Gut colonising microbiota in early life as a crucial step in the acquisition of tolerance to food antigen in the first months of life

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Keywords

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Abstract

Perinatal bacterial colonisation of the newborn's intestine is an essential key stage in the development of local immunity. The quality of its initiation on a still immature digestive mucosa and submucosa will eventually enable the acquisition of an immune balance between defence mechanisms and tolerance to antigenic epitopes of all kinds, firstly locally and then in the whole body. Failure to acquire this immune balance, favoured by complex epigenetic reactions linked to inadequate environmental modifications, can have a lasting impact on the development of subsequent general immunity. Exclusive and prolonged breastfeeding as stated by the World Health Organization (WHO) remains the only way of ensuring not only the quality of the colonising microbiota in the first few months of the infant's life, but also the quality of the development of this immune balance. This is linked to the completeness of human milk, rich in perfectly bioavailable constituents and complex, multiple immunomodulatory factors, which together enable it to be described as a functional food par excellence.

Introduction

Our understanding of the immune mechanisms governing the acquisition of tolerance to food antigens, firstly in the digestive epithelium, with secondary effects on other epithelia, has made enormous progress in recent years (1-13). Thanks to the various experimental studies carried out in this field, in which the similarities between intestinal immune responses in humans are becoming increasingly apparent, it is now clear that the perinatal microbiota that colonises the newborn's intestine plays a key role (14, 15). This colonising microbiota of a mucosa that is still immature enables both the initiation of immune defence mechanisms against invading bacteria and those that are set up in parallel and govern the tolerance of a progressively selected commensal microbiota (16). This balanced immune response will also provide the means to ensure good memory tolerance to environmental antigens, including food. This immune initiation takes place within the intestinal mucosa and submucosa. Without this colonising microbiota in the newborn, none of these defence and tolerance immune responses can be properly established (8, 12). Although these responses are at first sight opposites, they complement each other to achieve a balance that must be maintained throughout life (Figure). Failure to establish this all-important balance correctly at an early age can then have repercussions both locally and throughout the body, increasing the risk of immune deviations of all kinds. Preventing these problems involves firstly finding the best way of ensuring the quality of this colonising microbiota, and secondly improving the way in which food antigens are administered at a very young age.

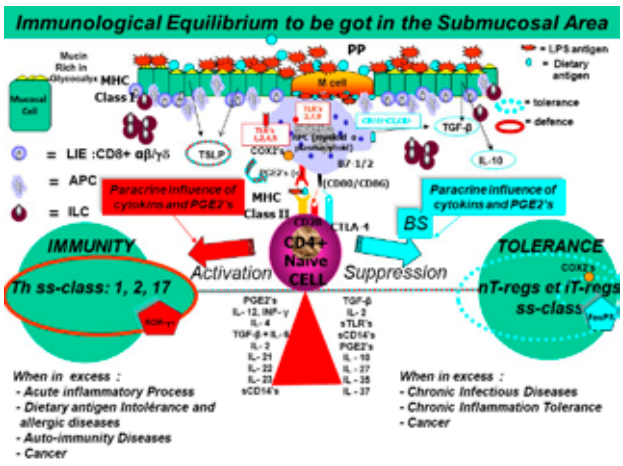
The neonatal enterocyte appears to be particularly well prepared for the initial establishment of a reciprocal biochemical dialogue with invading bacteria (17, 18). The colonising microbiota enables gene modulation of this mucosal cell and the creation of specific niches through specific glycosylation of the glycocalyx under the action of bacterial enzymes. It is this interaction between the host and the invading microbes that will gradually lead to the establishment of this all-important interface between the cells of innate immunity and the cells of adaptive immunity, the essential cellular organs of this digestive immunity and of essentially paracrine expression (from the Greek (παρα = beside) and (κρίηο = to secrete). This is done through various cytokines, i.e., the small proteins secreted by the cells of innate and adaptive immunity (Figure), with either pro-inflammatory or suppressive anti-inflammatory cascade actions.

There can be no doubt that exclusive and prolonged breastfeeding in early childhood is the only way not only to ensure the quality of the intestinal microbiota gradually tolerated by the child, but also to optimise this interface between the host and its microbiota, enabling locally induced immune defence mechanisms and long-term mechanisms for tolerance of environmental antigens, including food. The WHO is constantly reminding us of this, as it is responsible for ensuring the ideal nutritional recommendations for young children throughout the world (19). However, the human milk microbiome is largely influenced by perinatal maternal environmental factors (20).

Critical period: immune priming of the antigenic epitope on an immature digestive mucosa, de facto immaturity during the first year of life.

