Lifson (14) showed in dogs that elevations of venous pressure as low as 4–6 cm H_2O caused "secretory filtration" by both providing a driving force for secretion and increasing the hydraulic permeability of the epithelium, *without which the driving force would be ineffective*. The resulting pores, which are reminiscent of those induced by TNF and LIGHT, are large enough for proteins to pass.

How might anti-CD3, or TNF in particular, affect the hydrostatic pressure difference between lamina propria and gut lumen? TNF is known to increase vascular permeability (15, 16). The resulting extrusion of fluid from small blood vessels into lamina propria would likely increase pressure there, since the latter is a relatively confined space limited by the epithelium in one plane and smooth muscle in the other. The contractile smooth muscle response to cholinergic stimulation has been shown to increase following anti-CD3 administration (2). In conjunction with increased vascular permeability, this increase in muscle tension may further increase interstitial pressure.

In summary, since the phenotypic changes in the intestinal epithelium of mice resulting from their treatment with anti-CD3 or TNF do not, in themselves, explain concomitant fluid secretion and diarrhea, a secretory driving force other than active anion secretion must be postulated. The likely candidate is an increase in interstitial pressure, the influence of which is facilitated by the associated increase in epithelial permeability. The postulated pressure increase could result from the effects of TNF and perhaps other cytokines on vascular permeability and smooth muscle action. The role of interstitial pressure in cytokine-induced intestinal secretion needs to be explored. Such secretion, unlike that activated by cAMP, should be especially sensitive to reversal by an increase in intraluminal pressure (assuming that pressure on the bulk luminal solution is transmitted to intestinal crypts, where much of the fluid leakage is likely to occur).

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Pili prove pertinent to enterococcal endocarditis

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***Enterococcus faecalis* is an important agent of endocarditis and urinary tract infections, which occur frequently in hospitals. Antimicrobial therapy is complicated by the emergence of drug-resistant strains, which contribute significantly to mortality associated with *E. faecalis* infection. In this issue of the *JCI*, Nallapareddy and colleagues report that *E. faecalis* produces pili on its surface and that these proteinaceous fibers are used for bacterial adherence to host tissues and for the establishment of biofilms and endocarditis (see the related article beginning on page 2799). This information may enable new vaccine strategies for the prevention of *E. faecalis* infections.**

Enterococcus faecalis — snapshots of the pathogen

Enterococcus faecalis, a commensal bacterium of human biliary and gastrointestinal tracts, is a leading cause of surgical site, bloodstream, and urinary tract infections (1). Furthermore, *E. faecalis* is also a causal agent of bacterial endocarditis, whose complications — including congestive heart failure, septic emboli, and glomerulonephritis — result in mortality (2). Current management of enterococcal endocarditis involves administration of a combination of antimicrobials; however, the emergence of strains with multiple antibiotic resistance, including vancomycin resistance (e.g., vancomycin-resistant enterococci), present continuously increasing problems (3). Epidemiological studies show that more than 20% of enterococci isolated from infections of intensive care unit patients display vancomycin resistance (4). Thus, research that has the potential to unveil new therapies or preventive measures that disrupt the
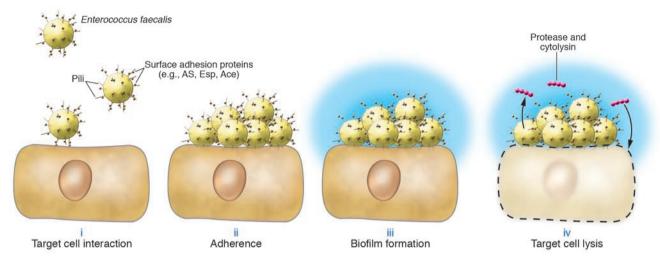
Figure 1
Role for pili in the establishment of *E. faecalis* infection. Enterococci may use pili (surface fibrils) for microbial attachment to host tissues (i). Surface proteins (e.g., AS, Esp, and Ace) are involved in establishing tight bacterial adherence to host cells (ii). Enterococcal aggregation induces biofilm formation (iii) and elaboration of exopolysaccharide matrix (blue surface layer). Quorum-sensing–controlled expression of protease and cytolysin mediate host cell death and spread of infection (iv). Schematic modified with permission from ASM Press (1).

pathogenesis of enterococcal endocarditis is urgently needed.

