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# The battle with the host over microbial size

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An eponymous feature of microbes is their small size, and size affects their pathogenesis. The recognition of microbes by host factors, for example, is often dependent on the density and number of molecular interactions occurring over a limited surface area. As a consequence, certain antimicrobial substances, such as complement, appear to target particles with a larger surface area more effectively. Although microbes may inhibit these antimicrobial activities by minimizing their effective size, the host uses defenses such as agglutination by immunoglobulin to counteract this microbial evasion strategy. Some successful pathogens in turn are able to prevent immune mediated clearance by expressing virulence factors that block agglutination. Thus, microbial size is one of the battlegrounds between microbial survival and host defense.

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## Introduction

Members of the microbial world span a great range of shapes and sizes. The selective value of microbial shape has been reviewed elsewhere [1]. Differences in size are used to distinguish species and impact many aspects of microbial physiology and lifestyle. Bacterial cells, for example, range from 0.15 to 700 μM in length. In addition, for single-celled organisms, modulation of cell division or separation may significantly impact their effective size. For microbes residing in a mammalian host, size may be a determining factor in an infectious agent's success or its clearance [2–5]. Many successful pathogens have evolved strategies to modulate their effective size to accommodate these challenges [6]. The host in turn appears to target the ability of microbes to escape its defenses with their small size. By analyzing bacteria differing only in effective size it is possible to sort out some of the independent contributions of size to pathogenesis. These studies reveal that microbial size is a battleground in the interaction between pathogen and host.

## Why microbial size matters

Most microbes are generally small to minimize their cellular volume and grow most efficiently. While prokaryotic species with large or giant cell morphology may be found in nature, these forms are generally not observed within mammalian hosts. However, pathogens that are typically small may form larger individual cells under certain growth conditions (Table 1). When cell division or septation is inhibited, long filamentous forms that may be 10–50 times longer than usual may result. Filamentous forms may occur, for example, during cultivation in sublethal concentrations of antibiotics. Filamentation by *Escherichia coli* during infection of the bladder has been shown to inhibit predation by neutrophils and macrophages, presumably because the extremely long size of the target particle exceeds the capacity of the professional phagocytes to surround and internalize it [2,7•]. A similar inhibitory effect has been noted when bacteria aggregate to form biofilms, densely packed communities of sessile cells encased in an extracellular matrix, which are more resistant to clearance mechanisms than individual planktonic cells [8].

Filamentous forms and biofilms, however, are extreme examples of bacteria multiplying their effective size. More moderate inhibition of cell division, as demonstrated during growth of *Staphylococcus aureus* or *Klebsiella pneumoniae* in sublethal concentrations of antibiotics, may lead to enlarged cells that are more readily phagocytosed and killed [9]. Experiments with 0.2–3 μM coated beads have shown that larger particles, if not too long to be internalized, enhance recognition by phagocytic cells, the formation of a phagocytic cup, and delivery to lysosomes [10]. The greater surface area of larger targets optimizes recognition by allowing for more adhesive events and receptor–ligand interactions per particle. This velcro-like effect increases the stability of attachment to host cells. For phagocytic cells, this facilitates host–bacterial interactions that lead to killing and clearance. In contrast, more efficient attachment of microbial cells with a greater surface area could also promote adherence to nonphagocytic host cells. Such interactions could promote the persistence of an organism within its environmental niche. For instance, *Streptococcus pneumoniae*, which colonizes the mucosal surface of the upper respiratory tract, grows in chains that vary in length or numbers of cells depending on the completeness of peptidoglycan cleavage between daughter cells after cell division. Mutants that form longer chains and natural chain-forming variants show increased adherence to airway epithelial cells in

**Table 1**

**Factors affecting effective microbial target size**

| Increasing | Decreasing |
|---|---|
| Filamentation and chaining (M)[a] | Cell division, septation and separation (M) |
| Sublethal concentration of antibiotics (H)[b] | |
| Formation of biofilm communities (M) | Cell dispersion and motility (M) |
| Agglutination by immunoglobulin (H) | IgA1 proteases (M) |
| Containment in molecular traps or nets (H) | DNases, nucleases (M) |

[a] (M) Microbial strategy.
[b] (H) Host strategy.

culture [11•]. Accordingly, these chaining mutants outcompete their shorter parent, which displays mostly diplococcal forms, *in vivo* during nasopharyngeal colonization in a mouse model.