At this stage in the general debate on how to help prevent all these immune deviances, which could be linked to the type of food given at

Figure:



Legends : PP: Peyer's patches; ILL: intraepithelial lymphocyte; APC: antigen-presenting cell or dendritic cell; CD: Cluster of differentiation; TLR: Toll Like Receptor; LPS: Lipopolysaccharide; MHC: Major Histo-compatibility Complex; IL: Interleukin (or cytokine); TSLP: Thymic stromal lymphopoietin; ILC: Innate Lymphocytic Cell; PGE2: Prostaglandin E2; COX: Cyclooxygenase; TGF : Transforming Growth Factor; CTLA: Cytotoxic-T-Lymphocyte- Antigen 4 protein ; nTreg: Native regulatory lymphocyte (thymic origin (n for native)); iTreg: Induced regulatory lymphocyte (peripheral origin (p) or induced (i)); INF : Interferon; BS: Bystander Suppression; MHC: Major Histocompatibility Complex; ROR-γτ: retinoic acid-related orphan receptor-γt transcription factor; FoxP3: forkhead box P3 transcription factor.

an early age, the problem that some people do not seem to understand sufficiently is the risk of sub-optimal *priming*, i.e. the risk of an inadequate imprint left on the intestinal immune system by the dietary antigenic epitope when it is administered inadequately for the first time. This is particularly true when the exogenous environmental antigenic load is too rapidly excessive, and not reproduced in a progressive increase. This notion of the importance of repetition of the antigenic epitope to mark the immune acceptability of tolerance had previously been highlighted in experimental models, and subsequently confirmed (3). Although these data are mainly based on experimental animal studies, it has become clear in recent years that the same principles of response by the innate and adaptive immune cells of these digestive mucous membranes and submucosa apply to all vertebrates and specifically to humans (14). However, the nomenclature of the immune cell receptors involved may sometimes be slightly different (15).

At an early age, this sub-optimal *priming* of the environmental antigen protein on the digestive mucosa may in fact be the source of induced immune deviations which will only manifest themselves when the antigenic epitope in question is reintroduced at a later stage. The risks of intolerance to this epitopic antigenic food imprint on the immature digestive immune system in infancy are all the greater now that there has been a change in the birth paradigm in recent years, which clearly impacts on the quality of the colonising microbiota of the newborn's intestine (16). Indeed, there has been a marked increase in the hyper-medicalisation of the birth process, as well as an increase in antibiotic therapy, but also a repetitive increase in the administration of certain xenobiotics at an early age, an increase in Caesarean section births, and an excessively marked decrease in prolonged exclusive breastfeeding (21, 22, 23).

We now know that this excessive and unique antigenic load in infancy, i.e. not repeated, on a mucosa that is still partially immature and not stabilised at the level of its interface with a colonising microbiota that is often disturbed by our modern perinatal medicine, creates tolerance essentially by stimulation of innate immunity, with apoptotic cell deletion and/or anergy of innate immunity cells, but without optimal solicitation of adaptive immunity (3). Only by stimulating the adaptive immune system can this tolerance be permanently memorised and acquired centrally.

Basic mechanisms involved: complex optimal interface at the digestive level between innate and adaptive immunity.

This immune tolerance to environmental antigens in the broadest sense, acquired locally at first and then memorised throughout the body, can only be achieved through the intervention of innate immunity cells which correctly control the development of adaptive immunity cells, first locally and then more generally (10,11,13,24). This local innate immunity is, first and foremost, represented by the mucosal cell itself, and the M cells which overlie Peyer's patches, submucosal lymphoid organs extremely rich in naive T and B lymphocyte cells (7). This submucosal region also contains many other innate cells, and particularly $CD8^{+}\gamma\delta$ intraepithelial lymphocytes (IELs), innate lymphocytic cells (ILCs) in submucosal clusters and their respective main specific cytokines (IL's-2,22) (24). These cells express type I histocompatibility factor (MCH-I), which expresses their limitations. The relay of information within the submucosal chorion on the nature of the antigenic epitope is then essentially conveyed by a wide variety of other multifunctional and highly specific innate cells, present in very large numbers at this level from an early age. They play a truly crucial role. Through direct contact and co-stimulation, these key cellular organs enable the immune orientation of naive $CD4^{+}$ effector lymphocyte cells, which are the linchpins of adaptive immunity: these cells driving all immune signals to the $CD4^{+}$ naïve lymphocyte are the dendritic cells, also known as antigen-presenting cells (10, 13, 25). These dendritic cells, still classically classified in the broad category of macrophages and therefore part of innate immunity, express histocompatibility factor type II (MHC-II), which makes their multiple respective functions clearly visible, depending on the information received via some of their specific receptors (Figure) (26). This cellular pluripotentiality of immune function will contribute, through their different receptors expressed on their surface, to the optimal analysis of the nature of the antigenic epitope presented to them by the intestinal mucosa (mucosal enterocytic cells, goblet cells and M cells) but also by these other innate immune cells (7).

Proper initiation of innate cellular immunity in the chorion-submucosa optimises the messages it transmits to the local adaptive immune system.

