CLINICAL MICROBIOLOGY REVIEWS, Oct. 1990, p. 335–344
0893-8512/90/040335-10$02.00/0

Vol. 3, No. 4

Is There a Role for Lactobacilli in Prevention of Urogenital and Intestinal Infections?

GREGOR REID,[1,2]†* ANDREW W. BRUCE,[1] JACQUELINE A. McGROARTY,[1] K.-J. CHENG,[3] AND J. WILLIAM COSTERTON[4]

Division of Urology, Department of Surgery,[1] and Department of Microbiology,[2] University of Toronto, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario M5G 2C4, Agriculture Canada, Research Station, Lethbridge, Alberta T1J 4B1,[3] and Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4,[4] Canada

INTRODUCTION

The purpose of this review is to discuss the relatively recent revival of interest in bacterial interference, particularly the use of *Lactobacillus* strains for the treatment and prevention of disease. To put the concept in perspective, it is important to clarify terminology, briefly mention the formation and structure of microbial communities, and then discuss the factors that influence the process. The scope of this review is rather extensive, and the reader will be provided with some appropriate references for more detailed coverage of certain areas.

Terminology

The review will concentrate primarily on specific examples of bacterial interference, a term that extends to interactions between two or more bacteria which lead to the establishment of a noninfected state in the host. We are in agreement with Tannock (120), who used the term interference to avoid confusion with numerous other terms. We use the term bacterial interference, as this review will concentrate primarily on bacterial species.

Although pathogenic organisms can interfere with the normal flora and cause infection, we have avoided use of the term negative interference, realizing that factors other than interference induce infection. Therefore, the interference terminology will cover only positive reactions, which lead to prevention of infection. As this process can be homologous, heterologous, natural, or artificial, each of these aspects will be discussed. Although the term competitive exclusion has often been used synonymously with interference, in this review it will be used only to describe blockage of bacterial adhesion to a surface in vivo and in vitro. Other terms, such as bacteriotherapy and bacterioprophylaxis, describe the mode of employment of bacterial interference and therefore are considered to be contained within the general term.

The terms normal, autochthonous, and indigenous will be used to describe the bacterial flora normally existing in a healthy host. Although Tannock (120) avoided the term flora for its botanical origin, we believe that it still merits usage here.

FORMATION AND STRUCTURE OF MICROBIAL COMMUNITIES

To discuss bacterial interference, it is important to mention briefly how microbial communities are formed. Extensive microbiological and morphological studies have provided valuable information on the structure of microbial communities. The primary determinant governing their establishment on the surface of tissues is adhesion. Clearly, in a system in which bulk fluids are mobile, bacterial adhesion initiates the sessile growth that will eventually result in the formation of bacterial biofilms on tissue surfaces (17). Although much emphasis is placed on very specific adhesin-mediated mechanisms (92), the simple truth is that most bacteria probably adhere to most surfaces by means of less

* Corresponding author.
† Present address: University Research Office, Stevenson-Lawson Building, University of Western Ontario, London, Ontario N6A 5B8, Canada.

avid, less specific mechanisms. In addition, chemically neutral surfaces in chemically neutral environments soon acquire hundreds of adherent bacterial species in available spaces (20).

Bacterial ecology differs profoundly from eucaryotic ecology in that very large numbers of species can establish themselves in a given niche. In addition, individual cells that thrive in that locus can soon come to completely dominate the ecosystem. The structure of the human vaginal flora is a case in point (90, 97). There, numerous bacterial species can adhere, but lactobacilli produce such an acidic niche that they survive and proliferate the best. The final microbial balance in these ecosystems is dependent on physiological factors. Changes in tissue surface chemistry, such as those mediated by hormone fluctuations, can alter selective pressures and change the predominant autochthonous flora of the site. Therefore, host changes, such as menopause, can profoundly alter the predominant microbial flora of the vagina and urethra (65). In addition, other factors, such as antibiotic therapy (81) and age (65), can distort the outcome of natural competition within a given ecosystem and radically alter the climax population (standing crop) of bacteria on tissue surfaces. Notwithstanding these effects, the tissue-associated bacterial populations of surfaces are relatively consistent and are usually dominated by the same genera and species in almost all individuals in the host species. In this way, the homeostasis of the host provides a consistent environment for the tissue-associated microbial flora, which often plays a vital physiological role in the effective function of the colonized site (15).

Microbial adhesion is a generalized phenomenon, as mentioned above, and perhaps the most important determinant in this process is survival and replication in the tissue surface environment. Adhesion assists organisms to survive secretion, peristalsis, or voiding motion, depending upon the host site. To adhere but not to replicate is, for bacterial species, a defect that could make them disappear from the tissue surface niche. Also, various nutritional requirements must be satisfied to maintain generation and existence. In preferred microbial habitats, survival is the paramount factor and small numbers of species usually predominate (e.g., campylobacters in the acidic stomach, lactobacilli in the vagina, small numbers of species in the bile-salt-rich biliary system). On the other hand, competition is the paramount factor in tissue surface ecosystems that generally support bacterial growth, and a large variety of species usually predominate (e.g., in the intestine and the oropharynx).

