

Review article: the role of the gut flora in health and disease, and its modification as therapy

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Accepted for publication 21 April 2002

SUMMARY

The gut flora is a vast interior ecosystem whose nature is only beginning to be unravelled, due to the emergence of sophisticated molecular tools. Techniques such as 16S ribosomal RNA analysis, polymerase chain reaction amplification and the use of DNA microarrays now facilitate rapid identification and characterization of species resistant to conventional culture and possibly unknown species. Life-long cross-talk between the host and the gut flora determines whether health is maintained or disease intervenes. An understanding of these bacteria–bacteria and bacteria–host immune and epithelial cell interactions is likely to lead to a greater insight into disease pathogenesis.

Studies of single organism–epithelial interactions have revealed the large range of metabolic processes that gut bacteria may influence. In inflammatory bowel diseases, bacteria drive the inflammatory process, and genetic predisposition to disease identified to date, such as the recently described NOD2/CARD15 gene variants, may relate to altered bacterial recognition. Extra-intestinal disorders, such as atopy and arthritis, may also have an altered gut milieu as their basis. Clinical evidence is emerging that the modification of this internal environment, using either antibiotics or probiotic bacteria, is beneficial in preventing and treating disease. This natural and apparently safe approach holds great appeal.

INTRODUCTION

There has been a re-emergence of interest in the relationship between the gastrointestinal flora and gut function, fuelled in part by the recognition of the potential value of probiotics and other means of modifying gut flora as therapeutic modalities.

This review aims to consider our existing knowledge of the nature of the gut flora and new techniques that allow enhanced characterization of this flora. It highlights emerging methods that allow bacteria–epithelial interactions to be evaluated. Finally, it considers some of

the disease areas in which bacterial modification has been shown to be of therapeutic benefit.

THE NORMAL FLORA

The flora of the gastrointestinal tract is complex and should be considered as a functionally active organ, the full potential of which remains to be elucidated.^{1–4} Each of us has about 10^{12} viable bacteria per gram of large bowel content, which is equivalent to more bacteria in one person's gut than there have ever been humans on the planet. This ecosystem is vast, both quantitatively and in terms of diversity, with the presence of at least 400–500 different species.⁵ A single organism, for example *Escherichia coli*, is known to have huge metabolic capability. Extrapolating from this, the metabolic

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capability of the entire gastrointestinal flora is immense. The magnitude of this organ is underlined when we consider that the total number of genes contained in the microflora is about 50–100 times that in our 'own' genome.⁶ A further level of complexity is the temporal and spatial diversity of the flora. The bacterial distribution varies greatly at different levels of the gastrointestinal tract,⁷ ranging from $<10^3$ colony-forming units/mL (CFU/mL) in the stomach, where the number of ingested bacteria is dramatically reduced by contact with gastric acid, to 10^{11} – 10^{12} CFU/mL within the colon, where anaerobes outnumber aerobes by a ratio of 1000 : 1. The species composition varies as we develop and age, and is influenced by our environment.

Methods of identifying the gastrointestinal flora are limited by this complexity, the inaccessibility of parts of the ecosystem and the temporal and spatial diversity. Furthermore, the definition of 'normality' of the gut flora needs to be clarified. How should normality be defined, in terms of different sites in the gut, different conditions and the population under evaluation? Much of the work has focused on Western subjects with known diagnoses, many of whom are on medications or have received anaesthetics or antibiotics. It is difficult to extrapolate from this to healthy individuals world-wide and to encompass the extremes of age. Furthermore, most of the studies undertaken to define the normal flora have used faecal samples, which may not be representative of the mucosal microenvironment. There are technical problems of sampling, transport and storage, in addition to cultivation, enumeration and identification, all of which may cause changes in the flora.⁸ Much of the work published over the last 40 years needs to be interpreted with caution.

