
# Dr. Jekyll and Mr. Hide: How *Enterococcus faecalis* Subverts the Host Immune Response to Cause Infection

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## Abstract

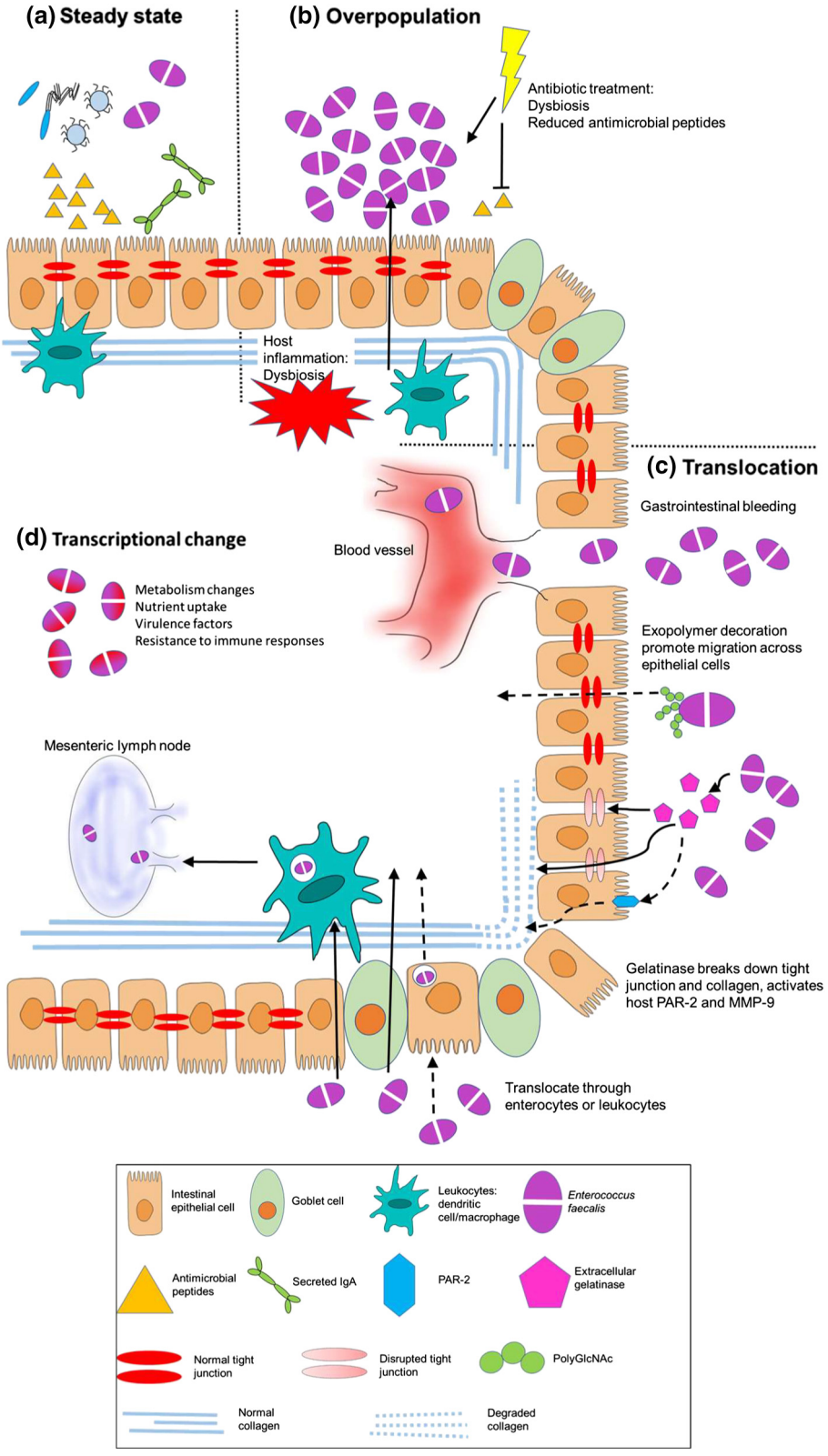
*Enterococcus faecalis*, a ubiquitous member of the healthy human gut microbiota, is also a common opportunistic pathogen and leading cause of nosocomial infections. This tenacious microbe is well adapted to infect and persist in multiple niches within the mammalian host and can rapidly tune its metabolism to respond to new environments, enabling infection in sites including the gastrointestinal tract, urinary tract, wounded epithelium, heart, and blood. In order to withstand and persist in the face of host immune responses, *E. faecalis* has an arsenal of strategies to suppress, evade, or inactivate innate and adaptive immune mechanisms. In this review, we present the variety of ways *E. faecalis* modulates the immune response, enabling this otherwise innocuous gut commensal to transition and persist as a pathogen.


## Introduction

Enterococci are common members of the healthy gastrointestinal tract (GIT) microbiome. However, since 1970, enterococci have emerged as a significant public health concern, frequently causing opportunistic infections in susceptible hosts and in hospitals, associated with their increasing antibiotic resistance [1]. In addition, enterococci possess multiple attributes that enhance its virulence in some host environments. For these reasons, the prevalence of enterococcal infections is on the rise [2]. Enterococci are a significant cause of nosocomial infection, ranking among the top three most prevalent healthcare-associated infections including catheter-associated urinary tract infection (CAUTI), central line-associated bloodstream infection, and surgical site infection [3]. Enterococci are also responsible for 7% of infections among intensive care unit patients and are frequently transmitted from patient to patient [4]. In addition to acquired antibiotic resistance, enterococci are also intrinsically resistant to many antimicrobial agents, together giving rise to a variety of infections with limited therapeutic options and are therefore a growing public health concern.