BACTERIAL INTERFERENCE

To place recent research findings in perspective, we will start with a few comments on some important historical landmarks, prior to the 1960s, that have contributed to the area.

Historical Perspective

A historical documentation of bacterial interference has been presented fairly comprehensively by Bibel (4, 5) and Florey (27). Some of the key stages are discussed below.

More than a century ago, in 1885, Arnoldo Cantani (10) treated pulmonary tuberculosis by spraying *Bacterium termo* into patients' lungs. This first report of bacteriotherapy showed that the approach could be effective. Two years later, Rudolf Emmerich (24) used streptococci to prevent death from anthrax in rabbits. This work proved that even the most virulent bacterium could be interfered with. Clinical tests continued in Europe, as witnessed by the work of David Newman in Scotland in 1915 (72). He used lactobacilli to treat bladder infections and claimed that the basis for this treatment was that the lactic acid produced by lactobacilli had antiseptic properties which could clear infecting organisms. However, his parameters of success were not clearly presented, and with a small sample size and lack of knowledge of the *Lactobacillus* strain, it is difficult to fully interpret how (or if) this therapy worked.

Elie Metchnikoff was perhaps the most renowned of the bacterial interference advocates. In 1894, he showed that cholera could be prevented by the presence of antagonistic organisms in the intestine (69). He was renowned for implying an important function for the normal flora, whereby by-products of the flora somehow affected the health status at points other than the intestinal colonization site. Metchnikoff's work with lactobacilli stimulated Yale University researchers to identify *Lactobacillus acidophilus* strains that could survive transmission through the stomach and intestine (93). Oral administration of this organism was found to help minor disorders such as constipation and diarrhea, presumably via colonization and interference.

In 1956, Sears et al. (106) implanted a gelatin capsule containing live cells of avirulent *Escherichia coli* into the dog intestine. This treatment repelled a challenge with a virulent strain of *E. coli* and set the stage for more extensive interference studies.

Studies during the Last 30 Years

In looking at work performed since 1960, we must ask why bacterial interference therapy has not had a more widespread acceptance in the medical world and why many more products are not commercially available. There are many examples of bacterial interference (achieved through natural and artificial means) that has established a normal flora that interferes with the infection process. However, with a few exceptions, mainly a group of health food products, bacterial interference does not form the main part of a physician's armamentarium. Possible reasons for this will be discussed in the next three sections.

Gastrointestinal tract. The bacterial flora of the gastrointestinal tract has been the subject of investigation for many years. This is a site of a large proliferation of microorganisms, with domination of different strains being influenced by many factors, and competition and interference being widespread (120). Some gastrointestinal tract studies have focused on bacterial interactions and their impact on anatomic, physiologic, and immunologic parameters (95). Antagonistic and cooperative reactions, which lead to interference of the establishment of pathogenic organisms, are known to occur. This process, whereby the microflora resists incursion by harmful microorganisms, has been referred to as colonization resistance (34).

The aerobic and anaerobic floras as a whole (132) have a protective role against bacteria and also fungal species such as *Candida albicans* (53). Interference with the effect of *Candida* species was linked to suppression of candidal growth and hence inhibition of its dissemination from the intestine. Dissemination, also referred to as intestinal translocation, has been studied by Wells et al., (130) who showed that *E. coli* translocation can be limited by enterococci and certain anaerobic bacteria. Therefore, systemic infection from an intestinal source can potentially be prevented by bacterial interference within the intestinal tract.

Specific members of the intestinal flora have been identified for their role in the process of interference, notably bifidobacteria (33, 71), enteric bacilli and anaerobes (44, 45), and lactobacilli (30, 120, 122). Selective colonization of the intestinal flora by using dietary supplements has been attempted since the work of Metchnikoff. For example, yogurt and milk beverages containing "commercially available *L. acidophilus*" have been used, often with no lasting colonization and with no significant effects on coliform numbers (31). To evaluate the various clinical studies, two questions must be examined: (i) Whether therapy involved specially selected microbial strains and (ii) whether randomized double-blind studies were undertaken in a prophylactic or treatment manner and whether the outcomes were well investigated.

(i) Therapeutic agents. The use of commercially available lactobacilli must be carefully investigated. In our experience, some of these preparations are unreliable. This point should be emphasized repeatedly, so that investigators and commercial companies do not flood the field with clinical results that are based upon inadequate microbial selection. Numerous *L. acidophilus* health food products are presently available commercially. Many of these are designed to be taken orally with a view to colonizing the intestine and establishing a balanced ecosystem. An examination of the dominant organism present in a selection of these preparations surprisingly showed a tendency toward *L. casei* rather than *L. acidophilus* (G. Reid, unpublished data). The reason for this is unclear. However, it emphasizes the need for caution in drawing conclusions from some existing therapeutic modalities. Hawley et al. (43) have previously stressed concern over "valueless lactobacillus preparations" which contained nonviable organisms or strains foreign to the intestine. This emphasizes strain selection geared to application. Until strains with specific properties are scientifically selected, characterized, and used carefully in commercial preparations (with clinical evidence and government approval), they are unlikely to prevent infection in the intestine or nearby mucosal sites (vagina, urinary tract). This may be one explanation for failures with *Lactobacillus* therapy.

