

Early bacterial colonisation of the intestine: why it matters

La colonizzazione batterica intestinale precoce: perché è importante

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Summary

The birth process allows the progressive formation of complex intestinal microflora composed of myriad bacteria, leading to this recently identified host-bacterial mutualism in the human intestine. This kind of cross-talk originating from birth is opportunistically used by the young host to initiate its own immune system. Recent epidemiological data support the hypothesis that some increasing immune deviances observed in the last two decades could have originated from a modification of the bacterial environment in young populations. Our modern approach to perinatal care may, to some extent, have modified inadequately the overall quality of this bacterial-host interface. The international medical community has to be made aware of the increasing importance that initial colonising intestinal microflora could have on the health and well-being of the host later in life. It is of great concern to decrease these possible negative influences and to discover in the near future the possible means of helping to manipulate positively the gut microbiota of infants.

Riassunto

Subito dopo la nascita il rapido e progressivo sviluppo della microflora intestinale è in grado di regolare diverse funzioni gastrointestinali ed immunitarie comprese nel cosiddetto mutualismo batterio-organismo ospite. Questa specie di interscambio inizia alla nascita e regola diversi aspetti del sistema immunitario del neonato. Recenti dati epidemiologici accreditano l'ipotesi che alcune possibili alterazioni immunitarie emerse dal punto di vista epidemiologico negli ultimi vent'anni potrebbero derivare da modificazioni della flora batterica intestinale. Diverse azioni terapeutiche comunemente attuate in epoca perinatale potrebbero essere potenzialmente in grado di alterare il normale interscambio tra microflora intestinale ed organismo ospite. La comunità medica internazionale deve essere consapevole della crescente importanza che la colonizzazione iniziale della microflora intestinale può avere sulla salute ed il benessere dell'organismo nell'arco della sua vita. È importante diminuire le possibili influenze negative e scoprire quanto prima il modo di aiutare a manipolare positivamente la microflora intestinale dei bambini.

Key words

Neonate • Immune induction •
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Parole chiave

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Introduction

Micro-organisms of different origins lived on the earth long before multi-cellular species. Along with the progression of life over millions of years, these emerging different, more complex species have learnt much about the ways to ensure their ability to fight against this potentially aggressive microbial environment. Consequently, at the top of this pyramid of species, mammalians, among whom primates constitute the most complex representatives, have developed a very sophisticated immune system. There is increasing evidence that the invasive microbial environment, first encountered during the birth process, is opportunistically used by the host to initiate its own defence mechanisms, which are still immature at that stage¹⁻³. In fact, birth is the only time in our life when the gastro-intestinal tract, while sterile, is colonised by bacteria originating from the maternal flora as well as by that of the close direct birth environment^{4,5}. The host-bacterial interface, originating from this

first contact, occurs in a perfectly satisfactory way in most cases⁶. The birth process allows the progressive formation of complex intestinal microflora composed of *myriad bacteria*, leading to this recently identified host-bacterial mutualism in the human intestine^{7,8}. Indeed, while some pathogens can be harmful to the host, the general relationship with components of the intestinal microbiota is increasingly seen as advantageous for both partners. This means that both partners can influence each other's health.

This initial microbial colonisation during the birth process and its consequent effect on the mucosal interface with the host has attracted increasing attention over the two last decades^{1-9,12}. The interest emerging from the scientific community on this matter is linked to newly published epidemiological data, which support the hypothesis that some increasing immune deviances observed in the last two decades could have originated from a modification of the bacterial environment in young populations. Indeed, an inverse relationship has been observed in developed countries between the general decrease in the overall prevalence of infectious disease in the early stage and the concomitant dramatic increase in immune deviances, in either allergic or auto-immune forms, later on in life¹³⁻¹⁹. This correlation has served to focus attention on the initial bacterial colonisation of the intestine, highlighting a suspected modification of the quality of the original bacterial-host interface. Early life events and this first bacterial invasion appear indeed to be crucial steps for the future well-being of the human host²⁰. Our modern approach to perinatal care may, to some extent, have modified inadequately the overall quality of this bacteria-host interface.

