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MINIREVIEW

Bacterial Perturbation of Cytokine Networks

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INTRODUCTION

During the past decade, the emphasis in the study of the pathogenesis of infectious diseases has shifted from determining the functions of the cellular players in the inflammatory response to the mediators which orchestrate this response. Cytokines, the most important class of such mediators (12), are signalling molecules which can behave as classic endocrine hormones but are better known for their autocrine and paracrine behavior, acting as integrating signals over short cellular distances. They integrate the activities of target cells by binding to specific high-affinity receptors and, historically, have been subdivided into families such as the interleukins, chemokines, and interferons, etc. However, this classification is not particularly useful and, in the case of the interleukins, is misleading. Indeed, one of the characteristic features of cytokines is their pleiotropy, although this should not be interpreted as redundancy, as it so often is. In the immunoinflammatory mechanisms which underpin the host response to infection, the most appropriate subdivisions of cytokines are (i) those that modulate leukocytes to produce proinflammatory responses and (ii) those that have the capacity to downregulate inflammatory cells (macrophages and lymphocytes, etc.). However, it is now realized that cytokines rarely, if ever, act in isolation but rather act to induce, or inhibit, other cytokines, creating a population, or network, of cytokines to which cells respond. In recent years it has become apparent that bacteria produce many molecules which have profound effects on the capacity of leukocytes and tissue cells to produce selected cytokine networks. Thus attention will have to switch from the current view of the host cell as the only controlling factor in cytokine biology, once the bacterium has stimulated this cell, to one in which the bacterium, by modifying its exported or structural molecules or by direct interaction with the cell, can directly modify cytokine networks. Thus an additional level of complexity in cytokine networks in infectious diseases is postulated.

CYTOKINE NETWORKS

One of the least understood concepts in cytokine biology is the cytokine network. Formally, a network consists of a set of entities (e.g., cells) connected by one or more binary relations, which determine the influences (signals) between entities. In addition to the possibility of multiple signals between pairs of entities, autosignalling is also possible. Each signal also has a strength parameter, representing the relative importance of the signal to the recipient, as in the much-studied neural nets of artificial intelligence and neuroscience (19). Cytokine networks are not static but are dynamic networks in which connections, or their strengths, and perhaps the entities themselves, are changing in time. Our own studies at University College London are utilizing the approach of continuous time dynamical systems, i.e., a system of (usually nonlinear) differential equations to model the simple cytokine networks likely to be induced in acute infectious states. It is envisaged that cytokine networks are the main controlling elements in the inflammation and immune reactions which occur in infections. Dynamic relationships between pro- and anti-inflammatory cytokines, their rates of production, and quantity and the rate of internalization and release of cytokine receptors will be among the variables which will control the induction, perpetuation, and collapse of a particular cytokine network and the consequent cellular events that it controls. One network which deserves mathematical attention is the network of cytokines which are believed to control the interactions of Th1 and Th2 lymphocytes (Fig. 1). Perturbation of this dynamic network(s) could have serious consequences for the ability of the organism to cope with infections. It is established that certain strains of mice infected with *Leishmania major* are unable to induce the correct Th1 cytokine network and thus are unable to activate parasitized monocytes to clear the infection (4). A similar problem occurs in patients with lepromatous leprosy whose immune systems appear to make an inappropriate Th2 response which is unable to control growth of *Mycobacterium leprae* (61).

The development of appropriate cytokine networks to combat infections will depend on the nature of the infecting organism and on the genetic makeup of the individual. A number of cytokine genes have now been found to have polymorphisms in noncoding regions. There is evidence that such polymorphisms can control the rate of production of the cytokine. Thus, in population terms, given a particular set of environmental signals (e.g., infectious agents), there can be a spectrum of cytokine networks. It is assumed that these different cytokine networks would render individuals more or less resistant to particular infections (56).

Our conception of cytokine networks in infection is, of course, missing a major part of the real network interactions, namely, the input of the cytokine-controlling molecules from the infecting organisms. A number of viruses have been shown to produce proteins able to modulate host cytokine networks. Such proteins include inhibitors of the protease interleukin-1β (IL-1β)-converting enzyme (ICE, now known as caspase 1) which catalyzes the production of the 17-kDa active form of IL-1β, soluble forms of cytokine receptors, growth factors, and homologs of IL-10 (an anti-inflammatory cytokine) (40). These proteins have been termed virokines and may have been cap-