What makes *E. faecalis* a pathogen? Unlike group A streptococci or *Staphylococcus aureus*, which are virulent microbes that secrete a plethora of hemolysins and toxins to battle innate immune responses (5, 6), *E. faecalis* pathogenesis relies on fewer virulence factors (7). Two protein adhesins, enterococcal surface protein (Esp) and adhesion to collagen from *E. faecalis* (Ace), are thought to mediate enterococcal attachment to host tissues (8, 9), while aggregation substance (AS) promotes aggregation of replicating microbes (10). Enterococci regulate protease expression (a secreted virulence factor involved in tissue degradation) via the *E. faecalis regulator* (*fsr*) quorum-sensing locus, including *fsrC* sensory kinase, *fsrA* response regulator, and *fsrB*-derived autoinducer (11). Once an infectious nidus harbors threshold numbers of enterococci, Fsr signaling activates protease secretion (11). Esp, Ace, Fsr, and protease are involved in the formation of a biofilm, a surface-attached bacterial community enclosed in extracellular matrix where enterococci assume a slower growth rate, increased antibiotic resistance, and elevated frequency of lateral gene transfer (11). Finally, cytolysin, a toxin secreted by enterococci, derives its cytolytic activity from 2 peptides that possess modifications characteristic of lantibiotics (compounds with thioether amino acids that are derived by posttranslational

modification from ribosomally synthesized precursors) (12, 13). Together these peptides lyse different host cells including polymorphonuclear leukocytes, a critical immune defense against *E. faecalis* infection (Figure 1) (14).

Pili occur even in gram-positive bacteria

The presence of pili on the surface of gram-positive bacteria has only recently been reported for several strains of *Corynebacterium* spp., *Actinomyces* spp., and *Streptococcus* spp. (15–17). By using antibodies as reagents for detection, adhesins at the pilus tip as well as major and minor pilin subunits were discovered (18). Structural genes encoding pilin subunits in *C. diphtheriae* and *A. naeslundii* are located in close proximity to genes encoding sortase-like enzymes (18–20). Sortases are transpeptidases that cleave sorting signals of surface proteins to immobilize their C-terminal carboxyl group via amide bond formation in the cell wall envelope of gram-positive bacteria such as staphylococci, listeria, or bacilli (21, 22). Mutations that abrogate sortase or pilin subunit gene expression abolish the formation of pili (18). A pilin motif sequence with a conserved lysine residue is found in all major pilin subunit genes, and this lysine residue is also essential for the formation of pili (18). Biochemical experiments demonstrated that gram-positive pili cannot be dissolved with SDS or organic solvents, but are cleaved by protease (23). Together these data suggest

a model whereby sortases promote pilus assembly via the formation of transpeptide bonds between the C-terminal carboxyl group of cleaved pilin precursors and the side chain amino group of lysine residues in pilin motif sequences (24).

Several predictions can be derived from this model. First, gram-positive microbes whose genome sequences harbor clusters of genes that encode sortases as well as surface proteins with sorting signals and pilin motif sequences must produce pili (24). This prediction is fulfilled for enterococci in this issue of the *JCI* by Nallapareddy et al. (25) but also in other recent publications identifying pili on the surface of group B streptococci or pneumococci (26, 27).

Enterococcal pili

In a previous study, Sillanpää and colleagues queried enterococcal genome sequences for LPXTG motif–containing sorting signals (where X is any amino acid) and structural features of surface proteins such as 160- to 500-aa segments containing Ig-like folds (28). Three open reading frames, ef1091–ef1093, displayed such features, and antibodies against these proteins were found more often in sera of individuals infected with *E. faecalis* than in the sera of noninfected control patients (28). In this issue of the *JCI*, Nallapareddy et al. (25) further characterize ef1091–ef1093 and their associated *sortase C* (*srtC*), thereby revealing that these 3 open reading frames and *srtC* are part of an operon encoding

endocarditis and biofilm–associated pili (*ebp*). The authors used immunogold electron microscopy to demonstrate the presence of pili composed of EbpA, EbpB, and EbpC on the surface of *E. faecalis*. Biofilm assays were used to show that a nonpiliated mutant was unable to form biofilms. When recovering microbes from aortic vegetations in a rat endovascular infection model, pilus mutants could not adequately compete with wild-type enterococci, suggesting that pili are required for the establishment of enterococcal infections. Using specific probes, the genes *ebpA*, *ebpB*, and *ebpC* were detected in 408 *E. faecalis* isolates from 4 continents, leading the authors to conclude that the formation of pili may be a ubiquitous trait of *E. faecalis*. Independent of this work, Tendolkar and colleagues recently identified a cluster of sortase and surface protein genes involved in biofilm formation (including *biofilm enhancer in Enterococcus* [*bee*]), although this study does not unveil the ability of enterococci to form pili (29).

If one assumes that pili — fibrous tools for bacterial adhesion to host tissues — are important for virulence, one could also speculate that immunization of animals or humans with pilin subunits may prevent microbial adhesion or the pathogenesis of infection. Two recent reports corroborate this contention, demonstrating that immunization of animals with pilin subunits prevent group B streptococcal neonatal meningitis or group A streptococcal infection (30, 31). Thus, the discovery of pili and pilus assembly in gram-positive bacteria not only promises new technology but may also generate public health measures that affect humans.

Conclusion

Sortase-anchored pili have been found in *Corynebacterium* spp., *Actinomyces* spp., group A and group B streptococci, pneumococci, and now in enterococci. In the current study (25), *E. faecalis* pili were found to play a major role in biofilm formation and appear to be important in mediating endovascular infection. As these ubiquitously encoded surface proteins are antigenic in humans during endocarditis infection, this study raises the possibility of potential new preventive measures that may disrupt the pathogenesis of enterococcal endocarditis.

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