms by other hydrolases in acidic environments (Rijnboutt *et al*. 1992; van Weert *et al*. 1995). Cathepsin D is synthesized as a 51–55 kDa proenzyme that is cleaved to yield a 49 kDa immature form in early endosomes and is subsequently processed to a two-chain 31/17 kDa enzyme in dense lysosomes. Therefore by immunoblotting isolated intracellular compartments and analysing the relative amounts of the different forms one can glean information regarding both the hydrolytic competence of the vacuole and the intracellular origin of cathepsin D. Immunoblotting of IgG-bead phagolysosomes demonstrate the 49 kDa and 31/17 kDa forms of the hydrolase; however, *M. avium*-containing vacuoles isolated at either 120 min or 9 d post-infection possess only the 55 kDa proenzyme and the 49 kDa form of cathepsin D (figure 3) (Sturgill-Koszycki *et al*. 1996). Acidification of isolated *M. avium* phagosomes by incubation in acetate buffer (pH 4.5) facilitated processing of the enzyme to forms with a lower molecular mass, indicating that pH alone was limiting proteolysis.

These data indicate two important points concerning mycobacterial vacuoles. First, the limited processing

provides graphic illustration of the restricted hydrolytic capacity of the vacuole. Second, and more importantly, it demonstrates that the cathepsin D delivered to the vacuole has emerged recently from the cell's synthetic pathway, and is not derived from fusion with preformed acidic compartments. This would suggest that the mycobacterial vacuole, which behaves like an early endosomal compartment, is sustained with material derived from the host cell's synthetic pathway. This interpretation presents a significant departure from current models of phagosome formation and it is unclear whether these proteins are routed directly from the transGolgi network or through a novel 'pre-endosome'. The central question arising from these results is whether or not this cellular compartment is peculiar to a mycobacterial infection or merely represents the stabilization of a compartment that is transitory in normal phagosome biogenesis. To test this, IgG beads were bound to macrophages on ice; the cells were then warmed up and the phagosomes were isolated at various time points after internalization. At early time points (3 min) the vacuoles had acquired LAMP 1 and cathepsin D in its pro- and 49 kDa forms before their accumulation of detectable concentrations of proton-ATPase (Sturgill-Koszycki *et al*. 1996). This profile was comparable to that of mycobacterial vacuoles and suggested that, as LAMP 1 and cathepsin D precede proton-ATPase, delivery of these components must be via an novel family of vesicles that are heterogeneous with respect to these three proteins. The data suggest that early remodelling of phagosomes is with components derived recently from the transGolgi network, either directly or via a 'pre-endosome', and not from fusion with preformed, acidic endosomes, which would contain both proton-ATPase and processed cathepsin D.

6. INTERSECTION OF MYCOBACTERIAL VACUOLES WITH THE RECYCLING ENDOSOMAL NETWORK

The ready accessibility of mycobacterial vacuoles to gangliosides from the cell's surface, and the reported presence of transferrin receptor (Clemens & Horwitz 1995) beg the question of whether or not mycobacterial vacuoles lie within the cell's recycling endosomal pathway. Higher eukaryote cells internalize iron-loaded transferrin via transferrin receptors, which traffic to the sorting endosome. Here, at pH 6.3, the iron comes off the transferrin, which is sorted into the recycling endosome and delivered to the cell surface, where it is released (Dautry-Varsat *et al*. 1983).

Clemens & Horwitz (1996) employed immuno-electron microscopy on *M. tuberculosis*-infected human monocyte-derived macrophages to detect transferrin within these vacuoles, indicating that they lay within the recycling pathway. In the studies on *M. avium*-infected murine macrophages, digoxigenin-labelled transferrin was used to increase sensitivity; in addition to the demonstration of transferrin in the vacuoles, it was shown that it could be outcompeted with unlabelled transferrin. This result indicated that uptake was receptor-specific (Sturgill-Koszycki *et al*. 1996). Moreover, the kinetics of appearance and disappearance of transferrin through the vacuole proved that mycobacterial vacuoles behave like integral components of a functional recycling pathway. The overall properties of mycobacterial vacuoles are illustrated and described in figure 4.

