Now they eat them, now they don't: phagocytes and *Borrelia burgdorferi* in Lyme disease

Ruth R. Montgomery

The course of natural infection

Lyme disease, the most common vector-borne disease in North America, is caused by infection with the tick-transmitted spirochaete *Borrelia burgdorferi* (*Bb*) and is characterized initially by the skin lesion, erythema migrans. Subsequent disease reflects the *in vivo* migration of the spirochaete in its host and includes arthritis, neurological symptoms and carditis. Even though most patients are readily cured by antibiotic therapy, rare patients have persistent or recurring illness.

Skin is the initial site of entry of spirochaetes into the host, and in established disease, the skin can provide a haven to *Bb* for extended periods without eliciting an immune response. *Bb* can be recultured from non-lesional human and murine skin and, although dormant in the original host, are still infectious and pathogenic when transferred to a naïve animal. In both human and murine Lyme borreliosis, intact spirochaetes persist in infected tissues and are not completely eliminated by phagocytic innate immune cells.

A vaccine for Lyme disease was developed in mice, targeting the prominently *in vitro*-expressed outer-surface protein A (OspA). The vaccine was effective by blocking transmission from the tick vector to the host and the protective immunity waned rapidly. Notably, OspA is down-regulated in the vertebrate host. Patients required three vaccinations and annual boosters; despite several years of successful use, it was withdrawn from the market by the manufacturer.

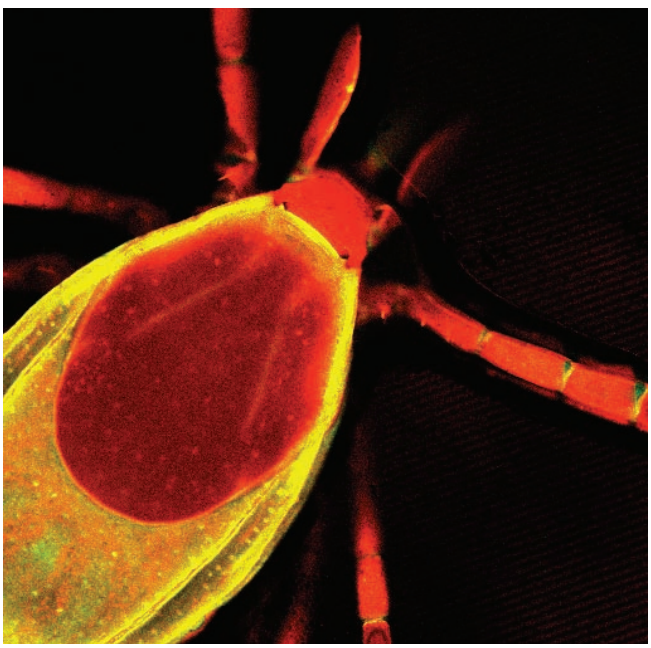
Innate immune cells and spirochaetes *in vitro*

Mature macrophages *in vitro* ingest and kill spirochaetes avidly and in large numbers. Spirochaetes attach at their ends, independently of the Fc receptor, and are delivered to lysosomes for degradation with a $T_{1/2}$ of about 20 minutes. Nearly all of the ingested spirochaetes are killed, but occasional cell-associated spirochaetes persist. Spirochaete stimulation of macrophages initiates a cascade of pro-inflammatory signals leading to the production of potent inflammatory mediators, including reactive oxygen and nitrogen intermediates, arachidonic acid metabolites, proteases, and in addition, cytokines and chemokines that elicit adaptive immune responses. *Bb* lipoproteins are highly inflammatory and incite this response via pattern recognition receptors CD14 and Toll-like receptors (TLR) 1 and 2; the latter are from a family of highly conserved transmembrane receptors that have an essential role in the innate immune defence against pathogens. Macrophages are the most efficient cellular defence against spirochaetes during the initial innate immune response; monocytes are less effective.

