Intracellular Survival of Microorganisms and Human Disease

E. N. Wardle


The biochemistry and immunology of phagocytosis and intracellular killing is reviewed. Illustrative details are given of the ways by which common microbial pathogens enter and survive within macrophages and other cells. Attention is drawn to the role of interferon-gamma as a macrophage-activating factor and to the role of other cytokines in subsequent intracellular killing. Without physician intervention, such killing is often suppressed or thwarted by the invading organism.

Ingestion of Microorganisms: Phagocytosis

The phagocytic cells which ingest and kill microorganisms comprise the 'microphages' better known as the polymorphonuclear leukocytes (PMNs) and the 'macrophages' that arise from monocytes. Commonly invading microorganisms have lectin-like means of attachment to host cells. Thus many bacteria have fimbriae, pili or molecular size 'adhesins' that attach non-specifically via glycoprotein interactions to the glycoalyx of the cells. For example, the outer membrane protein of *Chlamydia trachomatis* has the properties of an adhesin, and *Heliotoboctor pyloridis* sticks to the epithelial cells of the antrum of the stomach because there is adhesion to certain surface glycolipids. Also *Haemophilus influenzae* adheres to and enters epithelial cells and so it can persist in the nasopharynx.

Opsonins and Integrins

Phagocytosis by PMNs and macrophages is helped by specific opsonins such as the C3b complement component, and IgM and IgG antibodies to the surface antigens of the invading microorganisms. There are also non-specific opsonins like fibrinectin and C-reactive protein (CRP). CRP opsonizes the capsules of virulent pneumococci and then such organisms are cleared rapidly from the blood by the spleen.

Furthermore, the surfaces of PMNs and macrophages are covered by 'leukocyte adhesion proteins' or 'integrins'. They are a family of glycoproteins named CR3 (Mo1), LFA-1 and gp 150/95. Engagement of C3bi on the surface of a microorganism with the CR3 receptors on phagocytosing cells will trigger intracellular release of calcium ions. Also when antibodies that are attached to a microorganism interact via their Fc heavy chains with Fc receptors on the surface of the phagocytic cell, then via the intermediary of G proteins, there will be activation of phospholipase C (PLC) at membrane level. That leads to hydrolysis of phosphatidylinositol (PIP2) on the inner aspect of the cell membrane and so there is formation of inositol trisphosphate (IP3) and di-acyl glycerol (DAG). IP3 is a calcium ionophore, and DAG activates protein kinase C which is the intracellular messenger molecule that activates many intracellular proteins and events.

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So adherence to the phagocyte triggers several important processes:

1. ‘Receptor mediated endocytosis’ which means ingestion of a bacterium into a phagocytic vacuole called a ‘phagosome’ in the cytoplasm.
2. That phagosome fuses with neighbouring lysosomes that contain digestive enzymes such as lysozyme or the cathepsins. Phagosome–lysosome fusion is calcium-mediated.
3. Activation of the ‘respiratory burst’ follows, so that there is production of active oxygen radicals for the killing of microorganisms within the vacuoles. Likewise, there is formation of nitrogen oxides or nitrothiosol from L-arginine for the purpose of killing intracellular organisms.
4. Also there is mobilization of cationic peptides “the defensins” which induce membrane permeability of bacteria so that they die.
5. There is release of ‘cytokines’ such as interleukin 1 and Tumour necrosis factor (TNF), from macrophages so that their pro-inflammatory effects aid anti-microbial responses.

Exploitation of normal endocytotic pathways for internalization is used by many invasive microorganisms, e.g. Shigella, Salmonella, Yersinia and E. coli. Often these bacteria produce enzymes (exo- and endo-glycosidases) which remove hydrophilic groups from cell surfaces and so there is then hydrophobic adhesion.

Organisms that Survive within Macrophages
The discussion will centre on those microbial agents that commonly survive within macrophages (Table 1). What particular characteristics do they have?

Means of Survival
There are some general means of survival that can be illustrated from amongst the various microorganisms that commonly establish themselves within macrophages.

