How Can We Impact the Immune System with Pre- and Probiotics?

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Background

In recent years there has been a growing interest in understanding the influence of intestinal microbiota on the physiology of the body. Moreover, with the available genomic studies, it is now possible to analyze how components of the intestinal microbiota modulate features of human postnatal development and physiology [1]. An area of major interest has been the relationship between the gut bacteria and the immune system, both at the intestinal and systemic level [2].

Changes in the microbiologic content of the intestine can be induced by the administration of selected bacterial inoculums as part of a normal diet or as dietary supplements. The health-promoting microorganisms are called probiotics. The administration of specific fibers in the diet called prebiotics can also modify the intestinal ecology by promoting the growth of some particular components of the intestinal microbiota, such as bifidobacteria.

On the one hand, there is an immune activation which is associated with improved mucosal defenses against pathogens and responses to oral vaccines. On the other, a modified immune reactivity which preserves homeostasis in mucosal tissues confronted with a constantly changing environment. Not only does the latter avoid an excessive reaction and inflammatory damage in the local environment, it also influences the homeostasis of the systemic immune system and prevents the development of allergic or autoimmune diseases. It is difficult to provide a simple mechanistic explanation for the underlying cellular and molecular events that support these apparently opposing effects. However, a brief overview of the evolving models that have been postulated to explain basic immune function, may help us understand how intestinal bacteria effect the mucosal and systemic immune systems [3, 4]. The most important of these are the following.
(1) Burnet proposed the self-non-self theory in which antigen-specific immune cells (single cells) are activated following recognition of non-self. The model explains activation at the single cell level but fails to explain the mechanisms underlying the maintenance of homeostasis.

(2) The Janeway model subsequently proposed that initial events in immune activation depend on stimulation of accessory cells or antigen-presenting cells (APCs) [5]. APCs are not antigen-specific cells. They can recognize highly conserved pathogen, or commensal, microbial-associated molecular patterns through a limited number of germ-line encoded molecules, called pattern recognition receptors (PRRs). Recognition and subsequent activation are part of the innate response. Thus, the innate immune system is able to discriminate between ‘infectious non-self’ and ‘noninfectious self’. APCs in this model are preferentially dendritic cells (DCs) which upon activation can present antigen and activate naive antigen-specific T cells. This model marked the progression from the self-non-self theory to the infectious non-self model.

(3) The most recently proposed is the ‘danger model’ by Matzinger [3]. This model proposes that resting tissue APCs, particularly DCs, have a ‘sentinel’ function and detect not only infectious non-self but also danger signals generated by, for example, damaged cells of the host [3]. Highly conserved, ancient PRR molecules called toll-like receptors (TLRs) are expressed on DCs and allow recognition of conserved bacterial motifs present on pathogens and commensals, and endogenous danger signals [3, 4, 6, 7]. This process results in maturation and activation of the DCs [5, 8] that depend on the nuclear translocation of the transcription factor NF-κB and the subsequent activation of gene products involved in defenses, innate response and inflammation.

In this last controversial model, the innate activation of the ‘sentinel’ DCs may be transient and resolve after clearance of infectious non-self or components of damaged self. This short-lived innate response is probably sufficient to protect against the majority of invading microorganisms and is not accompanied by an adaptive immune response since immature DCs, resident in peripheral tissues, are unable to induce effector responses of naïve T cells. Another possibility is that incompletely activated DCs produce interleukin (IL)-10 and thereby induce or prime regulatory T cells [9] instead of effector T cells. In either case, immune tolerance results.

However, if pathogens or endogenous cell injury leads to persistent inflammatory reactions, the DCs become fully mature and activated [4, 10]. In turn, they activate naïve T cells to initiate the antigen-specific immune response through effector and memory T cells. Furthermore, they can integrate different stimuli and elicit Th1 or Th2 responses depending on the environmental signals [11].

In conclusion, DCs represent a heterogenous lineage of cells that attain different levels of maturation and activation depending on the nature of the stim-
uli in their microenvironment. This diversity in DC functional status elicits different, and often contradictory, types of T-cell responses which vary from deletion in the thymus to generation of effector and memory T cells, either Th1 or Th2, and T regulatory cells that participate in peripheral tolerance [12].

Application of the general models of immune function described above to the immune system of the intestinal mucosa has received little attention. No other place in the body has probably such an important interaction with non-self, or even with ‘infectious-non-self’, and yet, for the most part, it remains tolerant to commensal bacteria.