This short overview will remind the reader of some fundamental information regarding normal human intestinal microflora, how it develops in the early stage and by which means. The paper will also consider the fundamental role of intestinal microflora in the host's well-being. In addition, it illustrates some factors that could be at the origin of some disturbances in the early stages of intestinal flora formation. It also considers the means by which it could be possible to ameliorate the crucial invasive step of bacterial colonisation of the intestine and its possible consequence for the host.

Intestinal microflora: what is it?

An adult's whole intestine is colonised by a significant number of bacteria. This number (10^{14}) has been said to be higher than the total number of cells in the body (10^{13}). Streptococci and gram-positive filaments constitute the predominant oral flora, alongside various species of gram-negative cocci (*Branhamella*, *Neisseria* and *Veillonella*) and other anaerobic gram-negative bacilli, including *Bacteroides*, *Fusobacterium*, *Vibrio* and *Spirillum*. The stomach may contain a small number of bacteria: 10^1 - 10^3 colony forming units (CFU) per ml of stomach juice (Streptococci, Lactobacilli and

other yeasts). Contrary to general belief, the jejunum and ileum are not sterile. The upper small bowel usually contains less than 10^4 CFU per ml, although wide variations are reported; the distal ileum usually contains more bacteria. At this level, up to 10^8 CFU per ml have been reported with more diversified species (Lactobacilli, Streptococci, Bifidobacteria, Enterobacteriaceae, *Bacteroides*, and *Fusobacteria*). The study of this microflora is rendered more difficult by the location of the small intestine. Nevertheless, the beneficial effects of this flora are crucial in terms of the priming of the host's mucosal immune response.

Bacterial intestinal colonisation involves mainly the large intestine. It is generally accepted that 10^{11} CFU can be found per g of colic content. To date, over 400 separate species of bacteria have been discovered. The composition of the flora is quite stable in healthy individuals. Anaerobic microflora is by far the most important, and is dominated by: *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Peptostreptococcus*, *Fusobacterium*, *Clostridium*, *Lactobacillus*, *Streptococcus*, etc. The first four categories alone represent 80% of the total anaerobic microflora. Assessing *in situ* samples, it has been demonstrated that facultative anaerobic microorganisms represent about 25% of the total flora in the proximal part of the large intestine and that this proportion remains quite stable along the colon. By contrast, the number of fully anaerobic microorganisms, as well as of Bifidobacteria and *Bacteroides*, increases by more than 100-fold from the proximal to the distal part of the large intestine.

In the absence of either infectious diseases or antimicrobial drugs, the intestinal microflora remains very stable. However, some factors may influence bacterial distribution in the large intestine, even though the total number of microorganisms remains the same. Among these factors, the amount and type of growth substrate, which is undigested in the upper part of the gastrointestinal tract, represent the most influential one. Dietary fibre, as well as other resistant starches, oligosaccharides, food sweeteners and other non-absorbed sugars constitute the main fermentable dietary substrates in the adult gut. These products interact with endogenous materials, such as mucins, sloughed epithelial cells and bacterial lysis products, and contribute to create an optimal gut environment, allowing a more varied and fermentable flora. This very important and diversified microbiota of the large intestine influences health and well-being.

What is the normal development of the intestinal microflora in the early stage?

When the process of birth occurs the natural way, the colonisation pattern of the infant's intestine in the first weeks of life has, until now, always been thought to be typical. However, some recent data highlight four more formal steps in breast-fed infants⁵. The bacteria first

encountered are facultative anaerobics, mainly Streptococci, Enterobacteriaceae and Staphylococci. Few full anaerobic bacteria are found at this stage. The most determinant factor of this initial colonisation is the mother's vaginal flora and, even more so, her faecal flora. After 48 hours, the number of bacteria is already very high (10^4 - 10^6 CFU per ml of intestinal content). *This first phase* of colonisation is independent of how the baby is fed. Various factors can influence the type and quality of bacteria found in the baby. Of these, antibiotics given to the mother represent one of the most important ones, particularly in terms of bacterial multi-resistance. Later, Bifidobacteria and Lactobacilli are observed to reach a stable concentration of about 10^9 CFU per ml on day 10. This increase in full anaerobic colonisation accounts for *the second phase*. The phase of intestinal colonisation is further associated with an increased number of *Escherichia coli*, *Bacteroides spp.* and *Clostridia*, although the increase in the number of *Clostridia* is less important than that of the other two. At the same time, a decrease in the number of Staphylococci is also observed.