Among enterococci, *Enterococcus faecalis* is the most common species associated with human disease, responsible for twice as many healthcare-associated infections as caused by *Enterococcus faecium,* and it is the primary focus of this review. While *E. faecium* is associated with higher mortality rates than *E. faecalis* in some infections due to its higher prevalence of vancomycin resistance, *E. faecalis* has a higher incidence for most infections and the case numbers have been increasing in recent decades, likely due to its greater ability to persist in healthcare facilities and its higher abundance in the human gut microbiome [3–7]. In addition, *E. faecalis* is more frequently associated with community-acquired bacteremia, whereas *E. faecium* is primarily associated with hospital-acquired bacteremia [5]. Moreover, 94% of enterococcal infective endocarditis is caused by *E. faecalis*, which is dependent on its ability to attach to collagen [8]. The ability to adhere to and to form biofilms on a variety of surfaces, important in many

*E. faecalis* infections, confers phenotypic antimicrobial tolerance to the biofilm-associated bacteria [9–12]. In addition, biofilms can shield bacteria from immune detection or phagocytosis, serving as an effective mechanism of immune evasion [13]. After engulfment by a macrophage, *E. faecalis* can



also survive intracellularly for a longer period compared to *E. faecium*, as it possesses various immune evasion mechanisms that *E. faecium* does not [14,15].

In this review, we focus on the duality and transition of *E. faecalis* from commensal to pathogen. We compile recent literatures investigating interactions between *E. faecalis* and the host immune system, and survey how these host-pathogen dynamics contribute to pathogenesis. Finally, we highlight key findings regarding how *E. faecalis* adapts to different host environments and propose potential targets and strategies for immunotherapy to limit recalcitrant *E. faecalis* infections.

## The Duality of *E. faecalis*

### *E. faecalis* as a commensal

The origin of enterococci can be traced back to the time when animals started walking on land, when they were already well adapted to life within their animal host, and their subsequent speciation paralleled the diversification of their hosts [16]. Among enterococci, *E. faecalis* is a ubiquitous member of the human gut microbiota, colonizing 60%–80% of infants within the first week of life and 80% of adults [17–19]. *E. faecalis* is also a part of the normal flora in the human oral cavity, found in approximately 20% of healthy individuals [20,21]. Recently, *E. faecalis* was also identified as a member of the urinary tract microbiota [22–24]. GIT commensal microbes face many challenges including the high turnover rate of intestinal contents and mucosal immunity, which must be overcome in order to survive and prosper in this niche. For example, mucin, the main component of the mucus layer, is usually shed within 24 h of production and replaced with newly synthesized mucin [25]. In addition, the mucus layer contains multiple antimicrobial peptides and antibodies to prevent infection, posing a serious threat to nearby microbes [26]. Despite these innate defenses, *E. faecalis* can colonize the entire GIT close to the epithelium, illustrating the highly adapted commensal lifestyle of this organism [27]. One of the strategies enterococci take advantage of is biofilm formation, which both associates with the mucus layer and confers tolerance to antimicrobials [28]. *In vitro*, *E. faecalis* biofilm induces a lower level of proinflammatory cytokines (TNF-α and IL-6) and promotes survival within dendritic cells and macrophages as compared to planktonic *E. faecalis*, further enhancing *E. faecalis* persistence in the host [29,30]. In general, *E. faecalis* takes advantage of mechanisms to adhere, to aggregate, and to form biofilm in order to colonize various niches inside the host [31].

Probiotic is defined as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [32]. The beneficial effects are often categorized as (1) influencing other microbes, (2) modulating the host immune responses, (3) promoting the function of the epithelial barrier, and/or (4) providing essential nutrients [33]. *E. faecalis* has many traits associated with probiotics. For example, commensal *E. faecalis* produces a heptapeptide pheromone and a bacteriocin that kill pathogenic *E. faecalis* V583, competing for the niche and preventing the colonization of pathogenic *E. faecalis* [34,35]. Furthermore, *E. faecalis* can interact with other microorganisms to protect the host. *E. faecalis* are found in human breast milk, and these isolates can inhibit the growth of *Staphylococcus aureus in vitro*, potentially conferring protective colonization resistance from a very early stage of life [36,37]. *E. faecalis* isolated during asymptomatic bacteriuria is associated with lower recurrence rates of urinary tract infection (UTI), suggesting a potential protective role in preventing symptomatic UTI [38,39]. In a nematode model, *E. faecalis* inhibits *Candida albicans* hyphal morphogenesis, which is crucial for *C. albicans* pathogenicity; thus, *E. faecalis* can protect the nematode host from *C. albicans*-mediated killing [40]. *E. faecalis* also inhibits the adhesion of other pathogens, such as *Listeria monocytogenes*, to host epithelial cells, possibly by competing for binding sites [41]. To the canine helminth parasite *Toxocara canis*, *E. faecalis* possess larvicidal activity both *in vitro* and *in vivo*, which leads to a reduced worm load recovered from infected mice [42]. Finally, when combined with *Bifidobacterium* and *Lactobacillus, E. faecalis* helps to prevent intestinal epithelial barrier leakage by promoting tight junction protein expression and reducing the production of proinflammatory cytokines [43]. Together, these studies demonstrate the probiotic activities of *E. faecalis*, which include