(ii) Clinical studies. In a study of 94 patients, treatment of acute-onset diarrhea by using a cocktail of bacteria (35 to 45% lactobacilli) did not shorten the course of the infection (77). The preparation used contained mainly *Streptococcus thermophilus*, and no indication of its effectiveness against a range of enteric pathogens was presented. Therefore, evaluation of the effectiveness of this therapy cannot be completed.

Another commercially available "*L. acidophilus*/*L. bulgaricus*" preparation was tested in a randomized, double-blind trial to prevent traveler's diarrhea in Mexico (80). This prophylactic approach has merits in theory. However, there was no net prevention of diarrhea. Again, analysis of the data shows that it is unclear whether the infected patients had viral pathogens (which would probably not be affected by lactobacilli) or bacterial pathogens resistant to lactobacilli. In addition, the degree of colonization afforded by the dosage was not tested, and the exposure of patients to the pathogens was not monitored.

Other studies, however, support the use of bacterial interference for gastrointestinal tract disorders. Using a dosage of two lyophilized Bacid lactobacillus capsules (Fisons Corp., Rochester, N.Y.) four times daily, Settel (108) treated patients with various intestinal disorders, including pruritus ani, postantibiotic diarrhea, spastic and mucous colitis, and excessive flatus. He reported an improvement in patient condition in 39 of 48 (81%) patients. The mechanisms responsible for success were not discussed. Another study with Bacid showed similarly successful results in treating gastric problems, although placebo therapy was not assessed (3). The Bacid capsules again tested favorably in the treatment of symptomatic enteric infection (23). The authors concluded by stating that "no explanation can be offered for this phenomenon." The mechanism of action of lactobacilli is clearly open to investigation, but as with bifidobacteria (79), acid production and competition appear to play a role. Clinical studies which examine the mechanisms of disease prevention will be of great value in assessing and understanding bacterial interference.

(iii) Possible mechanisms of action. Once microbial communities have become well established in the intestine, it could prove difficult for lactobacilli to avoid swift passage through the tract. This may be one reason why *Lactobacillus* therapy is not always successful in treating infection. Rather, selective use of antimicrobial agents to clear infection, followed by implantation of indigenous organisms to recolonize and prevent recurrence, may prove a more fruitful avenue.

It has been proposed that for lactobacilli to colonize a mucosal surface, they must possess certain properties including adhesion, competitive exclusion ability, and inhibitor production (12, 86). Antagonistic activity appears to be important, in addition to adhesion (66). *Lactobacillus* sp. strain GG, which has been shown to have antimicrobial activity against a wide range of enteric bacteria (111), has been successfully used in the treatment of relapsing *Clostridium difficile* colitis (35). Continuous-flow and gnotobiotic-mouse studies by Itoh and Freter (50) have demonstrated that lactobacilli can compete with *E. coli* in the stomach and small intestine, but are less effective in the large intestine. However, clostridia have been found to control *E. coli* in the large intestine, indicating how organisms other than lactobacilli have a role in the interference process (50).

The mechanisms used by indigenous bacteria to interfere with potential pathogens may also be used by pathogens to induce disease. Production of bacteriocins is a case in point. These are a heterogeneous group of substances produced by many bacteria, as reviewed extensively elsewhere (116, 119). They are antibioticlike substances that have a narrow spectrum of activity, inhibiting only homologous species of bacteria. They consist of a biologically active protein moiety, have a bactericidal mode of action, and attach to specific cell receptors. There is a wide variation in their chemical composition and specific mode of action. Bacteriocins have been isolated from both gram-negative and gram-positive bacteria, for example, colicin E3 from *E. coli* and lactacin B from *L. acidophilus* (2). Only a few investigators have succeeded in demonstrating bacteriocin production in vivo (109). However, extrapolating from the in vitro data, it seems reasonable that many bacteria are capable of displacing or suppressing the growth of established resident bacteria in the indigenous flora. It has been suggested that in addition to their postulated role in population dynamics, bacteriocins may function as a recognition system related to cross-fertilization between different strains of bacteria (83).

