# Commensal Communism and the Oral Cavity

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**Abstract.** The world we live in contains unimaginable numbers of bacteria, and these and other single-celled creatures represent the major diversity of life on our planet. During the last decade or so, the complexity and intimacy of the interactions which occur between bacteria and host eukaryotic cells during the process of infection have begun to emerge. The study of such interactions is the subject of the new discipline of cellular microbiology. This intimacy of bacteria/host interactions creates a major paradox. The average human being is 90% bacteria in terms of cell numbers. These bacteria constitute the commensal or normal microflora and populate the mucosal surfaces of the oral cavity, gastrointestinal tract, urogenital tract, and the surface of the skin. In bacterial infections, much of the pathology is due to the release of a range of bacterial components *(e.g.,* modulins such as lipopolysaccharide, peptidoglycan, DNA, molecular chaperones), which induce the synthesis of the local hormone-like molecules known as pro-inflammatory cytokines. However, such components must also be constantly released by the vast numbers of bacteria constituting the normal microflora and, as a consequence, our mucosae should constantly be in a state of inflammation. This is patently not the case, and a hypothesis is forwarded to account for this "commensal paradox", namely, that our commensal bacteria and mucosal surfaces exist in a state of bio-communism, forming a unified "tissue" in which interactions between bacteria and epithelia are finely balanced to ensure bacterial survival and prevent the induction of damaging inflammation. Evidence is emerging that bacteria can produce a variety of proteins which can inhibit the synthesis/release of inflammatory cytokines. The authors predict that such proteins are simply one part of an extensive signaling system which occurs between bacteria and epithelial cells at mucosal surfaces such as those found in the oral cavity.

**Key words:** bacteria, cytokines, periodontal disease, modulins, cytokine networks.

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## Introduction

Ideas are generally the children of their time—or in the jargon of philosophers of science—the prevailing paradigm (Kuhn, 1962). Francis Fukuyama's book **The End of History and the Last Man** (Fukuyama, 1992) has proposed that the progression of history as suggested by Hegel and by Marx, leading inexorably to liberal democracy or communist states, respectively, has come to an end with the demise of communism. Communism as a political philosophy appears to have had its day. Nevertheless, could the ancient concept of communist societies—providing for equal sharing of all work, according to ability, and all benefits, according to need—be a paradigm for the interactions which exist in the oral cavity (and indeed, at all mucosal surfaces) between its microflora and the host tissues?

## A new view of the microbial world

The *fin de siècle* finds us rapidly moving from a state of complacency about bacterial infections to a state of panic, as more and more bacteria become resistant to antibiotics. Bacterial diseases such as tuberculosis and outbreaks of infections with bacteria such as *Neisseria meningitidis* and *Escherichia coli* fill our daily papers.

Just as we, in the developed world, begin to regain our fear of 'microbes', recent advances in molecular genomics are revealing just how enormously diverse the microbial world is (Roberts, 1996; Pace, 1997). Most readers will be familiar with the five Kingdoms of life: animals, plants, fungi, protists (protozoa), and monera (bacteria). However, during the past decades, with the determination of the ribosomal RNA (rRNA) gene sequences of many species, a new view of the relatedness of all organisms has emerged. Three primary lines of evolutionary descent are now recognized and grouped into so-called "superkingdoms" or "domains": Eucarya (eukaryotes), Bacteria (initially eubacteria), and Archaea (initially archaebacteria) (Fig. 1). This new molecular view of evolution demotes fungi, plants, and animals (including *Homo sapiens*) to a side street in the "superkingdom" Eucarya (Fig. 1). The sequencing of rRNA genes is also revealing the presence of unsuspected microbial diversity in the biosphere. It is well-known that