**Fig. 1.** Transition from commensal to pathogen in the GIT. (A) At steady state, mucosal immunity and the healthy microbiota achieve homeostasis. (B) Antibiotic treatment and inflammation can cause dysbiosis which can lead to overpopulation by *E. faecalis*. (C) High numbers of *E. faecalis* increase the risk for translocation from the intestinal lumen into tissues or circulation. In individuals with cirrhosis, gastrointestinal bleeding provides a route for bacteria to cross epithelial barriers. And with proper surface expression protein, *E. faecalis* can directly penetrate epitheial monolayers. On the other hand, gelatinase produced by *E. faecalis* can degrade collagen in the basement membrane and activate host PAR-2 and MMP-9, which in turn increases epithelial permeability. Alternatively, *E. faecalis* can also pass the epithelial barrier *via* intracellular routes. (D) After translocation and exposure to new environments, *E. faecalis* undergoes rapid transcriptional changes to adapt to new niches. Dashed arrows indicate mechanisms described in *in vitro* models that are not yet validated using *in vivo* models.

promoting colonization resistance and enhancing barrier function. *E. faecalis* also has many immunomodulating effects that are beneficial to the host, which are discussed below.

## Transition from Commensal to Pathogen

Although *E. faecalis* can live peacefully in the GIT of the host (Fig. 1a), if it grows unchecked in the gut or gains access to extra-intestinal sites, especially in susceptible hosts, it can transform into an opportunistic pathogen [2]. *E. faecalis* overgrowth in the GIT is often associated with antibiotic treatment and host inflammation, which can lead to subsequent translocation to other sites (Fig. 1b). In individuals with inflammatory bowel diseases (IBD), which include ulcerative colitis and Crohn's disease, the frequency of *E. faecalis* colonization and bacterial load in feces is higher than in healthy individuals [44]. The population density of *E. faecalis* in mucosal tissue is also positively correlated with disease severity, but not duration, in ulcerative colitis [45]. However, in experimental animal models, inoculation of *E. faecalis* into the GIT enhances colon inflammation only in interleukin-10 (IL-10) knockout mice but not in wild type hosts, suggesting that *E. faecalis* alone is not capable of inducing IBD [46]. IL-10 is an anti-inflammatory cytokine that can activate the TGF-β/Smad signaling pathway to attenuate immune activation induced by *E. faecalis* colonization, so mice lacking this cytokine fail to induce this anti-inflammatory response [47]. While more studies are required to determine whether *E. faecalis* overpopulation is a cause or consequence of gut inflammation, it is clear that *E. faecalis* can survive in an inflamed GIT environment.

In chronic endodontic and periodontal infections, *E. faecalis* is one of the most prevalent species and persists even after antibiotic treatment [21,48,49]. In these cases, higher *E. faecalis* numbers result from inflammation and antibiotics eliminating other commensals, allowing the more tolerant *E. faecalis* to overpopulate. It is also possible that the inflammatory environment favors the growth of *E. faecalis*, but more studies are required to understand the contributions of these possible mechanisms.

Overgrowth of intestinal bacteria is often associated with diseases such as spontaneous bacterial peritonitis and irritable bowel syndrome (Fig. 1c) [50,51]. In addition, the use of antibiotics can induce the translocation of *E. faecalis* and *Escherichia coli* from the GIT to mesenteric lymph nodes, which in turn induces inflammation [52]. Antibiotics also promote expansion of vancomycin-resistant *Enterococcus* by reducing the normal microbiota and/or by impairing antimicrobial peptide production, leading to dysbiosis and higher risks of bacterial sepsis [53,54]. Individuals with hemorrhagic cirrhosis often develop peritonitis, likely a result of bacterial translocation from the GIT due to the gastrointestinal bleeding [55]. *E. faecalis* transmission can also occur *via* mechanical transfer. *E. faecalis* can adhere to and can form biofilm on abiotic surfaces, making it prevalent in peritoneal dialysis-related peritonitis, CAUTI, and prosthetic valve infective endocarditis [8,11,56–58].

*E. faecalis* can also promote its own transit out of the GIT *via* intrinsic factors. Decoration of extracellular polysaccharides with β-1,6-linked poly-*N*-acetylglucosamine (polyGlcNAc) enable non-motile *E. faecalis* to penetrate epithelial monolayers, providing a potential mechanism for translocation from the intestinal lumen into tissues [59]. On the other hand, gelatinase (GelE), a metalloprotease produced by *E. faecalis*, can directly compromise epithelial tight junctions and degrade collagen in the intestinal tissue, giving rise to translocation [60,61]. During inflammation, host immune responses break down the basal membrane and tight junctions for leukocyte infiltration. *E. faecalis* GelE confers a similar outcome, activating host protease-activated receptor 2 (PAR-2) and matrix metalloproteinase 9 (MMP9) to disrupt the intestinal barrier and to increase permeability [61–64]. Altogether, *E. faecalis* both passively and actively take advantage of all of these barrier changes, which open a portal on the epithelial barrier.