Bacteriocinlike substances are similar to bacteriocins in nature, in that they have a narrow spectrum of antimicrobial activity. However, they do not inhibit homologous species of bacteria. They are believed to play a similar role to bacteriocins in bacterial ecology. By-products of bacterial metabolism such as hydrogen and hydroxyl ions, ammonia, free fatty acids, and hydrogen peroxide can influence the

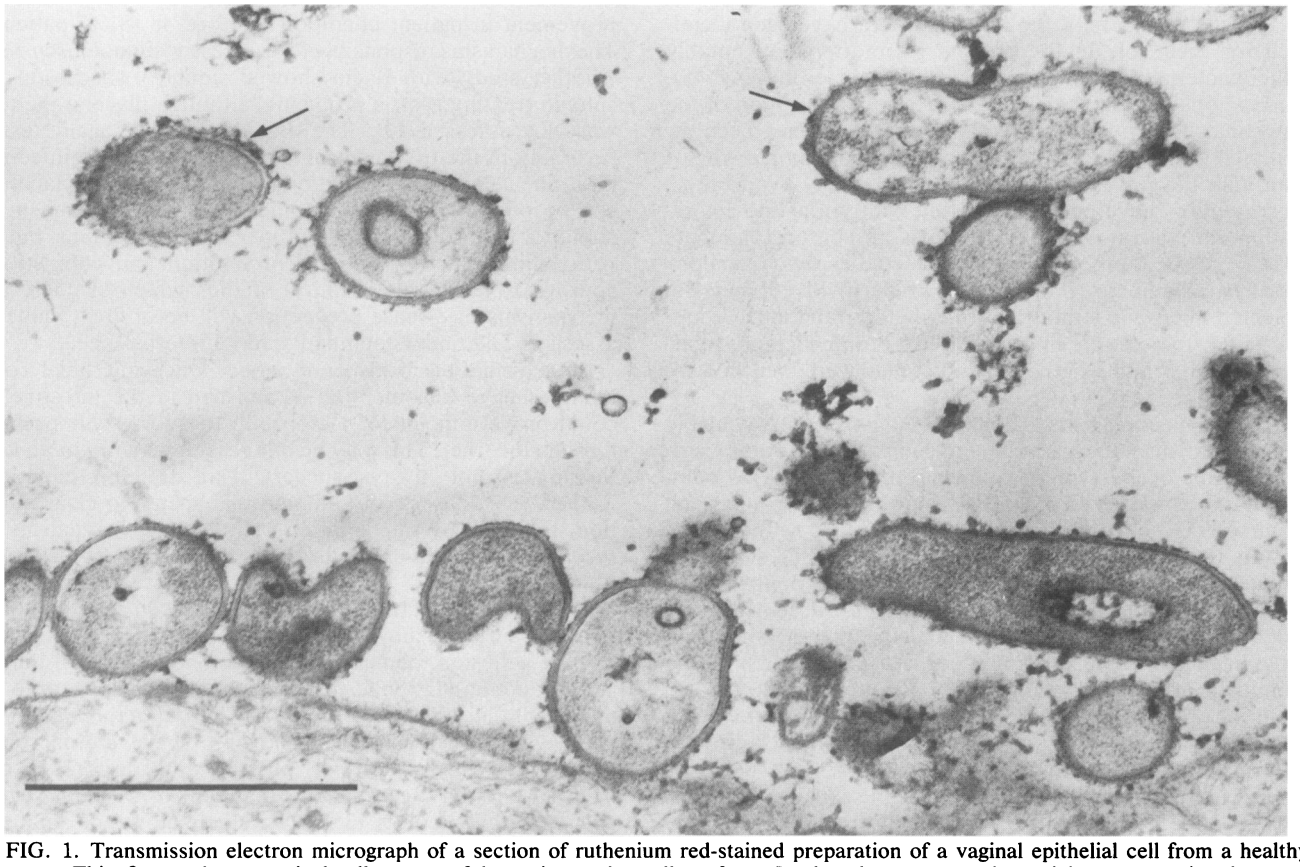

FIG. 1. Transmission electron micrograph of a section of ruthenium red-stained preparation of a vaginal epithelial cell from a healthy woman. This figure shows typical adherence of bacteria to the cell surface. It also demonstrates bacterial coaggregation between gram-positive and gram-negative (arrows) indigenous bacteria. Bar, 1.5 μm.

chemical composition of microenvironments and hence the bacterial flora that inhabits these niches.

Ileal (9) and cecal (61) tissues, which have been used to form a urinary conduit, overlap the gastrointestinal tract with the genitourinary tract. In human studies, it has been shown that naturally occurring bacterial interference can prevent infection. In particular, *Lactobacillus* colonization was correlated with an infection-free state in ileal conduits (9). Interestingly, the ileal colonization occurred with adhesion of the bacteria to mucus, and not to the ileal cells themselves. The mechanisms that control *Lactobacillus* interference remain to be fully elucidated. The conduit models represent a case in which bacteria had been eradicated by antimicrobial therapy before surgery and then natural recolonization had occurred following antibiotic therapy. Clearly, the order in which organisms colonize this tissue greatly influences whether there is infection or establishment of a healthy state. This fact is worthy of note if we are to use bacterial interference successfully in the clinical setting. As Dubos et al. (22) and Savage et al. (100, 102) have rightly pointed out, the close association of lactobacilli, anaerobic streptococci, and other microorganisms in the intestine emphasizes that a balanced microbial population must be the end product of interference processes, whether artificial or natural.