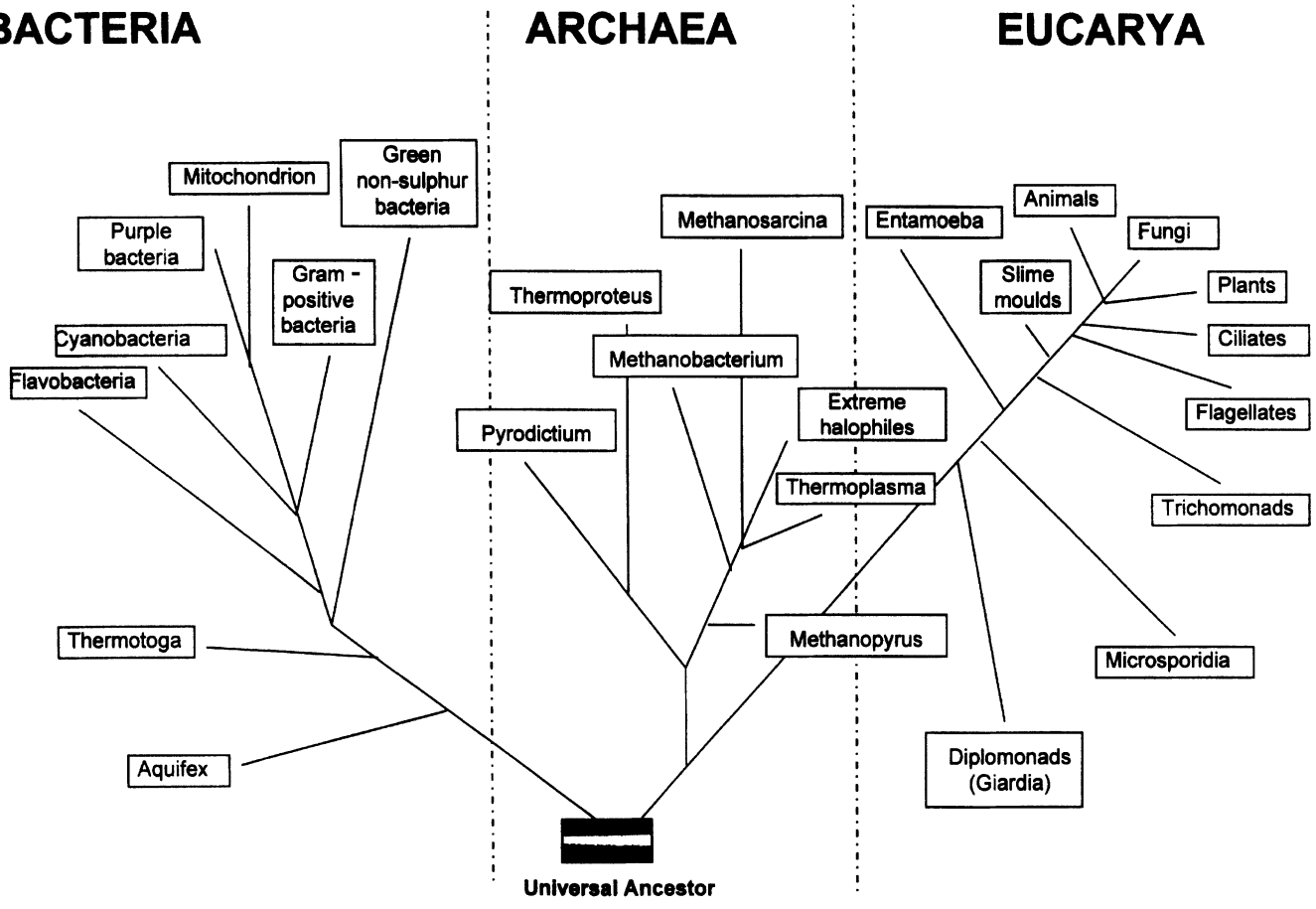


**Figure 1.** The three domains or superkingdoms of life as defined by comparative ribosomal RNA sequencing. This phylogenetic tree shows that Eucarya are not of recent origin but are as ancient as the prokaryotic lineages.

more than 99% of organisms seen microscopically are not able to be cultivated (Amann *et al.*, 1995). Sampling of environments worldwide, with rRNA sequencing used to detect novel organisms, has revealed the enormous diversity of Bacteria and Archaea. The oceans are alive with Bacteria and Archaea, as is the very crust of the earth. Thus, the conclusion from these ribosomal RNA safaris is that we know almost nothing about the microbial life inhabiting our planet. Only about 5000 non-eukaryotic organisms have been formally described, and this is probably only a fraction of a percent of all the microbial species present on earth (Pace, 1997).

It is now abundantly clear that we live in a world dominated by micro-organisms which include unimaginable numbers of bacteria. Bacterial diseases probably kill between 10 and 20 million people worldwide each year. In spite of this, only a relatively small number of bacteria cause infection in man. However, a very large number of bacteria, the so-called normal or commensal microflora, colonize multicelled animals and live in harmony with them. In *Homo sapiens*, it is estimated that up to 1000 different bacterial species live in concord with the epithelial mucosal surfaces of the body (Tannock, 1995), and given what has been said above, this is probably an underestimate. The major unanswered question, which arises from the finding of such a diverse microflora in man, is, what maintains the harmony between the 'superkingdoms' Bacteria and Eucarya?

## The paradoxical normal microflora

One of the most stunning statistics is that the average human body, composed as it is of $10^{13}$ eukaryotic cells, contains $10^{14}$ bacteria (the normal, or commensal, microflora). Thus, purely on numbers of cells, we are 90% bacteria, and perhaps Linnaeus should have named us *Homo bacteriens* (Henderson and Wilson, 1996). These commensal bacteria are present on the epithelial surfaces of the skin and on the mucosal surfaces of the oral cavity, respiratory tract, esophagus, gastrointestinal tract, and urogenital tract (Tannock, 1995). The oral cavity contains a large proportion (an estimated 300 to 500 bacterial species) of the body's commensal bacteria. A number of bacteria which populate the normal microflora, including those of the oral cavity, are opportunistic pathogens capable of injuring or even killing the carrier, if conditions permit—organisms like *Staphylococcus aureus, Haemophilus influenzae, Neisseria meningitides,* and *Streptococcus pneumoniae.* A number of the species implicated in the periodontal diseases can also be classified as opportunistic pathogens. There is growing evidence that oral bacteria may contribute to systemic

diseases. The best-known example is the involvement of the Gram-positive oral organisms *Streptococcus sanguis* and *S. oralis* in infective endocarditis (Durack, 1995). However, organisms involved in the periodontal diseases may also be linked to the development of coronary heart disease (Beck *et al.*, 1996).