Methods of identification

New methods of identification have enabled a renewed assessment to be made of the gut microflora. The traditional microbiological techniques of culture and microscopy are being supplemented by genotype analysis. This has been driven by the fact that traditional methods are unsuitable for high-throughput analysis and there is the added problem that a large proportion of the gut microbial residents are non-culturable by traditional techniques. Techniques that can detect and semi-quantify both culturable and non-culturable gut bacteria are now available and have

encouraged a renewed interest in the analysis of the intestinal flora.

The techniques that have found most use to date employ analysis of the small ribosomal subunit RNA, 16S rRNA in the case of bacteria.⁹ 16S rRNA contains hypervariable regions that contain signatures of phylogenetic groups or species. Polymerase chain reaction (PCR) amplification using universal primers allows the identification of bacterial species by sequencing of hypervariable regions or by the less laborious approach of denaturing gradient gel electrophoresis (DGGE).¹⁰ Alternatively, species-specific primers from the hypervariable sequences can allow the direct identification of particular organisms. There are currently in excess of 16 000 16S rRNA sequences stored in databases and the development of bioinformatic tools required to efficiently exploit such information is paralleling the advances in molecular biology and genetics.¹¹

These molecular techniques are very powerful but they do have limitations. For instance, DNA/RNA may not be extracted with equal efficiency from all bacteria; some techniques, such as PCR/DGGE, may not be very sensitive; and PCR amplification, particularly for large numbers of cycles, may introduce mutation artefacts. Future developments of identification technologies will probably include the use of high-density DNA microarrays as an alternative to PCR/DGGE for high-throughput analysis, and real-time PCR analysis for quantitative assessment of mRNA levels. In addition, microarray analysis will be combined with matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Using such technology, it is possible to determine the DNA sequence of an 80-base pair fragment present in an individual array spot in milliseconds and make the transition from genomics to proteomics.

The functional definition of bacterial populations has primarily involved immunochemical strategies. Antibody responses to local immunogenic microbial cells can be useful to identify microorganisms participating in the disordered environment. Expression cloning using antibody targets and subtractive cloning using genomic representational difference analysis, cDNA suppression subtractive hybridization and differential display hybridization offer functional methods of identifying the gut flora.

In a period of about 30 years, the identification of intestinal bacteria has developed a long way from 'culture and phenotype' to 'no culture, just genotype'.

The role of the normal flora in health

There is a great deal of evidence supporting the view that what is generally considered as normal gut structure and function is the end-point of a complex set of interactions between the host and microorganisms colonizing the gut. Such normal features include motility, secretion, absorption, cell composition, mitotic activity, villous length and crypt depth. This has been known for decades from observations in germ-free animals.³ The molecular basis for these host–flora interactions is now being studied. For example, a prototypic commensal, *Bacteroides thetaiotaomicron*, has been used to colonize germ-free mice, and the effect on epithelial cell gene expression has been assessed by DNA microarrays and laser capture microdissection. This commensal has been shown to influence many genes dictating nutrient absorption, mucosal barrier function, xenobiotic metabolism, angiogenesis, the enteric nervous system and postnatal intestinal maturation. Furthermore, different species have been shown to elicit different changes in gene expression and to participate in different physiological functions.¹² This is a reductionist approach and it will be the cumulative summation of the entire flora that will determine the end result. Nonetheless, it makes it possible to use *in vivo* systems to quantify the impact of a microbial population on host cell gene expression.

HOST IMMUNE RESPONSES TO THE NORMAL FLORA

A relationship between the normal flora and the host immune system exists with a mutual dependency between the two. Successful coexistence with a complex microflora presents a particular challenge to the immune system of the host. On the one hand, the host needs to avoid an overly aggressive response to this microbial population that would lead to the elimination of beneficial organisms and would almost certainly result in inflammation and extensive tissue damage. On the other hand, the capability to limit the spread of bacteria from the lumen into underlying tissues and to mount an effective response to intestinal pathogens needs to be maintained. A generalized hyporesponsiveness in the intestine would leave the host highly vulnerable to a wide range of intestinal pathogens.