An original feature of the intestinal mucosa is that the epithelial cells are in permanent contact with the luminal contents and, as such, bacterial products. Thus, intestinal epithelial cells (IECs), in addition to the ‘sentinel’ DCs, may also sense bacterial signals in the lumen, participate in innate immune responses and, through their secretory products, indirectly influence the stimulation of a subsequent adaptive immune reaction.

Are the mechanisms underlying mucosal immune activation and immune homeostasis opposing processes or rather a range of responses that need to be finely tuned in order to cope with danger and yet prevent an overreaction to harmless or even beneficial, non-self symbionts? It is likely that in this exquisite physiological process, both innate and adaptive responses are operating in a coordinated manner which is in symbiosis with the intestinal microbiota.

**Microbiota–Host Interactions**

Anatomical, Cellular and Molecular Bases of Microbiota–Host Interactions

The interactions between the intestinal bacteria and the host occur in different mucosal environments. In very general terms, bacteria–eukaryotic cell interactions take place at the absorptive mucosa, in the areas devoid of lymphoid-organized structures and in the gut-associated lymphoid tissue (GALT), such as the Peyer’s patches in the small bowel. At both sites, bacterial cells or their components interact preferentially with IECs or DCs. In the GALT, commensals and pathogens target specialized epithelial M cells, which are present only in the follicular-associated epithelium and which facilitate their uptake across the epithelial layer. Once in the dome of the GALT, the bacteria interact with immature DCs.

Whether pathogenic or not, bacteria, virus and other bugs have a number of molecular signatures, from proteins to nucleic acids, that are ligands for TLRs [13]. Epithelial cells and DCs are positioned to detect bacterial signals from both the complex variety of normal commensal microbiota and the superimposed pathogenic bacteria. Bacterial molecular signatures recognized by epithelial cells [14, 15] and DCs, although conserved and
of a limited repertoire, can induce different types of cellular and host responses [16].

The IECs, either primary cultures or cell lines, constitutively express TLRs, such as TLR2 and 4, which can bind bacterial products like peptidoglycans and lipopolysaccharide (LPS), respectively [16], and TLR3 and TLR5 which recognize double-stranded viral DNA and bacterial flagellin. The nature of the TLRs engaged influences the type of epithelial response [14, 16].

Cellular signalling initiated by non-pathogenic bacterial products binding to TLRs leads to nuclear factor κB (NF-κB) and mitogen-activated protein kinase activation [10], and expression of inflammatory genes such as chemokines. In normal situations, this physiological challenge and its response are of short duration and, as such, are well tolerated. They lead to a state of hyporesponsiveness. The length of the inflammatory response is apparently linked to the limited duration of the NF-κB nuclear translocation [17]. Other molecular mechanisms of epithelial tolerance to commensal challenge have been suggested. They depend on low surface expression of TLRs and the MD-2 adaptor molecule, as well as upregulation of the regulatory intermediate Tollip, which inhibits TLR expression [18]. Furthermore, anti-inflammatory downregulatory signals such as IL-10 or transforming growth

Fig. 1. Interaction of the mucosal surface with commensals or probiotics. Bacterial cells come in contact with immune cells preferentially at the Peyer's patches through the epithelium of M cells. T cells differentiate into regulatory T cells, which produce IL-10 and/or T helper (TH) 3 cells, which produce TGF-β. B cells produce secretory IgA that will result in immune exclusion of bacteria and reinforcement of the mucosal barrier to prevent bacterial translocation. Overall the immunological consequences are local IgA production, systemic tolerance and local immune homeostasis.

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factor-β (TGF-β), originating from other mucosal cell types present in the lamina propria, may also be involved [19, 20]. However, hyper-reactivity may also be prevented by the production of soluble TLRs, specifically sTLR-2 [21], which are released upon stimulation with bacterial products such as LPS.

Intestinal DCs can come in contact with commensal bacterial cells or their products (a) in the Peyer’s patches, (b) in the lamina propria due to ‘physiological’ bacterial translocation, or (c) through an active sampling by transepithelial DCs [22, 23]. Thereafter, the DCs can either participate in an innate reaction to prevent infection, initiate a protective secretory immune response, or participate in the induction and maintenance of immunological tolerance (peripheral tolerance) towards the organism [22]. DCs can also participate in the initiation and perpetuation of a proinflammatory immune reaction (fig. 1).