Aggregation substance (AS) is an *E. faecalis* adhesin that mediates adhesion of bacteria to mammalian cells and to extracellular matrix proteins, and therefore may promote adherence to injured epithelium (where ECM are exposed) and penetration through mucosal layer [65–67]. In addition, AS promotes internalization by enterocytes and phagocytosis by macrophages, where *E. faecalis* can survive for extended periods of time [68]. It has been speculated that this intracellular population could be a mechanism for movement of *E. faecalis* from the GIT to the blood and lymph circulation [52,68,69].

### *E. faecalis* as a pathogen

Coincident with the emergence of *E. faecalis* as one of the leading causes of bacterial infections in the 1980s, repeated mutations have arisen which allow *E. faecalis* to withstand the immune response and survive in different host environments [70]. Upon transition from the GIT to the bloodstream or tissues, *E. faecalis* senses and responds to the new environment in a manner that promotes virulence (Fig. 1d). When cultured in human urine, *E. faecalis* alters the expression of genes related to metabolism, cell envelope modifications, and trace metal acquisition to enhance survival, allowing them to readily grow in this condition [71]. Similarly, *E. faecalis* isolated from the infected mouse peritoneal cavity display induction of genes involved in glycerol

catabolism which promote peritoneal persistence, as well as induction of genes encoding virulence factors such as cytolysin, GelE, and AS [72]. Growing *E. faecalis* in horse blood for only 30 min results in a changed transcriptome, enhancing protection against oxidative stress and lysozyme, as well as expression of some virulence factors, demonstrating how rapid and easy the transition from commensal to pathogen can be [73]. Finally, some strains of *E. faecalis* produce hemolysin, which can lyse mouse erythrocytes and phagocytes, such as neutrophils and macrophages [74].

## The Fine Line between Commensal and Pathogen

The most well-studied pathogenic features of *E. faecalis* are adhesion, aggregation, and biofilm formation [75–82]. However, these same features also contribute to commensalism. Moreover, many virulence factors shown to contribute to pathogenesis are often encoded and expressed in commensal isolates of *E. faecalis*, such as those from healthy infants [83]. In addition, a comparison of commensal and infective endocarditis isolates found no difference in the presence of known virulence factor genes between the groups, although endocarditis isolates possessed stronger *in vitro* biofilm forming ability so likely encode or express genes differently [82,84]. Multi-locus sequence typing clusters most *E. faecalis* isolates involved in hospital outbreaks into distinct clonal complexes, suggesting that certain gene complexes can promote *E. faecalis* adaptation to or persistence in hospital environments [85]. The absence of CRISPR loci also correlates to multidrug resistance, serving as another genomic indicator for virulence in *E. faecalis* [86]. However, there have been no shared set of pathogen-specific genes identified to date and strains causing infections can also be found in healthy human and animals, together suggesting that the host–microbe interaction might be a more important determinant of disease than any specific virulence factor or lineage [87]. For example, *E. faecalis* can induce aggregation of human platelets, which promotes the formation of vegetations and is an essential step in the pathogenesis of infectious endocarditis. The capability to activate platelets is comparable among *E. faecalis* isolates from normal flora, blood or urine disease samples, and endocarditis; however, the platelet activation level varies from donor to donor, indicating that the difference is host-dependent rather than virulence factor-driven [84,88].

### *E. faecalis* and the immune system

As members of the commensal microbiota, *E. faecalis* can go undetected by the immune system under normal circumstances. Upon overgrowth in the GIT or translocation to other sites, however, *E. faecalis* can induce the production of pro-inflammatory cytokines, the activation of leukocytes, and inflammation. At the same time, *E. faecalis* must evade immune surveillance or resist immune-driven killing in order to successfully persist at infection sites. In this section, we will focus on the interactions between *E. faecalis* and the immune system, and how these interactions can tip the balance between commensal or pathogenic states.

### The immunomodulating effects of *E. faecalis*

The GIT constantly faces foreign antigens in the form of food or microbiota, requiring the immune system to tolerate harmless antigens while still surveilling for potential pathogens. Many members of gut microbiota participate in the development and maintenance of immune tolerance by promoting the production of anti-inflammatory cytokines such as IL-10 and TGF-β [89,90]. Heat-killed *E. faecalis* stimulates a subset of lamina propria-residing macrophages to produce IL-10, and abolishing this population of macrophages increases disease severity in a DSS-induced colitis model [91]. This finding suggests that *E. faecalis*, as a gut commensal, can promote the homeostasis of local immunity.

On the other hand, it is important to maintain a strong front-line defense in the GIT to prevent pathogen invasion. Secretory IgA is an integral part of mucosal immunity because this immunoglobulin can not only block the entry of bacteria into intestinal tissues but also fails to activate strong inflammatory responses and plays an important role in oral tolerance [92,93]. Inoculating *E. faecalis* intragastrically can induce a higher proportion of IgA secreting cells in the mouse intestine without strongly increasing pro-inflammatory cytokine production [94]. *E. faecalis* can also direct the T-cell response toward IFN-γ production, instead of IL-13 and IL-10, indicating a Th1 skewing phenotype. This immunostimulatory effect drives dendritic cells to induce adaptive immunity and suggests a possible adjuvant function of *E. faecalis,* which further indicates its role in establishing mucosal immunity [95].