Control of bacterial translocation

Translocation or penetration of intestinal bacteria into the underlying tissue or mesenteric lymph nodes can be demonstrated in animals and humans,¹³ but occurs without serious consequence in most healthy individuals. The acquired immune response appears to play only a minor role in limiting bacterial penetration. Although translocation is increased in T-cell-deficient mice,^{14, 15} these animals remain healthy. Similarly, there is evidence for increased translocation in immunoglobulin A (IgA)-deficient mice,¹⁶ but commensal-related sepsis is not observed in IgA 'knockout' mice or in IgA-deficient humans. In contrast, gp91^{phox-/-}/NOS2^{-/-} mice, whose phagocytes have severely impaired antimicrobial activity due to the inability to make both reactive oxygen and reactive nitrogen intermediates, develop massive abdominal abscesses containing enteric commensals.¹⁷ On the C57BL/6 genetic background, all such mice die by the age of about 4 weeks despite the use of prophylactic antibiotics. Mice with a deficiency in only one enzyme pathway (gp91^{phox-/-} mice or NOS2^{-/-} mice) do not develop abscesses. These results demonstrate the importance of phagocyte killing for limiting the consequence of bacterial translocation from the gut, and illustrate redundancy in the cellular pathways involved. They also indicate a constant exposure of host tissues to bacteria and their products, which occurs despite a fully functional acquired immune system.

Adaptation of the immune system to the normal flora

Whilst not playing the dominant role in the control of bacterial penetration from the intestine, it is clear that those parts of the host's immune system that mediate acquired responses are not ignorant of the gut microflora. Comparison of germ-free animals with those with a normal flora clearly demonstrates adaptation of the immune system to the presence of bacteria. Germ-free animals have reduced numbers of lamina propria or intra-epithelial T cells,¹⁸ and these are increased following the restoration of a normal microflora.^{19, 20} The turnover of lamina propria T cells, but not intra-epithelial T cells, is estimated to be rapid,²¹ and this process of lymphocyte activation, homing and death may be flora driven. Peyer's patches are small in germ-free animals, but cryptopatches, tiny sites of lymphopoiesis in the crypt lamina propria of

the intestine,²² do not appear to require a flora for their development.²³ Levels of secretory IgA and numbers of mucosal IgA-producing plasma cells are both low in germ-free mice,^{12, 16} but they increase rapidly upon introduction of even a single commensal bacterial species, such as *Morganella morganii*²⁴ or *Bacteroides thetaiotaomicron*.¹²

Some of the prodigious IgA production that occurs in the intestine of non-germ-free mice has no obvious specificity for microbial (or food) antigens. This 'natural' antibody may result from polyclonal activation by microbial products, such as lipopolysaccharide. However, some of the IgA can be shown to have specificity for components of the bacterial flora. Recent work suggests that at least some of this bacteria-specific IgA is produced, in a T-cell-independent fashion, by the B1 lineage cells in mice, derived from the pleuroperitoneal cavity.¹⁶ B1 cells, characteristically expressing CD5 and high levels of IgM, arise early in ontogeny and reside originally in the peritoneal and pleural cavities. Currently, it is not clear whether intestinal B1 cells are activated directly, perhaps by multimeric bacterial antigens, or whether other cell types, such as antigen-presenting cells, are involved. The contribution of B1 cells to human IgA responses is unclear. Another unanswered question is whether the intestinal flora influences the primary repertoire of B cells — that is the constellation of binding specificities available in a naive (non-pathogen-exposed) animal.

One of the special features of immunoregulation at mucosal sites is the ability of orally administered soluble antigens to induce a profound state of systemic non-responsiveness.²⁵ This experimentally induced non-responsiveness, also termed oral tolerance, is presumably related to physiological responses invoked by dietary proteins and perhaps commensal bacteria.²⁶ The mechanisms that underlie oral tolerance remain somewhat controversial, but could include active cytokine regulation, anergy or clonal deletion; different mechanisms are probably favoured at different antigen doses. Some aspects of oral tolerance are deficient in germ-free mice, and this deficiency can be reversed by reconstitution with *Bifidobacterium infantis* in the neonatal period;²⁷ reconstitution of adult mice is not effective. These data suggest that the presence of bacterial flora during the neonatal period is required for the development of a properly regulated immune system in which oral tolerance can be fully induced.