7. IMMUNE REGULATION AND THE DISPLACEMENT OF THE MYCOBACTERIAL VACUOLE

In infections *in vitro* with either *M. tuberculosis* or *M. avium* the bacilli grow unchecked in their host macrophages. It is obvious that this cannot be the case in infections *in vivo*. Much of the body of literature dealing with immune regulation of mycobacterial infections describes expansion of CD4+ T cells of the TH1 phenotype capable of releasing such macrophage activating cytokines as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Activation of infected and bystander macrophages with these cytokines leads to stasis and death of infecting bacilli, although the route to this mycobactericidal response is a matter for debate. In murine macrophages most studies implicate the activity of the inducible nitric oxide synthase (Bermudez 1993; Chan *et al*. 1995; Hanano & Kaufmann 1995); however, because many microbicidal mechanisms function in concert, it is not easy to separate the effects of individual mycobactericidal responses (O'Brien *et al*. 1996).

To document the mechanisms behind the mycobactericidal responses of activated macrophages, the physiological alterations in mycobacterial vacuoles were examined in activated macrophages. When macrophages were activated with IFN-γ and lipopolysaccharide before uptake of *M. avium*, the macrophages were able to acidify the mycobacterial phagosomes to below pH 5.0. Two hours after internalization there was a rebound towards neutral pH with marked population spread suggestive of a 'struggle' between the bacilli and the macrophage (Schaible *et al*. 1997). When these vacuoles were isolated and characterized biochemically they revealed an accumulation of proton-ATPase and were no longer accessible to transferrin delivered from outside the host cell. This physiological alteration indicated a translocation of the vacuole from the recycling pathway deeper down the endosomal pathway to an acidic, hydrolytic compartment. Concomitant analysis of the viability and survival of *M. avium* in resting macrophages indicated that the bacilli underwent an initial drop in viability before entry into exponential growth phase. In activated macrophages the infecting bacteria never establish exponential growth and are slowly killed by their host cells. Maintenance of mycobacterial vacuoles within the early endosomal machinery appears to require metabolic activity because dead bacilli are internalized into vacuoles that acidify and fuse with lysosomes (S. Sturgill-Koszycki *et al*., unpublished data).

Electron microscopy indicates that one of the first phenotypic alterations in activated macrophages is the coalescence of individual *M. avium*-containing vacuoles into communal vacuoles with many bacilli (figure 5). At

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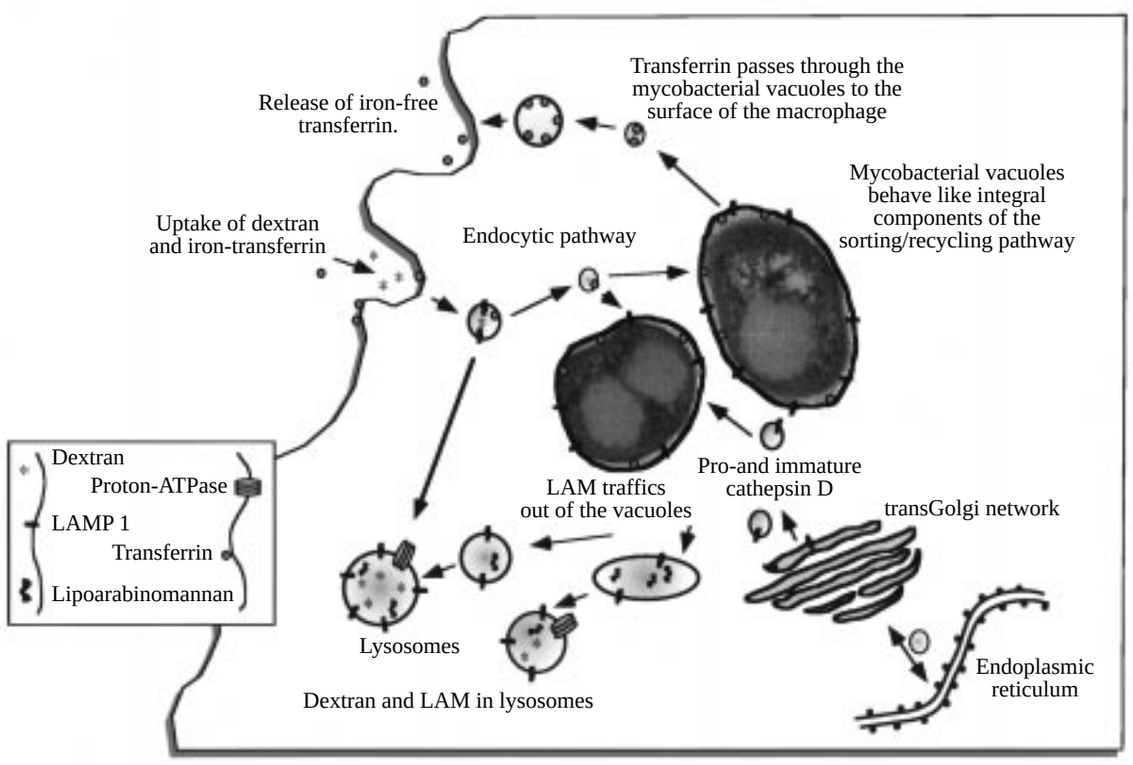