1. Bacteria can adhere specifically to MHC surface molecules, to the T cell antigen receptor or to immunoglobulin receptors and so they circumvent the defence mechanisms.
2. Means of stopping acidification of the phagocytic vacuoles, and thereby avoidance of killing by acidic proteases, are used by Legionella pneumophila, Toxoplasma gondii or Nocardia and others.
3. Other organisms are able to prevent phagosome-lysosome fusion, or they can use a lysin to disrupt the walls of the phagosomes so that they

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<th>Intracellular microbial pathogens</th>
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<td>Shigella</td>
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can then grow in the cytosol. Thus Shigella, Listeria and Rickettsia are noted for their ability to produce a haemolysin,10 and Legionella and others produce their own cytotoxins. 4. Salmonella, Yersinia or Coxiella burnetti have their own means of neutralizing anti-bacterial agents like the defensins.

5. One common feat is to avoid triggering the respiratory burst. In fact it is established that the receptors for C3b and C3bi trigger the process of phagocytosis but not the release of oxygen metabolites.11 So the many organisms that enter phagocytes via the CR3 receptor will avoid killing by reactive oxygen. Leishmania promastigotes enter macrophages via the CR3 receptors, and so do Mycobacterium tuberculosis, Mycobacterium leprae, Histoplasma capsulatum, Legionella pneumophila and Toxoplasma gondii. Conversely, entry by disturbance of Fc receptors would certainly lead to killing by oxidative processes.

6. Chlamydiae have means of inhibiting the NADPH oxidase of the respiratory burst that produces the superoxide anions, from which killer hydroxyl radicals OH• arise. Also organisms like Shigella, Nocardia and Listeria produce a superoxide dismutase that removes any superoxide anions. Staphylococcus aureus and Neisseria produce catalase that will inactivate any hydrogen peroxide that is formed from the superoxide anions.

7. Some organisms like Leishmania can invade macrophages in such a way that the release of cytokines, interleukin 1 and TNF does not occur. The macrophages stay in their resting state and produce prostaglandin E2 with the result that surrounding white cells like the T lymphocytes are not activated. In fact this is why the T cells do not produce adequate interferon-gamma (IFNγ) (see below).

Gastrointestinal Pathogens

Salmonella species

The genus that has attracted particular attention because of its clinical importance, which in turn depends on its capacity for intracellular survival, is Salmonella. Salmonella species pass through the epithelial cells of the ileum into deeper tissue, survive in mononuclear phagocytes, and disseminate so producing the typical typhoid illness. The highly pathogenic species has a plasmid that confers this form of virulence. Recently there has been clarification that survival in macrophages depends on:

(i) inhibition of the respiratory burst (NADPH oxidase);
(ii) inhibition of phagosome-lysosomal packet fusion;
(iii) escape from the phagosomes into the cell cytoplasm;
(iv) resistance to the microbicidal peptides, the defensins. In fact Salmonella typhimurium carries a gene Pho-P that is responsible for resistance to the defensins.12

Obviously cell-mediated immunity is important in controlling Salmonella infections. Increased bactericidal activity of the infected macrophages can be induced by IFNγ,13 which we know is a principal 'macrophage activating factor' (MAF). Recombinant IFNγ treatment of infected macrophages is followed by phagosome-lysosome fusion and killing of the organisms by oxygen-independent means. That increased killing capacity is unaffected by the addition of superoxide dismutase or catalase, and thus is not mediated via the respiratory burst (NADPH oxidase). Rather, IFNγ induces the enzyme indoleamine 2:3 di-oxygenase in mononuclear phagocytes and the result is degradation and depletion of the amino acid tryptophan.14 In fact tryptophan depletion allows oxygen-independent killing of organisms like Salmonella, Toxoplasma gondii, Chlamydia or Leishmania donovani when they have become established in macrophages.

Shigella

Shigella enters cells by induced endocytosis and so it enters a phagocytic vacuole, but its plasmid encoded haemolysin then enables it to break free in the host cytoplasm. There it inhibits protein synthesis by the host cell. Shigella produces a superoxide dismutase and so is protected from damage by superoxide anions.

Yersinia enterocolitica

Yersinia enterocolitica produces an acute gastroenteritis or terminal ileitis with mesenteric adenitis. It adheres to microvilli on the surfaces of intestinal cells or macrophages and is taken up by receptor-mediated endocytosis. Then it multiplies in intracellular vacuoles because it has a 70 kb plasmid that determines resistance to killing by the oxidative burst.