These dendritic cells will therefore transmit this information about the nature of the antigenic epitope to the naive lymphocyte cells of the adaptive immunity, which is composed primarily of naïve $CD4^{+}$ (effector) and CD8+ αβ (suppressor) lymphocytes present in large numbers in lymphoid nodules and Peyer's patches. This transmission occurs by direct contact, giving rise to a paracrine secretion of cytokines of corresponding quality and number, in response to the type of co-stimulation recorded. For bacterial and/or fungal and/or parasitic antigens of all kinds, as well as for environmental antigens, this transmission of information takes place in parallel via co-stimulation of their respective cell receptor (between dendritic cell and naive $CD4^{+}$ lymphocyte cell) (Figure). This optimisation of the immune information transmitted to the naïve $CD4^{+}$ lymphocyte by parallel co-stimulation in close cell contact, via one of its receptors (the CD28 receptor), followed by the specific paracrine secretion of these immunomodulating proteins, the cytokines, will initially enable an adaptive immune defence response. This will be balanced between naïve $CD4^{+}$ lymphocytes (Th1 and/or Th2 and/or Th17 type effector response, and their respective essential cytokines (IL-12, IL-4, Il-17). This will be followed, on the one hand, by the mature $CD4^{+}$ Th1 lymphocyte (cytokine INF-gamma, Il-2), and the appropriate initiation of the CD8+ αβ lymphocyte (killer), allowing this latter immune pathway to control delayed hypersensitivity (DHS), and, on the other hand, the parallel maturation of the naïve $CD4^{+}$ lymphocyte into a Th2 lymphocyte, under the impetus of the Il-4 cytokine, which immune pathway will promote, by subsequent direct contact, the transformation of the naïve B lymphocyte into a plasmocyte cell. After maturation in the lymphoid organs, this plasmocyte cell can synthesise the IgGs for which it has been programmed. The recolonisation of the submucosal chorion by IgA's-secreting plasmocyte cells, and even more so by secretory IgA's (sIgA's), i.e. IgA's which include the joining chain and secretory component (SC), plays a vital role. These sIgA's are found not only in the intestinal lumen

but also in exocrine glands, in this case and in adulthood the mammary gland in breast-feeding women. Of outstanding interest, the mother thus distributes not only her own microbiota to her child from birth, but also, through her milk, these corresponding sIgA's. In other words, sIgA's do represent the best immune means for the newborn to protect himself from the perinatal invading microbiota, as he is not yet capable of synthesising them correctly.

Following this defensive immune stimulation, in a recurrent situation of bacterial receptor saturation, marked in particular by their consequent partial paracrine extrusion, in the form of soluble glycoproteins (sTLR's (*Toll Like Receptors*) and sCD14's for the essential ones, ...), the newly generated local paracrine immune climate will enable a paradigm shift in the immune response of dendritic cells, via some of their specific receptors ($CD103^+$, for the most part). This will promote the adaptive transformation of the same naive $CD4^+$ lymphocyte, but this time, through another type of co-stimulation of one of its receptors (the CTLA-4 receptor), into an iTreg induced regulatory suppressive lymphocyte ($CD4^+$ $CD25^+$ $FoxP3^+$), capable of continuing to secrete the tolerance suppressing cytokine TGF-β) and, by bystander suppression (or direct proximity suppression), into a peripherally induced suppressor lymphocyte iTreg Tr1, with secretion of the tolerance suppressor cytokine, (IL-10) and Th3 $FoxP3^-$, with secretion of the tolerance suppressor cytokine, (TGF-β).

Our general immune health will in fact depend on the balance shown in the Figure, progressively acquired at local level and then maintained at the level of all the other mucous membranes, between, on the one hand, the effector response of adaptive immunity, capable of providing this defence against foreign micro-organisms, and, on the other hand, the tolerance suppressive response of adaptive immunity, capable of providing tolerance of the commensal microbiota as well as tolerance of the environmental antigen, including the food antigen.

Although this process is still immature at an early age, it is now clear from current experimental models that the inadequacy of this intestinal mucosal response at an early age, through complex epigenetic mechanisms is likely to have a negative impact on the quality of general immunity to this same environmental antigen, including in other mucous membranes (particularly respiratory) (27, 28). This can lead to subsequent severe immune deviations, which may be more marked if a genetic background conducive to their development is also present.

In practice, what attitudes should be recommended for all nutritionist clinicians working in maternity wards: their role in preventing subsequent immune deviations is crucial.

In practical terms and based on a general principle, it is essential to avoid at all costs giving the newborn single supplements of formula milk, i.e., with large quantities of foreign protein epitopes, at least when the mother's intention is to breastfeed over the long term (29). Human milk given exclusively is Nature's way of repeatedly distilling all the mother's environmental antigenic epitopes, including all the food antigens present in her own milk in small quantities (30,31). Furthermore, human milk contains large quantities of soluble immunomodulatory factors described in the digestive submucosal chorion, which help the newborn to acquire immune tolerance within its digestive tract (Table). The microbiome content of human milk is also largely dependent of maternal perinatal factors (20).