Importance of antibody for clearance by polymorphonuclear leukocytes (PMN)

PMN *in vitro* bind *Bb* by conventional phagocytosis as well as via tube and coiling phagocytosis. Antibody is critical for the efficient clearance of *Bb* by PMN: while unopsonized spirochaetes are poorly eliminated, PMN clear opsonized spirochaetes rapidly. There is no intracellular co-localization of PMN granule products

Lyme Disease is an important spirochaete infection which is transmitted by ticks. Ruth Montgomery describes the complex, and still mysterious, interaction between the host's phagocytes and these bacteria.



TOP LEFT:
Ixodes dammini tick. Ticks were microinjected in the anal aperture with fluorescent dyes to label surface structures and midgut cells. The ticks were imaged live 24 h after injection by laser scanning confocal fluorescent microscopy.
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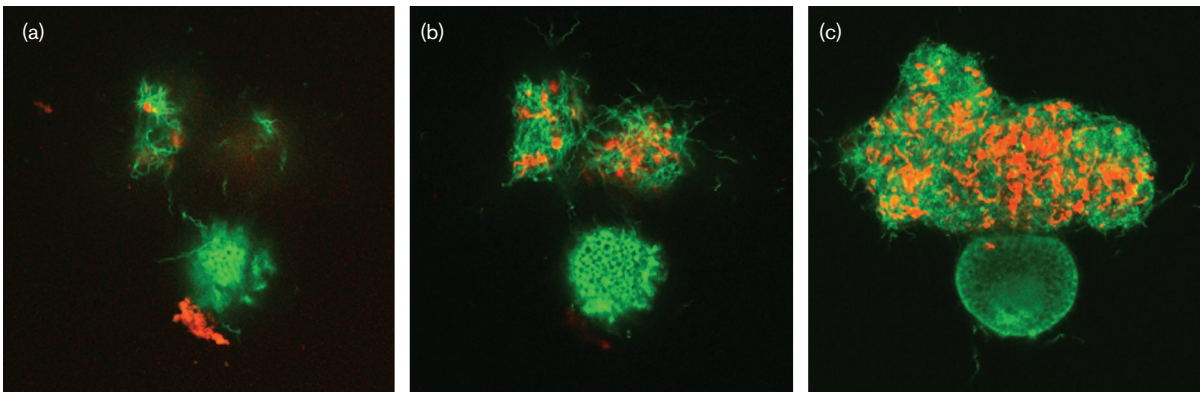

LOWER LEFT:
Killing of spirochaetes by macrophages. Human macrophages were incubated with unopsonized *Bb*. After 60 min incubation at 37 °C, samples were stained with live/dead dye, red indicating killed organisms. Note the extensive killing by macrophages evident at the top surface of the cells (a), the middle level (b) and adherent to the coverslip (c).
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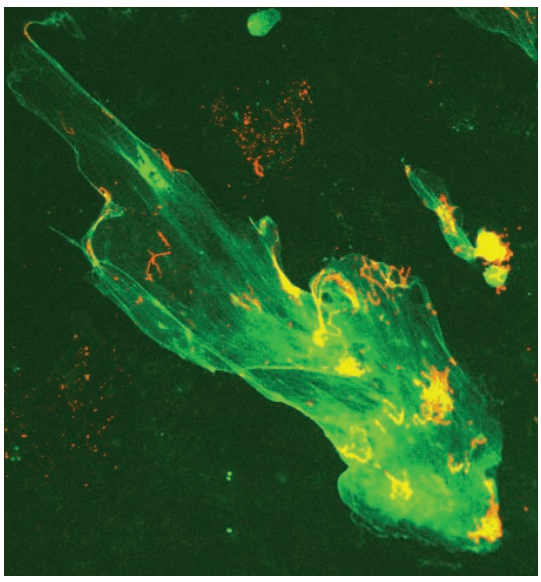
with spirochaetes; significant killing of spirochaetes by PMN occurs extracellularly. PMN have abundant mechanisms for killing spirochaetes and eliminate *Bb* using both oxidative and non-oxidative killing mechanisms. PMN lysates kill *Bb* as effectively as do intact PMN given opsonized spirochaetes, highlighting the importance of granule proteins. In addition, the abundant cytosolic protein calprotectin is also bacteristatic, probably through chelation of Zn^{2+}, an essential cation for *Bb*. In contrast to the granule components, which may require the environment of the phagolysosome, calprotectin can function as an extracellular anti-microbial factor. Indeed, elevated levels of calprotectin (also known as S100, MRP 8/14) are noted in arthritic joints.