Other Organisms Using Similar Mechanisms

Legionnaire's disease

This is an atypical pneumonia, so called because a Gram-stained smear of sputum does not show the typical organisms. Yet Legionella is present in tissue macrophages. Organisms are taken up by 'coiling phagocytosis' which means that a pseudo-pod of the phagocyte coils round the bacterium and
it is then drawn into a phagocytic vacuole. At the time there is adherence to CR3 receptors and so there is no triggering of the respiratory burst. Survival within macrophages is ensured because the organism inhibits phagosome–lysosome fusion, there is no acidification of the phagosome, and *Legionella* produces a cytotoxin. Restoration of killing capacity can be achieved by injections of IFNγ.15

**Brucella**

*Brucella* species also grow within mononuclear phagocytic cells. Cattle that are resistant have microbicidal macrophages but this is not the case for susceptible animals. If IFNγ is provided, it enhances MHC class II antigens and Fc receptors on the macrophages and at this stage their ability to initiate an immune response is much improved.16

**Listeria**

This is an aerobic Gram-positive rod that grows successfully in mononuclear phagocytes on account of its ability to produce a haemolysin.10 Yet, as it grows, the macrophages release the cytokine TNF, tumour necrosis factor, and later IFNγ arises from T cells. A good TNF response followed by IFNγ helps in the cure of listeriosis;17 IFNγ enhances the production of oxygen radicals and nitric oxide that stops *Listeria* spreading into the cell cytoplasm.18

**Tuberculosis**

*Mycobacterium tuberculosis* enters mononuclear phagocytes via the complement receptors and so avoids activation of the cells. There is a 25 kDa fraction of *M. tuberculosis* that inhibits macrophage hexose monophosphate shunt activity, inhibits hydrogen peroxide production and inhibits release of lysosomal enzymes. There is also inhibition of phagosome–lysosomal fusion. Accordingly, it is not surprising that tubercle bacilli readily establish infection in human macrophages and spread around the body inside them. Effective chemotherapy will help reverse this situation. If there is a refractory state IFNγ could help because it has an action, to which vitamin D is synergistic, in killing the intracellular invaders.19

**Leprosy**

In tuberculoid leprosy, in which there is a high degree of cell-mediated immunity, there are epithelioid cells surrounded by lymphocytes. Indeed in the reactive centres of lymph nodes there are many CD4 helper and memory cells, and there are CD8 cells in the mantle areas. Conversely, in lepromatous leprosy there are many foamy histiocytes full of leprosy bacilli because cell-mediated immunity is ineffective. The lymph nodes contain a preponderance of CD8 suppressor cells. Indeed, immunosuppression in the presence of antigen excess is characteristic of lepromatous lesions. That situation can be reversed by injections of rIFNγ, which increase the killing capacity of macrophages.20

It has been shown that in macrophages that have been invaded by leprosy bacilli there is failure of phagosome–lysosome fusion as a consequence of inhibition of movements of the lysosomal vacuoles.21 Also there is inhibition of the oxidative burst and of superoxide anion production because mycobacterial phenolic glycolipids are free radical scavengers.22 They also inhibit lymphocyte proliferation.23

**Leishmaniasis**

Promastigotes of the protozoan *Leishmania* when inoculated into warm blooded animals are phagocytosed by skin macrophages and within them they become aflagellar amastigotes. At the time of entry a glycoprotein gp 63 on the surface of the parasite interacts with the CR3 (C3bi) receptors in macrophages.24 That avoids cell activation. Also a highly anionic surface lipophosphoglycan25 on *Leishmania* mops up C3 cleavage products, scavenges superoxide anions and inhibits the action of lysosomal hydrolases.26 Indeed, when *L. donovani* is deficient in lipophosphoglycan, it is destroyed within the macrophages. In spite of the fact that phagosome–lysosome fusion does occur in *Leishmania* infection, the parasites survive because there is suppression of lysosomal enzymes.26 Also *Leishmania* produces superoxide dismutase and catalase, and a novel reducing agent trypanothione to detoxify oxygen radicals.

Injection of rIFNγ has demonstrable efficiency in killing.27 In fact in the natural healing phase IFNγ producing T cells are generated at the sites of infection. Another macrophage activating factor GM-CSF is also involved. How are the parasites killed? The indications are that nitric oxide derived from l-arginine is the principal mediator. IFNγ boosts this killing process.28 It has recently been appreciated that another cytokine TNFα acts synergistically with IFNγ to boost the non-specific defence that is mediated by nitric oxide.29,30

**References**