Bacteriology has naturally focused on those organisms that cause disease. With the realization that antibiotic resistance is becoming a major problem, there has been a renaissance of interest in how bacteria actually cause pathology. This has given rise to a new "interface" science called Cellular Microbiology (a combination of microbiology, molecular biology, and cellular biology), which is shaping a new paradigm of bacterial/host interactions, in which bacteria interact intimately with eukaryotic cells to produce pathology. With our increasing understanding of the closeness of the relationships between bacteria and eukaryotic cells, the question arises, What prevents the organisms constituting the normal microflora from causing disease? This, in the authors' opinion, is a major paradox whose solution, we believe, lies precisely in the growing understanding of the interactions which must occur between bacteria and host cells for disease to be caused.

## Cytokine networks and homeostasis

The homeostatic control of multicellular organisms depends on the integration of individual cell activity. Such integration, in turn, depends on effective information transfer at local, regional, and 'global' levels. Global signaling is the province of the nervous system and the endocrine hormones, while regional and local communication is controlled by a growing population of protein and peptide signals known as cytokines. Well over 100 cytokines have now been described (Henderson *et al.*, 1998a). Cytokines, unlike enzymes, have no inherent biological activity but must bind to specific high-affinity cell-surface receptors to produce their effects. These proteins rarely, if ever, work individually, and the common denominator in cytokine biology is the cytokine network, which describes the interaction of multiple cytokines with cells. It must be emphasized that some cytokines have pro-inflammatory actions (*e.g.*, interleukin [IL]-1, tumor necrosis factor [TNF]-α), and such activity needs to be balanced by cytokines, such as IL-10 and transforming growth factor (TGF)-β, which have anti-inflammatory actions (Henderson *et al.*, 1998b). Indeed, many cytokines fulfill the contradictory functions of being both the controlling factors in host defense against infection and the major cause of tissue pathology in infection (Henderson *et al.*, 1996a,b, 1998a). A good example is the often-lethal condition of septic shock, in which the rapid production of the pro-inflammatory cytokine TNF-α—which is unable to be controlled by the counter-regulatory cytokine IL-10—results in hypotension, end-organ failure, and death (Rietschel and Wagner, 1996). Septic shock is a good example of what happens if there is a failure of the homeostatic network regulation of cytokines (Henderson *et al.*, 1998b).

Septic shock is caused by the release of lipopolysaccharides (LPS), the bacterial components that most readers would recognize as cytokine inducers. However, during the past decade, many bacterial components—intracellular and exported proteins, cell wall proteins, carbohydrates, lipoproteins, and low-molecular-mass components—have been found to be able to induce eukaryotic cells to produce cytokines (Henderson and Wilson, 1995; Henderson *et al.*, 1996a,b, 1998a). Even bacterial DNA appears to be a potent inducer of cytokine synthesis (Sparwasser *et al.*, 1997). This range of components is certainly produced by oral bacteria (Wilson *et al.*, 1996). Surprisingly, the most potent cytokine-inducing molecules produced by bacteria are the exotoxins, which are generally thought of in terms of eukaryotic cell cytotoxicity. However, many exotoxins are more active as cytokine inducers than they are as cell toxins (Henderson *et al.*, 1997).

We initially termed such bacterial cytokine-inducing molecules 'modulins', since the induction of host cell cytokine synthesis by such molecules modulates cell behavior, and classified them as virulence factors in the same way as, for example, aggressins and impedins produce cellular changes which aid microbial virulence. Such cytokine-inducing molecules (*e.g.*, LPS, DNA, secreted proteins, *etc.*) are clearly produced by members of the normal microflora which exist in contact with mucosal epithelia. In turn, the epithelial cells of mucosal surfaces are able to produce a wide range of pro-inflammatory cytokines and, indeed, have been proposed to act as the watchdogs of the immune defenses against bacteria (Eckmann *et al.*, 1995). Given the ability of the normal microflora to produce and release cytokine-inducing molecules, coupled with the ability of epithelial cells to respond to such activating substances by producing pro-inflammatory cytokines, what prevents the surface epithelia from being in a constant state of inflammation? This is a paradox whose solution could have profound consequences for our understanding of bacteria/eukaryote interactions and, in consequence, for the treatment of infectious diseases.

## Cytokine network control by infectious organisms

Further insight into the interactions among the normal microflora, mucosal surfaces, and cytokine networks has come from cytokine transgenic knockouts. Mice in which the gene for the lymphokine, IL-2, has been inactivated suffer from a lethal colitis and produce auto-antibodies to constituents of the colon. However, if animals are bred in gnotobiotic conditions, they fail to develop colitis (Sadlack *et al.*, 1993). This suggests that the failure to produce and perpetuate the correct cytokine network(s) in the colonic epithelium and mucosa results in the development of an inflammatory response to the normal microflora. It is not known if this inflammatory response is due to the ability of IL-2-deficient mice to respond to bacterial constituents which are released normally, or whether the alterations in cytokine network homeostasis in the colon of the knockout animals cause a switching-on of virulence factor production (or, indeed, a switching-off of inhibitory factor production) by the commensal bacteria in the colon (Fig. 2).