Mechanisms of controlled inflammation

The presence of large amounts of immunogenic and pro-inflammatory molecules in the intestinal lumen and the evidence for the interaction of the host's immune system with this material have led to the concept that the gut mucosa is in a state of restrained immune reactivity driven by the intestinal flora. This has been termed 'controlled' or 'physiologic' inflammation. It is clear that such a restrained response must be very tightly regulated. In support of this concept, many knockout mouse strains, in which the genes involved in immune regulation are targeted (e.g. interleukin-10, T-cell receptor α or β chain, interleukin-2, interleukin-2 receptor), develop intestinal inflammation.²⁸ Similarly, perturbations in T-cell development or lymphocyte subpopulations can also result in inflammatory gut disease. Where tested, this inflammation was shown to be driven by the intestinal flora.

Control of this highly regulated response is probably achieved by multiple mechanisms acting at different levels; a network of interactions between bacteria, the immune system, epithelial cells, stromal elements and components of the nervous system is likely to be involved. From the perspective of the immune system, it is often argued that the gut displays an inherent bias towards anti-inflammatory (Th2) or regulatory (Th3/Tr) T-cell responses, with the production of 'regulatory' cytokines such as interleukin-10 and transforming growth factor- β . This concept has its origins in studies on the massive IgA production associated with the gut and on the propensity of T cells from gut-associated lymphoid tissue to support IgA responses. It has been adapted to include concepts of Th1 vs. Th2/Th3/Tr responses, as our understanding of functional T-cell subsets has developed. However, a simple model of a default pathway away from Th1 responses is clearly an oversimplification. Interferon- γ is produced in response to fed antigens, even when they are administered at tolerogenic doses,²⁹ and human Peyer's patch cells display a T-cell type 1 cytokine profile in response to dietary antigens.³⁰ Indeed, a bias away from Th1 responses may be a feature of mice, but not humans, and it could be a consequence of a reduced flora (quantitatively and qualitatively) in young, specific pathogen-free mice.³¹

Despite the complexities of Th cell differentiation in the intestine, animal models provide convincing evidence that regulatory T-cell populations are involved in restraining the response to the intestinal flora.^{32, 33}

Local immunosuppression by these cells is mediated by interleukin-10 and transforming growth factor- β and is dependent on signalling through the cytolytic T lymphocyte-associated antigen-4 receptor for members of the B7 family of co-stimulatory molecules. The conditions that favour the induction of these regulatory T cells are poorly understood, but at least some regulatory T cells are present in germ-free animals, suggesting that they are not specific for, or dependent on the presence of, components of the flora.

Dendritic cells are one of the first cell types of the immune system to come into contact with components of the flora, and are likely to play a crucial role in shaping the type of response that develops. Gut dendritic cells can acquire antigens such as ovalbumin when these are fed to an animal and, upon isolation, these dendritic cells can stimulate ovalbumin-specific T cells.³⁴ Sampling of the luminal contents by dendritic cells could occur by several routes. Firstly, dendritic cells in the subepithelial dome of Peyer's patches could acquire materials transported across M cells. Secondly, recent evidence suggests that dendritic cells in the lamina propria are able to interdigitate between epithelial cells and directly sample luminal contents;³⁵ expression of tight junction proteins by the dendritic cells ensures that mucosal integrity is preserved. Thirdly, some gut dendritic cells appear to contain apoptotic epithelial cells,³⁶ and this may represent an indirect pathway by which dendritic cells sample materials derived from the lumen that have been taken up by the epithelial cells. Dendritic cells may also take up materials that have been transported across epithelial cells or gained access through transient breaches in mucosal integrity. Recognition and response to bacterial products by dendritic cells will be determined by molecules such as pattern recognition molecules and toll-like receptors that are expressed on the surface of dendritic cells.³⁷ It is conceivable that dendritic cells may also express the bacteria-sensing molecule NOD2, genetic variants of which have recently been reported to be associated with Crohn's disease.^{38, 39}