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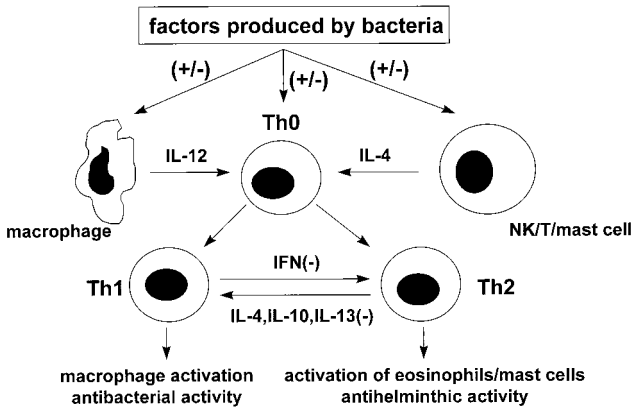

FIG. 1. Interrelationship between Th1 and Th2 CD4 T lymphocytes and the networks of cytokines that they produce. Th1 lymphocyte responses are largely aimed at intracellular parasites in macrophages. Th2 responses are aimed at activating mast cells and eosinophils for anti-helminth defenses. The cytokines produced by both sets of T cells can have inflammatory actions and can also inhibit the actions of the opposite cell type. Thus, IL-10 can block the actions of Th1 lymphocytes. The key question still requiring an answer is the role that microbial products play in determining the nature of CD4 T-lymphocyte cytokine networks and thus in determining the quality and quantity of Th1 and Th2 responses.

tured by viruses from host genomes. We have proposed that bacteria will have evolved similar molecules with the power to modulate cytokine networks. We have suggested the term bacteriokine to describe these proteins, and the generic term microkine is appropriate for any molecule from a microorganism able to modulate cytokine networks (21). The consequence of this argument is that there may be supernetworks of interacting host and microbial molecules which determine the nature and effectiveness of host defenses.

CYTOKINE INDUCTION BY BACTERIA

Interaction between bacteria and host cells invariably results in the release of one or more cytokines, the actual cytokines produced depending mainly on the nature of the bacterium and host cells involved. The resulting cytokine network, of course, constitutes an important part of the innate immune response and represents the host's attempt to deal with that particular organism. As such, therefore, the ability of bacterial components (or bacterial activities) to induce cytokine release from host cells can be regarded as an aspect of bacterial virulence only when this response results in pathology due to its intensity and/or chronicity.

Modulins. It is widely known that lipopolysaccharide (LPS) is a potent inducer of cytokine release from a variety of host cell types. What is not so widely appreciated is that LPS is not the only bacterial component with this ability, and as many as 15 classes of bacterial surface components or secretory products are known to stimulate cytokine release (22, 58). It has been suggested that this chemically diverse group of compounds be recognized as a separate class of virulence factors, modulins, so called because of their ability to modulate the behavior of cells due to the induction of cytokine synthesis (20). Such molecules include peptidoglycans, teichoic acids, and fimbrial proteins, etc. (Table 1). As the biology of these molecules has recently been reviewed (24), this review will concentrate on other ways in which bacteria can interfere with cytokine networks.

Adhesion-induced cytokine release. McCormick et al. (39) reported that adhesion of *Salmonella typhimurium* to human intestinal epithelial cells induced the release of IL-8. Subsequently, it was shown that adhesion of this organism to murine macrophages increased the levels of mRNA for IL-1β, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokines, such as MIP-1β and MIP-2, and melanocyte growth stimulatory activity (59). Yamamoto et al. (60) have also reported that binding of *Legionella pneumophila* to the surface of murine macrophages (treated with cytochalasin D to prevent phagocytosis) results in increased levels of mRNA for IL-1α, IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), and GM-CSF. At least two ligand-receptor systems were involved in cytokine induction, one being α-methyl-D-mannoside (αMM) dependent and the other being αMM independent. Hence, induction of macrophage mRNAs for IL-1β, IL-6, and GM-CSF was inhibited by αMM while the levels of mRNA for the chemokines MIP-1β and MIP-2 were unaffected.

Cytokine release by epithelial cells following adhesion of uropathogenic strains of *Escherichia coli* has received considerable attention. Intravesicular inoculation of mice with P-fimbriated strains of *E. coli* has been shown to cause release of IL-6 into the urine (36). Cytokine release was dependent on the presence of the PapG adhesin on the fimbriae (which is specific for Galα1,4Gal residues), as no IL-6 was released by strains bearing S fimbriae or type 1 fimbriae. The receptors for the adhesin were shown to be glucosphingolipids (49). Adhesion of an *E. coli* strain with type 1 and P fimbriae to a bladder epithelial cell line was found to induce the release of IL-6, IL-8, and IL-1α while a kidney cell line released only IL-6 and IL-8 (1). Thus, different cells appear to respond to identical bacterial signals with the production of distinct cytokine networks. In vivo, the IL-6 released could prime neutrophils to the chemotactic peptide fMLP, as well as induce the secretion of immunoglobulin A (IgA) from IgA-committed B lymphocytes. IL-8 is a potent chemoattractant for polymorphonuclear leukocytes (PMNs), and IL-1–IL-8 can prime PMNs for the re-