In summary, both IECs and DCs are involved in the interaction with the intestinal microbiota, and play a role in the defense and homeostatic responses. Although both cell types participate in innate responses, only DCs play a direct role in the initiation of antigen-specific responses. A limited, inflammatory innate response to commensals is crucial to prevent mucosal damage. In addition, it has been suggested that commensals may play an active role in the modulation of sentinel reactivity to pathogens and proinflammatory molecules (see below).

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*Differential Innate Response to Intestinal Bugs: From Pathogens to Different Types of Commensals*

Pathogenic microbes subvert nonspecific host-defenses to reach specific cellular receptors on host tissues. They thereby attain a niche which provides ecological advantages and improved survival [24]. The strategies and cellular receptors exploited by pathogens to interact with intestinal mucosal cells are varied. They bind to the same PRRs that are involved in the interactions with commensals. However, due to either the different cellular signalling or cytopathic effects they induce, they stimulate a different innate response. For the most part, the pathogenic response involves a persistent activation of NF-κB [25], chemokine production and leukocyte recruitment with inflammatory tissue damage. Working together, the innate and adaptive immune responses ultimately clear the pathogen. Although under physiological conditions, interactions between commensals and the host can be varied, these organisms do not alter the integrity of the intestinal mucosa.

Commensals are primarily embedded in the mucous layer, are coated by secretory antibodies, and very seldom do they seem to have direct contact with enterocytes. When this rare event happens, host epithelial cells detect or sense bacterial signals and react to them. It is possible that bacterial sensing by the host is in part independent of direct contact with the epithelial membrane since soluble PRRs, such as sCD14 and sTLRs, may play a sentinel role in the lumen of the gut [26]. Consequently, the normal flora seldom has direct contact with enterocytes.
The immune responses of the mucosal surface induced by interaction with commensal microbiota can be considered as antipathogenic and immunomodulatory.

*Bacteroides thetaiotaomicron* has been used as a model of symbiotic bacteria in several experimental studies. Colonization with this organism reinforces the mucosal barrier innate defenses through the secretion of a bactericidal product, Ang4, by Paneth cells [27]. Furthermore, both Gram-negative and Gram-positive bacteria, LPS, lipoteichoic acid, lipid A and muramyl dipeptide, elicit antimicrobial peptides, such as α-defensins, that keep bacterial populations in check [28]. In addition, it has recently been reported that Paneth cell antimicrobial cryptdins play a role as paracrine regulators of the intestinal innate response to bacteria [29]. Thus sensing bacteria by cells of the mucosal surface at some compartments initiate a defensive cellular response and also a coordinated innate host reaction.

The result of bacterial changes at the intestinal level can result not only in local responses but they can also have an influence on the systemic innate reactivity of the host. It has been observed that probiotic administration to healthy adult volunteers increased the phagocytic capacity of blood granulocytes and monocytes [30, 31].

In addition to the aforementioned antipathogenic effects commensals play an immunomodulatory activity. In vitro studies have shown that single epithelial cells responded differently to Gram-negative non-pathogenic enterobacteria and Gram-positive lactic acid bacteria (LAB). Only the former were able to clearly induce inflammatory gene activation probably through NF-κB nuclear translocation [32].

More recent studies, performed with IEC-6 and primary rat epithelial cells confirmed a proinflammatory response of epithelial cells challenged by Gram-negative bacteria and a lack of response to Gram-positive nonpathogens [33]. Furthermore, it was shown that nonpathogenic Gram-negative bacteria had the capacity to signal IECs through TLR-4 and the NF-κB system, specifically through phosphorylation and nuclear translocation of the RelA subunit.

Studies using Caco-2/PBMC cocultures showed that epithelial cells responded differently to nonpathogenic bacteria and produced a transient, proinflammatory response to Gram-negatives [34]. When pathogenic *Escherichia coli* were used for comparison, the inflammatory response lasted longer than that induced by nonpathogenic *E. coli*. If an inflammatory response was induced to LAB using the same model, the reaction was transient. Of note, some specific strains of LAB did not induce an inflammatory response but increased expression of the gene for TGF-β. TGF-β is central to the maintenance of mucosal immune homeostasis.