In the steady state, dendritic cells may play a part in mediating non-responsiveness to intestinal flora, but can be induced to stimulate a response by cytokines that signal tissue damage or the presence of a pathogen.⁴⁰ Non-responsiveness could be induced by constitutive antigen presentation by immature dendritic cells, resulting in the induction of anergic or regulatory populations,⁴¹ or by activation of T cells by a 'tolero-

genic' dendritic cell subpopulation. The dendritic cells bearing apoptotic epithelial cells could be a candidate for such a population. Additional mechanisms or combinations thereof are also possible.

It is important to emphasize that dendritic cells do not function in isolation and will be influenced by signals other than the bacteria themselves. Prime amongst such signals will be those provided by epithelial cells, which can produce a range of cytokine and chemokine signals from the basal surface in response to interactions with bacteria at their apical surface. Enteric epithelial cells act as sensors, particularly for the presence of pathogenic organisms, and signal the onset of mucosal inflammation. Epithelial signalling by a number of different pathogens appears to converge on activation of the transcription factor NF- κ B, and some non-pathogenic components of the flora appear to attenuate these pro-inflammatory responses by blocking the degradation of the counter-regulatory factor I κ B.⁴² Thus there may be fundamental differences in the ways in which pathogens and non-pathogens interact with enterocytes. Chemokine production by gut epithelial cells is, at least in part, likely to orchestrate the recruitment and spatial distribution of cells of the immune system. For instance, constitutive production of thymus-expressed chemokine (TECK)/CCL25 by small intestinal epithelium preferentially recruits α 4 β 7⁺ T cells, expressing its cognate receptor CCR9, to the small bowel.⁴³ In contrast, colonic epithelial cells do not express TECK/CCL25, but produce mucosae-associated epithelial chemokine/CCL28, whose ligand is CCR10. Thus, immune cells expressing CCR10 may be preferentially recruited to the colon.⁴⁴

It is evident from the above discussion that the selective recruitment of cells and their spatial organization, as well as their function upon contact with bacterial products, will contribute to the tightly regulated response to the intestinal flora.

THE ROLE OF THE FLORA IN DISEASE

We need only consider the impact that the eradication of *Helicobacter pylori* has had in the management of peptic ulcer disease to see the potential for the enteric flora to cause disease.

With regard to inflammatory bowel disease, the aetiology appears to involve three overlapping elements: genetic susceptibility, priming by an environmental

factor, which appears to be part of the normal enteric flora, and immune-mediated tissue injury. The genetic susceptibility may determine the dysregulated immune response, a leaky mucosal barrier or even an imbalance in the enteric flora.⁴⁵ It is not clear whether the barrier function is primarily compromised by intrinsic defects in epithelial integrity, by infection with pathogens, or by the loss of or changes in commensal-dependent signals necessary to maintain it.

The search for a single organism as the causative agent has yielded inconsistent results. Enterococcal strains of *Escherichia coli* in the ileal mucosa have been found in patients with Crohn's disease.^{46, 47} More recently, a novel bacterial sequence has been reported in association with intestinal lesions in patients with Crohn's disease. This sequence represents a novel bacterial transcription factor which, through genomic homologue evaluation, appears to originate from *Pseudomonas fluorescens*.⁴⁸ However, whether these changes are primary or secondary to the disease process is not clear.