Figure 4. A summary diagram illustrating the properties of the mycobacterial vacuole. The vacuoles have a relatively high pH, possess few proton-ATPase molecules, and show a marked reluctance to fuse with lysosomes. Furthermore, the lysosomal hydrolases, such as cathepsin D, are not fully processed; this result demonstrates the limited hydrolytic capacity of the vacuoles. The occurrence of cathepsin D in its immature form(s) indicates that it has been derived recently from the cell's synthetic machinery and not through fusion with preformed acidic endosomes or lysosomes. This hypothetical pathway from the transGolgi network either direct to mycobacterial vacuoles, or via 'pre-endosomal' compartments, could explain the segregation of the proton-ATPase from endosomal constituents with which it usually colocalizes.

The relatively high pH, and the ready access of Ctx-B–GM1 (ganglioside M1) complexes and digoxigenin–transferrin, all support the contention that these vacuoles are stabilized within the sorting and recycling endosomal network of their host cells. Retention of these bacilli within this region of the endocytic continuum would undoubtedly prevent their exposure to the full hydrolytic capacity of their host cell while providing access to a constant supply of nutrients.

the time of fusion the majority of these bacilli show few signs of damage or degradation and there is little change in bacterial viability measured by colony-forming units (Schaible *et al*. 1997). These observations indicate that the functional transition of mycobacterial vacuoles to acidic endosomes precedes the drop in microbial viability. Furthermore, it suggests that the acidic lysosomal environment would then augment or potentiate the microbicidal mechanisms of the host macrophage.

Loss of mycobacterial viability appears to be a gradual process subsquent to the functional translocation of mycobacterial vacuoles to acidic compartments. The transition will transfer mycobacteria from a relatively non-hostile environment to one accessible to low pH, reducing conditions, acid hydrolases, toxic peptides and the potentiated effects of O and NO radicals at low pH (O'Brien *et al*. 1996). Gauging the relative contributions of these various microbicidal mechanisms awaits a fuller understanding of the physiology of macrophage activation and its effects on the segregation of components in the endosomal–lysosomal continuum.

8. AVOIDING THE INDUCTION OF A PROTECTIVE IMMUNE RESPONSE

Considering that the outcome of a productive immune response is death of the infecting bacilli, there is significant pressure on mycobacteria to develop strategies that avoid the induction or consequences of a cellular immune response that leads to release of macrophage-activating cytokines. This pressure is common to all intracellular parasites; there are many reports that imbue microbial pathogens with the ability to subvert or divert protective immune responses. The literature discussing mycobacterial infections is particularly rich with reports of non-responsiveness and the suppression of cellular immunity. Nash & Douglass (1980) noted that, despite their heavy bacterial load, up to 25% of patients with pulmonary tuberculosis have negative skin tests to purified protein derivatives of mycobacteria. Other researchers have reported a lack of delayed type hypersensitivity response and decreased proliferation of lymphocytes *in vitro* in mice infected with *Mycobacterium bovis* BCG. This effect was