At the end of the first month, differences are clearly observed between babies, according to how they are fed. There are indeed differences in the faecal flora between exclusively breast-fed and formula-fed infants, with a dramatic increase in the populations of Bifidobacteria in the former group, whereas these represent only 30-40% in the latter group. This kind of optimal microflora in the intestine of breast-fed infants has been attributed to the presence of "bifidogenic" growth factors in human milk. Of these, we would highlight kappa-casein, which is a highly glycosylated human milk protein. The proteolysis of its C-terminal part represents a strong bifidogenic factor. Carbohydrates are another example of the bifidogenic compounds found in human milk. Of these, mono-oligosaccharides, fucose and other carbohydrate units added to lactose are certainly the most important one. However, the more specific growth factors that can influence the bifidobacteria content in the faeces of breast-fed infants are the low protein and phosphorus contents and the high lactose concentration in human milk. These factors account for the reduced buffering capacity of this milk and may allow the growth of Bifidobacteria, which in turn maintain the relative acidity of the intestinal lumen, due to acetic and lactic acid formation.

However, conflicting data are observed in the literature when analysing the Bifidobacteria content of faeces from breast-fed babies. Taken together, these differences are related to: 1) different methodologies used in assessing the type of bacteria, 2) the heterogeneity of the studied population and 3) environmental factors, such as maternal hygiene¹. *The third phase* of colonisation starts when dietary supplementation is given. At this time, the differences observed between exclusively breast-fed and formula-fed infants disappear. Enterobacteriaceae, as well as Streptococci and *Clostridia spp.*, increase along with a more diversified fully anaerobic flora (*Fusobacterium*, *Eubacterium*, etc.).

At the end of the first year of the baby's life and into the second year, the microflora can be said to be equivalent to the microflora in adults. The most important observation at that time is the further increase in full anaerobic bacterial diversity. This is *the fourth phase of colonisation*. A higher content of phosphorus and protein, increased buffering capacity and reduced lactose intake are responsible for the disappearance of the acetic buffering system observed in breast-fed infants. As demonstrated by one study, some variations can, however, still be found during the second year, when the faeces are compared with those of older children and adults²¹. This can explain the differences observed at that time in terms of metabolic activities, despite the fact that insufficient data are available in that study regarding hygiene and nutritional habits. However, it is probably aspects such as hygiene and nutritional habits that explain the differences observed when the faecal flora from infants of different countries is compared¹⁸²². It is interesting to observe that, whatever the country, the intestinal microflora of allergic infants appears different compared to that of non-allergic infants²².

Which roles played by intestinal microflora are likely to be crucial since the early stages?

Two main roles of intestinal microflora in terms of the host's health are extremely important, but they remain only partially known: 1) promoting a local innate immune induction, giving rise to an optimal innate-adaptive immune interface in order to get antigen oral tolerance. Paralleled with this immune reaction, a resistance to further bacterial colonisation is progressively created – the so called "barrier-effect"; 2) participating in dietary metabolic biotransformation²³⁻²⁷.

MUCOSAL IMMUNE SYSTEM INDUCTION

Scientific knowledge regarding the mucosal immune system comes largely from studies performed in germ-free animals in which the following have been demonstrated: a small development of the lymphoid nodules called "Peyer's patches", a lower count of local lymphocytes (CD4+ T-cells and intraepithelial alpha-beta TCR CD8+ cells) and an inability to gain a normal oral diet antigen tolerance²⁸. Of concern is the finding that restoring a bacterial flora in the intestine of older germ-free animals does not enable them to regain an antigen diet tolerance, highlighting the importance of the bacterial-gut interface in initiating the innate immune system and its adaptive counterpart in the very early stages²⁸. Invasive gut commensal microbes play indeed an important role in the development of GALT (*Gut-associated Lymphoid Tissues*), through the information transmitted to enterocytes and M cells. The GALT system, which is still immature in early stages, will allow two apparently opposite effects: the progressive induction of a defence mechanism against viral and bacterial pathogens through a not excessive, well-controlled

