REVIEW ARTICLE

Modulation of host responses by oral commensal bacteria

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Immunomodulatory commensal bacteria are proposed to be essential for maintaining healthy tissues, having multiple roles including priming immune responses to ensure rapid and efficient defences against pathogens. The default state of oral tissues, like the gut, is one of inflammation which may be balanced by regulatory mechanisms and the activities of anti-inflammatory resident bacteria that modulate Toll-like receptor (TLR) signalling or NF-κB activation, or influence the development and activities of immune cells. However, the widespread ability of normal resident organisms to suppress inflammation could impose an unsustainable burden on the immune system and compromise responses to pathogens. Immunosuppressive resident bacteria have been isolated from the mouth and, for example, may constitute 30% of the resident streptococci in plaque or on the tongue. Their roles in oral health and dysbiosis remain to be determined. A wide range of bacterial components and/or products can mediate immunomodulatory activity, raising the possibility of development of alternative strategies for therapy and health promotion using probiotics, prebiotics, or commensal-derived immunomodulatory molecules.

Keywords: *immunomodulation*; *homeostasis*; *immunosuppression*; *NFκB inhibition*; *resident microbiota*; *dysbiosis*; *probiotics*

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Many human tissues support large, resident microbial populations (1) which confer significant benefits. While much of the evidence for the beneficial and homeostatic activities of the resident microbiota is derived from studies of the gut, this also informs our understanding of oral ecology and oral host–microbe homeostasis. Although beneficial under normal circumstances, imbalances in the resident human microbiota, or our responses to them, (dysbiosis) make a major contribution to the incidence of some significant, multifactorial diseases (2, 3). The role of dysbiosis in the development of periodontal diseases and dental caries has long been recognised, in that they are due to alterations in the balance and composition of the resident plaque communities. In periodontal diseases, tissue damage occurs due to the failure of the immune system to limit both the microbial community and the local host immune response (4, 5). In health, heavily colonised tissues do not normally enter a state of permanent damaging inflammation, and retain the ability to respond adequately to pathogenic challenges. It is proposed that this balance is maintained in health by homeostatic mechanisms that include regulation or modulation of host responses by commensal organisms.

Immunomodulation by commensal bacteria

Commensal bacteria display pro-inflammatory and anti-inflammatory activities, and both are important in maintaining host–microbe homeostasis at heavily colonised sites. Some immunomodulatory commensals in the gut (termed autobionts) are able to regulate the activities, development, and/or deployment of host immune cells, providing subtle effects on immune responses and immune status (6).

Effects on cells of the immune system

Multiple commensal species induce tolerance within the gut, limiting inflammatory responses by ensuring an appropriate balance of intestinal T cell populations (7, 8). In the case of *Bacteroides fragilis*, extracellular polysaccharide (PSA) stimulation of CD4+ T cells via TLR-2 is the mechanism, whereby tolerance is induced through initial TREG expansion. Commensal lactic acid bacteria regulate communication between NK cells and dendritic cells, thereby helping to direct the adaptive immune response in the gut (9). In the mouth, neutrophils are key in the defence of the gingival tissues, and chemokines such as CXCL1, 2, and CXCL8 (IL-8) establish a gradient of neutrophils in gingival tissues and gingival crevicular fluid.

The balance of expression of neutrophil chemokine receptors such as CXCR1, 2, and 4 can also be modulated by chemokines and cytokines to favour neutrophil recruitment to mucosal tissue or homing to bone marrow. Resident bacteria in subgingival plaque may influence neutrophil deployment by regulating low levels of expression of intracellular adhesion molecule 1 (ICAM-1), E-selectin, and CXCL8; oral commensals also promote expression of IL-1β mRNA in the oral mucosa (10, 11). An absolute requirement for CXCR2 in periodontal neutrophil recruitment was recently reported, and commensal colonisation increased the recruitment of neutrophils to gingival tissues via the up-regulation of the CXCR2 ligand, CXCL2 (11).

Effects on inflammatory responses
Many gut commensal bacteria initiate pro-inflammatory responses and contribute to health by stimulating and 'priming' the immune system (12). Conversely, other commensal organisms inhibit or suppress epithelial cell inflammatory responses by a functional modulation of immunity through Toll-like receptor (TLR) or NOD-like receptor (NLR) expression and signalling, while others suppress inflammatory responses by inhibiting activation of NF-κB or by increasing the secretion of anti-inflammatory cytokines, such as IL10 (2, 13, 14).

The default position in the gut is thought to be one of inflammation, balanced by regulatory mechanisms and the activities of anti-inflammatory, or immunosuppressive members of the microbiota (3, 15). However, the widespread possession of anti-inflammatory ability by resident mucosal bacteria could be detrimental, by imposing an unsustainable burden on the host immune system and compromising the ability to respond effectively to pathogens (16). Indeed, suppression of host inflammatory responses is also a strategy employed by the red complex periodontopathogens *Porphyromonas gingivalis* and *Treponema denticola* (4, 17). However, *P. gingivalis* utilises multiple mechanisms to cause extensive inhibition of local immune responses, while the limiting, immunomodulatory effects of oral commensals are more subtle (11, 18).

Gingival tissues, in a similar manner to the gut, are probably normally mildly inflamed (19). Certain strains and species of commensal oral streptococci suppress epithelial cell cytokine expression (13, 20–23). The probiotic *Streptococcus salivarius* K12 down-regulated CXCL8 secretion from bronchial, skin, and oral keratinocytes (cell lines and primary cells), via inhibition of activation of NFκB (13). Oral strains of *S. salivarius* were later isolated that suppressed inflammatory responses in pharyngeal cells (24). A wide range of *S. salivarius* and *S. vestibularis* strains suppressed responses of intestinal epithelial cells and monocyte-like cells via NFκB inhibition, and *S. salivarius* inhibited inflammation *in vivo* (21, 22). *S. cristatus* also inhibited CXCL8 secretion by keratinocytes by modulating the activity of IκB-α, an inhibitor of NFκB (25).