Urogenital tract. The urogenital tract (vagina, cervix, periurethra, and urethra) is a haven for many bacterial species. The balance between maintenance of a healthy state and emergence of infecting bacteria probably involves many factors. In vivo studies have demonstrated that members of the vaginal autochthonous flora coaggregate and colonize the epithelial cell surfaces (90) (Fig. 1). In vitro experiments can duplicate these interactions to an extent, although the existing methodologies are limited. It is clear that lactobacilli are the dominant members of the healthy adult female flora and that they are able to coaggregate with other bacteria (89). When this coaggregation is combined with inhibitor production by the lactobacilli, the reaction leads to the demise of uropathogenic *E. coli* in vitro (67). The coaggregation effect may be one method whereby the flora neutralize uropathogens and inhibit their infection of the urinary tract.

(i) Uropathogenic properties associated with disease. During urinary tract infection (UTI), the uropathogens are present in larger numbers in the flora of the urethra and vagina compared with healthy controls (64, 78). However, it is still unclear how uropathogens emerge from the urogenital mucosa and infect the urinary tract.

Numerous studies have demonstrated that uropathogens express many virulence factors (49, 94, 115, 116), such as adhesins (92), hemolysin (11, 57), siderophores (92), and certain O antigens (7, 92). Their adhesion to urogenital cells and to each other (autoaggregation) enhances their ability to infect (88, 92). However, their adhesion to other organisms (coaggregation) is less well understood. Just as this is an important phenomenon in maintaining a balanced flora, it would also seem to be relevant to pathogenesis. The inability of a uropathogen to survive and compete with other bacteria for nutrients, space, and habitat would have adverse effects on its pathogenicity. Coaggregation has been investigated in the oral cavity, where there is a correlation between plaque

formation and interaction of *Bacteroides gingivalis*, *Actinomyces viscosus*, and streptococci (18, 56). This serves as a useful model to investigate other potentially interactive processes. Several subsequent studies have shown that bacterial aggregation and coaggregation can indeed occur and may be associated with disease in the urinary tract (88) and peritoneal cavity (6, 91). The intriguing finding that type 1 fimbriated *E. coli* organisms coaggregate with lactobacilli (90) and *B. fragilis* (88) could suggest a means whereby *E. coli* is able to integrate into the urogenital flora before ascending into the bladder. This hypothesis remains to be validated, but is an interesting possibility.

(ii) *Lactobacillus* by-products aiding interference. A number of metabolic by-products of lactobacilli are believed to contribute to their ability to aid in the maintenance of a healthy urogenital tract. Lactic acid and hydrogen peroxide are toxic to a number of bacterial species and have been demonstrated to inhibit potential pathogens (30, 124). In addition, a number of bacteriocins produced by lactobacilli have been described that are active against a wide range of bacteria and fungi (2, 51, 111, 127). A bacteriocinlike substance described by McGroarty and Reid (67, 68) that is derived from lactobacilli showed activity in vitro against uropathogenic *E. coli* and *Enterococcus* species. The role of these substances in vivo remains to be elucidated.

(iii) Clinical studies of bacterial interference. Specific *Lactobacillus* strains have been selected for clinical use to restore the protective flora of women suffering from recurrent UTI (8). In one study, *L. casei* GR-1 was implanted directly into the urinary bladder in postmenopausal patients who had suffered persistent colonization and infection with uropathogens (37). The lactobacilli were found not to adhere to the bladder, indicating that the origin of administration (the bladder rather than the vagina) affects the outcome of a study.

The clinical study demonstrated that even under invasive circumstances, this *Lactobacillus* strain did not infect the urinary tract. Only rarely have lactobacilli been found to infect the kidneys (as can most bacterial species) when given access directly via obstruction or under exceptional clinical situations (62).

An interesting finding of the work by Hagberg et al. (37) was that avirulent *E. coli* strains (i.e., strains with few virulence markers of adhesion, specific O and K serotypes, hemolysin production, and resistance to serum effects) rather than virulent strains from the patients' own fecal flora could be used as competitive colonizers of the bladder. When these organisms were instilled intravesically (6 ml of 10^9 bacilli per ml), they colonized the mucosa and apparently interfered with the onset of symptomatic infection. The possibility was raised that a genetically engineered avirulent strain could prove useful in high-risk patients who continually fail to respond to antibiotic therapy.

The use of *L. casei* GR-1 in a small, uncontrolled study of pre- and postmenopausal women met with more success than bladder instillation when the organisms were given intravaginally in a douche form. The lactobacilli colonized the vaginal mucosa, and patients correspondingly had more prolonged periods without recurrence of UTI (8). Because enterococcal breakthrough infections occurred in two patients, *L. fermentum* B-54 was added to the therapeutic agent. This strain had shown in vitro inhibitory activity against enterococci (68).

In a subsequent 1-year study of eight pre- and postmenopausal women, only two patients suffered enterococcal infections; this proportion is not abnormal for this population. There was an increased level of *Lactobacillus* vaginal colonization and a 77% overall reduction in incidence of UTI, compared with the prestudy period (A. W. Bruce et al., unpublished data).