One of the most successful groups of infectious organisms are the viruses. In the past decade, it has become

evident that many viruses rely on genes encoding cytokine-network-modulating proteins for their survival. These cytokine-modulating proteins have been termed virokines and fall into a number of classes (Table 1). One obvious mechanism for preventing the spread of viral infection is for infected cells to undergo apoptosis or programmed cell death. At least 12 proteases, termed caspases, are now recognized to induce apoptosis when overexpressed (Takahashi and Earnshaw, 1996). The first caspase to be discovered is also able to catalyze the cleavage of the inactive 31-kDa precursor of the pro-inflammatory cytokine, IL-1β (pro-IL-β) to the active 17-kDa form. This protease was originally termed ICE (interleukin-1β converting enzyme). Viruses encode serpin-like protease inhibitors which inhibit members of the caspase family. For example, the protein termed crmA inhibits ICE and decreases the host inflammatory response (Ray *et al.*, 1992). Other cytokine-network-modulating viral proteins (virokines) include soluble forms of cytokine receptors (*e.g.*, the IL-1 receptor [Alcami and Smith, 1992]), which act as cytokine antagonists and homologues of the anti-inflammatory cytokine, IL-10 (Smith, 1994). One important finding is that the deletion of certain of these virokine genes can produce a mutant virus which is lethal to the host (Alcami and Smith, 1992). This suggests that the virokines may have co-evolved to enable the host to survive its own inflammatory antiviral response. These virokines are part of a system which infectious organisms use to evade the immune defenses of the host (see Mims *et al.*, 1995).

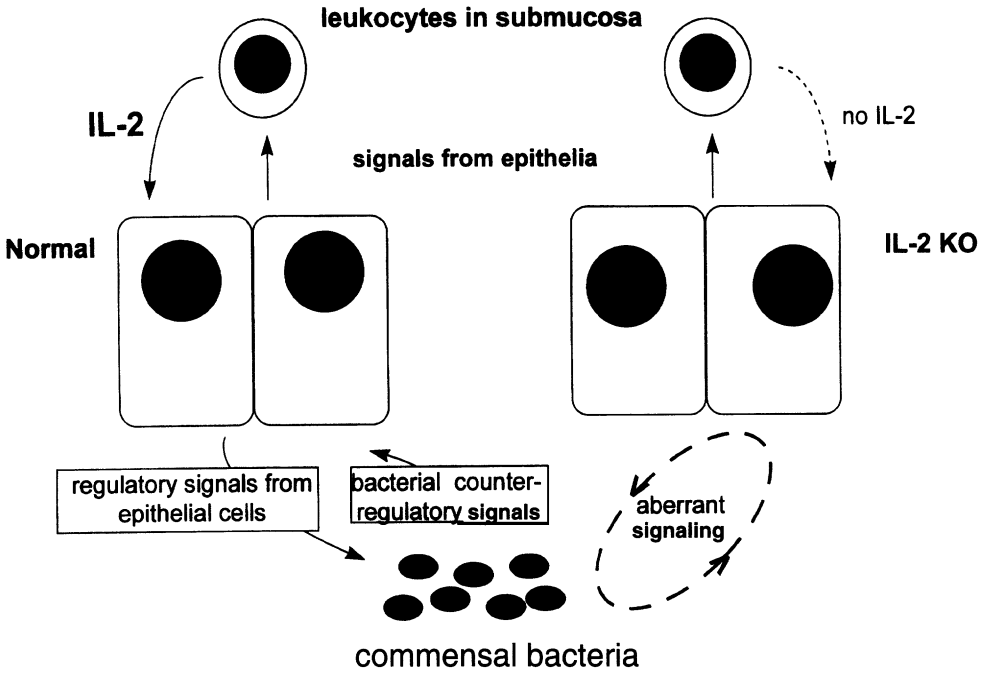


**Figure 2.** Possible interactions between the mucosal epithelium and commensal bacteria in normal and IL-2 knockout mice. In animals with normal levels of IL-2, the cytokine networks in the submucosa, which contains many lymphoid and myeloid cells, may feed back onto the epithelium to produce signals which interact with the commensal bacteria and either induce such bacteria to produce anti-inflammatory proteins or switch off their production of pro-inflammatory proteins. In the absence of appropriate submucosal cytokine networks, the epithelial/bacterial signaling is aberrant, and the result is the generation of inflammatory signals from the normal microflora of the bowel.