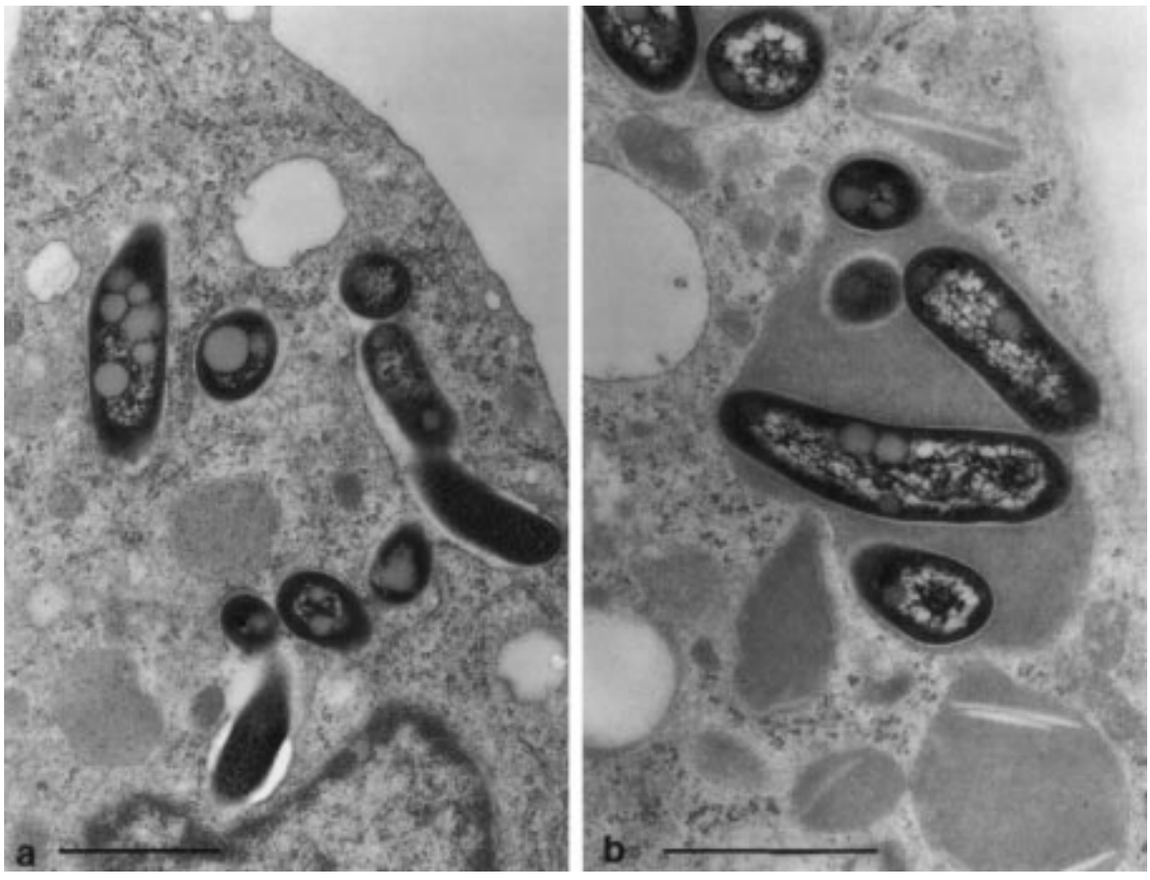

Figure 5. Electron micrographs of murine bone-marrow-derived macrophages (BMMO) infected with *M. avium*, revealing alterations in vacuole morphology after activation of macrophages with IFN-γ and LPS. (*a*) Resting BMMO 5 d after infection. The bacilli tend to be sequestered in individual vacuoles, which show little evidence of lysosomal fusion. (*b*) BMMO infected 5 d previously with *M. avium* and activated 1 d before examination. Bacteria are observed more frequently in communal vacuoles, which contain dense, lysosomal matrix. BMMO were activated with IFN-γ (400 U ml^{-1}) for 16 h and LPS (1 μg ml^{-1}) for 2 h. Scale bars: 1 μm.

mediated by macrophage-derived soluble factors, which could inhibit DNA synthesis and interleukin-2 (IL-2) production by T cells (Colizzi *et al*. 1984). More recently, Pancholi *et al*. (1993) demonstrated that human monocytes chronically infected with BCG were defective in their ability to present mycobacterial antigens, but were able to present antigens added exogenously. Despite these obvious compromises of immune responsiveness the mechanism(s) behind these modulations have yet to be elucidated.

To distinguish between passive avoidance and active suppression, the ability of BCG-infected bone-marrow-derived macrophages to process and present antigens to T cells *in vitro* was investigated (VanHeyningen *et al*. 1997). Although artificial, this model system permitted the manipulation of the bacterial viability, antigen source and physiological status of the antigen-presenting cells. The resulting data showed that infected macrophages were suppressed in their ability to stimulate a T-cell response. This suppressive effect could be transferred with conditioned medium to uninfected macrophages (figure 6). It had already been reported that mycobacteria-infected macrophages release copious amounts of interleukin-6 (IL-6); VanHeyningen *et al*. (1997) found that the inhibitory effect of conditioned medium could be completely ablated by immunodepletion of IL-6. Moreover, recombinant IL-6 had a comparable inhibitory effect on antigen-dependent responses of both primary T cells and T-cell hybridomas (VanHeyningen *et al*. 1997).