Instead, it may be that environmental factors are exerting their effects via the immune system. Indeed, the increasing prevalence of inflammatory bowel disease parallels an increase in atopy, asthma and insulin-dependent diabetes mellitus, all of which are immunologically mediated.⁴⁹ Differences in the neonatal gut microflora precede the development of atopy, suggesting a role for the balance of intestinal bacteria in the maturation of immunity.⁵⁰

There is increasing evidence that the antigens that drive the tissue-damaging response are derived from the normal bacterial flora. Proof of this concept has come from studies of at least 11 models of inflammatory bowel disease. In these models, inflammation is dependent on the presence of a normal flora and in its absence there is no disease. The phenomenon is seen in different species (mice, rats and guinea pigs) and occurs in manipulated systems, such as knockout or transgenic animals, as well as in induced models, such as indomethacin- or carrageenan-induced colitis. Moreover, in some models, colonization with normal flora rapidly results in T-cell-mediated gut inflammation,⁵¹ and disease can be transferred with effector T cells against enteric bacteria.^{52, 53} It appears that a constant bacterial stimulus is needed for the induction and perpetuation of inflammation,⁵⁴ and that all commensal bacteria are not equal in their ability to induce

inflammation,⁵⁵ but are influenced by the host genetic background.

Secondly, evidence comes from studies of patients with defects in phagocyte killing of bacteria, such as children with chronic granulomatous disease or glycogen storage disease type 1b. A subgroup of these children develops a disease similar to Crohn's disease. When the phagocyte defect is treated, the gut lesions resolve.^{56, 57}

Thirdly, it has been demonstrated that T cells from the lamina propria of Crohn's disease patients respond *in vitro* to the antigens of their own flora with a Th1 polarized response,⁵⁸ indicating a loss of tolerance to their normal flora. This is reflected by a serologic reactivity to enteric bacteria and cross-reactivity between autoantibodies, such as antineutrophil cytoplasmic antibodies and enteric bacterial antigens.^{59, 60}

In addition, diversion of the faecal stream after surgery prevents the recurrence of Crohn's disease and re-anastomosis results in inflammation.^{61–64} Furthermore, this effect has been demonstrated directly by infusion of luminal contents into excluded ileum.^{65, 66} The lesions occur in areas of the bowel with the highest bacterial counts, the terminal ileum and colon, and in ileal pouches formed after colectomy in patients with ulcerative colitis. Modulating the luminal bacteria with antibiotics has been beneficial in pouchitis⁶⁷ and Crohn's disease.^{68–71}

Finally, the recent identification of defective variants of a gene, NOD2/CARD15 on chromosome 16, that predisposes to Crohn's disease is an encouraging development.^{38, 39} The function of the protein encoded is partly known, detecting bacteria and playing a role in the inflammatory response to them. NOD2/CARD15 seems to function as an intracellular receptor for lipopolysaccharide via a leucine-rich repeat domain and is involved in the regulation of the NF- κ B pathway, which is the master modulator of the genes involved in inflammation. It is suggested that the NOD2/CARD15 gene product confers susceptibility to Crohn's disease by altering the recognition of bacterial components and/or by altering the activation of NF- κ B in monocytes. This clearly points to a link between microorganisms that could be recruited from the intestinal microflora and the innate immune system in triggering episodes of Crohn's disease.

Taken together, the evidence for bacterial flora driving an inflammatory process is compelling.

MODIFICATION OF GUT MICROFLORA — TREATMENT OF DISEASE

The role of the flora in disease has led to an interest in the use of probiotics as a therapeutic modality. Probiotics are defined as 'living organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition'. An added stimulus in this area has been the relentless use of antibiotics and the associated spread of antibiotic resistance.

The most extensively studied gastrointestinal condition treated with probiotics is acute infantile diarrhoea. *Lactobacillus* GG decreases the duration of diarrhoea in infants with acute rotavirus diarrhoea,⁷² a result that has been confirmed in similar⁷³ and different⁷⁴ population groups and in multicentre studies.⁷⁵ In addition, *Lactobacillus* GG is efficacious in the prevention of hospital-acquired diarrhoea.⁷⁶

There is increasing evidence from animal models and human studies that probiotics may be an important treatment in inflammatory bowel disease. In particular, the administration of *Lactobacillus reuteri* was effective in ameliorating acetic acid-induced colitis⁷⁷ and met-hotrexate-induced colitis⁷⁸ in rats. More recently, *Lactobacillus* sp. were able to prevent the development of spontaneous colitis in interleukin-10-deficient mice,⁷⁹ and continuous feeding with *Lactobacillus plantarum* attenuated an established colitis in the same knockout model.⁸⁰

