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Nutritional Support for the Infant's Immune System

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Newborn babies possess a functional but immature immune system as a defense against a world teeming with microorganisms. Breast milk contains a number of biological, active compounds that support the infant's immune system. These include secretory immunoglobulin A (IgA), which confers specific protection against enteric pathogens, as well as numerous other immunological, active ingredients. A number of these ingredients can be used as supplements for infant formulas based on cow's milk. Here, the strength of evidence regarding the immune-stimulating effects of selected minerals, vitamins, fatty acids, pre- and probiotics, and nucleotides is reviewed. An assessment of how these ingredients are used in infant-formula products currently available on the market is also presented.

Key words: breast milk, infant formula, infant immunity, immune stimulation, probiotics, nucleotides

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INTRODUCTION

Newborn babies and infants are exposed to a vast array of potentially infectious microorganisms. The immune system, which has the foremost function of protecting against infections, is developed at birth, but it is still immature and, therefore, not fully functional. The ability of the newborn to resist infections is consequently impaired, but this can be, and is, supported by passive immunity. Transferred from mother to child, passive immunity is provided by maternal IgG antibodies, which are transported transplacentally during the last trimester of pregnancy, and by IgA antibodies in breast milk. In addition to possessing IgA, breast milk contains a range of other nutritional components that can improve the infant's immune system.

The IgA antibodies in breast milk can not be reproduced in infant formula, but other nutritional components can. In this review, the development of the immune system in babies and infants is described, with an emphasis on the function of the immune system for protecting against infections. The nutritional components in breast milk and the evidence for an immunomodulatory role are also reviewed, based on a PubMed search for human trials performed during the last 10 years and other relevant literature. The data collected was examined and the evidence was graded. Finally, the results of a market survey are presented.

This survey was conducted to determine how the existing knowledge on immunomodulation by nutrition ingredients has been translated to incorporate these ingredients into the currently available infant formulas.

THE INFANT IMMUNE SYSTEM

Development of the Immune System

The immune system starts to develop during embryogenesis, when the first hematopoietic cells develop outside the embryo, in the yolk sac. In week 6 of gestation, committed hematopoietic stem cells develop in the mesoderm of the fetus, the so-called aorta-gonad-mesonephros.[1] Hematopoietic stem cells then migrate to the fetal liver where they initiate erythropoiesis.[2] During week 7 of gestation, cells seed the developing thymus. Seeding into the bone marrow occurs much later (by week 20).[3,4] In the thymus, T lymphocytes develop in a process that involves rearrangement of the T-cell receptors followed by selection for functionality and negative selection against self-antigens. Development of natural killer (NK) cells as well as various dendritic cell (DC)

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populations also takes place in the thymus. In bone marrow, B lymphocytes, granulocytes, monocytes, and DC develop. The development of lymphoid cells and organs is a complex process that requires the timely expression of growth factors (cytokines, chemokines) and receptors as well as adhesion molecules. Apart from maternal-fetal transfer, the development of the immune system is independent of antigenic stimulation (i.e., bacterial, viral, or allergenic).

Birth marks a fundamental change in the demand put upon the immune system. When children are born, they emerge from the relatively sterile environment of the uterus into a world teeming with bacteria. Within the first days of life, mucosal surfaces of the gastrointestinal as well as the respiratory tract become colonized with bacterial communities.[5]

At birth, the lymphoid system is not yet mature, even though it is developed. All T and B lymphocytes are naive, i.e., they have not yet encountered antigen and memory lymphocytes; therefore, they are not yet present. Some studies suggest that, especially for allergens, transplacental priming of fetal T lymphocytes may occur, but this is still controversial.[6] Activation of T lymphocytes results in a response that is dominated by TH2 cytokine production (IL-4 and IL-5) with relatively little production of the TH1 cytokine γ-interferon.[7] The reasons for this imbalance are unclear; it may be that the default differentiation pathway in the absence of antigenic stimuli is biased to TH2. Alternatively, the immature phenotypes of antigen presenting cells or differential expression of cell-signaling molecules or transcription factors may contribute to this bias.[8]

