

# CFTR: helping to acidify macrophage lysosomes

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**A new study shows that alveolar macrophages use the cystic fibrosis transmembrane-conductance regulator (CFTR) to maintain lysosomes at low pH and to restrict the growth of ingested bacteria. This may help to explain the persistent infections and chronic inflammation of the lungs that characterize cystic fibrosis.**

Cystic fibrosis is a complex, heritable disease attributable to defects in a single protein, CFTR. Severe pathology results from chronic inflammation due to poor control of bacterial infections in the lungs<sup>1</sup>. Defective secretion by epithelial cells in respiratory airways and submucosal glands creates an extracellular environment that allows bacteria, such as *Pseudomonas aeruginosa*, to flourish<sup>2</sup>. Persistent infections stimulate epithelial cells and neutrophils to release inflammatory cytokines that cause tissue damage and pathology. On page 933 of this issue, work by Di *et al.*<sup>3</sup> draws attention to another major sentinel of infection in the lung, the alveolar macrophage. They demonstrate that CFTR contributes to lysosome acidification in alveolar macrophages and, consequently, to limiting the growth of ingested bacteria. This role for CFTR in alveolar macrophage function suggests that the mutant CFTR of cystic fibrosis compromises an essential regulator of host defence in the lung.

CFTR is a cyclic AMP-regulated chloride channel, located in the plasma membrane and membranous organelles such as the trans-Golgi network (TGN) and endosomes<sup>4</sup>. Mutations that correlate with disease either alter CFTR channel activity or cause it to mis-sort inside epithelial cells, such that the newly synthesized molecules fail to reach their target membranes<sup>5</sup>. The consequent deficiency in chloride transport affects the ionic composition of epithelial cell secretions and the biosynthesis of mucus polysaccharides. The alterations of pH, ion concentrations, mucus chemistry or matrix hydration in cystic fibrosis slow bacterial-clearance mechanisms in the airways of the lungs and permit more extensive growth of bacteria, which eventually increases inflammation.

Alveolar macrophages maintain lung sterility by actively migrating across lung surfaces and ingesting microbes and particulates by phagocytosis<sup>6</sup>. In phagocytosis, extracellular

particles (such as bacteria) are enveloped by extensions of the macrophage plasma membrane, resulting in intracellular vesicles containing the particles<sup>7</sup> (Fig. 1a). Ultimately, the phagosome fuses with the lysosomes, whose low pH is maintained by the vacuolar proton ATPase<sup>8</sup>. The acidic pH and acid hydrolases of phagolysosomes usually degrade the ingested microbes. Macrophages are also an important source of inflammatory cytokines, which are released after interaction with bacteria, fungi or viruses, or as indigestible particles that accumulate in phagolysosomes. Therefore, a defect in lysosomal function that impairs the ability of a macrophage to degrade bacteria could compromise the mechanisms for maintaining lung sterility and possibly also trigger extraordinary inflammatory responses.

Di *et al.*<sup>3</sup> show that alveolar macrophages contain CFTR in their plasma membranes and lysosomes, and that lysosomal CFTR cooperates with the vacuolar proton ATPase to acidify lysosomes, probably by a chloride transport-based mechanism of charge compensation. By comparing macrophages from wild-type and *Cftr*-deficient mice, the group identified cyclic AMP-dependent chloride conductances consistent with the presence of CFTR in macrophage plasma membranes. Antibodies against CFTR detected the protein in macrophages, but not in neutrophils from mice and humans, and immunoelectron microscopy localized CFTR to late endosomes or lysosomes. Fluorescence microscopic measurements of pH in endocytic compartments, showed that lysosomes of *Cftr*-deficient mouse macrophages were one pH unit more alkaline than lysosomes of wild-type macrophages. Treatment of wild-type alveolar macrophages with reagents that inhibited *Cftr*-dependent chloride transport or the vacuolar proton ATPase led to alkalinization of lysosomes. Moreover, they showed that lowering cellular cyclic AMP concentrations also elevated lysosomal pH in wild-type alveolar macrophages, indicating a previously unknown role for cyclic AMP in the maintenance of lysosomal pH. The acute alkalinization caused by