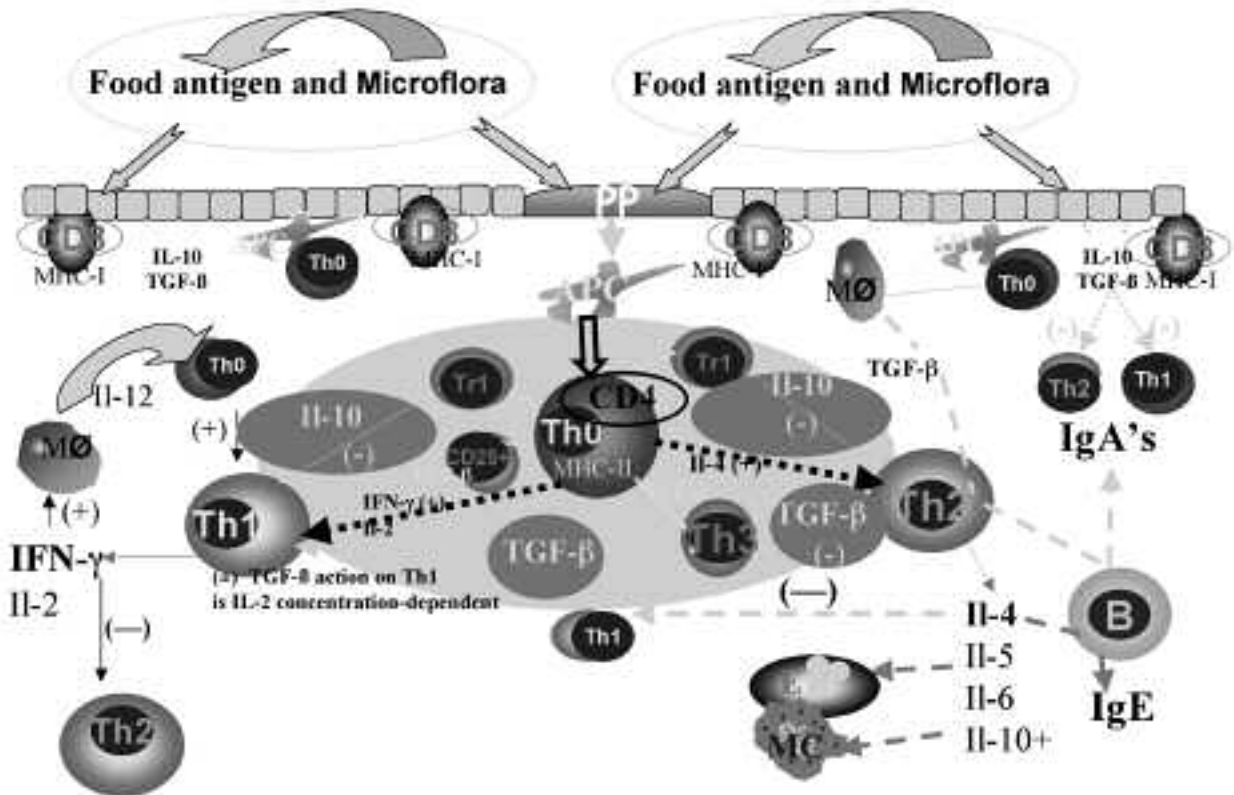
pro-inflammatory response, and the promotion of very complex immune mechanisms that will facilitate dietary antigen tolerance^{25 26 29-31}. The progressively constituted commensal flora, which in turn is also tolerated, will help immune system induction in both functions³².

Enterocytes, which express only class I major histocompatibility complex (MHC), are partly able to give information on the nature of the antigen and are thus the first active players in the normal homeostatic mechanisms of mucosal immunity. Their role in immune responses to luminal bacteria is important, as they act partly as a non-specific antigen-presenting cell. The interaction between enterocytes and intraepithelial lymphocytes, mostly CD8+ T cells, also appears fundamental, judging by the induced cytotoxic effects these lymphocytes are able to create³⁰. This interaction allows clonal deletion and anergy of the immune response by the lymphocyte apoptosis induced. It also partly helps in diet antigen tolerance after feeding with high doses of an antigen. In addition, through the restricted repertoire of MHC class molecules, the interrelationship between the intraepithelial lymphocyte

and the enterocyte, which exists even in the early stage, allows the latter to secrete IL-10, an anti-inflammatory cytokine, which permits some degree of bacterial tolerance.