Like T lymphocytes, B lymphocytes are naive and immature at birth, and memory B lymphocytes have not yet developed.[9] Nevertheless, the neonate is able to mount an antibody response upon primary infection or upon primary vaccination with protein-based vaccines; however, they are unable to respond to polysaccharide antigens, making them extra vulnerable to infections with polysaccharide-encapsulated bacteria such as group B streptococci and pneumococci. The polysaccharide-specific B lymphocytes are already present, but they fail to express a coreceptor (CD21), which is necessary for the response to these antigens.[10,11]

Postnatally, the gastrointestinal tract and the respiratory tract become colonized with microorganisms.[5] The spectrum of commensal and pathogenic microorganisms, and the corresponding pathogen-associated molecular patterns (PAMPs) to which the immune system is exposed, is immense. The immune system's broad repertoire renders it capable of responding to virtually every trigger. It is now realized, however, that the immune system does not respond to every stimulus; rather, it responds to danger signals from the environment.[12] The emerging mechanism is that PAMPs are recognized by a polymorphic repertoire of receptors of the innate and adaptive immune system,[13] and this shapes the direction of the immune system's development through child- and adulthood.

Infections Early in Life

During pregnancy, the immune system of the fetus operates in coexistence with the mother's immune system. After birth, the immune system of the newborn must switch its mode in order to protect the infant against invading pathogens and to develop a tolerance to harmless non-self antigens, such as food antigens. The competence of the newborn's immune system develops progressively during the first months of life. Specific features of the newborn's immune system and its development determine the newborn's susceptibility to different types of infectious diseases.

First, T cell-mediated immunity in newborns is predominantly naive. Memory T cells develop gradually in healthy infants during the first years of life. The response of T cells to specific antigens at birth or in cord blood is largely absent unless intrauterine exposure occurred. This indicates that every postpartum encounter with a pathogen can result in a primary infection. The neonatal immune system is able to clear pathogens, but neonates and young infants are prone to experience more morbidity from viral infections than older children due to the decreased responsiveness of T cell-mediated immunity. Viral infections such as respiratory syncytial virus, enteroviruses, and influenza virus are a few examples of pathogens that may cause severe illness. In a repeat encounter with the same pathogen, immunological memory is present and the resulting improved clearance results in less morbidity.

Second, antibody production in newborns and young infants is immature. As a result, newborns and young infants are more vulnerable to serious bacterial infections. Pathogens acquired from the maternal genital tract such as group B *Streptococcus* and enteric organisms like *Escherichia coli* are major causative agents related to bacterial infections in neonates. In young infants, especially, the production of antibodies to polysaccharide antigens is undeveloped. Therefore, polysaccharide-encapsulated bacteria such as *Streptococcus pneumoniae* are major causative agents of upper and lower respiratory tract infections such as pneumonia and otitis media in young infants. Routine immunization with conjugate vaccines for encapsulated bacteria such as *Haemophilus influenzae* type b and the pneumococcal conjugate vaccine (PCV7) have resulted in a decreased incidence of invasive disease in young infants.[14] In general, infants are at risk for serious infection due to their developing

immune system, and this warrants vaccination against pathogens associated with high morbidity and mortality.

Allergy

The immune system is tightly controlled by its own regulatory network to prevent inappropriate immune reactions from resulting in pathologic conditions. Regulatory system failure is believed to result in either allergy or autoimmunity. Genetic as well as environmental factors contribute to an individual's susceptibility to autoimmune and allergic disease. Autoimmune diseases are relatively rare in children. Atopic disease adds considerably to childhood morbidity. The cumulative prevalence during childhood is estimated to be 20% to 30%. The pathogenesis of allergic diseases is multifactorial. In addition to a positive family history, which adds considerably to the risk of a child developing allergic disease in life, many polymorphic genetic markers have been associated with allergic disease. Furthermore, among environmental factors, feeding and nutritional composition play an important role in gastrointestinal function, host defense, and exposure to food allergens.[15]