In contrast, once the requirements of epithelial attachment and airway colonization of the airway are bypassed during invasive infection, the virulence of *S. pneumoniae* mutants in the bloodstream is inversely correlated with their relative chain length [12••]. Evasion of the complement system, which is abundant in the serum and functions by either directly lysing its microbial target or by opsonizing it for uptake by phagocytes, is critical for a pathogen's survival in the bloodstream. In fact, many of the most common invasive pathogens, including *S. pneumoniae*, *Haemophilus influenzae* and *Neisseriae meningitidis*, are particularly small in size. Individuals with a deficiency in the complement pathway are more susceptible to a number of invasive infections, but in particular those caused by this group of organisms [13,14]. Following incubation in serum, activation of the alternative pathway by spontaneous hydrolysis of complement component 3 (C3) results in the covalent deposition of C3b on microbial surfaces through its reactive thioester bond. The density of C3 fragments deposited is proportional to the average length of chain-forming *S. pneumoniae* mutants. When wild-type strains were grown under conditions that affect average chain length, the deposition of C3 fragments was also proportional to average chain length. For a given surface, activation of the alternative pathway of complement is a stochastic process; once activated its spreads throughout the bacterial chain. Therefore, the greater the surface area of the particle, which in the case of the pneumococcus is seen with longer chain forms, the more likely that a focus of complement activation will be initiated. Accordingly, shorter chains that minimize their effective surface area are more virulent in a model of invasive infection, and this effect of size is complement dependent. The complement system, therefore, is an example of an important component of host defense for which the size of the target is an important factor in its effectiveness. Invasive pathogens that do not minimize their size are likely to depend on other means of evading complement, particularly the alternative pathway of complement activation. Likewise, bacteria within large communities may require additional mechanisms to evade complement activation [15]. The effect of surface area on complement activation demonstrates how small size may contribute to bacterial virulence. Because many other antimicrobial substances are directed to the microbial surface, its area could also be a factor in their activity.

## The host fights back

Although microbes may take advantage of their small surface area to evade key defenses such as the complement system, other aspects of the host response appear to circumvent this strategy. For example, the bivalent or multivalent binding of immunoglobulin agglutinates microbial targets to increase their effective size (Table 1). This process is demonstrated by the 'threading reaction', which refers to the inhibition of separation of daughter cells by the bridging of bound immunoglobulin during replication in the presence of cell surface antibody in convalescent serum [16]. These threads resemble chains that then fold on themselves to generate clumps. The clumping or agglutinating effect of antibody has been correlated with protection from systemic infection by *S. pneumoniae* and *Salmonella typhimurium* [17–19]. However, there has been little understanding of how agglutination of microbes contributes to host defense and neutralization of infectious targets [20]. The clumping of microbial particles, which occurs during mucociliary defense, enhances mechanical clearance mechanisms, and, therefore, prevents dispersion and motility from allowing microbial escape. The agglutination of microbes also increases the effective surface area that can be targeted by complement and, thus, the likelihood of attaining a focus of activation. In fact, the degree of agglutination correlates with both complement activation and complement-dependent microbial killing [12••]. This ability of agglutinating antibody to promote complement activation is independent of any direct interaction between complement and antibody. Immunoglobulin G lacking its C3 binding Fc portion [the bivalent $F(ab')_2$ fragment of IgG] still promotes complement activation and killing, but only if it agglutinates its target. Even though Fab fragments that also lack Fc bind equally compared to $F(ab')_2$, these are monovalent and consequently do not agglutinate their target or promote complement activation or killing. This effect of $F(ab')_2$, but not Fab, has been shown for *H. influenzae*, for which activation of the complement cascade results in direct lysis, and for *S. pneumoniae* and *Pseudomonas aeruginosa* where complement deposition causes opsonization and uptake by phagocytes [12••,21]. In this manner, agglutinating antibody is able to subvert virulence factors such as capsular polysaccharide that otherwise limit complement deposition on the cell surface. This could