*E. faecalis* is not only involved in local immune modulation, but its ability to shape immunity can also have systemic effects. For example, in an atopic dermatitis model, mice fed heat-killed *E. faecalis* have reduced levels of cytokines including TNF-α, important for inflammation induction, as well as IL-4 and thymic stromal lymphopoietin involved in allergy development, at the site of skin inflammation (the ear), as well as reduced systemic IgE levels in serum. In addition, the number of mast cells at the ear are lower, together resulting in an attenuated allergic response [96]. In another case, leukocyte influx induced by influenza virus in the lung can be

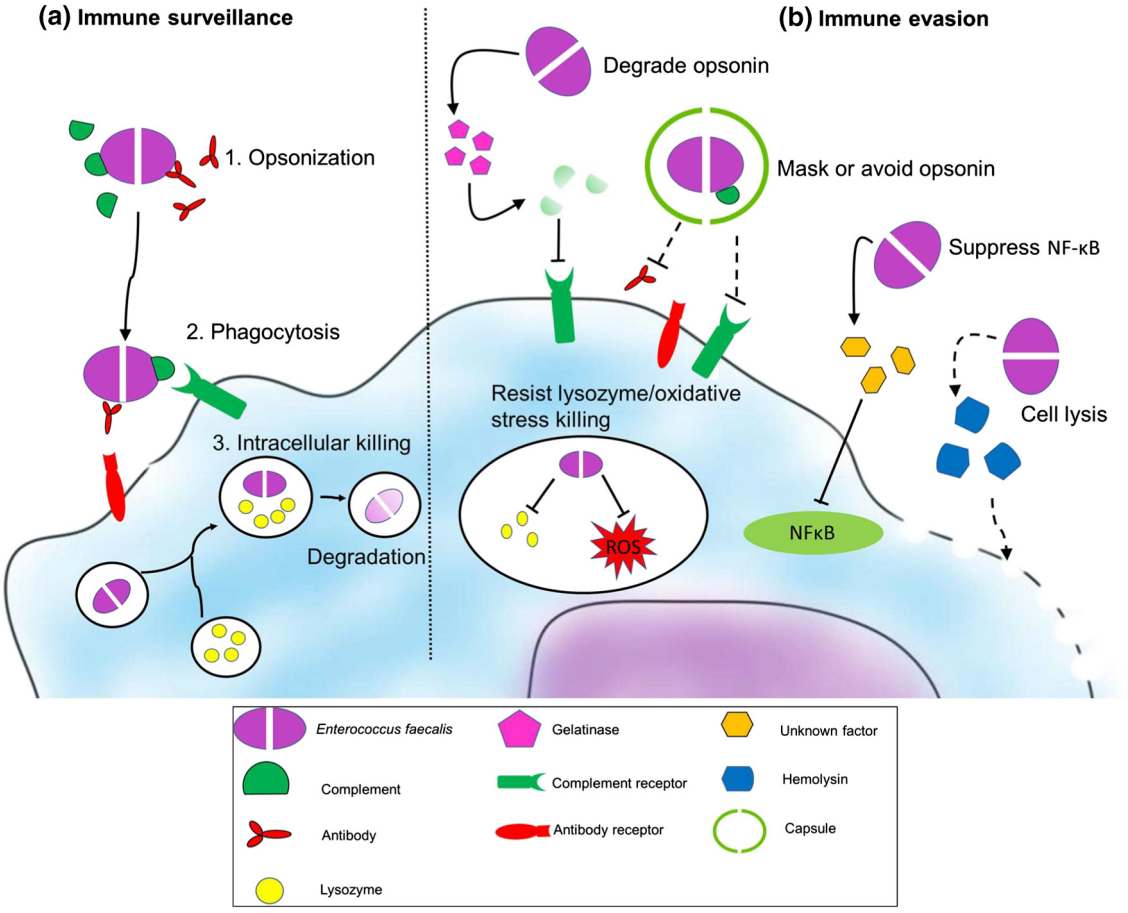



**Fig. 2.** *E. faecalis* evasion of immune surveillance. Many factors are involved in immune surveillance to promote bacterial clearance. (A) Typically, microbes are opsonized by antibody or complement, which promotes phagocyte recognition and intracellular clearance. (B) *E. faecalis* can escape immune surveillance by avoiding opsonization, either by degrading complement or masking the opsonin-binding site. *E. faecalis* also evades immune surveillance by suppressing immune activation, lysing immune cells, and surviving intracellularly. Dashed arrows indicate mechanisms described in *in vitro* models that are not yet validated using *in vivo* models.

attenuated upon oral administration of lysozyme-treated *E. faecalis*, a treatment that suppresses chemokine CXCL4 production, which is chemotactic for neutrophils and monocytes, and reduces alveolar capillary permeability which prevents leukocyte infiltration [97]. In both cases, the mice received bacteria orally, but displayed immune regulatory effects at distant organs, suggesting the exposure of *E. faecalis* to the gut immune system can result in a systematic response. The underlying mechanism is currently unclear. Some orally administered probiotics, such as *Lactobacillus*, can induce IgA production that is detectable in the serum, indicating a systemic immune response [98,99]. Since *E. faecalis* induces IgA secreting cells, it is possible that *E. faecalis* also promotes class switching toward IgA and subsequently reduces IgE production, thus attenuating allergic responses.