Breastfeeding may protect against the development of allergic disease, although this is still a subject of discussion. Breastfeeding appears to be protective against the development of food allergy. Sensitization to allergens only develops upon exposure. The cow's milk protein β-lactoglobulin, which is a major allergen in cow's milk protein allergy, can be detected in breast milk, but in much lower amounts than is present in cow's milk and infant formulas. In addition to the reduced exposure to cow's milk protein that breastfeeding affords, the oligosaccharides present in breast milk affect the intestinal bacterial flora by promoting the presence of bifidobacteria and lactobacilli. The reduced presence of bifidobacteria in infants' stool early in life is associated with the development of allergic disease later in life. When breast milk is insufficient or lacking, infant formula is needed. Feeding with hydrolyzed formulas, especially extensively hydrolyzed formulas, significantly reduces the incidence of cow's milk protein allergy compared with feeding with conventional cow's milk formulas.[16]

CONTRIBUTION OF BREAST MILK TO HOST DEFENSE IN INFANTS

An infant's immature immune system is supported by passive immunity transferred from mother to child. Passive immunity is provided via maternal immunoglobulins and breast milk.

During the last semester of pregnancy active transport of maternal IgG occurs across the placenta; thus, after full-term pregnancy, the IgG levels in the neonate equal that of the mother (Figure 1). IgM and IgA antibodies can not be transported transplacentally. The IgG from the mother disappears with a half-life of 21 days, so most of it is gone by 3 months. Prolonged support of passive immunity is provided by breastfeeding. On a global scale, it has been estimated that optimal breastfeeding behavior (defined as exclusive breastfeeding for at least 6 months and continuation for the first year) could prevent the deaths of 1.3 million children annually.[17] Breast milk contains 0.4 to 1.0 g/L secretory IgA, which is directed specifically against enteric and respiratory pathogens from the environment of the mother and the child. Breast milk contains many other components that directly or indirectly support the infant's ability to resist infection (Table 1).

Lactoferrin and lysozyme are proteins with direct antibacterial activity. Lactoferrin is a ferric iron-binding glycoprotein that inhibits the growth of pathogens by competing with bacteria for ferric iron. The amino-terminal peptide of lactoferrin, lactoferricin, also exerts chelation-independent bactericidal activity. Because lactoferrin resists digestion by proteolytic enzymes, most of the ingested lactoferrin survives passage through the gastrointestinal tract of infants. Lysozyme, an antimicrobial peptide that cleaves peptidoglycans in the cell walls of bacteria is present in a 300-fold higher concentration in breast milk than in cow's milk.

Breast milk contains at least 80 different oligosaccharides. Many of these function as receptor analogues that inhibit the binding of bacterial or viral pathogens, or toxins, to gut epithelial cells. The structure of the oligosaccharide determines the specificity of binding to adherence receptors of bacteria or bacterial toxins. GM1 gangliosides are receptor analogues for toxins produced by *V. cholerae* and *E. coli*, whereas lacto-*N*-fucopentaose II (Lewis X) prevents HIV-1 transfer. Furthermore, certain glycosylated proteins, such as the mucin MUC1,

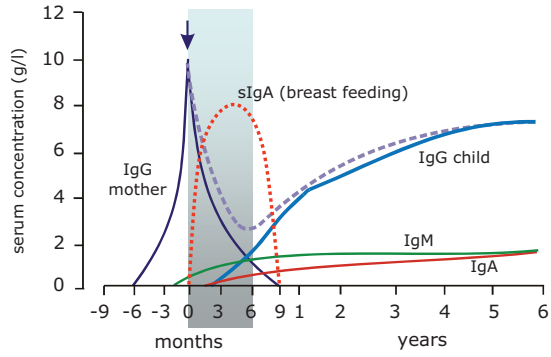

Figure 1. Development of serum immunoglobulins in early life. Note that secretory IgA delivered by breastfeeding remains confined to the intestine and does not contribute to IgA levels in serum. Partly based on data reported by Zinkernagel.[83]