Arthropod saliva modulates infection

In natural infection, spirochaetes are delivered via an Ixodid tick vector in the presence of saliva, which forms a plug at the inoculation site. The local concentrations of saliva in the skin may be quite high. The saliva of hematophagous arthropods is a pharmacological arsenal to aid in blood feeding by the vector (such vectors have been called 'invertebrate syringes'). Saliva contains potent activities that both reduce clotting and increase vasodilation, as well as potent anti-inflammatory activities that inhibit cellular immune responses, including antigen presentation and IFNγ-stimulated H_2O_2 release by human macrophages. The efficacy of the arthropod strategy can be seen as sandfly saliva allows a smaller inoculum of infecting *Leishmania* organisms to establish infection, encompassing a larger lesion in the mouse. Similarly, in early Lyme disease, there is a boost to infection via tick delivery (over syringe inoculation), altering the initial antibody response, presumably due to the presence of saliva.



BELOW:
PMN myeloperoxidase does not co-localize with spirochaetes. Fresh human PMN incubated with opsonized *Bb* were fixed after 60 minutes and double-labelled with antibodies specific for myeloperoxidase (green) and spirochaetes (red). Images obtained using confocal microscopy demonstrate the spread of granule components over an area far exceeding that of two neighbouring intact cells (top and right), consistent with the extracellular killing of spirochaetes.
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Tick saliva alters phagocyte function

PMN are the first cells of the innate immune system to arrive at the site of spirochaete deposition in the skin. The saliva of *Ixodes* ticks is known to inhibit in some way phagocytosis, granule release and superoxide production by PMN, as well as aggregation of platelets *in vitro*. *Ixodes* tick saliva also inhibits T cell proliferation and reduces the production of cytokines and nitric oxide and the killing of spirochaetes by macrophages. Inhibitory bioactivities of *Ixodes* tick saliva include an anti-coagulant, prostaglandin E2, kininase, an antioxidant and an anticomplement peptide. These salivary proteins are now being thoroughly characterized by several groups for potential therapeutic advantage.

The conundrum of persistent infection

The ease of elimination *in vitro* belies the persistence of spirochaetes in the infected host. We have at present no explanation for the persistence of spirochaetes in the host in prolonged disease and the apparent immune invisibility of *Bb* in tissue. Macrophages *in vivo* in infected animals display no global inhibition of function; rather they are appropriately activated *in situ* and remain in a resting state in unaffected tissues. In Lyme carditis, they produce increased levels of mRNA for pro-inflammatory cytokines, reflecting appropriate macrophage activation and display no evidence of pressure toward immune down-regulation.

Tick saliva contributes to the failure of phagocytes to clear organisms from the skin in initial infection. Saliva's inhibition of phagocyte function gives the infecting spirochaetes an initial advantage in evading the sentry phagocytes.

Persistence may depend on the spirochaetes themselves: there is evidence that spirochaetes down-regulate their antigenic surface proteins or coat them with host proteins. Freeze fracture electron microscopy of *Bb* reveals fewer surface-expressed proteins than on other spirochaetes, and an mRNA array analysis of *Bb* lipoprotein genes during the course of murine infection shows a reduction from 116 genes expressed to fewer than 40 genes remaining at 30 days of infection, after induction of adaptive immunity. The paradoxical persistence of spirochaetes in the host, despite appropriate activation (or non-activation) of phagocytes, remains mysterious.

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Further reading

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