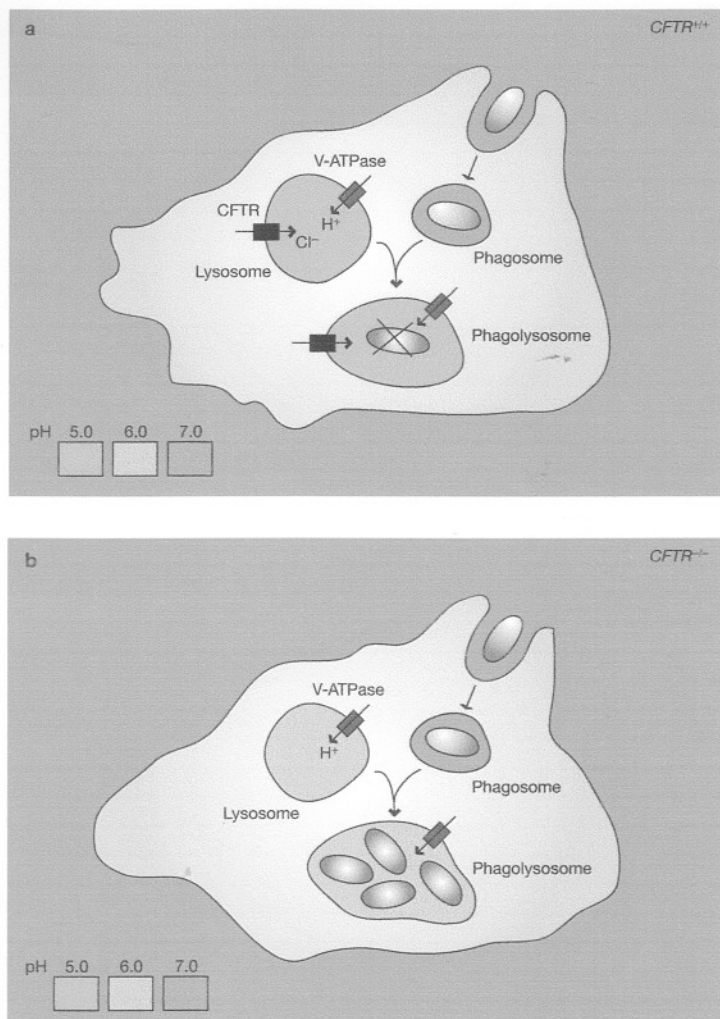
these inhibitors indicated that *Cftr* contributes directly to lysosomal acidification, and that the higher pH of lysosomes in *Cftr*-deficient macrophages was not an indirect consequence of missorting of other proteins that control lysosome acidification. The most likely mechanism for CFTR-dependent acidification is that chloride delivered into lysosomes through the CFTR allows the vacuolar proton ATPase (V-ATPase) to maintain larger gradients of protons across the lysosomal membranes (Fig. 1b).

The authors found that phagolysosomes containing ingested yeast or the bacterium *P. aeruginosa* were also more alkaline in macrophages from *Cftr*-deficient mice and that bacteria ingested by phagocytosis grew and divided inside those organelles — rather than being degraded as in phagolysosomes from wild-type macrophages. Other macrophage antimicrobial activities (oxidative burst, particle binding to cell surfaces and phagosome-lysosome fusion) were similar in wild-type and mutant macrophages. Thus, the failure to expose bacteria to the acid bath inside phagolysosomes allowed bacterial growth in *Cftr*-deficient macrophages. These findings suggest that the macrophage deficiency in bacterial clearance that results from mutant CFTR contributes to the poor control of bacterial growth seen in cystic fibrosis.

One of the mysteries about cystic fibrosis is that the opportunistic infections that exacerbate the disease are caused mostly by *P. aeruginosa*<sup>2</sup>. It is possible that this bacterium is normally held in check by the acid bath of macrophage phagolysosomes, and that when this defence is weakened, due to mutant CFTR, the bacteria are free to exploit the many other environmental changes that favour growth.

Infection and inflammation in cystic fibrosis is largely restricted to lungs. If defective CFTR affects lysosome acidification in macrophages, why then are so few systemic infections associated with the disease? One possibility is that CFTR only affects lysosome acidification in alveolar macrophages. Di *et al.*<sup>3</sup> observed that, in contrast with their findings in alveolar

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**Figure 1** CFTR and the microbicidal acid bath of macrophage phagolysosomes. (a) Through phagocytosis, alveolar macrophages ingest particles such as bacteria (grey ovals) into plasma membrane-derived phagosomes. Phagosomes fuse with acidic lysosomes to form phagolysosomes, wherein low pH and acid hydrolases kill or restrict the growth of bacteria. Lysosomes and phagolysosomes are acidified by the V-ATPase. In the absence of other ion conductances, proton transport by the V-ATPase generates a transmembrane electrical potential that precludes acidification below pH 6.0. Chloride delivered across lysosomal membranes by CFTR could provide charge compensation that would allow further acidification to pH 5.0. (b) In alveolar macrophages deficient in CFTR, the V-ATPase cannot acidify lysosomes below pH 6.0. Consequently, the growth of bacteria is no longer limited after their delivery into the phagolysosomes.

macrophages, peritoneal macrophages from *Cftr*-deficient mice were not impaired in their ability to restrict the growth of bacteria. This indicates that not all macrophages that express CFTR use it for lysosome

acidification. If CFTR normally affects only the lysosomal pH in alveolar macrophages then mutant CFTR should not compromise macrophage phagolysosomes in other organs of the body.

A role for CFTR in the regulation of organelle pH was suggested earlier as a mechanism for acidifying the TGN and endosomes of epithelial cells. Using electron microscopy to measure organelle pH, it was found that in cells with mutant CFTR, the normally acidic compartments (such as the TGN and endosomes) were more alkaline<sup>9</sup>. However, direct measurements of pH in the TGN of fibroblasts and epithelial cells expressing CFTR indicated either no effect of CFTR on pH<sup>10</sup> or hyperacidification, attributable to interactions between CFTR and sodium transport activities<sup>11</sup>. A role for CFTR in acidification of macrophage lysosomes is consistent with the original model of chloride-dependent charge compensation of proton ATPase activity.

Many questions about CFTR in macrophage biology need to be answered before new therapies for cystic fibrosis can be suggested. Do mutations in the *CFTR* gene that correlate with disease pathology also increase lysosomal pH? Does CFTR affect lysosomal pH in all of the various macrophages from humans and mice that express the molecule? In the larger context of disease pathology, how much does the inability of alveolar macrophages to control bacterial growth set up the initial inflammatory response that underlies the pathology? Perhaps therapeutic strategies for cystic fibrosis that aggressively treat early infections<sup>12</sup> succeed because they address early failures in the alveolar macrophage defence mechanism. □

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