Interestingly, it inhibits innate immune activation via TLR-4 [35]. Furthermore, it is able to inhibit LPS or bacterially induced recruitment of NF-κB to the IL-6 gene promoter through modulation of histone acetylation [20].
Attenuated Salmonella prevents nuclear translocation of NF-κB by inhibiting IkB-α degradation and, moreover, prevents proinflammatory gene activation induced by a wild-type Salmonella [36]. In these experiments colonization of the monolayers with the attenuated strains also inhibited IL-8 induction by TNF-α.

B. thetaiotaomicron, a dominant component of human anaerobic microflora, was able to attenuate the proinflammatory reaction induced in vitro and in vivo by Salmonella enteritidis as well as other proinflammatory agonists by regulating the nuclear export of RelA complexed to peroxisome proliferator-activated receptor-γ [17].

In in vitro IECs/immune cell coculture assays, secretory products, such as IL-10 produced by immune cells in the basolateral compartment, have also been shown to play a role in dampening the transient inflammatory reaction of epithelial cells.

Thus commensals and selected probiotic strains can induce a defensive reaction of the IECs and also downregulatory responses to proinflammatory stimuli.

**Adaptive Immune Response to Commensals**

Intestinal components of the microbiota reside in the lumen, embedded in the mucus covering the mucosal surface and coated by secretory immunoglobulin A (sIgA) [37]. Although a symbiotic relation between the host and its microbiota was thought to be based on immunological tolerance, antibody and cellular responses to commensal bacterial antigens have been reported by different authors.

Stimulation of IgA secretion by non-pathogens in the intestinal lumen may provide a shield against bacterial dissemination or translocation. It may also prevent the antigenic dissemination that could stimulate overwhelming immune reactions and autoimmunity. A large proportion of intestinal IgA are specifically directed to commensal cell wall antigens and it is independent of T-cell help [38, 39]. Moreover specific commensal antibodies are found in intestinal secretions but seldom in serum. Occasionally it has been reported that healthy human subjects can have serum antibody responses to the predominant intestinal lactobacilli and bifidobacteria of their own microbiota, thus although local responses seem to be prevalent, some leakage of non-pathogenic bacterial antigens cannot be excluded [40].

The induction of the local secretory immune response is initiated by DC sampling of commensal bacterial cells and triggering a local protective immune response in the lamina propria and in the mesenteric lymph nodes without systemic dissemination and immune activation. Thus symbiosis between the host and its microflora is supported by the competence of the host mucosal immune system [39]. It is not known whether this secretory immune response limits bacterial growth or also inhibits penetration of the mucosal surface. In any case the response does not result in prevention of intestinal colonization.
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The interaction of the intestinal microbiota and the host adaptive immune system depends on cell populations, mainly DCs, that can recognize dangerous and harmless environmental signals. In the absence of inflammation or another type of tissue damage, DCs in contact with commensal bacteria will attain a partial degree of maturation and stimulate regulatory T-cell differentiation, B-cell IgA production, immunological tolerance at the systemic level and homeostasis of the immune function [41]. In contrast if pathogens are encountered or an inflammatory reaction is ongoing, then DCs will prime effector cells, most probably of the Th1 subtype, and thereby contribute to further inflammation and breakdown of oral tolerance.

It has been observed that the administration of probiotics can have an immunoadjuvant effect during oral vaccination against *Salmonella* in healthy human subjects [42]. The cellular events of the adjuvant activity have not been characterized but were not associated with any inflammatory reaction. Recently it was reported that the administration of *Enterococcus faecium* to young dogs induced an increase in polyclonal fecal IgA and specific antibodies against canine distemper virus vaccination [43].

**From Cellular Events and Experimental Studies to Health Benefits**

The physiology of the innate and adaptive intestinal immune response cannot be evaluated independent of the multiple and complex interactions that the host entertains with its microbiota.

Epidemiological evidence suggests that an improved standard of life is associated with an increased incidence of diseases with underlying immunopathological mechanisms. Allergy, autoimmunity, inflammatory bowel disease are all mediated by pathological immune responses.

Very clean or ‘germ-free’ environments seem to contribute to a huge increase in diseases such as allergy and asthma.

In addition to this cleaner environment, it is possible that sterile foods, prescribed antibiotics or their inadvertent presence in our diet, all contribute to the reduced Trophism of the intestinal microbiota. As the latter can be considered a real ‘organ’ of the body, an effect of this magnitude modifies several important functions, most particularly the immune response.