TABLE 1. Bacterial factors capable of stimulating cytokine synthesis

Components of gram-positive species
Lipoarabinomannan, lipomannans, and phosphatidylinositol mannosides
Purified protein derivative
Mycobacterial heat shock proteins
Protein A
Lipoteichoic acid
Components of gram-negative species
Lipopolysaccharide
Lipid A
Lipid A-associated proteins
Outer membrane proteins
Porins
Chaperonins
Cell wall components of gram-positive and gram-negative species
Cell surface proteins
Fimbriae and pili
Lipopeptides
Lipoproteins
Muramyl dipeptide
Peptidoglycan
Polysaccharides
Extracellular products of gram-positive and gram-negative species
Toxins
Superantigens

TABLE 2. Induction of cytokine synthesis following adhesion of bacteria to host cells

Organism inducing cytokine synthesis	Cytokine-producing cell	Cytokine(s) produced	Reference(s)
Salmonella typhimurium	Human intestinal epithelial cells	IL-8	39
	Murine macrophages	IL-1β, IL-6, GM-CSF, MIP-1β, KC[a]	59
Legionella pneumophila	Murine macrophages	IL-1α, IL-1β, IL-6, TNF-α, GM-CSF	60
E. coli	Bladder epithelial cells	IL-1α, IL-6, IL-8	1, 36
	Kidney cells	IL-6, IL-8	1
H. pylori	Gastric epithelial cells	IL-6, IL-7, IL-8, IL-10, TNF-α	7, 62
Viridans group streptococci	Human epithelial cells	IL-8	53

[a] KC, melanocyte growth stimulatory factor.

lease of reactive oxygen products in response to fMLP. The combined action of the IL-8 and IL-1 would, therefore, be to recruit PMNs to the site of the infection and then activate them for bacterial killing.

A number of in vivo and in vitro studies have shown that *Helicobacter pylori* can induce cytokine expression in epithelial cells. Crabtree et al. (7), for example, reported that patients infected with the organism express much greater levels of IL-8 in their gastric epithelia than those patients in which the organism could not be detected. Levels of mRNA for IL-6, IL-7, IL-8, IL-10, and TNF-α have also been reported to be significantly higher in the gastric mucosae of patients infected with *H. pylori* than in those free of the organism (62). Strains of the organism which produce the immunodominant antigen *cagA* appear to be more frequently associated with duodenal ulcer disease, and it was found that the mucosae of patients infected with *cagA*$^+$ strains expressed higher levels of mRNA for IL-8 than those infected with *cagA* mutant strains. In vitro studies employing *cagA* isogenic mutants, however, revealed no difference from the wild-type parental strain with regard to their ability to induce secretion of IL-8 from epithelial cell lines (47), implying that the *cagA* protein itself is not responsible for stimulation of IL-8 production. *cagA*$^+$ strains of the organism also have an operon containing the *picA* and *picB* genes, which encode 36- and 101-kDa polypeptides, respectively. With mutants defective in the production of these polypeptides, it has been shown that the PicB protein is involved in inducing IL-8 secretion from gastric epithelial cells, while the PicA protein controls the expression of PicB (52).

In contrast to those of gram-negative bacteria, few studies have investigated cytokine induction due to adhesion of gram-positive bacteria to host cells. Nevertheless, adhesion to a human epithelial cell line of each of 11 strains of oral viridans streptococci has been shown to stimulate the release of IL-8 (53). In the case of one of these organisms, *Streptococcus mutans*, adhesion was mediated by protein I/IIf and a rhamnose-glucose polymer, and these isolated adhesins were also able to stimulate release of IL-8 from the epithelial cells.

It is obvious from these reports that a characteristic response of epithelial cells from a number of mucosal surfaces to bacterial adhesion is the release of a range of cytokines (Table 2). It is tempting, therefore, to hypothesize that, as well as serving as a barrier, the epithelium functions to alert the immune system to the presence of disease-inducing microbes (14). However, if this is the case, how do epithelial cells distinguish between such microbes and members of the normal microflora? While most attention has been focused on cytokine release induced by pathogenic bacteria, the study of Vernier et al. (53) has shown that a number of harmless members of the normal oral microflora can induce the release of IL-8 from epithelial cells. If this release occurs in vivo, what prevents the oral mucosa from being in a constant state of inflammation? Clearly, the interactions between epithelial cells and the normal microflora are complex and should not be viewed simply as involving the induction, by bacteria, of cytokine release from epithelial cells. We have already proposed that molecular cross talk between a bacterium and a host cell involves upregulatory and downregulatory processes induced by proinflammatory and anti-inflammatory molecules secreted by the bacteria and bacterium-regulating molecules secreted by the host cell (23). Later in this review we will discuss a number of ways in which bacteria can suppress the release of proinflammatory cytokines and possibly, thereby, produce cytokine networks which do not activate leukocytes and induce the mechanisms of innate and acquired immunity.