Whatever the nature of a milk formula supplement given to a baby whose mother is planning to breastfeed completely, this food supplement runs the risk of negatively modifying the beneficial protective action exerted on her child's digestive tract thanks to the perfect completeness of human milk. Only this completeness makes it a unique functional food par excellence, capable of promoting the establishment of an ideal digestive microbiota. Midwives in maternity wards have understood this well when, on the contrary, they favour the manual extraction of colostrum from breastfeeding mothers. This is particularly the case when spontaneous breastfeeding is less well initiated by the newborn itself and/or when its medical situation requires a very temporary nutritional supplement. Where there is a risk of hypoglycaemia and/or proven hypoglycaemia, for example, the use of dextrose gels should be recommended as a priority, based on the results of recent well-conducted studies (32). It also appears that it does not alter the quality of the neonatal colonising intestinal microbiota. In fact, in addition to the risk of an incorrect immune imprint on the newborn's digestive tract when given inadequately in a single dose with a high epitopic load, any milk formula supplement - which is therefore not of human origin - interferes negatively with the benefits provided by the famous bifidogenic triad in breast milk. This complementary triad of three specific constituents, enabling the establishment of a beneficial acidophilic microbiota, is recalled here: 1) the high lactose content; 2) the low protein content of human milk but with a high bioavailability value; 3) the low phosphorus content of human milk with an optimal calcium/phosphorus ratio for at term neonate. Together, with the interesting acidophilic bacteria's nutritional support of human

Nutrients	Amount	Function
Protein		
- sIgA (IgG, IgM)	50 – 100 mg/dL	Specific immune protection
- Lactoferrin	100 – 300 mg/dL	Bacteriostasis and bactericidy; iron carrier
- Lysozyme	5 - 25 mg/dL	Bactericidy
- α-Lactalbumin	100 - 300 mg/dL	Part of lactose synthase; anti-infective
- Hamlet Component	-	Anti-infective; bactericidy, tumoricidy
- sTLR2,4,5; sCD14, TGFβ	Variable	Anti-inflammatory action – specific receptors
- Lactadherin	Variable	Bacteriostasis – immune modulator
- β-defensin 2	Col ± 8.5; Mat ± 1;0 µG/ml	Bactericidy – negative modulation on TLR7
- Caseins	200 - 300 mg/dl	κ-Casein; bifidogen; inhibition bacterial adherence
Carbohydrates		
- Lactose	6.5 – 7.3 g/dL	Energy source
- Oligosaccharides	1.0 – 1.5 g/dL	Microbial ligands; 3'GL neg modulation on TLR3
- Glycoconjugates	-	Microbial and viral ligands
Fat		
- Triglyceride	3.0 - .4.5 g/dL	Energy source
- LC-PUFA	Variable	Essential for brain and retinal development
- FFA	Variable	Anti-infective as bacterial detergents

The essential immunomodulatory components of human milk. LC-PUFA: long chain polyunsaturated fatty acids; FFA: Free Fatty Acids; sTLR: soluble Toll Like Receptors; TGF-β: Transforming Growth Factor.

milk oligosaccharides (HMO's), because of their quantity and structural diversity, they maintain this low buffering capacity in the intestinal lumen and encourage the growth of this beneficial fermenting microbiota. This low buffering capacity, which is conducive to the establishment of an acidophilic microbiota composed essentially of bifidobacteria and acid-lactic bacteria (*Lactobacilli, Akkermansiae,...*), is only present when human milk intake is complete. In fact, it is the low buffering capacity of human milk that generates and maintains a fermenting microbiota. This microbiota, thanks to the local anaerobic metabolism it induces, is uniquely able to maintain a low pH in the intestinal lumen of exclusively breastfed newborn infants, thereby reducing the risk of intestinal invasion by more pathogenic germs (33). This maintained fermenting microbiota is also able to favour this ideal interface with the colonised host at the level of its digestive mucosa, for a high-quality local immune response.

The only indication to give the neonate, very temporarily and preferably, an advanced casein hydrolysate, or even an amino acid complex, could prevail when there is an absolute and well-considered (and therefore rare) need to give a single supplement - or one that is very rarely repeated. It does matter if the mother has a clear plan for prolonged breastfeeding. This may be understandable only because her breastfeeding does not start immediately and perfectly and there is not enough hand-extracted colostrum available. Only advanced casein hydrolysate - and better still, the complex elementary formula made up solely of amino acids - has shown some prevention of this inadequate priming in the case of the former - and therefore some preventive efficacy, but the level of evidence remains low (34). In fact, no hydrolysed milk given at an early age, whether partially or completely, is currently capable of preventing the development of long-term immune deficiencies, whatever their nature (allergic and/or autoimmune deficiencies) (35, 36). Only in well-conducted studies (and only in those studies) has prolonged exclusive breastfeeding shown to some limited extent a tendency to prevent immune deviations (37,38). The relative disappointment observed in the absence of long-term prevention of these immune deviations by prolonged breastfeeding, as also demonstrated in the PROBIT study, is linked to the existence of the early epigenetic reactions on the genome already mentioned which interfere with local digestive immunity, factors not yet sufficiently identified - and therefore not controlled - but probably linked to these inadequate repetitive environmental factors (27, 28, 39).