Several factors are prompting the medical community to examine ways in which to modify or to feed this ‘organ’ and thereby achieve health benefits.

There are emerging scientific and clinical evidence to support the use of probiotics to control diarrhea, allergy, chronic inflammatory bowel disease and gastrointestinal infections. The mechanisms are only partially understood, but immune function can be positively modified by the administration of carefully selected strains [44].
It has also been shown that prebiotics can modulate the host intestinal immune response. Their administration leads to an increment in the intestinal IgA response [45]. Furthermore, given to elderly humans they change the intestinal ecology and have an influence on the systemic inflammatory status that is frequently observed with increasing age [46].

Thus modulation of the innate immune response at the mucosal surface is not a total abrogation of reactivity, strong responses can still be initiated if the surface is exposed to proinflammatory cytokines or microbial products that may be ‘interpreted’ by the sentinel cells to be virulence factors or danger signals.

Immune regulation does not occur without activation. Some level of intermediate activation or priming is required if the host is to respond quickly to pathogens. Fortunately, this activation is tightly controlled and occurs in the absence of major inflammatory damage. It is achieved through the intervention of commensal or probiotic bacteria that induce the production of modulatory mediators such as TGF-β, IL-10, and IL1-ra.

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Discussion

Dr. Powell-Tuck: I think what one is hearing here is very little short of the very fundamental basis of gastroenterology. This is what gut disease and gut health is all about, and the further understanding of this will undoubtedly be the way forward in the future. Just going on though from that, if we are going to treat gastrointestinal disease, we have got to alter these responses, we have got to improve mucosal health. How can we beneficially influence the functions of these dendritic cells and all the cytokines, apart from probiotics which are going to have a modest effect? I am aware of the huge effects of antibiotics in hospital care which massively outweigh the effect of prebiotics and probiotics, but how else do you think we can influence this fundamental cellular function?

Dr. Schiffrin: I don't know very well where we are going. This is a critical moment for the whole subject because having jumped into probiotics and prebiotics some years ago, we honestly had the illusion that they were good for everything. But then the more we knew about them the more we realized that sometimes they can be pushing the immune system towards a Th1 or a Th2 response or promoting or not oral tolerance. I think this raises some concerns and also the necessity to make better research. I don't know if the industry will have the energy to support that or who is going to support that, but I think today the most rational intelligent selection of probiotics for specific problems will come from a genomic evaluation that a lot of people have done. We can see that bacteria that are taxonomically close can interact with cells or with animals or with humans in totally different ways. So this is the problem today, we don't know how to predict the biological activity, it is not like a single molecule such as glutamine, we are dealing with something complex and really at the moment the selection of probiotics still remains very empiric. Then of course we have to be sure that they are safe first, and then we do our best guess. But I don't think yet that we are doing really an intelligent selection of probiotics for specific problems.

Dr. Endres: You showed a lot of interactions of probiotics and the immune system. Concerning the prebiotics the data were more rough clinical results. Could you please outline a little bit more the mechanistic ideas about how prebiotics work? Is it via probiotics or is it a direct effect or possibly both?

Dr. Schiffrin: We have assumed, probably from a simplistic point of view, that immune modulation with nutrients such as probiotics is more achievable in the small bowel. Bacterial colonization is lower and there are major immune cell populations in
the mucosa. In contrast, to change the ecological condition of the colon containing massive bacterial populations and, in addition, poorer immune cell compartments seems a more difficult task. Any nutritional bacterial inoculum will probably have a minor consequence in changing the endogenous bacterial communities of the colon. Are probiotics able to grow and establish in an already overcrowded habitat? I think for this particular purpose that prebiotics may represent an interesting possibility. The example of the probiotic blend VSL3 as an efficient way to control pouchitis may in part be due to the lack of colon and thus ingested bacteria get to the distal reconstructed intestine faster. I don’t know if other bacterial blends could also have some beneficial effect in this particular condition. Another example of the successful use of probiotics is in the case of prevention or even treatment of the Th2-driven immune reactions in atopy, but our guess is that the action takes place in the small bowel. In the case of nutrition for a change in the colonic ecology, prebiotics are interesting candidates. It is true that I have shown more mechanistic explanations for the potential benefits induced by probiotics and more clinical evidence without a detailed mechanistic approach of how prebiotics work. Our first guess is of course that they can change bacterial communities and their metabolic activities in an altered ecological system due to the clinical conditions and their treatments, in particular antibiotics.