In humans, definitive evidence indicating the efficacy of probiotics is emerging. A non-pathogenic strain of *Escherichia coli* has been reported to have efficacy equivalent to that of mesalazine in preventing relapse in patients with ulcerative colitis.^{81–83} Perhaps the most impressive evidence for the efficacy of probiotics in inflammatory bowel disease has been the maintenance of remission and prevention of onset of pouchitis with a mixture of eight bacterial strains.^{84, 85} The high concentrations and multiple species of bacteria used may be more effective in dealing with the heterogeneity of disease than single strains. Nonetheless, the importance of the careful selection of single strains with particular physical, biochemical, genetic and immunological properties is clear, and their role in treating disease needs to be clarified.

Recently, the introduction of genetically engineered organisms to produce and deliver cytokines or other biologically relevant molecules to the mucosa offers further potential for probiotics.⁸⁶

A different approach to altering the balance of the beneficial and harmful bacteria in the gut is achieved through dietary supplementation. A prebiotic is a non-digestible food ingredient that beneficially affects the host by stimulating the growth or activity of a genus of bacteria, such as lactobacilli or bifidobacteria, that can improve the host's health. Fructo-oligosaccharides have a specific colonic fermentation directed towards bifidobacteria and their activity has been confirmed in laboratory and human trials.^{87–89} As prebiotics exploit non-viable food ingredients, their applicability in diets is wide ranging. The challenge will be to achieve finer control over the microflora and modulate at the level of the species rather than the genus. A further approach is synbiotics,⁹⁰ where probiotics and prebiotics are combined and, indeed, probiotics could be encapsulated within a prebiotic coating to enhance targeted distal delivery.

The ingestion of large numbers of viable bacteria requires an assurance of safety. If bacteria are used which are derived from the normal flora, their natural presence attests to their safety. Such bacteria have rarely caused disease through translocation, and their safety record through use in fermented milk, vegetables and cereals is excellent. In the case of genetically modified organisms, such as the *Lactococcus lactis* engineered to locally produce interleukin-10, the most important safety factor is the content of antibiotic resistance markers in the genetic constituents of the modified organisms. Another issue to consider is that a genetically modified component of the flora may acquire a growth advantage that will disturb the homeostasis in the gut ecosystem. It is already possible to assess these risks, for example, by using suicide vectors to limit the proliferation of these microbial populations.^{91, 92}

THE FUTURE

Life-long cross-talk occurs between the host and the intestinal flora and the outcome of this can determine whether health is maintained or disease intervenes. Our knowledge of the intestinal flora in health is limited, but a combination of sophisticated molecular techniques, in conjunction with traditional microbiological methods and an appreciation of their limitations, will pave the way to a greater understanding of this complex organ, which can then be explored in disease states. An understanding of the bacteria–bacteria and bacteria–host cell

interactions in the gastrointestinal tract will provide us with potential ways of modulating the flora.

The intestinal flora appears to have a role in driving inflammation. It remains to be seen which components of the flora are important in this process. Indeed, it may be that unique bacterial antigens are associated with disease in hosts with different genetic backgrounds.

Manipulating such a complex ecosystem is challenging. Therapeutic interventions are superimposed on a complex background with a high noise to signal ratio. Nonetheless, there is tantalizing evidence from animal and human studies that such manipulation may be effective in treating disease. More evidence-based medicine is needed, but the proof of principle has led to a requirement to establish the mechanism of action, so that treatments can be targeted and novel innovative approaches explored.

ACKNOWLEDGEMENT

This review includes highlights of a meeting held in April 2001, entitled 'Gut Ecology — To Explore the Role of the Gut Flora in Maintaining Health and in Disease and its Modification as a Therapy'. The meeting was sponsored by AstraZeneca.

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