In other situations, particularly those where mixed breastfeeding by choice of the mother is used for a longer period from the outset, and/or of course if the mother does not wish to breastfeed, it does not really matter which milk is given as a supplement, if it is not given all at once in large quantities, but rather repeated over several days, gradually increasing the antigenic load. This basic principle should also be borne in mind when improving antigen *priming* during the dietary diversification phase, which should ideally be initiated at around 6 months, as the WHO continues to recommend (19). Any introduction of food antigens during the first year should therefore ideally be initiated according to the same principle of gradually increasing epitopic load repetitions, in the same way between 6 and 12 months, on a digestive mucosa and submucosa that are still immature in terms of the immune response induced. This should happen for all newly introduced foods, ideally as a complement in addition to breastfeeding, which should be preferred for as long as possible. In fact, during this period, breastfeeding should be maintained as the essential milk intake. According to new concepts, this defined period of optimal diversification represents an ideal immune window to try to reduce the subsequent risks of allergy (40). It is therefore desirable to take greater responsibility for the immune *priming* of any food newly introduced during the first year, which is far from being the current approach. This new form of dietary diversification must (should) therefore also consist of a gradual and repeated increase in the antigenic load of this new food, whatever it may be, and whatever the possible genetic susceptibility of the atopic family.

This new way of proceeding would promote memorised immune tolerance to these newly introduced environmental antigens. This will allow the appropriate immune *priming* of the submucosal intestinal chorion, which is so important for the lifelong acquisition of the necessary memorised immune tolerance, which should make it possible to control any inadequate reaction to the introduced environmental antigen. This locally acquired tolerance is then transmitted to the other epithelia through the colonisation of all the lymphoid organs by immune cells with tolerance memory for this antigen. Experimental studies show that a fortnight's repetition, every day, of the dietary antigen is enough to obtain tolerance that is permanently anchored in the immune memory, but this remains to be proven in humans.

However, the latest studies show that, when this initial priming of the food antigen is inadequate, it is difficult to recover the tolerance induced by the oral route, even though certain recent trials using the same principles of repetition of a small stabilising daily dose with a progressive increase in load are beginning to give interesting results, i.e., a certain degree of recovery of the immune tolerance response. This highlights the lifelong impact of the inadequacy of the initial *priming*, which has led to a memorised immune intolerance response. It is precisely this inadequacy of *priming* at an early age, on this still immature mucosa ,that must be avoided in the first place to enable the induced tolerance to the antigenic food epitope to be initiated for life and not, on the contrary, to induce a memorised intolerance that is difficult to recover later on (16).

What are we to make of the recommendations recently published by French paediatric allergists?

French paediatric allergists now seem to have understood the importance of primary prevention of food allergy by improving the immune *priming* of the food antigen at a very early age in the mucosal and submucosal intestinal system, a *priming* that is optimally obtained as described briefly above in terms of the complex immune mechanisms involved (41). They understand the importance of this form of introduction of food antigens, a form of introduction which was raised long before their observation as requiring well-conducted studies to try to improve this memorised optimal acquisition of tolerance to the food antigen and, consequently, to try to prevent immune deviations of intolerance (42).

However, what these paediatric allergists are currently tending to do, and what poses a major ethical concern in the way they are progressing, is to recommend the introduction of small quantities of formula milk at a very early age, in case of familial atopic diseases, even in newborns who are perfectly and completely breastfed by their own mothers. What's more, this is being done without any validation support in the form of prospective, randomised scientific research that has demonstrated the merits of this practice. The European *Academy of Allergy and Clinical Immunology* (EAACI) had suggested that further in-depth studies should be carried out (43). In addition, the *American Academy of Pediatrics* (AAP) has also taken a position on two occasions (2008 and 2019), concluding that there was insufficient evidence to suggest changing the timing and type of dietary diversification. It invited stakeholders to carry out further randomised prospective studies (44). This debate in Europe on the very early introduction of food antigens during the breastfeeding phase is not new (45). It was vehemently opposed in the past, when it was first recommended for children exclusively breastfed by their mothers (46). In so doing, French paediatric allergists are ignoring what Nature itself spontaneously ensures by repeated distillation of all the mother's environmental antigens present in her own milk (30, 31). Even if these data still need to be clarified, as they are essentially based on experimental models, this 'neglect' of what Nature seems to do in all mammals to promote food tolerance in their offspring is also being done with the 'benevolence' of the firms that manufacture milk formulas and infant products. On the other hand, they have found here an opportunity to promote their product in the very early administration of milk supplements to mothers who wish to breastfeed their babies completely.