## Bacterial control of cytokine networks

Many structural bacterial macromolecules, such as LPS, peptidoglycan, and DNA, can induce cytokine synthesis (Henderson *et al.*, 1998a). Other intracellular molecules, some of which are obligatory for cell survival, and therefore common to all bacteria, also promote cytokine production. Perhaps the most fascinating example of such molecules are the chaperonins. During studies to define the nature of the osteolytic surface-associated activity of the oral bacterium *Actinobacillus actinomycetemcomitans*, the authors identified this activity as the molecular chaperone, chaperonin 60. These chaperonins are potent inducers of bone resorption (Kirby *et al.*, 1995; Meghji *et al.*, 1997) and of cytokine synthesis (Tabona *et al.*, 1998). Importantly, even peptides derived from the chaperonins are capable of inducing bone resorption and cytokine synthesis (Meghji *et al.*, 1997; Tabona *et al.*, 1998). Thus, even after proteolysis, the chaperonins are still capable of inducing the synthesis of cytokines. Molecular chaperones are generally regarded as intracellular proteins responsible for inhibiting protein denaturation in cells (van Eden and Young, 1996). However, there is increasing evidence that the chaperonins can exist at the surfaces of both bacteria and eukaryotic cells, and can even be released by cells (Henderson *et al.*, 1996c; Herzberg, 1996). It is conceivable that the intracellular protein-folding molecular chaperones have a completely different range of biological functions once released from cells. We also found a cytokine-modulating 2-kDa peptide on the surface of *A. actinomycetemcomitans* which potently stimulated human gingival fibroblasts to transcribe the gene for IL-6. However, such transcription did not require the prior transcription of IL-1 or tumor necrosis factor (TNF), as is normally the case. Thus, this peptide appears to be unique in its capacity for independent regulation of the transcription of the IL-6 gene (Reddi *et al.*, 1996), and, as is described below, this may play a role in controlling the antibacterial response of the host.

Another mechanism by which bacteria may exert control over cytokine networks is by the release of proteinases (reviewed in Henderson *et al.*, 1996b). We have recently shown that *Porphyromonas gingivalis* releases proteases able

**Table 1.** Cytokine-modulating genes and gene products produced by viruses

| Virus | Viral Protein | Host Homologue | Function |
|---|---|---|---|
| Cowpox | crmA | serpin-type protease inhibitor | inhibits ICE/blocks IL-1β synthesis |
| Baculovirus | p35 | protease inhibitor | inhibits ICE/blocksIL-1β synthesis |
| Vaccinia | B15R | IL-1β receptor | inhibits IL-1β activity |
| HVSa | ORF78 | soluble IL-8 receptor | inhibits IL-8 activity |
| HCMV | US28 | soluble IL-8 receptor | blocks chemokine activity |
| Myxoma | T2 | soluble TNF receptor | blocks TNF activity |
| Myxoma | T7 | soluble IFNγ receptor | blocks IFNγ activity |
| Myxoma | SERP-1 | a serine proteinase inhibitor | inhibits ICE? |
| Myxoma | Myxoma growth factor | epidermal growth factor-like proteins | stimulates cell growth |
| SFV | T2 | soluble TNF receptor | blocks TNF activity |
| EBV | BCRF1 | IL-10 | anti-inflammatory |
| EHV2 | IL-10-like protein | IL-10 | anti-inflammatory |
| Pox viruses | | EGF, TGFα | cell growth promoters |
| KSHV | v-chemokines | MIP-I, MIP-II | ? |
| KSHV | v-IL-6 | IL-6 | inhibits apoptosis? |
| KSHV | v-IRF | IRF | ? |

[a] Abbreviations: EBV, Epstein Barr virus; IRF, interferon regulatory factor; EHV2, equine herpes virus type 2; MIP, macrophage inflammatory protein; HVS, herpesvirus saimiri; HCMV, human cytomegalovirus; KSHV, Kaposi's sarcoma-associated herpesvirus, also known as human herpes virus-6; SFV, Shope fibroma virus.

to neutralize the activity of both pro- (IL-1β) and anti-inflammatory (IL-1 receptor antagonist, IL-1ra) cytokines, and cytokines (*e.g.*, IL-6) with both actions (Fletcher *et al.*, 1997, 1998). Thus, bacteria implicated in the periodontal diseases appear to have the capacity to modulate cytokine networks.

Non-oral bacteria have also produced proteins which can modulate cytokine networks. The regulation of intracellular cyclic nucleotides is believed to be a major controlling factor in the synthesis of certain cytokines, particularly IL-6 and TNFα (Zhang *et al.*, 1988). Increased intracellular levels of cAMP are thought to promote the synthesis of IL-6 but block the formation of TNF (Renz *et al.*, 1988). Certain bacterial toxins with ADP-ribosylating activity upregulate the activity of adenylyl cyclase and therefore raise intracellular cAMP levels. Cholera toxin is a potent stimulator of IL-6 production but inhibits TNF synthesis (Leal-Berumen *et al.*, 1996; McGee *et al.*, 1996). Pertussis toxin inhibits the B-cell and macrophage response to LPS and is a potent inhibitor of IL-1 synthesis (Jakway and DeFranco, 1986). Likewise, anthrax toxin edema factor, which is an adenylyl cyclase, stimulates monocyte IL-6 synthesis but inhibits TNFα synthesis (Hoover *et al.*, 1994). The role of IL-6 in inflammation and infection is still unclear. A study of IL-6 knockout mice has proposed that IL-6 has an overall protective effect in *E. coli* infections but no role in LPS-induced septic shock (Dalrymple *et al.*, 1996). Thus, it is possible that bacterial exotoxins selectively inducing IL-6 synthesis could act to increase the antibacterial actions of the host, and this may play some role in controlling the normal microflora. Other bacterial exotoxins have inhibitory effects on cytokine synthesis (reviewed in Henderson *et al.*, 1998a), although it is unlikely that these molecules will play a role in controlling normal microflora/host cytokine networks.