The most fundamental role of the intestinal epithelium is its contribution to triggering an optimal interface between the innate and the adaptive immune systems. The M cells specialising in internalising and trafficking bacteria, viruses and other macromolecules cover Peyer's patches in the follicle-associated epithelium (FAE), which are aggregates of lymphoid tissues. These cells participate in controlling the entry of antigens and larger particles through the intestine. Early in gestation, there are abundant dendritic cells (DCs) in the lamina propria, which will inform the naive CD4+ helper cells as to the nature of the antigen presented at the basal side of the M cell^{25-27 33}. Using cell-to-cell contact between both M cells and enterocytes on one side and DCs through specific receptors using MHC class II on the other side, information on the nature of the antigen is transmitted, allowing this very specific innate immunity to be initiated (Fig. 1). The activation of the naive T helper cells is later on highly dependent

Fig. 1. Food Antigen – Epithelial Cell – Microflora Interface: Towards a Th1/Th2 optimal equilibrium and a high level of T-reg Cells activation process.



CD: Cluster of Differentiation; IL: Interleukins; IFN: Interferon; TGF-β: Transforming Growth Factor-Beta; Tr1: T regulator Cell; M': Macrophages; APC: Antigen presenting Cell (Dendritic Cell); E: Eosinophils; MC: Mastocytes; MHC: Major Histocompatibility Complex; PP: Peyer Patches; TH1: inflammatory CD4 T cells; Th2: helper CD4 T cells; Ig: Immunoglobulin.

on the information these cells have received from the competent DCs after close contact with each other.

In the absence of co-stimulation from DCs to the lymphocytes, as in the case of dietary antigen for example, anergy and/or apoptosis of the immune cell will be well oriented. However, the DCs receptiveness of the nature of the antigen (diet or bacterial) makes them particularly efficient in activating the T effector cells when co-stimulation is effective. It will allow memorisation to achieve either tolerance in case of diet antigen, or rejection in case of bacterial material. In the latter case, it is done when the bacterial materials modulate the immune system *via* a family of molecules expressed by intestinal epithelial cells, but also by all adjacent cell components of the mucosal immune system. These pattern recognition receptors, called "Toll-like receptors" (TLRs), are able to recognise bacterial-derived molecules and to initiate the transcription factor NF-kappaB, leading to pro-inflammatory gene expression^{12 32-36}. In this particular case, lymphocyte activation will then ensure a priming immunologic central memory, also called adaptive response. The method of activation will depend on both the TLRs activated (to date, four members out of a total of thirteen have been identified in the gastrointestinal tract)³⁴⁻³⁶ and the type of DC activated (myeloid DCs [mDCs] or plasmacytoid DCs [pDCs])^{37 38}. The initial cytokine secretion will consequently follow either in terms of a Th1 (mDCs) or a Th2 response (pDCs)³⁶.

Given a well-balanced lymphocytic response issued through the DCs from the invading commensal microflora, the immune system primarily allows a balanced pro-inflammatory Th1 immune response through the secretion of different cytokines (IL2, IL12, interferon gamma, etc.). In addition, the Th1 response indirectly allows a progressive well memorised tolerance to dietary antigens and enables the control of delayed-type hypersensitivity (DTH) cellular immunity. Th1 activation, first induced by the intestinal bacterial material, subsequently helps control Th2 response through interferon gamma secretion, alleviating allergic deviance against food antigens^{30 31}. In the first months of life, there is a lower ability to secrete interferon gamma as well as IL12 cytokines, which explains the relatively low "physiological" tolerance of infants to food antigens. On the other hand, one of the final tools of the concomitant Th2 response is to ensure defence against pathogens by secreting IgA (IgM in the first weeks of life) through an efficient but not excessive immune response *via* IL-4, IL-5 and IL-10 cytokine secretion. While organised Peyer's Patches with primary follicle T cell zones and M cells are present in the human intestine at 19 weeks of gestation, the sub-mucosal area lacks plasma cells. After birth, there is a progressive activation of B secreting IgM cells in the follicle, and the gut immune system starts to respond to diet antigens and gut bacteria. The first plasma cells appear progressively in the blood and there is a switch from an IgM isotype to an IgA isotype after a few weeks. A relative deficiency of IgA plasma is, however, the rule until two years of age.