The primary function of IL-6 is the induction of terminal differentiation of activated B cells to immunoglobulin-producing plasma cells (Tosato *et al*. 1988). The cytokine also regulates acute-phase protein synthesis by hepatocytes, is necessary for the growth and function of T cells, and synergizes with IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in recruitment and differentiation of bone-marrow-derived cells (Hirano 1992). The classical cytokine cascade by which TNF-α upregulates IL-1, which in turn upregulates IL-6, does not appear to be causing the induction of IL-6 in mycobacterium-infected macrophages: Van Heyningen *et al*. (1997) found that neutralizing antibodies against IL-1α, IL-1β, TNF-α, or all three in combination had no effect on IL-6 expression. We also noted that mycobacteria had to be metabolically active to induce IL-6 production; this observation suggests that the bacillus must synthesize,

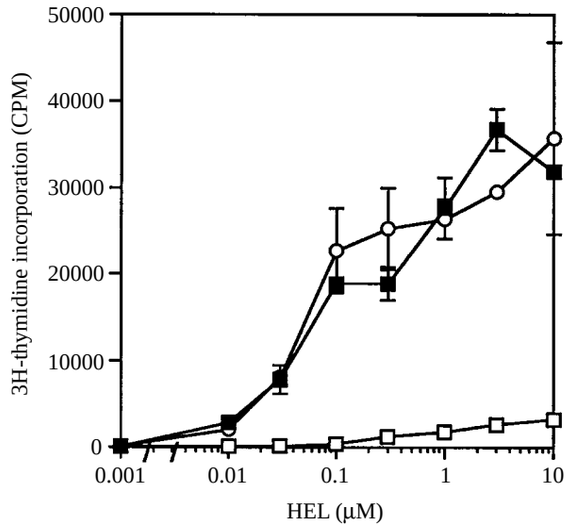

Figure 6. Inhibition of T-cell stimulation is mediated by a soluble factor produced by BCG-infected macrophages. Uninfected bone-marrow-derived macrophages (5×10^4 per well) were incubated with hen egg-white lysosome and 3A9 T-cell hybridomas (10^5 per well) in either control medium (filled squares), medium derived from infected macrophages (open squares), or a 1/8 dilution of supernatant from BCG grown *in vitro* (open circles). One day later, supernatants were collected and assayed for IL-2, which was shown to be depressed markedly in the hybridomas incubated in the presence of medium from infected macrophages. Results are expressed as mean ± SD of [^{3}H]thymidine incorporation of triplicate. Source: Van Heyningen *et al*. (1997).

or process, an effector molecule for the induction of IL-6. The difference in immune responsiveness to metabolically active and killed mycobacteria may also explain the apparent anomaly between the suppressive effects seen in productively infected individuals and the stimulatory effect of killed mycobacteria in adjuvants.

There are several reports detailing induction of IL-6 in infections *in vivo*; notably, Champsi *et al*. (1995) compared the concentrations of IL-6 induced after infection of mice with *M. avium* over a period of five weeks and found that IL-6 was produced by cultured spleen cells two weeks post infection and that its concentrations were maintained throughout the five-week infection period. Recently, the concentrations of IL-6 in bronchoalveolar lavage from patients with active pulmonary tuberculosis were also found to be elevated (Law *et al*. 1996). These data mesh well with the continuous concentrations of IL-6 observed by the present authors after infection of macrophages *in vitro*. An appreciation of how such an anomalous mechanism of T-cell suppression might function *in vivo* requires one to remember that many mycobacterial infections persist as macrophage granulomas. Infected macrophages are at the centre of these lesions and could generate an IL-6 gradient that would result in an anergic centre to the granuloma without compromising the cellular responsiveness of the periphery. Analysis of the kinetics of IL-6 accumulation and inhibition indicates that there is a sharp drop off in inhibition with falling IL-6 concentration. In support of this proposal is a recent report that IL-6 plays a role in the formation of type 2 giant cells from alveolar macrophages (Lemaire *et al*. 1996); giant cells are frequently observed in the centre of mycobacterial granulomas. Finally, although these data suggest one mechanism through which mycobacteria may induce a localized suppression of the immune response, they do not exclude the existence of other suppressive or avoidance strategies.

9. CONCLUDING REMARKS

The pathobiology of mycobacterium infections exhibits many different tiers of host–pathogen interplay and has evolved into an extremely stable interaction that, more frequently than not, results in cryptic infections with minimal damage to the host. This paper describes the interactions between bacteria and host cell, and between infected host cells and the immune system. It is a reflection of the interests that have occupied this laboratory for the past five years and is not intended to be encyclopaedic in coverage.

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