Invasion and cytokine synthesis. Bacterial invasion of a number of cell types is accompanied by cytokine synthesis. Examples of this phenomenon are given in Table 3.

(i) Epithelial cells. Eckmann et al. (13) reported that invasion of epithelial cell lines by *Salmonella dublin*, *Yersinia enterocolitica*, *Shigella dysenteriae*, and *Listeria monocytogenes* induced the release of the chemotactic cytokine IL-8. In contrast, no IL-8 was detected in supernatants of cells exposed to noninvasive strains of *E. coli* and *Enterococcus faecium*. In vivo, release of IL-8 after invasion of epithelial cells may serve as the signal for initiation of the acute inflammatory response. In order to help evade this response, *Y. enterocolitica* appears to have evolved a means of suppressing, to some extent, the amount of IL-8 released by cells, as it was noted that IL-8 levels induced by this organism were significantly lower than those induced by the other invasive strains tested. Schulte et al. (46) have shown that the suppression of IL-8 release is attributable to the Yop proteins.

A number of epithelial cell lines have been shown to secrete the proinflammatory cytokines IL-8, monocyte chemotactic protein 1 (MCP-1), GM-CSF, and TNF-α in response to inva-

TABLE 3. Cytokine synthesis induced by bacterial invasion of host cells

Target cell	Cytokine(s) produced	Invading organism	Reference
Epithelial cells	IL-8	*Salmonella dublin*, *Y. enterocolitica*, *Shigella dysenteriae*, *Listeria monocytogenes*	13
	IL-8, MCP-1, GM-CSF, TNF-α	*Salmonella dublin*, *Y. enterocolitica*, *Shigella dysenteriae*, *Listeria monocytogenes*, enteroinvasive *E. coli*	31
Human umbilical endothelial cells	IL-1β, IL-6	*S. aureus*	63
Human fibroblasts	IFN-β	*Shigella flexneri*, enteroinvasive *E. coli*	26
Macrophages	IL-1β	*Shigella flexneri*	64

sion by *Salmonella dublin*, *Shigella dysenteriae*, *Y. enterocolitica*, *Listeria monocytogenes*, and enteroinvasive *E. coli* (31). Noninvasive bacteria (*Enterococcus faecium*, *Streptococcus bovis*, and a noninvasive strain of *E. coli*) did not induce increased expression of these cytokines. The release of this particular combination of cytokines in vivo by the epithelium would be an effective means of initiating a mucosal inflammatory response. IL-8 and MCP-1 are potent chemoattractants for, and activators of, neutrophils and monocytes, respectively, while GM-CSF prolongs the survival of these cells and increases their response to other proinflammatory agonists. TNF-α can activate both types of cell and can stimulate further release of IL-8 and MCP-1 from them.

(ii) Endothelial cells. Most studies of bacterium-induced release of cytokines by endothelial cells have been carried out with purified cell components rather than whole bacteria. However, recently it has been shown that invasion of human umbilical vein endothelial cells by *Staphylococcus aureus* stimulates the release of both IL-1β and IL-6 (63). The inability of a noninvasive strain of the organism to induce IL-1β and IL-8 release implied that invasion rather than adhesion was the trigger for cytokine synthesis, and this possibility was supported by the finding that cytochalasin D inhibited the internalization of the invasive strains by, and cytokine release from, the endothelial cells. Internalization of as few as three bacteria per cell was sufficient to induce the expression of the cytokines, although maximal expression was achieved only following internalization of approximately 10^3 bacteria per cell.

(iii) Fibroblasts. Invasion of human fibroblasts by either *Shigella flexneri* or an enteroinvasive *E. coli* strain has been shown to induce the production of beta interferon (IFN-β) (26). Further studies of cytokine release induced by *Shigella flexneri* revealed that a noninvasive isogenic mutant was unable to stimulate IFN release from the fibroblasts and that neither LPS nor outer membrane preparations from the organism were active in this respect. These findings suggest that cytokine induction is a response to bacterial invasion. In a subsequent study it was found that an avirulent isogenic variant of the organism which invaded but failed to grow intracellularly did not induce IFN production and that the addition of rifampin (which blocks bacterial RNA synthesis) also inhibited cytokine synthesis (27). Cytokine induction would, therefore, appear to be dependent on the presence of actively growing bacteria within the fibroblasts.

(iv) Macrophages. *Shigella flexneri* invades and induces apoptosis in macrophages, which is accompanied by release of IL-1β (64). Recently, the mechanisms involved in this process have been elucidated, and it has been shown that the invasion plasmid antigen B (IpaB) is responsible for induction of apoptosis and the release of IL-1β (51). IpaB binds to and activates ICE, hence inducing the conversion of the proform of the cytokine to biologically active IL-1β.