Finally, the equilibrium between the Th1 and the Th2 responses is known to be partly obtained *via* a newly discovered subset of sub-mucosal T-regulatory cells (Tr1/Th3)³⁹. The activation process of this subset from a well balanced T helper cell response in the sub-mucosal area is called "Bystander suppression" (Fig. 1) and appears to be mediated directly by pro-inflammatory bacterial products *via* the TLR4 receptor⁴⁰. This mechanism seems essential in preventing an excess of both Th2 and Th1 adaptive responses. It is made through the secretion of two cytokines, IL-10 and TGF-beta, in the lamina propria. This seems crucial to achieve oral diet antigen tolerance and avoid an inflammatory process^{39 41}.

In summary, an appropriate commensal flora is able to produce a balanced T helper cell response between the Th1, Th2 and Th3/Tr1 T helper cell responses, alleviating both inflammatory and atopic disease.

METABOLIC DIET TRANSFORMATION

The metabolic activities of the microflora represent another crucial point of interest in studying intestinal microbiota. Bacteria contribute to make dietary antigens more tolerogenic, and thus to improve dietary tolerance. This very vigorous metabolic activity is the result of polyside degradation, ose fermentation, xenobiotic transformation to biliary product metabolism, mutagen production through proteolysis, amino acid fermentation and elimination of fermented hydrogen, etc.⁴²⁻⁴⁴. The quality of degradation products of this metabolic activity depends directly on the diet residues that reach the large intestine, and constitutes the substrate of bacterial enzyme activity. For example, in adults, an excess intake of animal fat and proteins is associated with increased amount of products harmful to the host's health^{45 46}. This metabolic activity varies according to its localisation in the large intestine and is much less diversified in the infant than in older children and adults^{47 48}.

BOTH THE ROLES OF THE MICROFLORA ARE CRUCIAL SINCE THE EARLY STAGES

An increasing amount of data has become available attributing to microbial flora a possible fundamental role in triggering immature intestinal immune function and preventing allergic disorders, as well as in auto-immune disorders^{2 3 14 20}. The development of the metabolic activity occurs very progressively, and is also related to environmental factors^{42 43 49-54}. The intestinal flora develops much more slowly in exclusively breast-fed infants⁴⁷. The true impact on later morbidity rates of formula fed infants not exposed to early dietary diversification, remains, however, to be determined^{55 56}. Nevertheless, it seems that Nature, through exclusive and prolonged breast feeding, has shown us the best way to keep the intestinal epithelium in contact with the best fermenting flora (Bifidobacteria and *Lactobacilli*) in order to progressively induce both optimal immune function and optimal metabolic activity^{5 49 50 57-60}. Optimising bacterial colonisation in the intestine could lead to better diet antigen tolerance and may pre-

vent allergic disorders^{61 62}. One recent study demonstrated an early colonisation by *Bacteroides sp* and the possibly positive role this kind of bacteria might have on the development of local and humoral immunity in humans⁶³.

Neonatal bacterial intestinal colonisation allows the immune system of the host to switch from a physiological Th2-biased response to a Th1-type response. The information given to the DCs by the colonising commensal microflora is crucial at this time^{26 27 35}. Through the TLRs already present in the early stage at the basolateral side of the enterocytes⁶⁴ and the resulting activation of NF-kappaB, the neonate will initiate the above described immune defence through specific cytokine secretion (IL2, IL12, etc.). Recent data highlight the dramatic importance of TLRs in initiating this host-commensal symbiosis, which is essential all through life to ensure adequate innate immune induction³². sCD14, the soluble form of the 55-kDa glycoprotein expressed mainly on the surface of monocytes/macrophages – the latter known to function as a receptor for bacterial lipopolysaccharide (LPS) – is a pattern recognition receptor present in breast milk but not on the gut surface. This helps maintain the integrity of the epithelial response after the interaction between the TLRs and the luminal microflora⁶⁵. It is interesting to highlight the fact that reduced sCD14 levels in amniotic fluid and breast milk are associated with atopic diseases⁶⁶. The first host-bacteria inter-relationships and their consequences have also recently been well illustrated^{8 67}. These two studies highlight the genetic modulation of the enterocyte by the microbe and the establishment of specific bacterial niches emerging from the orientated expression. It is made by the activation of cell glycosyltransferases and the consequent glycosylation on the glycocalyx. There is a real “mutual dialogue” between the bacteria and the host⁶⁸. It is highly conceivable that this kind of cross-talk would be beneficial for the host later on, if it were initiated by non-pathogenic bacteria less able to induce an excessive pro-inflammatory response⁵⁹. It is useful to remember once again that the best way to initiate an optimal and beneficial bacterial colonisation of the intestine (i.e. by Bifidobacteria and *Lactobacilli*), is to promote exclusive and prolonged breast-feeding of the human neonate. The perinatal development of an optimal host-bacterial inter-relationship certainly appears to be important, given the evidence that also *Lactobacillus rhamnosus* given to allergic mothers and their babies during the first six months of the baby’s life reduces the incidence of allergy at 2 but also at 4 years of age¹¹.