Table 1. Immunological active ingredients of human milk (per 100 kcal)

Class	Compound	Concentration*	Recommended[†,††] (min–max)
Immunoglobulins	sIgA	69–153 mg	
Antimicrobial proteins	Lactoferrin	139–264 mg	
	Lysozyme	260–347 mg	
Leukocytes		0.5×10^6/ml	
Cytokines, chemokines	IL-1, IL-6, TNF-α		
	γ-IFN, IL-12, IL-10		
	IL-8, CCL5		
Hormones	GM-CSF, EPO		
	Cortisol		
Fatty acids	DHA	9.8–12.5 mg	optional
	AA	16.7–23.6 mg	optional
Oligosaccharides	FOS	1.53–1.67 g	optional
	GOS	0.28–0.42 g	optional
Minerals	Zn	360–460 μg	500–1500
	Se	1.1–2.6 μg	1–9
	Fe	86–130 μg	30 (60)–130 (170)
	Cu	51–60 μg	35–80
	Mn	2–3.5 μg	1–50
	Ca	44–50 mg	50–140
Vitamins	Vitamin A	56–106 μg	66–198
	Vitamin E	0.39–0.54 IU	0.75–7.5
	Vitamin D^3	$<$0.01 μg	1–2.5 (3)
	Vitamin C	4.3–6.3 mg	10–30
	Vitamin B^{12}	0.01 μg	0.10–0.50
Nucleotides	Total	7.38–8.06 mg	–5 (optional)
	AMP	0.42–0.48 mg	–1.5
	CMP	2.19–2.37 mg	–2.5
	GMP	1.35–1.55 mg	–0.5
	IMP	1.23–1.29 mg	–1.0
	UMP	2.19–2.37 mg	–1.75
Various	Sialic acid	41.7–208 mg	
	Gangliosides	1.1–1.4 mg	
	L-carnitine	0.94–1.31 mg	1.2

*Concentrations are based on data reported by Labbok et al.[18]
[†]Recommendations are based on data reported by Koletzko et al.[54]
[††]Figures in parentheses represent follow-on formulas.
AA, arachidonic acid; CCL, chemokine (C-C motif) ligand; DHA, docosahexanoic acid; EPO, erythropoietin; FOS, fructo-oligosaccharides; GM-CSF, granulocyte-macrophage colony stimulating factor; GOS, galacto-oligosaccharides; IL, interleukin; TNF, tumor-necrosis factor; IFN, interferon.

interfere with bacterial or viral adherence. Lactadhedrin, a component of milk-fat globules, protects against rotavirus infections.[18] Free fatty acids and monoglycerides, generated by enzymatic digestion of triglycerides, can disrupt enveloped viruses.[18]

Oligosaccharides also promote the proliferation of commensal *Bifidobacterium* sp. and lactobacilli in the intestinal tract. These bacteria, which are termed probiotic bacteria, are generally believed to have a health-promoting effect, probably because, amongst other things, they produce organic acids that retard the growth of enteric pathogens.

Human milk contains leukocytes, including neutrophils (40–65% of total leukocytes), monocytes/macrophages (35–55%), and mainly activated $CD8^+$ T lymphocytes (5–10%). It is unknown whether any of these cells can transfer functional cellular immunity from mother to child during breastfeeding.

Cytokines and chemokines present in human milk include the following: the pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, and tumor-necrosis factor-α (TNF-α); the TH1 cytokines γ-interferon and IL-12; the TH2 cytokines IL-4, IL-5, and IL-13; the regulatory cytokines IL-10 and transforming growth factor-β (TGF-β); and the chemokines IL-8 and CCL5. Granulocyte-macrophage colony stimulating factor (GM-CSF), eryth-

ropoietin (EPO), and cortisol are also detectable in breast milk.