Host degradation products. The possibility that the end products of bacterially induced protein degradation may have cytokine-inducing activities has largely remained unexplored. Engel et al. (15) have reported that an 80-kDa protease from *Porphyromonas gingivalis* can generate Fc fragments from human IgG1 which are able to induce the release of IL-6, IL-8, and TNF-α from human peripheral blood monocytes.

Functional molecular mimicry. There has been much interest in the concept that bacterial components which resemble those of the host could give rise to immune responses that cause self-recognition and disease. However, the concept of functional molecular mimicry is less well developed. As has been described previously, IL-1β is synthesized as a 31-kDa inactive precursor (pre-IL-1β) which is cleaved by ICE (caspase 1) to the biologically active 17.5-kDa molecule. An exotoxin from *Streptococcus pyogenes*, streptococcal pyrogenic exotoxin B (SPE B), is a cysteine protease which mimics ICE in that it can also convert pre-IL-1β to IL-1β (32). SPE B, however, cleaves pre-IL-1β between His-115 and Asp-116 rather than between Asp-116 and Ala-117 as is the case with ICE, resulting in an IL-1β molecule with an additional amino acid. An enzyme which cleaves pre-IL-1β in the same manner has also been detected in human peripheral blood mononuclear cells (PBMCs). IL-1β with the additional amino acid was biologically active in that it induced nitric oxide synthase activity in vascular smooth muscle cells and killed cells of the human melanoma A375 cell line. As pre-IL-1β is known to be released from monocytes, the SPE B could convert this to the active form during the course of infection with *Streptococcus pyogenes*, thereby effectively acting to increase levels of this proinflammatory cytokine.

SUPPRESSION OF CYTOKINE SYNTHESIS OR ACTIVITY

The discussion so far has centered on the means by which bacteria can induce a particular cytokine network, or alter an existing one, by stimulating the release of a variety of cytokines from host cells. To a large extent, this induction can be regarded as being beneficial to the host unless a prolonged or overactive response results in tissue damage. However, bacteria have evolved a number of ways of manipulating cytokine networks to their advantage. They can be regarded as true virulence factors and will be the focus of the rest of the review.

A number of bacteria synthesize molecules capable of suppressing cytokine release from host cells (Table 4). A 14-kDa protein from *Actinobacillus actinomycetemcomitans* has been shown to inhibit the release of Th1 (IL-2 and IFN-γ) and Th2 (IL-4 and IL-5) cytokines from concanavalin A-stimulated murine $CD4^+$ T cells (34). Production of this molecule in vivo is likely to aid the survival of the organism by interfering with both humoral and cell-mediated immune responses. Inhibition

TABLE 4. Suppression of cytokine synthesis

Organism	Process inhibited[a]	Active component	Reference
Actinomyces actinomycetemcomitans	Release of IL-2, IL-4, IL-5, and IFN-γ from ConA-stimulated murine $CD4^+$ cells	14-kDa protein	34
Escherichia coli	Expression of mRNA for IL-2, IL-4, IL-5, and IFN-γ in PMA-stimulated PBMCs	8-kDa protein	33
Yersinia enterocolitica	TNF-α release from LPS-stimulated murine macrophages	YopB	3
Brucella suis	TNF-α release from *E. coli*-infected macrophages	45- to 50-kDa protein	6
Vibrio cholerae	TNF-α release from rat peritoneal mast cells	Cholera toxin	35
Bacillus anthracis	TNF-α secretion by LPS-stimulated human monocytes	Anthrax edema toxin	29
Pseudomonas aeruginosa	Synthesis of IL-1α, IL-1β, TNF-α, and IFN-γ by PHA-stimulated human monocytes	ADP-ribosylating toxin	48
	TNF-α and IL-12 release from LPS-stimulated human monocytes	3-Oxododecanoyl-L-homoserine lactone	58
Salmonella typhimurium	Synthesis of IL-2 by murine T lymphocytes	Protein	38

[a] ConA, concanavalin A; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin.

of lymphokine production has also been shown to be a property of lysates from certain strains of enteropathogenic *E. coli* (33). At rather high concentrations (>10 μg/ml) of the lysate, expression of mRNA for IL-2, IL-4, IL-5, and IFN-γ was greatly reduced in phorbol 12-myristate 13-acetate (PMA)- and pokeweed mitogen-stimulated PBMCs. Interestingly, levels of mRNA for cytokines synthesized mainly by monocytes (IL-1β, IL-6, IL-8, IL-10, IL-12, and RANTES) were unaffected. The active component was both protease and heat sensitive and had a molecular mass of less than 8 kDa.