The colonization data are based upon studies of vaginal pH, numbers of lactobacilli adherent to vaginal epithelial cells, and semiquantitative vaginal *Lactobacillus* counts. No simple and practical technology is yet available to absolutely identify *Lactobacillus* strains artificially administered to humans. Therefore, replication time and length of colonization remain to be established.

A randomized, double-blind study is currently under way to accumulate sufficient data on premenopausal women to allow statistical analysis. Lactobacilli are the predominant organisms in the urogenital tracts of healthy premenopausal women (64, 90); therefore, this age group has been selected for further study (64, 90). However, as indicated by previous results, this therapy could also potentially help postmenopausal women whose protective lactobacilli are absent as a consequence of hormonal changes.

The lactobacilli were prepared as freeze-dried suppositories and are self-administered weekly. The use of freeze-dried cultures ensures uniform delivery of lactobacilli per patient and per application. The results after 10 months for 20 patients show a 50% reduction in the incidence of UTI (A. W. Bruce and G. Reid, unpublished data). This decrease demonstrates a positive effect, but not until the end of the 2-year trial will the effects of active versus placebo (skim-milk) therapy be known.

(iv) Use of lactobacilli in postantibiotic therapy. Another potential role for bacterial interference is in patients after antibiotic therapy. It has been estimated that up to 25% of women will develop a UTI during their lifetime and as many as 80% of these will have a recurrence (52, 58). Although the actual recurrence rate at any given time is difficult to estimate, the population of affected females is substantial. For active infection and for prophylaxis, 3 to 7 days of antibiotic treatment and 6 months to 2 years of therapy, respectively, are, for the most part, effective in eradicating bacteriuria and preventing recurrences (25, 96, 103). However, UTI has been shown to recur within a few months of cessation of long-term antibiotic therapy (74, 75). Patients who fail therapy or are unwilling to suffer the side effects or prolonged use of antibiotics currently have few alternatives.

Careful selection of antibiotic regimens with fewer detrimental effects on the fecal reservoir can clear UTI and reduce reinfections (40, 117). However, upon cessation of antibiotic therapy, the urogenital flora is not restored immediately to a normal population as found in healthy women (87). Infections in the bladder and vagina following antibiotic therapy are not uncommon (85, 87, 117). In addition, studies have shown that uroepithelial cells lose their adherent microbial population upon administration of antimicrobial agents, but retain their receptivity for bacteria (85). Therefore, after therapy for UTI, the potential exists for recolonization and reinfection by uropathogens. The fact that not all cystitis patients have postantibiotic infection implies that their urogenital flora must somehow be restored to a stable equilibrium. This fascinating sequence of events remains to be investigated, although there is evidence to show a reemergence of large numbers of lactobacilli in the urethra and vagina 4 weeks after cessation of antibiotics (87). It would appear that there is still an opportunity for bacterial interference to be applied, postantibiotic therapy, particularly in a select group of patients. A study of this type is currently being completed by our group. *Lactobacillus* suppositories

containing *L. casei* GR-1 and *L. fermentum* B-54 are given vaginally to patients after 3 days of norfloxacin or trimethoprim-sulfamethoxazole therapy in an attempt to artificially restore the normal flora. Although the blinding has not been lifted, the preliminary data on 18 patients shows a twofold increase in the number of patients colonized with lactobacilli (G. Reid and A. W. Bruce, unpublished data). This suggests that *Lactobacillus* domination of the urogenital flora postantibiotic therapy may be possible by using artificial implantation.

(v) Therapy for vaginitis. A number of investigators have used lactobacilli with varying success for the treatment and prevention of bacterial (28) and yeast (99) vaginitis. The mechanisms of action may involve inhibitory substances; in vitro studies have shown that lactobacilli inhibit a range of aerobic and anaerobic bacteria from the vaginal flora of women with and without bacterial vaginitis (63, 112). The inhibitory substances were not identified or characterized, but the activity was influenced by pH. Tolino et al. (123) found that topical and oral administration of an *L. acidophilus* suspension reduced bacterial and mycotic vaginal infections.

Protective effects of lactobacilli against *Neisseria gonorrhoeae* have also been reported and are associated with the production of inhibitory substances (98). Significantly more uninfected women exposed to gonococci harbored lactobacilli (76%) than did women infected with gonococci (54%). The lactobacilli were able to inhibit gonococci in vitro; however, the study did not verify that inhibitory substances were produced in vivo or that they prevented gonococcal infection. In addition, the study did not control for differences in exposure rates of patients to gonococci or for differences in the type and quantity of lactobacilli present at any given time.