**Dr. Morley:** That was a brilliant lecture and I really enjoyed it although I am not claiming I understood all of it. If we go back historically to about the early 1900s, Medziekov decided that because Bulgarians ate a lot of yogurts and seemed to live forever, though they didn’t, that basically we could actually get longevity by altering gut flora. The problem I have as I look at the literature is that we are almost at the same stage now as we were 105 years ago, and I am wondering if we are tackling the wrong problems. Whether the evidence is true or not, I think many of us will believe that giving yogurt will certainly make a difference, or taking antibiotics particularly in older people as far as diarrhea is concerned. But the situations where I would assume that we are liable to see a major effect would be in irritable bowel syndrome and gas production, things which are very dependent upon changes in gastrointestinal motility in which an alteration in cytokines could easily alter the system. Many years ago we showed that exorphins in the food fed to a person will basically alter gastrointestinal motility and therefore one would assume that you can do the same sort of thing with pre- and probiotics depending on how you alter the cytokine milieu that has been produced in the gut. I wonder how much data there are to support this sort of concept. Certainly there were data many years ago, which nobody ever followed up as far as I know, showing that with bacterial overgrowth you actually get severe anorexia and weight loss which is curable by antibiotics. I wonder if that area is not where we should be going a little bit and are there data to support going down that sort of pathway? Do we have good animal models to perhaps look at gastrointestinal motility in a different biotic structure at this moment in time?

**Dr. Schiffrin:** I think this is a very good point and irritable bowel syndrome is a major problem in gastroenterology. There is a group in France that is working along these lines, trying to relate bacterial colonization or some probiotics and intestinal motility [Bueno L., personal commun.]. Nowadays we have irritable bowel syndrome with and without inflammation; we have a heterogenous picture with different underlying mechanisms. Cytokine production is playing a role. I think that in very few months we will see this information in the literature showing the interest in this field.

**Dr. Bowling:** You have shown us an awful lot of animal data. Of course if this is going to translate into useful products along these lines, we have got to have in vivo human data. The problem with a lot of the studies, and certainly some that you
presented, is that we are looking at short-chain fatty acid concentrations in the stools. However, the most important part of the colon is the ascending colon, and what is coming out of the bottom end is not necessarily any kind of reflection of what is going on physiologically much further into the system. I am just wondering how much we actually know of what is going on in that part of the human colon in vivo?

Dr. Schiffrin: I agree with you. We know that we will be losing information analyzing the fecal short-chain fatty acids instead of going to the place where their generation is taking place. There are some studies that have tried to sample using magnetic capsules that can be given to the volunteer, followed by radioscopy and sampling at the right place, and analysis of short-chain fatty acids was then made. Macfarlane et al. [1] did studies like that. So it is true, those are very complicated studies, you have to have the volunteers for a couple of days and of course give the diet many days before, but then at the moment of sampling you have to be very careful. So these things are going on, I don’t expect to see many studies trying to clarify these points. I guess that we will have to be satisfied with just a few but of course these points were raised.

Dr. Armstrong: This is highly speculative. I was wondering if you could speculate on how important prebiotics and probiotics are going to be in early life in the immediate postnatal period. I remember a person saying that our gut flora is pretty well determined within a few days of birth, or within a few weeks, and we also heard this morning that there are differences between twins with respect, for example, to calcium and vitamin D status decades into life. So my question is, is the behavior of our gut and the way that it deals with its microflora and the consequences determined by the nutrient status, the initial colonization? Do you think that there is a role for investigating or dealing with people’s problems very early on with prebiotics and probiotics?

Dr. Schiffrin: I think that your question is fascinating because we don’t yet understand the physiology of neonatal colonization. I guess that what we were discussing earlier today regarding the challenge to have nutritional effects despite gene diversity is also applicable to the immune system. However, in this latter case the possibility of immune education seems to exist. The immune system has a memory, so if you really do the real good points at the beginning of life or in the critical moments early in life, I don’t know when this time is, perhaps 7 days after birth or at weaning, then I think that you can improve a lot of things in the immune system despite the fact that you will always be dealing with genetic diversity. Now the question is do we intervene already in children, in neonates? I think a lot of people are starting to do that with reasonable success. I still think though that we need to do some homework to understand the physiological events of neonatal colonization with breast feeding. We still don’t know what the relevant points are there, and I think until we understand that, the temptation to intervene without having the frame of what is physiological is not the right way to do things. Of course there are such artificial situations in the neonatal intensive care unit in which we know antibiotics will be used. The neonates will not have breast milk, they will be colonized by necrotizing enterocolitis promoting bacteria, then in that case of course the benefit could be so good that you are tempted to do something. But for the general population I think that we only partially understand the problem for the moment.