In mice infected with *Y. enterocolitica* it has been shown that no mRNA for TNF-α can be detected in the main site of bacterial invasion, the Peyer's patches. In vitro studies have shown that this effect is probably attributable to the 41-kDa outer membrane protein, YopB, as this protein is able to inhibit the production of TNF-α from LPS IFN-γ-stimulated murine macrophages (3). Additional evidence in support of the role of YopB in suppression of TNF-α production comes from the finding that intraperitoneal injection of mice with anti-YopB antibodies before and after infection with *Y. enterocolitica* resulted in an appreciable increase in the level of TNF-α in Peyer's patch homogenates. This increase was accompanied by a pronounced decrease in the number of bacteria that could be isolated from the Peyer's patches. These findings demonstrate the importance of TNF-α in host defense systems and the advantage to the pathogen of having the capacity to suppress the synthesis of this cytokine. Another organism able to inhibit TNF-α production by macrophages is *Brucella suis*. A protein (with a size of 45 to 50 kDa) secreted by this organism inhibits the release of TNF-α from *E. coli*-infected macrophages (6).

A number of bacterial toxins, as well as being potent inducers of cytokine synthesis (25), can also function as inhibitors of cytokine synthesis or release. Both cholera toxin and its B subunit, for example, can inhibit the synthesis of TNF-α by rat peritoneal mast cells at concentrations as low as 1.0 ng/ml (35). This suppression of TNF-α production may be due to the rise in intracellular cyclic AMP elicited by the toxin in that forskolin, which is also able to elevate cyclic AMP levels, had a similar effect on TNF-α production. Interestingly, anthrax edema toxin, which has adenylate cyclase activity, did not induce TNF-α production by human monocytes but inhibited LPS-induced TNF-α secretion by these cells at concentrations as low as 2 ng/ml (29). The ADP-ribosylating toxin of *Pseudomonas aeruginosa*, exotoxin A, has been reported to inhibit the synthesis of IL-1α, IL-1β, TNF-α, and IFN-γ by phytohemagglutinin- or *S. aureus*-stimulated human monocytes (48). An immunosuppressive protein from *Salmonella typhimurium* (salmonella-derived inhibitor of T-cell proliferation) has been shown to inhibit the synthesis of IL-2 in murine splenic T lymphocytes activated with anti-CD3 antibodies and PMA (38). The ability of bacterial proteins to modulate in vivo immune responses has been reported. Administration of the B subunit of the heat-labile *E. coli* enterotoxin was able to inhibit the induction of collagen-induced arthritis in mice (57). Protection was associated with a shift in the Th1/Th2 balance as well as a reduction in the extent of the anti-type II collagen immune response.

A quorum-sensing molecule from *Pseudomonas aeruginosa*, 3-oxododecanoyl-L-homoserine lactone, has been shown to suppress the release of TNF-α and IL-12 by LPS-stimulated macrophages (58).

CYTOKINE DEGRADATION

Not surprisingly, cytokines have been shown to be susceptible to degradation by bacterial proteases, and the first reports of this appeared in the late 1980s (Table 5). Theander et al. (50) showed that both the alkaline protease and the elastase of *Pseudomonas aeruginosa* were able to inhibit IL-2-induced proliferation of murine lymphocytes. That this inhibition was due to proteolysis of IL-2 was supported by the detection of cytokine degradation products and reduced binding to IL-2 receptors following incubation of the cytokine with each of the enzymes. The alkaline protease of this organism has also been shown to degrade and inactivate IFN-γ (30). IFN-γ is the

TABLE 5. Degradation of cytokines by bacterial proteases

Organism	Cytokine(s) degraded	Enzyme(s) responsible	Reference(s)
Pseudomonas aeruginosa	IL-2, TNF-α	Alkaline protease, elastase	42, 50
	IFN-γ	Alkaline protease	30
Legionella pneumophila	IL-2	Zn-metalloproteinase	41
Porphyromonas gingivalis	IL-1β, IL-6, IL-1ra	Gingipain	16, 17

principal T-cell product which activates macrophages, so its destruction in vivo would seriously impair host defense mechanisms.

The susceptibility of other cytokines to the proteases of *Pseudomonas aeruginosa* has been investigated by Parmely et al. (42). Neither human recombinant IL-1α nor IL-1β showed any decrease in bioactivity or in molecular mass following exposure to the enzymes. In contrast, human recombinant TNF-α was susceptible to hydrolysis by both proteases. A 38-kDa zinc metalloproteinase from *Legionella pneumophila* has been shown to cleave human IL-2 and to inhibit IL-2-induced proliferation of mouse CTLL-2 cells (41). Supernatants from cultures of *Porphyromonas gingivalis* have been shown to hydrolyze IL-1β, IL-6, and IL-1 receptor antagonist (IL-1ra), and after exposure to such supernatants the IL-1β was unable to stimulate the release of IL-6 from human gingival fibroblasts, demonstrating that it had lost biological activity (16). The purified proteases, Arg-1 and Arg-1A, from this organism were also able to degrade IL-1β. We have also shown that biofilms of *Porphyromonas gingivalis* are able to degrade the above-mentioned cytokines and that proteolysis can take place even when the biofilms are immersed in 100% serum (17).