A recent study suggested that *L. acidophilus* is not effective in treating bacterial vaginosis (B. Fredericsson, K. Englund, L. Weintraub, A. Olund, and C. E. Nord, Abstr. 4th Eur. Congr. Clin. Microbiol., abstr. no. 179, p. 91, 1989). However, the *L. acidophilus* strain used in these studies was not carefully selected for specific properties that might interfere with the infecting species. This lack of selectivity does little to test the concept of bacterial interference. Another study, from Israel, also used an uncharacterized *L. acidophilus* strain, but found an improved clinical outcome in the treatment of vaginitis (28). Interestingly, this latter group added oral vitamin B to the treatment and suggested that this compound stimulated *Lactobacillus* growth and colonization and improved bacterial interference.

The number and type of organisms in the urogenital flora vary over the menstrual cycle (107). Hormonal influences (84, 104) can potentially affect uroepithelial cell receptivity for bacterial adhesion, including *Lactobacillus* adhesion (12). Animal studies confirm that estrogens affect *E. coli* and vaginal flora colonization (59, 114).

Some recent attention has focused on the effects of spermicidal agents on urogenital flora. The advent of acquired immunodeficiency syndrome has led to increased usage of barrier methods of contraception, in particular, condoms used in conjunction with spermicide. The active ingredient of most spermicidal preparations is Nonoxynol-9, used at 5% in creams and 12.5% in foam. It is a surface-active agent that has potent antibacterial and antiviral activity (76). In vitro studies have indicated that spermicidal preparations have the potential to alter the composition of the urogenital flora and thus may contribute to an increased incidence of recurrent UTI in women using this form of contraception (66a). In these studies, doubling dilutions of 0.1 to 25% Nonoxynol-9 were made and the MIC was determined for a number of vaginal isolates of lactobacilli and uropathogenic bacteria in appropriate growth media. A total of 18 fresh, vaginal *Lactobacillus* isolates were tested; growth of 67% was inhibited in the presence of <1.0% (wt/vol) Nonoxynol-9, and the remaining 33% flourished in the presence of 25% Nonoxynol-9, which is twice the maximum concentration used for contraceptive purposes. The growth of gram-positive and gram-negative uropathogens, as well as *Candida* spp., was unaffected by up to 25% Nonoxynol-9. Therefore, the in vitro evidence suggests that spermicide has the potential to increase the risk of acquiring a UTI or yeast infection by altering the ecological balance of the urogenital tract. In vivo studies support these observations, showing that uropathogens and yeasts are isolated two to three times more frequently from the vaginas of women using a diaphragm plus spermicide for contraception than in those using other forms (26, 32, 46, 118, 129), whereas it is inferred from increased vaginal pH that numbers of lactobacilli drop (26, 46). It remains to be determined whether the use of a *Lactobacillus* preparation which is resistant to Nonoxynol-9 (such as *L. casei* GR-1 and *L. fermentum* B-54) will prove effective in vivo against recurrent UTI and vaginitis associated with spermicide use.

Probiotics. Many of the complex issues being addressed here in relation to humans also have relevance for other animals. Much can be gained from examining the use of probiotics in animals, particularly in relation to the effectiveness of gastrointestinal applications. Probiotics is a term more often associated with the veterinary than the medical field and is used here to mean prophylactic use of microorganisms to help protect the host animal from disease. The following discussion will illustrate that carefully selected cocktails of bacteria may well have a place in animal disease prevention.

The autochthonous microbial populations of animal tissues, like any stable and mature populations within any ecosystem, are remarkably resistant to manipulation by the introduction of new species. Generally, an autochthonous microbial population must be either absent or profoundly disturbed by physiological factors before extraneous organisms can be recruited as members of these very complex adherent communities (14, 17, 101). Despite this well-established principle of microbial ecology, hundreds of probiotic preparations are presently marketed on the premise that they somehow introduce beneficial bacteria into economically important ecosystems. As with *Lactobacillus* preparations for humans, such claims must be vigorously screened to ascertain that these extraneous organisms are actually integrated into the autochthonous mucin and tissue-associated microbial populations of the organ system concerned.

When extensive tissue-associated microbial populations were first discovered and when the importance of direct adhesion in the pathogenesis of many bacterial infections (17) was first realized, it was logical to suggest that vigorous autochthonous populations at the surface might reduce tissue access by pathogens and thus protect treated animals from disease. In the simplest of these experiments, fowl feces were fed to chickens to accelerate their acquisition of tissue-associated bacterial populations as a protection from infection by *Salmonella* spp. (36, 47, 48, 82, 105, 113). In more refined studies, pure cultures of single organisms, such as *Enterococcus faecalis*, *L. acidophilus*, *Enterococcus faecium*, *Bacillus toyoi*, *L. sporogenes*, *Bifidobacterium thermophilus*, *Bifidobacterium pseudolongum*, and *Bacillus*

nato, have been fed to newborn and adult animals (both ruminants and monogastrics) to preclude the adhesion of bacterial pathogens (16, 29, 38, 39, 54, 55, 70, 71, 105, 133). Cheng and Costerton (16) have inoculated newborn ruminants with a complex combination of pure cultures of autochthonous organisms from healthy animals of the same species. They showed that this procedure accelerates colonization of the tissues of the treated animals and provides a measure of resistance to infection by enterotoxigenic *E. coli*. Generally, all of these treatments have provided some success, with accelerated or improved tissue-associated populations protecting the animals to a certain extent against infection by specific adherent pathogens (73). In addition to adhesion, inhibitory effects possibly related to acid production, for example by lactobacilli, may aid in the bacterial interference process (128).

