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Sizing up microbes

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The size range of life forms is dictated by basic principles of physics. Large microorganisms, with sizes approaching a millimeter, have compensating features that address the immutable laws of physics. For pathogens, size may impact a range of functions, such as adherence and immune evasion. We review several recent studies on factors impacting, and impacted by, the size of microorganisms.

Mismatched adversaries figure prominently in our mythological and fictional imaginations. David fought Goliath, Ulysses escaped the one-eyed Polyphemus, and Gulliver was rendered powerless by the minute Lilliputians. "Pick on someone your size" is as much a school yard admonition as an acknowledgment of the distinct advantages conferred by a large size. The political scientist Ivan Arreguín-Toft found that in wars fought over the past 200 years, the Goliaths, armies ten times stronger than their opponents, won 71.5% of the encounters.[1] Notably, the underdogs came ahead in nearly a third of the conflicts, and a majority of those wars were won by weaker armies that opted for unconventional strategies. In the asymmetric warfare between man and microbe, the latter appear to have always had an advantage. Microbes have killed more human beings than all our wars combined, and for the greater part of our history, we were not even aware of the existence of our miniscule tormenters.

In a delightful little essay, Stephen Jay Gould discusses the biological improbability of fictional giants and outsized insects, staples of science-fiction movies.[2] (Gould would have been fascinated, but undeterred, by the 71-g giant weta insect discovered in New Zealand last year.) Gould explains the "geometry of space," whereby increases in volume are more rapid than increases in the surface area of an object [since volume increases as the cube of length (l^3), and surface only as the square of length (l^2)]. This simple truth has implications for such diverse issues as the shape of medieval churches, the necessity of folds in the intestine, and the invaginations on insect bodies that serve to increase the surface area (and aid in "breathing").

At the scale of bacteria, gravity is irrelevant, viscosity is the dominant external force (in the typically aqueous environments), and diffusion of molecules, outside as well as inside cells, imposes limits on size. The behemoths of the bacterial domain, *Thiomargarita namibiensis* (750 µM dia.) and *Epulopiscium fishelsoni* (80 × 600 µM), are visible to the naked eye and have biovolumes approximately 10 orders of magnitude greater than the runts of the prokaryotic world (e.g., Mycoplasma sp, 0.2 µM dia.).[3] *T. namibiensis* is a free-living marine organism, while *E. fishelsoni* is a symbiont that lives in the intestines of surgeonfish. These and other giant bacteria have two conserved features that help offset the physical problems engendered by their large size. First, most large bacteria have expansive inclusion bodies that effectively curtail the volume of the cytoplasm and limit diffusion distances within the cell.[3] Second, large bacteria are polyploid, with tens of thousands of genomes per cell, possibly allowing the distributed chromosomes to tend to local needs.[4] The mechanism by which such distributed processes are coordinated is not entirely clear at present.

Size is not merely an issue for the very large bacteria. Even the relatively

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puny *Escherichia coli* (1 × 2 μM) actively monitors its size. One reason that *E. coli* mutants severely deficient for lipopolysaccharide production are non-viable, it appears, is that they are too large for their all-too-critical membrane coats.[5] A second mutation (Δ*fabH*) that reduces the size of the bacteria permits them to survive in spite of the LPS deficiency. FabH catalyzes the first step in fatty acid biosynthesis, and mutants lacking this enzyme grew 75% slower, had half the surface area and less than a third the volume of wild type cells and could therefore tolerate a mutation in LPS biosynthesis that severely limits the size of the cell envelope. While wild type bacteria can alter their size according to nutrient availability, Δ*fabH* maintained their anorexic size irrespective of growth conditions. The authors propose a new model whereby nutrient availability regulates fatty acid production which, in turn, dictates the size of the cell envelope.

Not surprisingly, size has implications for pathogenesis. When the human fungal pathogen, *Cryptococcus neoformans* lodges in the human lung, up to 20% of the cells enlarge 5 to 10-fold to form titan cells (30–100 μM in diameter). Not only are titan cells too large to be phagocytosed by alveolar macrophages (10–20 μM in diameter), they also have capsule and cell wall differences that impede complement deposition and opsonization. The presence of titan cells also inhibited phagocytosis of neighboring normal-sized cells, though titan cell size was not the sole parameter responsible for this property.[6] While mutants that overproduced titan cells fared well in the lung, they did not disseminate to the nervous system as well as the wild type. In a mouse model of infection, mutants that made fewer titan cells were less virulent, and failed to colonize or disseminate as efficiently as wild type cells.[7]

Where bluster is not possible, stealth and teamwork may be worthwhile strategies. *Streptococcus pneumoniae* (~0.7 μM dia.) contends with the distinct challenges of airway colonization and systemic infection by altering its chain length (which offers similar benefits as an increase in size, but bypasses the messy internal adjustments). *S. pneumoniae* cultured in tryptic soy broth has a distinctive diplococcal morphology but displays both short- and long-chain morphologies when grown in human nasal airway surface fluid. Long-chain forms attached better to epithelial cells, presumably due to increased contact with host receptors.[8] Mutants lacking cell-wall hydrolases that preferably formed chains fared significantly better than the wild type in a mouse colonization model.

In a systemic infection, however, longer chains are more vulnerable to opsonization and subsequent immune-mediated clearance. Many strains isolated from a mutant screen selecting for increased opsonophagocytosis exhibited longer chain-lengths.[9] The increased chain lengths enhanced complement-mediated killing by neutrophils in vivo, and longer-chain forms were out-competed by the wild type strain in a mouse model of systemic infection. Life at the other end of the microscope appears to be more bizarre than our mythic imaginations, and it is only natural that we feel dwarfed by microbial inventiveness!

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