BACTERIAL BINDING TO CYTOKINES

A number of studies have reported the ability of cytokines to bind to bacteria, which can, in some cases, affect the growth of the organism (Table 6). This binding may occur when the organism is either outside or inside a host cell.

Effect of cytokines on growth of extracellular bacteria. Porat et al. (43, 44) have shown that IL-1β, IL-2, GM-CSF, and epidermal growth factor (EGF) not only bind to bacteria but also stimulate their growth. For example, IL-1β at concentrations as low as 10 ng/ml stimulated the growth of virulent strains of *E. coli*, and this effect could be abolished by IL-1ra. Radioiodinated IL-1 was found to bind to virulent, but not avirulent, strains of the organism in a cell density-dependent manner, and binding of the cytokine was saturated at a concentration of 20 pg/ml. The kinetics of desorption suggested that the organism contained 2×10^4 to 4×10^4 binding sites per cell, a number significantly greater than the number of IL-1 receptors present on human or murine cells. Cytokine-elicited growth stimulation of *E. coli* has also been reported by Denis et al. (9). IL-2 and GM-CSF (but not IL-4) were able to enhance the growth of a virulent, but not an avirulent, strain of the organism. The enhanced growth was accompanied by a decrease in IL-2 in the medium and could be abrogated by anti-IL-2 antibodies. Although the growth enhancement (approximately a threefold increase in the number of cells) may not have serious consequences for the host, the resulting local depletion of the cytokine may. Hence, an immunosuppressive effect would be induced by the consequent decrease in T-cell proliferation and decreased immunoglobulin production by stimulated B cells. *Mycobacterium avium* is another organism whose growth has been shown to be affected by cytokines (8). In this case, IL-6 stimulated growth and this effect was abrogated by heat inactivation of the cytokine or by anti-IL-6 antibodies. Scatchard analysis of receptor interaction revealed that the organism had a single receptor with a K_d of 50 nM and that the number of receptor sites per bacterium was approximately 15,000.

A recent report has established that both *Mycobacterium tuberculosis* and *M. avium*, cultured extracellularly, can be stimulated to grow by recombinant human EGF, with significant enhancement of growth occurring at a concentration of 50 ng/ml (2). Radioiodinated EGF bound to bacteria and could be inhibited by cold EGF, and Scatchard analysis revealed that the receptor on *M. avium* had a K_d of 2×10^{-10} M and that there were 450 ± 60 receptors per cell. The affinity of this bacterial EGF receptor is similar to that of the high-affinity EGF receptor found on mammalian cells. Luo et al. (37) reported that *E. coli*, *Salmonella typhimurium*, and *Shigella flexneri* can bind TNF-α. Although binding of the cytokine to gram-positive bacteria (*Listeria monocytogenes*, *S. aureus*, and *Streptococcus mitis*) was also observed, the amount bound was appreciably less than that to gram-negative organisms. This phenomenon was investigated in more detail with *Shigella flexneri*, and it was found that the organism had high-affinity receptors for the cytokine with 276 binding sites per cell and that binding was not inhibited by TNF-β. Binding of the cytokine had a number of consequences. First, uptake of the bacterium-cytokine complex by macrophages was considerably greater than uptake of the cytokine-free bacteria. Second, invasion of HeLa cells by the bacteria was enhanced by the bound cytokine. Enhanced phagocytosis of the bacterium-cytokine com-

TABLE 6. Binding of cytokines to bacteria and growth stimulation

Cytokine(s)	Organism(s)	Effect	Reference(s)
TNF-α	*E. coli*, *S. typhimurium*, *Shigella flexneri*	Binding	37
IL-1β	*E. coli*	Growth stimulation	43, 44
IL-2, GM-CSF	*E. coli*	Growth stimulation	9
TGF-β	*M. tuberculosis*	Growth stimulation in macrophages	28
IL-6	*M. avium*	Growth stimulation in macrophages and in culture fluid	10
CSF-1, IL-3	*Listeria monocytogenes*	Growth stimulation in macrophages	11
EGF	*M. avium*, *M. tuberculosis*	Growth stimulation	2

plex would be of obvious benefit to the host whereas the increased invasive capacity of the complex would enable the bacteria to escape host defense mechanisms.