How can it be imagined that a mother could be involved in this 'sorcerer's apprentice' approach when each of them considers that her milk, given exclusively and over a long period of time to her newborn baby, is reputed - quite rightly - to be the best food for him at an early age. This subliminal "benevolence" on the part of milk companies was virulently denounced in a recent special issue of the Lancet (https://www.thelancet.com/series/Breastfeeding-2023) (47). By acting in this way, these French paediatric allergists are bypassing the essential research stages required to confirm the validity of these practices in humans and are playing the sorcerer's

apprentice. Worse still, they are ignoring WHO recommendations, which stress the importance of exclusive breastfeeding for 6 months and, if possible, breastfeeding in addition to dietary complements thereafter (19). Let's not forget that the WHO publishes recommendations and provides the impetus for optimal public health research for the world's children, first and foremost. It is not a question of giving small quantities of formula milk to babies breastfed by their own mothers, in countries where food safety is more precarious, with the possibility of reconstituting the formula milk with contaminated water. Just imagine the damage this could cause, as we have seen in the past! What is just as serious mistake in this article recently published by French paediatric allergists is that they recommend repeating ultra-processed foods from the age of 4 months, which is precisely what needs to be combated in terms of obesity prevention (48).

Whatever the atopic familial status, the point here is therefore to reiterate the importance of not giving milk formula supplements in maternity wards, and as a single administration, an importance that must be asserted vigorously, giving preference to all other known means, including manual extraction of colostrum to get through any difficult stage at the start of breastfeeding. As explained above, these one-off supplements, with their high antigenic load from the outset, are precisely the source of possible subsequent immune deficiencies when cow's milk protein is reintroduced. The one and only indication for repeating small doses of formula milk would be when the paediatrician and/or clinical nutritionist is confronted with newborns who have had to receive a single supplement of formula milk for medical reasons, and where natural supplements following colostrum extraction from the mother could not be provided.

It may be possible to discuss with French paediatric allergists the merits of introducing diversification at around 4 to 5 months of age, which is often the case in our so-called civilised countries, if possible, also in addition to breast milk. However, there can be no question at this stage of systematically giving formula milk supplements to mothers who full breast feeders are! This is quite simply nonsense at this stage, nonsense that runs counter to *Evidence Based Medicine* (EBM) and risks having serious consequences for infants the world over!

In conclusion

At this point, it is important to reiterate in the strongest terms the importance of prolonged and exclusive breastfeeding during the first 6 months of life: this is the only way to ensure an optimal colonising microbiota in the newborn's intestine and, consequently, an immune response initiated, and defined as optimal, in the intestinal mucosa and submucosa. This is Nature's way of ensuring a good balance in the immune response, between defence and tolerance, to counteract as best as possible any inadequacy in the *priming* of the environmental antigen on the neonatal digestive tract. While this new concept of repeating the antigenic load of a food given for the first time is perfectly acceptable, if the doses are increased gradually and in in addition to breastfeeding, it will first be important to demonstrate its value through well-conducted randomised trials from 6 months of age up to 12 months, which seems to be the ideal immunity window. However, the WHO recommendations on exclusive breastfeeding for up to 6 months should be respected.

In absolute terms, and in keeping with the scientific rigour that must be maintained, we should avoid recommending practices that have not yet been tried and tested, and we should not act like sorcerer's apprentices. Above all, it is important for all doctors and others involved in perinatal health to think of the interests of all the world's newborns, some of whom are born in conditions that are sometimes more difficult in terms of their health care. Nature provides them with protection through well-managed, exclusive, and prolonged breastfeeding, a natural protection that should not be disrupted.