## *E. faecalis* and immunosurveillance

The immune system uses multiple mechanisms to detect, identify, contain and eliminate pathogens, many of which are involved in clearing *E. faecalis* infection (Fig. 2a). These mechanisms include antibody recognition and complement binding, capture by phagocytosis, as well as recruitment and activation by leukocytes through chemokine and cytokine production. However, like many bacteria, *E. faecalis* has evolved opposing strategies to evade immune surveillance and killing, enabling a successful transition from commensal to pathogen (Fig. 2b).

### Neutrophils

Neutrophils are the most abundant leukocytes in human peripheral blood, are the first responder to

most infections, and have a variety of antimicrobial properties such as degranulation, phagocytosis, and neutrophil extracellular traps. Neutrophils are also equipped with numerous antimicrobial components including myeloperoxidase (MPO), elastase, and defensins and can efficiently clear enterococci by phagocytosis [100,101]. Multiple strains of *E. faecalis* are highly susceptible to neutrophil-mediated killing, resulting in less than 10% survival rate within an hour of co-culture. This efficient killing is strongly dependent on opsonization, however, and is substantially reduced when the opsonins, such as antibodies or complement, are removed or inactivated [101,102].

In the absence of opsonization, *E. faecalis* can resist phagocytic killing despite neutrophil activation, and several bacterial factors contribute to this resistance. Enterococcal polysaccharide antigen (EPA), for instance, is encoded by a cluster of genes that is found among all examined *E. faecalis* strains and provides resistance to neutrophil-mediated killing partly due to the avoidance of phagocytosis and resistance against antimicrobial compounds [103,104]. On the other hand, AS promotes survival within neutrophils by providing tolerance to MPO, although it also augments adherence to neutrophils [105,106]. Neutrophils are recruited to the site of *E. faecalis* infection in many experimental animal models, yet the infiltrating neutrophils are not always able to clear *E. faecalis* [107–109]. *E. faecalis* possesses several strategies to compromise opsonization, such as degrading or avoiding antibodies and complement, which may contribute to its ability to evade neutrophil-mediated clearance. These mechanisms are discussed below.

## Macrophage

Macrophages are the janitors of the body with strong phagocytic activity to engulf a variety of particles and respond rapidly to infections. *Via* recognition of pathogen-associated molecular patterns, NF-κB signaling in macrophages can be activated by very few of *E. faecalis* (at a multiplicity of infection of 1) [110]. However, upon co-culture with higher numbers of *E. faecalis* (multiplicity of infection 100), instead of inducing a stronger inflammatory response, macrophages fail to become activated. High numbers of *E. faecalis* can also suppress agonist-induced macrophage activation. The immune suppressive effects of *E. faecalis* have yet to be deciphered, but only live bacteria exhibit this activity while heat or ultraviolet killed bacteria do not. Furthermore, culturing macrophages in conditioned medium after *E. faecalis* infection also inhibit LPS agonist-induced activation. These *in vitro* findings of macrophage suppression can be recapitulated in an experimental UTI model, where *E. faecalis*-mediated suppression promotes the survival of co-infecting *E. coli* [110]. Some strains of *E. faecalis* express a TIR domain-containing protein that can inhibit the interaction between Toll-like receptors (TLRs) and downstream adaptor proteins MyD88, to suppress the activation of NF-κB [111]. The lipoteichoic acid of *E. faecalis* can also suppress the immune response within an explanted tissue cell mixture from human periodontal ligaments by upregulating IL-1 receptor-associated kinase-M (IRAK-M), a negative regulator of TLR signaling, which is an additional potential mechanism of macrophage suppression [112]. Another strategy to evade immune surveillance is to avoid engulfment by macrophages. The enterococcal leucine-rich protein A (ElrA) belongs to the internalin family of proteins and prevents *E. faecalis* from phagocytosis or sensing by macrophages, and its genetic presence positively correlates with disease severity in a mouse model of peritonitis [113,114]. Upon phagocytosis, *E. faecalis* can survive intracellularly in macrophages from hours to days, which can be further

**Table 1.** *E. faecalis* immune-evading mechanisms in host infections

| Type of infection | Immune evading mechanisms | Involved bacterial factors | *In vivo* relevance | Ref. |
|---|---|---|---|---|
| Peritonitis | Inhibit phagocytosis by leukocytes and intracellular killing | Unknown | Promote polymicrobial infection | [109] |
| | Resist macrophage-mediated killing | OatA homolog | Persistent infection | [115] |
| | | PgdA homolog | Resist lysozyme | |
| | | MnSOD | Persistent infection<br>Resist oxidative stress | [116] |
| | Suppress autophagy | Unknown | Tested *in vitro*, implied *in vivo* | [117] |
| UTI | Suppress cytokine production | Unknown | Promote polymicrobial infection | [110] |
| | Resist neutrophil-mediated killing | Unknown | Persistent infection | [107] |
| Wound infection | Modulate cytokine production | Unknown | Persistent infection | [108] |
| IE | Avoid antibody detection | Biofilm formation | | [118] |
| | Degrade complement | GelE | | [119] |
| Colitis | Survive inflamed environment | Unknown | *E. faecalis* overpopulation | [44,45,120] |
| Bacteraemia | Resist oxidative stress | MnSOD | Stronger enzymatic activity in strains isolated from blood | [73,121] |

OatA, *Staphylococcus aureus* peptidoglycan *O*-acetyltransferase; PgdA, *Streptococcus pneumoniae N*-acetylglucosamine deacetylase; MnSOD, manganese-containing superoxide mutase; GelE, gelatinase; UTI, urinary tract infection; IE, infective endocarditis.

prolonged for biofilm *E. faecalis* [14,30,68]. *E. faecalis* is intrinsically resistant to lysozyme, oxidative stress, and low pH (Table 1), which contributes to the prolonged survival inside macrophages [115–117,121,122]. Similar to intracellular *E. faecalis* in neutrophils, intracellular survival in macrophages has also been proposed as a means of translocation from the GIT into the lymph system or bloodstream [68].