Effect of cytokines on growth of intracellular bacteria. A number of studies have shown that cytokines can affect the growth of a variety of bacteria inside host cells. Transforming growth factor β1 (TGF-β1) and TNF-α, for example, stimulate the growth of *M. tuberculosis* in macrophages and human monocytes, respectively (5, 28). IL-6 has been shown to increase the growth of *M. avium* in macrophages regardless of whether it was added before or after bacterial uptake (10), and treatment of macrophages with either CSF-1 (macrophage-CSF) or IL-3 enhances the growth of *Listeria monocytogenes* (11).

INDUCTION OF RECEPTOR RELEASE

Cell surface receptors for many ligands can be shed by proteolytic cleavage, and cytokine receptors are not different in this respect. This effect can have several consequences. (i) The receptor may act as a competitive inhibitor of the corresponding cytokine, (ii) the receptor-cytokine complex may act as a depot for the cytokine, and/or (iii) the receptor may bind to a cell which normally does not have such a receptor, thereby rendering it responsive to this cytokine. All of these possibilities have been shown to occur (45). Proteases from a number of organisms including *S. aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Serratia marcescens* have been shown to release the IL-6 receptor (IL-6R) from human monocytes (54). Experiments with the purified metalloproteinase from *Serratia marcescens* revealed that the shed receptor was biologically active as it was able to render human hepatoma cells (which do not express IL-6R) responsive to IL-6. The pore-forming toxins streptolysin O and *E. coli* hemolysin have also been shown to induce the release of IL-6R from human monocytes and macrophages (55). The induction of an IL-6-mediated response in human hepatoma cells indicated that the liberated IL-6R retained its biological activity. The release of the IL-6R was inhibited by a specific inhibitor of the endogenous mammalian shedding protease, suggesting that shedding is mediated by this enzyme following its activation (by an unknown mechanism) by the toxin. Because up to 50% of the IL-6R can be liberated by low concentrations (nanograms per milliliter) of the toxins within 10 min of exposure, the biological consequences would be expected to be dramatic. Not only would normally unresponsive cells be rendered responsive to IL-6 but also cells from which the IL-6 had been shed may no longer be capable of reacting to the cytokine.

SUMMARY

From the preceding discussion it is obvious that bacteria have evolved a number of means of inducing and manipulating host cytokine networks. Some mechanisms appear to be very straightforward, involving the production by the organism of potent modulins, such as LPS or exotoxins, which can induce the release of large quantities of proinflammatory cytokines and thereby creating a strong impetus towards the establishment of a cytokine network with a net proinflammatory effect. Others are more subtle, as exemplified by the induction of the release of soluble cytokine receptors. Regardless of the sophistication of the means by which bacteria induce cytokine synthesis, there is overwhelming evidence that bacteria can stimulate the release of (mainly) proinflammatory cytokines. This finding raises the important question mentioned earlier: why are we not in a state of continual inflammation? Although this review has mentioned that bacteria can also suppress cytokine release, the organisms involved have been mainly traditional pathogenic species. What then is the effect of the normal microflora? These organisms certainly have a range of modulins which are potent inducers of proinflammatory cytokine release. It is our view that members of the normal microflora must have surface components (or secreted products) with the ability to downregulate the synthesis of proinflammatory cytokines, to upregulate the release of anti-inflammatory cytokines, or to neutralize the biological activities of proinflammatory cytokines. Alternatively, host cells must be able to affect the activities, or production, of bacterial modulins. The results of a recent study of the ability of a number of gram-positive and gram-negative bacteria to induce the release of certain cytokines from whole human blood are of interest (18). Most of the gram-negative species tested (*E. coli*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae*) were between 100-fold and 1,000-fold more potent than the gram-positive species (*S. aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*) in inducing the release of the proinflammatory cytokines IL-1β and IL-6. Although the gram-negative bacteria also produced more of the anti-inflammatory cytokine IL-1ra, the difference in potencies between the two groups of organisms was slight. So, are these differences due to the different potencies of the modulins of gram-positive and gram-negative bacteria, or is the difference due to the different abilities of the organisms to neutralize or counteract the activities of proinflammatory cytokine-inducing components? Of particular interest was the finding that *Bacteroides fragilis*, one of the dominant members of the normal colonic microflora, behaved in a manner identical to that of the gram-positive species (the majority of which are also members of the normal microflora); i.e., it induced very low levels of proinflammatory cytokines. We hypothesize that the reason that *Bacteroides fragilis*, *S. aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* produced such low quantities of proinflammatory cytokines is because, being members of the normal microflora, they have evolved means of downregulating the synthesis of proinflammatory cytokines so as to enable them to live (usually) in harmony with their host in contrast to the classic pathogens, *N. meningitidis* and *N. gonorrhoeae*. It is our view that understanding the pathology of infectious disease would benefit greatly from fuller investigation of the means by which members of the normal microflora (usually) fail to induce a chronic inflammatory response in their host. An additional benefit may be the discovery of whole new families of anti-inflammatory compounds.

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