A well-controlled pro-inflammatory Th1 response facilitated by this optimal epithelial-microbial interface will progressively allow the neonate to gain an optimal diet antigen tolerance through the induced secretion of Th1 main cytokine interferon gamma, which blocks an excess of Th2 response. It must be mentioned that a premature baby’s enterocytes could, however, be less able to control a pro-inflammatory response originating from bacterial stimulation⁶⁹. An uncontrolled excess

secretion of pro-inflammatory cytokines (IL-1beta and IL-8) by these immature intestinal cells could explain one of the mechanisms that exposes these babies to the risk of contracting necrotising enterocolitis (NEC)^{64 69}.

What are the factors influencing the type of initial microbial colonisation encountered in the neonate?

As discussed, nutritional habits may affect the type of bacterial colonisation after 10 days. In this way, exclusive and prolonged breast feeding can optimise both neonatal intestinal colonisation with a rich fermenting microflora, but also the epithelial-microbe interface. However, initial bacterial intestinal colonisation is also directly dependent on both type of delivery and environment. In this way, intestinal microflora from babies born in suburban areas is quite different when compared with that of babies born in urban areas⁷⁰. The same difference is observed when comparing babies born at home with those born in hospital^{71 72}. Different hygiene methods and the use of antibiotics could both explain these differences. Other studies have shown that the primary gut flora in infants born by caesarean delivery may be significantly disturbed for up to 4 months after birth^{54 73}. The clinical relevance of these findings remains to be demonstrated, but could be of interest in terms of mucosal immunity induction^{74 75}. Further evidence that perinatal events can influence bacterial colonisation is given by a study of faeces in premature babies^{71 76}. Previous treatment with antibiotics and being nursed in an incubator have been shown to be significantly associated with a lower rate of anaerobic flora^{71 76}. In addition, an increase in potentially harmful bacteria is often seen. Among these bacteria, *Clostridiae spp.* are certainly the most important group, well known for their associated morbidity. Moreover, these babies are at risk of being colonised with multi-resistant bacteria (*Klebsiella spp.*, *Citrobacter spp.*, *Enterobacter spp.*, etc.) and of developing nosocomial infections and/or NEC.

How can we ensure optimal bacterial intestinal colonisation?

Given the influence of delivery mode on bacterial colonisation in the neonate, it is important to underline the dramatic increase in the prevalence of abdominal deliveries observed in developed countries over the last two decades. Clearly, this observation cannot be interpreted by itself as an amelioration of perinatal care⁷⁷⁻⁷⁹. Obstetricians should be made aware of the possible adverse influence of the increased prevalence of caesarean deliveries on the early immune system induction of the immature being.

The best way to favour optimal bacterial colonisation of neonatal intestine would be to minimise, as far as possible, any alteration of the mothers’ recto-vaginal