*Yersinia enterocolitica* produces a TNF-inhibiting protein called Yop B (Beuscher *et al.*, 1995), *Salmonella typhimurium* produces a protein that inhibits IL-2 synthesis (Matsui, 1996), and *Brucella* species produce a TNF-inhibiting protein (Caron *et al.*, 1996). These proteins are presumably involved in the evasion of host immune responses.

Thus, bacteria can stimulate or inhibit cytokine production through various released components. The latter have presumably evolved to downregulate immune and inflammatory mechanisms. Is there any evidence that bacteria comprising the normal microflora can do the same? It must be borne in mind that, since such bacteria do not cause disease, they have received much less attention than classic pathogenic species.

Klapproth and co-workers (1995) have reported that certain pathogenic *E. coli* strains produce a protein or proteins which inhibit lymphokine production (IL-2, IL-4, IL-5, and IFN-γ) by peripheral blood mononuclear cells. However, there was no inhibition of the pro-inflammatory cytokines IL-1β, IL-6, IL-12, or RANTES or of the anti-inflammatory cytokine IL-10. Indomethacin did not block the cytokine-inhibitory activity, showing that the mechanism was not *via* prostaglandin-induced upregulation of intracellular cAMP levels. The cytokine-modulating activities of *A. actinomycetemcomitans* have been described earlier, and a 14-kDa lymphokine-inhibiting protein has been isolated by Kurita Ochiai and Ochiai (1996). In addition to these proteins, *A. actinomycetemcomitans* also produces a leukotoxin, which kills neutrophils and monocytes and, in consequence, is immunosuppressive (Rabie *et al.*, 1988), and a 60-kDa immunosuppressive protein which appears to act on B-lymphocytes (Shenker *et al.*, 1990). The various bacterial proteins which act to inhibit cytokine synthesis have been tabulated in Table 2.

While the above studies reveal that certain bacteria produce proteins able to downregulate immune and inflammatory mechanisms, few studies have determined whether these molecules are effective when they are associated with intact organisms. A recent comparative study of the cytokine-inducing activity of Gram-negative and -positive bacteria is therefore of interest (Frieling *et al.*, 1997). Most of the Gram-negative species tested (*E. coli, N. meningitides, N. gonorrhoeae*) were between 100-fold and 1000-fold more potent than the Gram-positive species (*Staph. aureus, Strep. pyogenes, Strep. pneumoniae, Enterococcus fecalis*) in inducing the release of IL-1β and IL-6. Gram-negative bacteria preferentially induced the production of the anti-inflammatory cytokine, ILI receptor antagonist, although the differences in potencies between the two groups of organisms were less marked. Such differences may be attributable to variations in the potencies of either pro- or anti-inflammatory molecules produced by the bacteria. Of particular interest was the finding that *B. fragilis*, one of the dominant members of the normal colonic microflora, behaved in a manner similar to that exhibited by the Gram-positive species (the majority of which are also members of the normal microflora), in that it induced very low levels of pro-inflammatory cytokines. We would hypothesize that the reason for the low pro-inflammatory cytokine-inducing activity of *B. fragilis, Staph. aureus, Strep. pyogenes, Strep. pneumoniae,* and *Enter. fecalis* is that, as members of the normal microflora, they have evolved means of down-regulating pro-inflammatory cytokine networks to live in harmony with their hosts.

It is expected that these few bacterial proteins described above will simply be the tip of an extremely large 'iceberg' of bacterial cytokine network-modulating proteins. Such proteins may have evolved initially by particular bacteria to evade host immune responses. Over time, the bacterium may have further evolved from a parasite into a commensal organism, and the cytokine-modulatory proteins may then assume a homeostatic function, acting to control host cytokine networks. We postulate that in addition to tissue networks of cytokines acting in a regulatory manner, there will be interacting supernetworks of cytokine-like molecules at epithelial surfaces which will act to regulate the tissue responses to the commensal bacteria. It is likely that other epithelial-derived proteins and peptides will play roles in these supernetworks (Fig. 3).

**Table 2.** Bacterial proteins inhibiting cytokine synthesis

| Protein | Cytokine whose Synthesis is Inhibited |
|---|---|
| Cholera toxin | TNF (not IL-6 or TGFβ) |
| Pertussis toxin | IL-1 |
| Anthrax edema toxin | TNF (stimulates IL-6) |
| *Pseudomonas aeruginosa* exotoxin A | IL-1, TNF lymphotoxin, γ-IFN |
| Botulinum toxin type D | TNF |
| *Yersinia enterocolitica* YopB | TNF (no effect on IL-1 or IL-6) |
| *Proteus mirabilis* 39-kDa protein | IL-1 |
| *S. typhimurium*-derived inhibitor of T-cell proliferation (STI) | IL-2 (stimulates γ-IFN) |
| *Brucella*-derived protein | TNF (not IL-1 or IL-6) |
| Enteropathogenic *E. coli* proteins | IL-2, IL-4, IL-5, γ-IFN (not IL-1, IL-6, IL-12, or RANTES) |
| 14-kDa *A. actinomycetemcomitans* protein | IL-2, IL-4, IL-5, γ-IFN |