### Antibody

Antibodies are an integral part of humoral immunity, capable of both blocking adhesion and entry of a pathogen into tissues and opsonizing to promote phagocytosis by leukocytes and microbial clearance. Antibodies binding to *E. faecalis* can be found in healthy humans, possibly because *E. faecalis* is part of the commensal microbiota so the host immune system is naturally educated to recognize it. For instance, secreted IgA reactive to *E. faecalis* can be detected in infant saliva within the first month of life [123]. Naturally acquired antibodies against four serotypes of *E. faecalis* capsular polysaccharides have been reported in healthy human sera [124]. Most of these antibodies are cross-reactive to other serotypes or even other bacterial species such as *Streptococcus mutans*, and it is not clear which species stimulated antibody production in the first place. Nonetheless, these studies indicate that humoral immunity plays a protective role in an *E. faecalis* model of infection [125].

However, *E. faecalis* can also evade antibody-driven immunity. *E. faecalis* secrete an endoglycosidase that degrades human IgG, but its role in infections has not been investigated [126]. Certain types of capsule can mask *E. faecalis* lipoteichoic acid from antibody detection, reducing both phagocytosis by macrophages and pro-inflammatory cytokine production [127,128]. In infectious endocarditis, *E. faecalis* has multiple strategies to avoid or even hijack the functions of antibodies for its own benefit. In a large percentage of enterococcal infectious endocarditis, vegetations can be found [8]. A vegetation is a biofilm-like community that develops on heart valves containing bacteria, host platelets, and fibrin. Once enclosed by the vegetation, *E. faecalis* can avoid antibody recognition and therefore grows safely within the protected vegetation [118]. *E. faecalis* expressing AS, on the other hand, utilizes antibodies to enhance the bacterial aggregation, and this interaction may contribute to infective endocarditis severity [79].

### Complement

The complement system is another important part of humoral immunity that can opsonize microbes and, in some cases, directly drive cytotoxicity toward bacteria. Neutrophil-mediated clearance of *E. faecalis* strongly depends on a functional complement system and neutrophil bactericidal activity is significantly reduced when serum is heat-treated to inactivate complement [101]. *E. faecalis* capsular polysaccharides limit opsonization by C3, a complement component required for the activation of all three complement pathways, thus preventing macrophage activation and reducing TNF-α production [120]. It is worth noting that the level of C3 deposition is comparable among all strains that have been tested, but two out of four serotypes avoid antibodies that detect bound C3, suggesting that C3 is somehow masked in these serotypes [127]. *E. faecalis* can also degrade C3 by secreting extracellular GelE, preventing complement activation and opsonization, and reducing phagocytosis by human polymorphonuclear leukocytes [129]. A similar effect occurs in an experimental infective endocarditis model where *E. faecalis* expressing GelE display reduced neutrophil infiltration, possibly by degrading C5a, leading to an eventual increase in enterococcal numbers in the heart [119]. Moreover, *E. faecalis* inserts certain wall teichoic acids into the cell wall, reducing the deposition of two key lectin pathway components, mannose-binding lectin and mannose-binding lectin-associated serine protease-2 (MASP-2), which results in a lower opsonophagocytic killing by human neutrophil [130].

## The impact of immune evasion in *E. faecalis*-mediated infections

*E. faecalis* causes a variety of infections, most of which share some common traits. One feature of *E. faecalis* infections is they are often persistent and hard to eliminate (Table 1). *E. faecalis* is one of the predominant microbes identified in chronic wound infections (wound that fails to heal within the normal trajectory), which include diabetic foot ulcers (11.6%–29%) and chronic venous leg ulcers (71.1%) [131–134]. This frequency may be partly due to the ability of *E. faecalis* to survive in inflamed environments. In IBD patients, For instance, macrophages exhibit a pro-inflammatory profile with increased expression of IL-12, IL-23, TLR-2, and TLR-4 along with lower levels of IL-10 [135]. IBD patients also have higher IgG levels in serum than healthy individuals, which enhance the respiratory burst of neutrophils against *E. faecalis* [120]. Despite such immune activities, the number of *E. faecalis* still increases in the gut of IBD patients [45]. Catheter implantation during CAUTI also elicits a strong neutrophil infiltration and induces the production of several pro-inflammatory cytokines (IL-6, G-CSF, KC), yet *E. faecalis* can overcome the implant-induced inflammation and colonize the catheter and urinary tract organs [107]. In an excisional mouse wound infection model, *E. faecalis* infection induces production of chemoattractant chemokines for

neutrophils (CXCL1, CCL3) and for monocyte and dendritic cells (CCL2), and elicits neutrophil and macrophage recruitment, yet *E. faecalis* can persist at the wound site for up to 7 days. Surprisingly, production of proinflammatory cytokines such as IL-1α, IL-1β, TNF-α, and IL-17 are comparable to mock-infected animals 3 days after infection, although up to $10^5$ *E. faecalis* can be detected [108]. These reports indicate that *E. faecalis* can resist the immune responses and may even be favored in certain situations, while other microbes are cleared by immune activities.