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REFERENCES

1. Alpan O, Rudomen G, Matzinger P. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. J Imunol, 2001, 166, 4843-4852. https://doi.org/10.4049/jimmunol.166.8.4843.
2. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science, 2005, 307, 1915-1920. https://doi.org/10.1126/science.1104816.
3. Friedman A. Induction of anergy in Th1 lymphocytes by oral tolerance. Importance of antigen dosage and frequency of feeding. Ann N Y Acad Sci, 1996, 778, 103-110. https://doi.org/10.1111/j.1749-6632.1996.tb21119.x.
4. Hooper LV, Midvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr, 2002, 22, 283-307. https://doi.org/10.1146/annurev.nutr.22.011602.092259.
5. Kelsall B, Strober W. Gut-associated Lymphoid Tis¬sue. Antigen Handling and T-Lymphocyte Responses. In : Mucosal Immunology. Eds: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR. 2nd Ed. Academic press, 1999, New-York.
6. Li MO, Rudensky AY. T cell receptor signalling in the control of regulatory T cell differentiation and function. Nat Rev Immunol 2016; 16: 221-233. https://doi.org/10.1038/nri.2016.26.
7. McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012; 14: 345-349. https://doi.org/10.1038/nature10863.
8. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. Cell, 2004, 118, 229-241. https://doi.org/10.1016/j.cell.2004.07.002.
9. Ruane DT and Lavelle EdC. The role of $CD103^{+}$ dendritic cells in the intestinal mucosal immune system Front Immunol 2011; 2:1-6. https://doi.org/10.3389/fimmu.2011.00025.
10. Scott CL, Aumeunier AM, Mowat A Mcl. Intestinal CD103+ dendritic cells: master regulators of tolerance? Trends in Immunol 2011; 32: 412-419. https://doi.org/10.1016/j.it.2011.06.003 .
11. Strobel S, Mowat A Mcl. Immune responses to dietary antigens: oral tolerance. Immunol Today, 1998, 19, 173-181. https://doi.org/10.1016/s0167-5699(97)01239-5.
12. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol, 1997, 159, 1739-1745.
13. Trabanelli S, Ocadlikova D, Evangelisti C, Parisi S, Curti A. Induction of regulatory T Cells by dendritic cells through indoleamine 2,3-dioxygenase: a potent mechanism of acquired peripheral tolerance Curr Med Chem 2011; 18: 2234-2239. https://doi.org/10.2174/092986711795656054.
14. Allen TM, Brehm MA, Bridges A, Ferguson S, Kumar P, Mirochnitchnenko O et al. Humanized immune system mouse models: progress, challenges and opportunities. Nat Immunol 2019 20:770. https://doi.org/10.1038/s41590-019-0416-z.
15. Gibbons DL and Spencer J. Mouse and human intestinal immunity: same ballpark, different players; different rules, same score. Mucosal Immunol 2011 ; 4 : 148-157 https://www.mucosalimmunology.org/article/S1933-0219(22)01374-5/fulltext#:~:text=DOI%3A-,https%3A//doi.org/10.1038/mi.2010.85,-PlumX%20Metrics.
16. Torow N, Marsland BJ, Hornef MW, Gollwitzer ES. Neonatal mucosal immunology. Muc Immunol 2017; 10: 5-17. https://doi.org/10.1038/mi.2016.81..
17. Levy O. Innate immunity of the newborn : basic mechanisms and clinical correlates. Nature Rev Immunol, 2007, 7, 379-390. https://doi.org/10.1038/nri2075
18. Newburg D, Walker A. Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr Res, 2007, 61, 2-8. https://doi.org/10.1203/01.pdr.0000250274.68571.18
19. World Health Organization UNCsf. Global strategy for infant and young child feeding. Geneva: World Health Organization; 2003. https://www.paho.org/en/documents/global-strategy-infant-and-young-child-feeding.
20. Moossavi S, Sepheri S, Robertson R, Bode L, Goruk S, Field CJ et al. Composition and Variation of the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. Cell Host & Microbe 2019; 25: 324–335. https://doi.org/10.1016/j.chom.2019.01.011.
21. Langhendries JP, Allegaert K, van den Anker JN, Veyckemans F, Smets F. Possible effects of repeated exposure to ibuprofen and acetaminophen on the intestinal immune response in young infants. Med Hypoth 2016; 87: 90-96. https://doi.org/10.1016/j.mehy.2015.11.012.
22. Marra F, Marra CA, Richardson K, Lynd LD, Kozryrskyj A, Patrick DM et al. Antibiotic use in children is associated with increased risk of asthma. Pediatrics 2009; 123: 1003-1010. https://doi.org/10.1542/peds.2008-1146.
23. Sevelsted A, Stokholm J, Bonnelykke K, Bisgaard H. Cesarean section and chronic immune disorders Pediatrics 2015; 135 : e92-8. https://doi.org/10.1542/peds.2014-0596.

24. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science 2014 ; 343 ; 1439. https://doi.org/10.1126/science.1249288.

25. Zigmond E and Jung S. Intestinal macrophages: well educated exceptions from the rule. Trends Immunol 2013; 34: 162-168. https://doi.org/10.1016/j.it.2013.02.001.

26. Guilliams M, Ginhoux F, Jakubzick C, Naik SA, Onai N, Schraml BU et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol 2014; 14: 571-578. https://doi.org/10.1038/nri3712.

27. Canani RB, Di Constanzo M, Leone L, Bedogni G, Brambilla P, Cianfarani S et al. Epigenetic mechanisms elicited by nutrition in early life. Nutr Res Rev 2011; 24:198-205. https://doi.org/10.1017/s0954422411000102.

28. Huehn J, Polansky JK, Hamann A. Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? Nat Rev Immunol 2009; 9: 83-59. https://doi.org/10.1038/nri2474.

29. Urashima M, Mezawa H, Okuyama M, Urashima T, Hirano D, Gocho N et al. Primary Prevention of Cow's Milk Sensitization and Food Allergy by Avoiding Supplementation With Cow's Milk Formula at Birth A Randomized Clinical Trial. JAMA Pediatr 2019 ;173 :1137-1145. https://doi.org/10.1001/jamapediatrics.2019.3544.

30. Verhasselt V. Neonatal tolerance under breastfeeding influence: the presence of allergen and transforming growth factor-beta in breast milk protects the progeny from allergic asthma. J Pediatr 2010. 56 : S16-20. https://doi.org/10.1016/j.jpeds.2009.11.015.

31. Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandie E et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. Mucosal Immunol 2010; 3:461-74. https://doi.org/10.1038/mi.2010.23.