Microbial ecologists (14) have shown that tissue-associated bacteria often carry out physiological functions that are important or essential for domestic ruminants. This work suggests that acceleration of the natural microbial colonization of both the rumen and gut of newborn ruminants would improve the performance of these organs. A comparison of the anatomical and physiological development of gnotobiotic and normal newborn ruminants clearly shows that bacteria interact with the tissues of the digestive tract in their development and physiological function. When Cheng and Costerton (16) inoculated newborn lambs with their complex mixture of autochthonous ruminal and gut bacteria, treated animals gained 20% more weight than did untreated vivarium-reared controls. However, when the performance of inoculated lambs was compared with that of control animals reared by their mothers, the inoculated animals showed no better protection against infection. These data suggest that bacterial colonization is very important in the physiological development of young ruminants but that the use of probiotic inocula may not be more efficient than the natural acquisition and competitive selection of cooperative bacteria. Recently the physiological performance of adult ruminants and monogastric animals has been shown to be improved by simple feeding of specific yeasts and other fungi (1, 19, 21, 41, 42, 131). These organisms do not integrate into the autochthonous populations and must be fed continuously to the treated animals. They alter fermentation patterns, increase fiber digestion, decrease methane production, and control ruminal pH to improve performance by increasing feed uptake. As our knowledge of native anaerobic fungi increases, we expect to find several species that serve as additives to improve the performance of ruminants on low-quality feeds.

In summary, it is now clear that the autochthonous microbial flora is beneficial to a wide variety of animals, in both disease resistance and physiological functions. Generally, mature and vigorous autochthonous populations resist manipulation and extraneous microorganisms are rarely integrated into the complex communities. However, in cases of persistent and recurrent infection the microbial populations have been disturbed, and therefore artificial implantation of organisms, such as lactobacilli, may be useful. Newborn animals lack these beneficial populations, and their acquisition may be accelerated or improved by inoculation, if this process is more rapid or superior to natural acquisition from the mother. The deleterious effects of ecological disturbances may be mitigated by microbial manipulation, but the use of all probiotics must depend on proof that the extraneous organisms actually integrate into the tissue-adherent autochthonous populations and exert a beneficial effect on the host. Most of the currently available veterinary probiotic products fail to meet these criteria and offer little usefulness beyond modest success. For bacterial interference to be successful, therefore, strains and target animals must be carefully selected. As indicated above, this goal is clearly attainable.

Future Directions

Although certain acute diseases caused by single species will probably be controlled by vaccination and antibiotic therapy, the same is not true of a large number of diseases, such as pseudomembranous colitis, vaginitis, pneumonia, and UTI. Because ecological factors such as those discussed above (antimicrobial agents, hormones, etc.) may be of prime importance in these diseases, we must understand the base-line microbial ecology of the organs concerned and mobilize the concepts and techniques routinely used by ecologists in industrial and environmental circles. Primarily, the future of bacterial interference will depend on a greater understanding of the mechanisms involved. More basic information on the properties of microorganisms which enable them to interfere with pathogenic species is required. For *Lactobacillus* species, this means understanding genetic mechanisms. How are the bacteriocins and adhesins encoded? Are they transmissible? Can we genetically engineer a hardy *Lactobacillus* species with optimum adherence and inhibitor production? Will this strain survive and prosper in vivo? How can we verify the presence and replication of implanted organisms? This is particularly important because morphological and growth characteristics of lactobacilli can vary within strains. Conjugal transfer of a plasmid encoding antibiotic resistance between certain *Lactobacillus* and *Enterococcus* species has been demonstrated in vitro (110, 121, 122). Certainly, various species of urogenital bacteria can coaggregate, creating the opportunity for genetic transfer of material, as found in vitro between gram-positive and gram-negative bacteria (13, 60, 125). What effect does this have on microbial communities?

An important finding by Tuomanen (126) showed that adhesins produced by *Bordetella pertussis* could be used by strains of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus*. In this case, bacterial cooperation was correlated with disease initiation, indicating ways in which bacteria within ecosystems can interact to affect the host. This finding raises the question of how indigenous organisms interact with each other and with potential pathogens. By understanding these mechanisms, we will be better placed to implement bacterial interference technology.

We believe strongly that bacterial interference plays a major role in preventing disease and that well-researched artificial interference can provide an added weapon in preventative care. However, many questions remain unanswered, and the next decade should see an exciting period of investigation.

ACKNOWLEDGMENTS

This work received support from the Medical Research Council of Canada and from the Ontario Ministry of Health and University Research Incentive Fund of Ontario. G.R. is an Ontario Ministry of Health Career Scientist.

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