Dr. Endres: It is known that breast-fed infants have a predominance of bifidobacteria in their feces [2–5] and it is also known that infants fed with a whey-predominant formula have some bifidobacteria [3], whereas those fed with a casein-predominant formula have particularly no bifidobacteria [6]. So the industry is trying to imitate this status seen in breast-fed infants, and so far we are trying to reach this goal by using Bifidobacterium lactis in infant formulae. A positive effect is that with this you can
reduce the prevalence of diarrhea, the prevalence of constipation, but when it comes to the very critically ill preterm babies there are two studies, one with *Lactobacillus GG* [7] and the other with the *Lactobacillus acidophilus* [8] showing that there is a colonization, which is not really a colonization but counting the bugs in the stools, and the authors have shown that the bacteria given are found in the feces but the negative bacteria are not diminished. There are two unsuccessful clinical studies in preterm infants trying to reduce the rate of necrotizing enterocolitis, one using *Lactobacillus GG* [9], the other using *B. lactis* [10]. I think this is really the group of patients who could have benefited from a formula or expressed breast milk fortified with probiotics.

*Dr. Schiffrin:* Yes, in addition, there is a study in Colombia with about 1,200 preterm babies given *Lactobacillus GG* with less incidence of necrotizing enterocolitis [11]. So absolutely, this population seems to be very candidate.

*Dr. Ockenga:* Are you aware of any major clinically relevant side effects when using probiotics?

*Dr. Schiffrin:* I know a few cases. For example there are reports of fungemia with *Saccharomyces boulardii* in critically ill patients [12]. *S. boulardii* was given to prevent diarrhea, it has been used with good efficacy, and apparently they don’t know whether this was really a translocation of the yeast or if it was a spray when facets were opened. There are some doubts about the use of live bacteria in the intensive care unit due to the spray possibility. Sometimes we don’t want even to think of probiotics or how to use a probiotic in the intensive care unit because if you can culture from a catheter or if during the manipulation there is a mistake then the use of probiotics will be questioned. Then there is a case report of a liver abscess with GG in a diabetic woman consuming enormous quantities of GG-containing products [13]. There are now very interesting studies going on in Finland. A study has recently been published in which they looked for sepsis due to gram-positive lactobacilli and in 5 years they had 26 cases due to *Lactobacillus rhamnosus* and in 11 of those cases they have a fingerprint with a profile which is similar to GG [14]. So there are things coming out. Of course sometimes there are the immunodepressed, the immunosuppressed patients, and safety considerations are always a priority.

*Dr. Labadarios:* As far as normality is concerned the side effects are actually very few. Regarding the immunocompromised patient that is another matter, and I think Dr. Schiffrin just shown that there is a lot of caution to be exercised.

*Dr. Schiffrin:* I think that the benefit that we could have in immunocompromised patients is potentially enormous using probiotics since we know that the wasting syndrome of immunocompromised patients depends on the colonization of *Escherichia coli* or translocation of *E. coli*. On the other hand 2 patients with short bowel had sepsis due to GG. So I think we could really have a benefit but of course who will dare to go first, the nutrition company or the pharmaceutical company, we are in a nutrition world so it is really difficult. We are always a little bit blocked, since nutritional interventions are usually not established to tackle very severe health problems.

*Dr. Roessle:* At Nestlé, we have seriously considered proposing products for enteral nutrition with probiotics. If you listen to the experts there are two typical reactions: most gastroenterologists are rather favorable, but the physicians in intensive care have problems handling the paradox of using alive bacteria in an environment which is meant to be germ-free or sterile, and adding probiotics to tube feeding which, by definition, is almost a sterile product is a kind of contradiction. Of course there are some occasional side effects reported in the literature [15, 16], but if you look at that you also have to discuss the risk-benefit potential and as soon as you have therapeutic means to fight against the side effects by using antibiotics then perhaps the benefits will overtake. So our opinion is still in favor of trying to use probiotics in selected clinical conditions.
References