32. Harris DL, Gamble GD, Harding JE, on behalf of the CHYLD Study Group. Outcome at 4.5 years after dextrose gel treatment of hypoglycaemia: follow-up of the Sugar Babies randomised trial. Arch Dis Child Fetal Neonatal Ed 2023; 108: F121–F129. https://doi.org/10.1136/archdischild-2022-324148.

33. Langhendries JP, Detry J, Van Hees JN, Lamboray J-M, Darimont J, Mozin M-J et al. Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. J Pediatr Gastroenterol Nutr 1995; 21: 177-181. https://doi.org/10.1097/00005176-199508000-00009

34. von Berg A, Filipiak-Pittroff B, Schulz H, Hoffmann U, Link E, Suβmann M et al. Allergic manifestation 15 years after early intervention with hydrolyzed formulas--the GINI Study. Allergy 2016 ;71 :210-219. https://doi.org/10.1111/all.12790.

35. Brand P. Partially hydrolysed whey and soy-based infant formulas did not prevent allergic disease in high-risk children. Arch Dis Child Educ Pract Ed. 2012 Jun; 97: 120. https://doi.org/10.1136/archdischild-2011-300992.

36. Lowe AJ, Hosking CS, Bennet CM, Allen KJ, Axelrad C, Carlin JB et al. Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial. J Allergy Clin Immunol. 2011; 128:360-365. https://doi.org/10.1016/j.jaci.2010.05.006.

37. van Odijk J, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA al. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. Allergy 2003; 58: 833-843. https://doi.org/10.1034/j.1398-9995.2003.00264.x.

38. Lodge C, Tan DJ, Lau MXZ, Dai X, Tham R, Lowe AJ, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. Acta Pædiatrica 2015; 104: 38–53. https://doi.org/10.1111/apa.13132.

39. Flohr C, Henderson AJ, Kramer MS, Patel R, Thompson J, Rifas-Shiman SL et al. Effect of an Intervention to Promote Breastfeeding on Asthma, Lung Function, and Atopic Eczema at Age 16 Years: Follow-up of the PROBIT Randomized Trial. JAMA Pediatr 2018 ;172: e174064. https://doi.org/10.1001/jamapediatrics.2017.4064.

40. ESPGHAN Committee on Nutrition. - Complementary feeding: a commentary by the ESPGHAN Committee on nutrition. J Pediatr Gastroenterol Nutr, 2008, 46, 99-110. https://doi.org/10.1097/01.mpg.0000304464.60788.bd.

41. Sabouraud-Leclerc D, Bradatan E, Moraly T, Payot F, Broué Chabert A, Pontcharraud R et al. Générale Prévention primaire en 2021 de l'allergie alimentaire du jeuneenfant : mise au point et propositions d'un groupe de pédiatresallergologues francophones appartenant au groupe de travail « Allergies Alimentaires de la Société française d'allergologie ». Revue française d'allergologie https://doi.org/10.1016/j.reval.2022.01.002.

42. Langhendries JP. La tolérance alimentaire chez le nourrisson : nouvelles données immunologiques et implications pratiques. Rev Med Liège 2009 ; 64 : 10 : 530-534.

43. Halken S, Muraro A, de Silva D, Khaleva E, Angier E, Arasi S et al. EAACI guideline: Preventing the development of food allergy in infants and young children (2020 update). Pediatr Allergy Immunol 2021; 32: 843-858. https://doi.org/10.1111/pai.13496.

44. Greer FR, Sicherer SH, Burks AW, COMMITTEE ON NUTRITION; SECTION ON ALLERGY AND IMMUNOLOGY, Abrams SA et al. The Effects of Early Nutritional Interventions on the Development of Atopic Disease in Infants and Children: The Role of Maternal Dietary Restriction, Breastfeeding, Hydrolyzed Formulas, and Timing of Introduction of Allergenic Complementary Foods. Pediatrics 2019; 143: e20190281. https://doi.org/10.1542/peds.2019-0281.

45. Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. J Allergy Clin Immunol 2010; 126 : 77-82. https://doi.org/10.1016/j.jaci.2010.04.020.

46. Koletzko S, Filipiak-Pittroff B, von Berg A, Grübl A, Heinrich J, Krämer U et al. Supplementation with cow's milk at birth is not recommended. J Allergy Clin Immunol 2011 ;127 : 836-838. https://doi.org/10.1016/j.jaci.2010.11.051.

47. Pérez-Escamillar R, Tomori C, Hernandez-Cordero S, Baker P, Barros AJD, Bégin F et al. Breastfeeding: crucially important, but increasingly challenged in a market-driven world. Lancet 2023; 401: 472–485. https://doi.org/10.1016/s0140-6736(22)01932-8.

48. Rauber F, Chang K, Vamos EP, da Costa Louzada ML, Monteiro CA, Millett C et al. Ultra-processed food consumption and risk of obesity: a prospective cohort study of UK Biobank. Eur J Nutr 2021; 60 :2169-2180. https://doi.org/10.1007/s00394-020-02367-1.