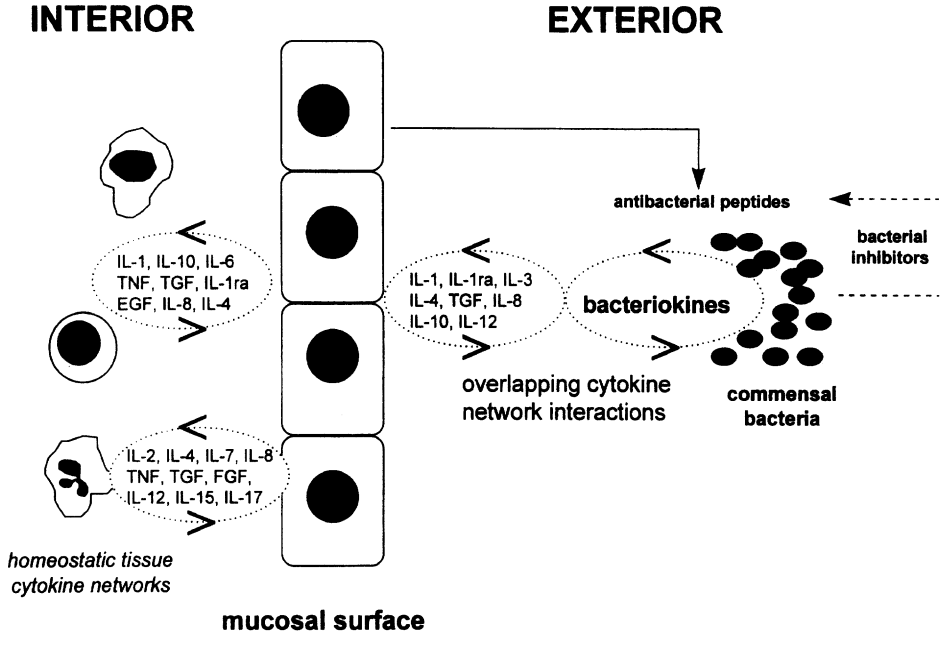


**Figure 3.** Homeostatic networks of cytokines exist in all tissues underlying epithelia, due to the presence of immune cells which make up the skin-associated lymphoid tissue (SALT), gutassociated lymphoid tissue (GALT), and, generically, the mucosa-associated lymphoid tissue (MALT). The authors propose that these homeostatic cytokine networks mesh with additional networks of interacting molecules coming from the commensal bacteria to produce supernetworks of interacting signals which are the reason for the failure of the mucosal surfaces to become inflamed. It is also predicted that the commensal bacteria will produce inhibitors of the antibiotic peptides as part of the communistic signaling networks at mucosal surfaces.

## Host control of bacteria and host susceptibility to bacteria

Thus far, our focus has been on the behavior of the bacterium. However, our hypothesis explaining the interactions between the normal microflora and the host proposes that we are dealing with a fully integrated system in which the host epithelial cells have a key role, yet to be fully defined, in the maintenance of a stable interactive network with the normal microflora. One example of this integration of eukaryotic and bacterial signaling networks appears in reports that certain bacteria can bind cytokines with high affinity and can use these proteins as growth factors. The first indication that cytokines could have an effect on the growth of bacteria was a report from Charles Dinarello, one of the pioneers of IL-1 research, that IL-1 stimulated the growth of "virulent" strains of *E. coli*. Surprisingly, avirulent strains did not respond to IL-1. IL-1 binding was saturable and could be inhibited by the natural antagonist of the IL-1 receptor—IL-1 receptor antagonist—suggesting that bacteria have specific IL-1 receptors on their surfaces (Porat *et al.*, 1991). Another group reported that IL-2 and GM-CSF were growth factors for *E. coli* (Denis *et al.*, 1991). Klimpel and coworkers (Luo *et al.*, 1993) have reported that certain Gram-negative bacteria (*Salmonella typhimurium, Shigella flexneri,* and *E. coli*) have receptors for TNFα. *M. tuberculosis* and *M. avium*, when cultured extracellularly, can be stimulated to grow by recombinant human epidermal growth factor (EGF). Scatchard analysis of EGF binding revealed that there were from 400 to 500 EGF receptors on *M. avium*, with a Kd of $2 \times 10^{-10}$ M, and cloning of this receptor revealed significant homology to the glyceraldehyde 3-phosphate dehydrogenase (GAPD) of group A streptococci (Bermudez *et al.*, 1996). These reports clearly indicate that host cytokines can have effects on bacteria, and Kaprelyants and Kell (1996) have introduced the term *microendocrinology* to describe the growth factors produced by other bacteria or by the host.