*E. faecalis* infections are also often polymicrobial in nature, as reported for peritonitis (45% are polymicrobial), UTI (110/114), and diabetic foot ulcer (60/65) [58,136–138]. When infecting dental pulp, *E. faecalis* induces stronger TNF-α and IL-1β production compared to *Streptococcus anginosus* alone, and the mixed-species infection of both *E. faecalis* and *S. anginosus* gives rise to an additive immune response compared to either single infection [139]. In the peritoneum, co-infection with a high inoculum of *E. faecalis* augments *E. coli* survival as long as 6 days after infection as compared to a low *E. faecalis* co-inoculum or to *E. coli* mono-infection. Increased *E. coli* titers occurred in both peritoneal fluid and in peritoneal cells, suggesting that *E. faecalis* can inhibit clearance of other pathogens, despite the fact that *E. faecalis* actually elevates inflammation and leukocyte infiltration in this niche [109]. In other niches, such as the catheterized bladder, *E. faecalis* can suppress innate immune responses and promotes colonization of co-infecting UPEC; however, the underlying mechanism is still unclear [110].

## Future Prospects

While immune-based therapeutic strategies are promising for *E. faecalis* infections, it is still unclear which components of the immune system are most important for bacterial elimination. In the intestinal environment, innate antimicrobial peptides are important for controlling colonization by vancomycin-resistant enterococci, and antimicrobial peptide gene expression can be enhanced by probiotics like *Lactobacillus paracasei* [140]. This demonstrates that augmenting immune responses may help prevent or clear increasingly antibiotic resistant enterococcal infections. Neutrophil-mediated killing is clearly important in other infections; however, the contradiction between strong *in vitro* killing of *E. faecalis* by neutrophils and weak *in vivo* clearance in the presence of neutrophils is not yet understood [102,107–109,124]. The antimicrobial factors within neutrophils that are responsible for *E. faecalis* killing also remain unidentified, although the bacterium is resistant to most of the components tested such as MPO, lysozyme, and low pH [105,115–117]. Another intriguing question is what are the deciding factors that determine the difference in survival rate of *E. faecalis* between opsonized and unopsonized neutrophils? Comparing the signaling pathways in differentially activated neutrophils may decipher which antimicrobial mechanisms are most efficient for *E. faecalis* clearance.

Considering the importance of antibodies in neutrophil-mediated killing, vaccines that induce protective antibody production could serve as an alternative treatment against the multi-drug resistant *E. faecalis* by augmenting opsonophagocytic killing [141]. By targeting highly homologous antigens shared between *E. faecalis* and *E. faecium*, such as shared capsular polysaccharides and metal ABC transporter lipoproteins, vaccines could provide protection against both members of the *Enterococcus* genus [125,142]. Another approach is to target *E. faecalis* adhesins as an anti-virulence strategy. Attachment of *E. faecalis* to catheter implants and subsequent biofilm formation is critical to CAUTI pathogenesis, a process that is improved if the catheter is first coated with fibrinogen. The *E. faecalis* endocarditis- and biofilm-associated pilus (Ebp) is responsible for binding to fibrinogen, so antibodies blocking Ebp binding domain can prevent the biofilm formation on the catheter surface [143]. Despite these promising results, more studies are needed to identify additional targets for protective antibodies to be generated, suitable for the production of multivalent vaccine strategies [144]. For instance, the immunoglobulin isotype induced by vaccination must be considered. To promote immunity against systemic *E. faecalis* infection, generation of IgG would be optimal since this is abundant in serum. IgA, on the other hand, can penetrate the mucus layer to bind *E. faecalis* to potentially block attachment and biofilm formation, preventing GIT overgrowth and infection. Either way, vaccination should not disrupt the homeostasis of microbiota since IgG is not secreted into intestinal lumen and IgA is more likely to serve as a neutralizing antibody rather than an immunogenic opsonin, which could otherwise result in dysbiosis [92,93].

There are still many open questions about the physiology of *E. faecalis* during infection. The intracellular lifestyle, for instance, has been described in several cell types, but remains unclear whether and how it contributes to disease development [69,77]. In addition, understanding how *E. faecalis* actively suppresses macrophage activation may inform immunotherapeutic strategies that could overcome this suppression and promote macrophage-mediated clearance. Enhanced *E. faecalis* growth in inflamed tissues is also not well understood, whether is there an environmental cue that promotes *E. faecalis* growth or whether it they continue to grow in this environment because they

are simply more resilient [45]. Understanding these mechanisms could provide insights that benefit the development of potential treatments or prophylaxis.

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GIT, gastrointestinal tract; CAUTI, catheter-associated urinary tract infection; UTI, urinary tract infection; IBD, inflammatory bowel disease; IL-10, interleukin-10; GelE, gelatinase; AS, aggregation substance; MPO, myeloperoxidase.

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