microflora. The latter has indeed been demonstrated as being the most important determinant of neonatal bacterial colonisation^{4 80}. It is important to remember that broad-spectrum antibiotics given to the mother in the perinatal period will modify her microflora and indirectly act against future neonatal optimal bacterial colonisation⁸¹. In addition, some studies have demonstrated an increased risk for preterm babies of being infected by multi-resistant bacteria when their mothers are treated with broad spectrum antibiotics^{82 83}. While no difference in this risk was demonstrated by Edwards et al.⁸⁴ when the intrapartum use of penicillin G was compared to ampicillin, a more recent study clearly shows that the risk of the infant being infected by multi-resistant bacteria during the first three months is more elevated when intra partum broad spectrum antibiotics are used by the mother⁸⁵. Once again, as nutrition can influence neonatal bacterial colonisation as early as by the end of the first week, exclusive and prolonged breast-feeding has to be claimed as the key-rule for optimising fermenting microflora, which will act optimally on enterocyte immune induction⁸⁶⁻⁸⁹. For neonates who are not breast fed, the concept of adding prebiotics to the infant formula could be relevant, as this could favour the implementation of a bifidogenic microflora⁹⁰⁻⁹³. Slow and late food diversification (not before 6 months of life) is certainly the best way to maximise the development of the intestinal microflora^{94 95}. In addition, progressively feeding low doses of an antigen results in active and memorised suppression by maximising the induction of regulatory T cells in Peyer's patches. This allows a better long term food tolerance through the control of DTH cell immunity²⁹⁻³². A mature intestinal microflora is considered very stable and more resistant to new bacterial colonisation⁹⁵. This is a very interesting and helpful "barrier effect" that emerges from both the above mentioned early genic modulation of the enterocyte and the specific glycosylation of the glycocalyx, which precede the development of the definitive intestinal microflora^{8 96}. For therapeutic purposes, some trials have been performed in older children and adults, using probiotics – which can be defined as live bacteria beneficial to health^{97 98}. A transient modification of the flora was observed in these trials only when the administration was maintained over time. However, one would expect different results if these probiotics were given in the perinatal period^{99 100}. Indeed, it is theoretically possible that an optimal initial bacterial colonisation with reputed "good" bacteria could play an interesting role in alleviating further immune deviances and rendering other pathogens less able to progressively implement their excessive pro-inflammatory action^{101 102}. A previous study has demonstrated that viable bifidobacteria given to neonates seem to be able to induce a bifidobacterial prevalence at 1 month of age, close to that of breast-fed infants¹⁰³. Perinatal administration of

Lactobacilli GG to allergic mothers appears promising¹¹ with regard to a subsequent decrease in clinical atopic disorders in infants. Along the same lines, a recent randomised study was able to demonstrate that giving the mother a mixture of probiotics before delivery, and continuing the administration to the infant may influence gut immunity¹⁰⁴. Even more interesting, the sCD14 concentration in colostrum correlated with IgA and IgM at 12 months of life. In the immediate postnatal period, there is also the potential to work on the bacterial colonisation of very preterm babies, who have been shown to be at increased risk of NEC. A recent study has shown that probiotics given orally to breast-fed very low birth weight (VLBW) infants significantly decrease their incidence and severity of NEC¹⁰⁵. An even more recent randomised study performed in neonates weighing less than 1,500 g was also able to demonstrate that probiotic supplementation reduced both the incidence and the severity of NEC¹⁰⁶. Another study with seven day *Lactobacilli GG* supplementation since the first feed in VLBW failed to find a significant beneficial effect in preventing NEC¹⁰⁷. Combining prebiotics and probiotics in a symbiotic approach has also been claimed as a potential means of optimizing neonatal bacterial colonisation of the intestine¹⁰⁸. Nevertheless, these studies should still be considered as experimental. Larger studies are still needed before these products can be administered routinely in the perinatal period. Of particular concern is the need to ensure that the bacteria administered are absolutely harmless. It is also important to obtain more information on their pharmacokinetics (dosage, intervals and length of administration, etc.). Nevertheless, these interesting data seem to demonstrate that the bacterial environment during the perinatal period could be of interest in terms of future intestinal microflora and that sub-optimal intestinal colonisation during that period could expose a person to a greater level of morbidity in advancing age^{2 3 20 109 110}.

Conclusions

The international medical community has to be made aware of the increasing importance that initial colonising intestinal microflora could have on the health and well-being of the host later in life. It is of great importance to know that the initial bacterial colonisation of the neonate appears to play a crucial role in inducing immunity in the immature human being, and that a sub-optimal process could have definite consequences. The optimal early interface between the microbes and the intestinal mucosa of the host may have been somewhat disturbed by modern perinatal care. It is fundamental to try to decrease these possible negative influences and to discover in the near future the possible means to help manipulate positively the gut microbiota of infants.

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