Another obvious participant in the interaction between epithelia and the commensal bacteria is the epithelial-derived antibacterial defense mechanisms. Probably the most important of the epithelial-derived antibacterial protective mechanisms are the antibacterial peptides, such as lingualassociated peptide (LAP) (Schonwetter *et al.*, 1995) and tracheal-associated peptide (TAP) (Russell *et al.*, 1996), which have been discovered in the last decade (reviewed by Boman, 1995). Antibacterial peptides are now known to be produced by all organisms, from bacteria to trees, and play a key role in invertebrate and, to a lesser extent, vertebrate antibacterial defense (Boman, 1995). Many of these peptides act by forming pores in the bacterial cell wall. The epithelial-derived antibacterial peptides are also upregulated by bacterial components such as LPS (Russell *et al.*, 1996). It is postulated that these antibacterial peptides act to control the growth of the normal microflora, although direct evidence for this is lacking, and the obvious test of this hypothesis would be to produce transgenic animals in which antibacterial peptide genes had been inactivated. In addition to their antibiotic-like activity, several of the antibacterial peptides have other actions. Some have cytokine-like activity. Thus, the defensins [human neutrophil peptide (HNP)-1 and HNP2 and CAP37 (Territo *et al.*, 1989; Chertov *et al.*, 1996)] and the cathelicidin proBac7 (Verbanac *et al.*, 1993) have been reported to have chemokine-like activity. Interestingly, the defensins are chemotactic for T-lymphocytes, and this finding ties this arm of the innate immune response together with the key cellular element of acquired immunity. This finding is interesting in light of the recent discoveries that certain chemokines can block the uptake of HIV into CD4 lymphocytes. Do antibiotic peptides have similar anti-retroviral activity? Other defensins have growth-factor-like properties (Murphy *et al.*, 1993; Gallo *et al.*, 1994). Another fascinating action of antibiotic peptides is their ability to potently inhibit cortisol synthesis by cultured adrenal cells, due to their ability to block ACTH action at the receptor (Zhu and Solomon, 1992). This may be a mechanism to prolong the inflammatory response, since cortisol, the main glucocorticoid, is a natural and potent antiinflammatory hormone.

A major question which must be addressed is, How do the epithelial-derived antibacterial peptides interact with the normal microflora? Do these peptides control the numbers of bacteria on epithelial surfaces, or have the commensal bacteria evolved mechanisms which enable them to survive in the presence of such peptides? For example, do commensal bacteria produce antibiotic peptide-binding/neutralizing proteins or low-molecular-mass neutralizing ligands? Boman (1995) has described experiments in which the administration of inhibitors of protein synthesis to insects results in their death due to overgrowth of the normal microflora. However, given the very large numbers of bacteria which constitute the normal microflora in mammals, are antibiotic peptides applying population control to commensal bacteria? Again, knockout of the genes encoding epithelial-derived antibiotic peptides would answer this question.

Having postulated that the normal microflora has evolved cytokine-modulating molecules to live in harmony with host mucosal surfaces, the question must be addressed as to what happens in the significant proportion of patients with periodontal disease, where there is an obvious lack of harmony between oral commensal bacteria and the gingivae. What changes to the oral microflora, the oral epithelium, or to the interactions which occur between both, take place in periodontitis? The development of a lethal colitis in mice lacking IL-2 has already been described. This is a monogenic change in a cytokine gene resulting in discrete tissue pathology. There is also emerging evidence that many inflammatory and infectious diseases may be polygenic diseases involving multiple cytokine genes (Daser *et al.*, 1996). This introduces the concept of cytokine polymorphisms as a contributory factor to tissue pathology. Polymorphisms have been found in the coding regions of a number of pro-inflammatory (*e.g.*, IL- 1, TNFα) and anti-inflammatory (*e.g.*, IL-1 receptor antagonist and IL-10) cytokine genes. Such alterations in the base sequence can modify the rate of gene transcription such that, for any given stimulus, individuals with polymorphisms produce greater amounts of cytokine. This could render such individuals more susceptible to infectious micro-organisms or could even mean that they recognize and respond to their own normal microflora. A recent publication suggests that

polymorphism in the IL-1β gene may be a contributory factor to susceptibility to the periodontal diseases (Kornman *et al.*, 1997).

## Conclusions

Communism is an ancient societal concept, derived from sources including Plato's *Republic* and early Christian communes, in which the major resources and means of production in a society are owned by the community rather than by individuals. This article started off with the communist creed, 'providing for equal sharing of all work, according to ability, and all benefits, according to need', and the suggestion that this idealized social mechanism may be a paradigm for what is happening at mucosal surfaces between epithelial (and post-epithelial) cells and the normal microflora which lives on these surfaces. This paradigm proposes that the mucosal surface and its associated normal microflora are not two distinct groups of cells in casual interaction but, rather, represent a balanced organic unit (commune), sharing all work according to cellular ability and the reciprocal benefits according to need. In other words, the prokaryotic and eukaryotic communities in our bodies are acting for the common good. To survive, each member of the community must be sensitive to the other and be able to respond in the appropriate manner. The epithelial cells obviously have mechanisms for inhibiting bacterial growth and killing bacteria, but these are not absolute, since our mucosal surfaces are not sterile. Bacteria contain an armamentarium of potent proinflammatory substances whose activity, we propose, is negated by bacterially produced cytokine-network-regulating molecules. In turn, these bacterial cytokine-modulating molecules (microkines) may be induced by signals from the host's epithelia. The new science of Cellular Microbiology has a major challenge in attempting to understand the interactions between the normal microflora and the host epithelia. We believe that when these interactions have been fully revealed, we will see that communism does work, at least at the molecular and cellular levels.

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