Effector molecules causing immunosuppression
Dissecting the mechanisms underlying the immunomodulatory, particularly anti-inflammatory, ability of commensal bacteria will increase understanding of host–microbe homeostasis and health, and may also provide opportunities for developing therapeutic or health-promoting immunomodulatory molecules based on microbial components.

Metabolites
Metabolites from commensal bacteria may mediate immunomodulation and contribute to the balance of pro- and anti-inflammatory responses. Commensal lactobacilli with tryptophanase activity generate indole derivatives that can function as aryl hydrocarbon (AhR) ligands; AhR activation promotes anti-inflammatory T$_{REG}$ development (26). Short chain fatty acids (SCFAs; e.g. butyrate, propionate, acetate) are produced by members of the resident microbiota, and a range of immune cells are targets for SCFA-mediated immunomodulation by activation of GRP43 (which is highly expressed by neutrophils, macrophages, and monocytes), epigenetic control via inhibition of histone deacetylases and regulation of autophagy (7, 27, 28).

Proteins and peptides
Cell-associated and secreted proteins or peptides from various bacterial genera have been linked with immunosuppressive abilities. The most studied genus in this respect is *Lactobacillus,* and their abilities to inhibit NFκB activation or promote IL-10 secretion have been attributed to cell-associated and secreted peptides (14, 29). The cellular product mediating immunosuppression by two *S. salivarius* strains was a secreted peptide of <3 KDa (21).

Nucleic acids
Bacterial and viral DNA motifs (detected by TLR3, TLR7, and TLR9) differ in their ability to produce pro- or anti-inflammatory responses. The genomes of commensal and probiotic lactobacilli can be enriched in sequences that are immunosuppressive (15). Unmethylated CpG motifs from the resident microbiota, recognised by TLR9, may have a role in maintaining an appropriate balance of Th1/Th2 cells, and in supporting mucosal functions in health (30). Double-stranded RNA from intestinal lactic acid bacteria induces interferon-β production by dendritic cells via TLR3 activation, thereby promoting anti-inflammatory effects (31). Clustered regularly interspaced short palindromic repeats (CRISPR) sequences are often adjacent to *cas* genes (CRISPR-associated), which encode enzymes that can degrade and inactivate nucleic acids. CRISPR/Cas systems can also affect gene expression in the host bacterium, thereby

indirectly affecting immunomodulation, for example, by down-regulating pro-inflammatory lipoproteins (32). Although CRISPR/Cas systems have been detected in genomes of the commensal *S. mitis*, they are not present in the closely related pathogen *S. pneumoniae* (33). Uracil is pro-inflammatory; it is proposed that commensal bacteria do not secrete uracil while pathogens do and that uracil secretion is significant in determining host–microbe homeostasis at tissues colonised by bacterial communities (34).

Concluding comments

Understanding of the gut microbiome in gastrointestinal health or disease has shaped our views of host–microbiome interactions at other body sites, but it is important to consider that microbiomes at various sites are distinct from each other and are determined by the unique properties of, and host responses at, each site (35, 36). Thus, control of the immune response by commensal populations is compartmentalised. Darveau (4) has highlighted the differences between the anatomy and biology of gut epithelial tissues compared with periodontal tissues, and the distinct host defence strategies used at each. The contribution of oral commensals to the structure and function of periodontal tissues is more subtle than those seen in the gut, and the gingival epithelium is more porous and more exposed to microbes and their products than gut epithelia (11, 18). Thus, while we should learn from data emanating from studies of host responses to the resident gut microbiota and probiotics, it is essential that further studies are carried out with relevant oral organisms and tissues in order to better understand oral host–microbe homeostasis.

The resident communities at each site contribute to tissue complexity and have coevolved with their host to tune host requirements at each site and establish a threshold of activation required for immune fitness (37). Immunomodulatory commensals are held to be beneficial via both immunostimulatory and immunosuppressive mechanisms; most likely, the relative balance of pro-inflammatory and immunosuppressive resident organisms is critical for appropriate immune responses in the mouth, and maintenance of host–microbe homeostasis in a manner analogous to that proposed for the gut.

Up to 30–40% of resident streptococci isolated from the tongue or plaque were able to inhibit CXCL8 secretion (largely via inhibition of NFκB) from cells stimulated by flagellin, LL-37 or by oral pathogens such as *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (Devine et al., unpublished observations). The impact of such immunosuppressive populations on host–microbe homeostasis in the mouth is unknown, although transient reductions in CXCL8 secretion in the GCF of individuals with mild gingival inflammation were demonstrated following use of chewing gum containing immunosuppressive probiotic lactobacilli (38). The beneficial effects of commensal or probiotic organisms extend beyond the ability to modulate immune responses, to also include enhancement of mucin production and barrier function, induction of antimicrobial host defence peptides, promotion of angiogenesis and wound healing. The oral probiotic *S. salivarius* K12, which secretes bacteriocin-like inhibitory substances, not only down-regulated epithelial cell inflammatory responses, but also up-regulated hepcidin (an antimicrobial and iron regulating peptide), actively stimulated beneficial pathways including type I and II interferon responses, and exerted significant effects on the cytoskeleton and adhesive properties of the host cells (13). An appropriate balance of immunomodulatory commensals capable of exhibiting a combination of such beneficial and homeostatic properties may be essential for health.

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There is no conflict of interest in the present study for any of the authors.

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