Apart from the components listed above, which have a direct effect on the immune system, breast milk contains a number of other ingredients that indirectly support the infant's immune system, including vitamins, minerals, and nucleotides (Table 1).

Breast milk has been termed an irreplaceable immunological resource because it supports passive and active immunity during the vulnerable first months and years of life.[18] A number of components of breast milk are, indeed, irreplaceable, notably IgA. Other components, however, can be added to infant formulas and are detailed below.

CONTRIBUTION OF INFANT FORMULA TO THE IMMUNE SYSTEM

Because the immune system is immature at birth, malnutrition during childhood might have long-term effects on health. Lack of adequate macronutrients or undernutrition impairs the development and differentiation of a normal immune system. The combination of chronic undernutrition and infection further weakens the immune response. Micronutrient deficiencies will affect the adaptive antibody and cellular immune response, as well as the innate immune response.[19] Concurrent micronutrient deficiency may attenuate or aggravate effects on the different components of the immune system. Therefore, when infants are deficient for one or more micronutrients, it is likely that their immune function is also impaired.

No single test can adequately define overall immune status, but measurements of several parameters of separate components of the immune system can be used in combination to assess functional capacity as follows: 1) measuring specific cell functions ex vivo; 2) measuring in vivo responses to challenge, e.g., change in antibody levels in peripheral blood or response to antigens; and 3) determining the incidence and severity of infection in target populations during naturally occurring episodes or in response to attenuated pathogens.[20,21]

The focus of this review is on the replaceable ingredients of human milk and their (possible) relationships with immune function. Primarily, formula designated for full-term infants between the ages of 0 and 6 months old, is discussed, but toddler formula (for 1–3 year olds) is also addressed where appropriate. Specific formulas designed for pre-term infants have been reviewed elsewhere[22] and are outside the scope of this review. General safety aspects of infant formula other than their negative effects on the immune system are also outside the scope of this review.

For the purpose of this review, we searched PubMed for human trials performed during the last 10 years as well as other relevant literature. Based on the different types of studies performed and their outcomes, we estimated the strength of evidence (in 4 categories) for the relationship between a specific nutritional ingredient and immune function. The highest ranking (category 4, +++) was assigned when the experimental evidence included data from randomized control trials (RCTs) in infants performed by different study groups (see Figure 2).

IMMUNE STIMULATION THROUGH SELECTED INGREDIENTS

Table 1 summarizes data from studies performed on immune stimulation through selected ingredients. It should be noted that RCTs are usually short-term, so not many long-term RCTs are available for evaluation. Also, in the absence of data from RCTs in infants, data from in vitro and animal studies as well as human adult trials have been evaluated, since they provide some information about the activity of different ingredients.

The role of individual nutritional factors on immunocompetence is difficult to quantify for the following reasons: 1) different ingredients may affect different components of the immune system; 2) numerous interactions between separate nutrition factors exist; 3) dose-effect relationships are difficult to establish in young children because of methodological aspects (i.e., significant non-nutritional confounding variables) and because of ethical problems in designing intervention trials in infants; 4) the relevance of a subclinical deficiency of a given nutrient is often difficult to judge (i.e., subclinical inflammation or disease may result in a lower plasma micronutrient concentration that may be misinterpreted as a deficiency). Low concentrations of other nutrients, such as ascorbate and iron, may not necessarily impair immune function.[23] The same holds true for the relevance of the effects of short-term supplementation with supraoptimal doses as compared to the amounts usually contained within infant formulas. For many micronutrients, excessive intake is associated with impaired immune function.

Minerals

A number of minerals, especially zinc, but also selenium, copper, and iron are essential for normal immune function. It should be noted that some minerals can compete for absorption in the body and abundance of a given micronutrient might lead to deficiency of another. Therefore, potential interactions between minerals should be taken into account.[24] Note as well that viruses and infectious microorganisms also require iron and other trace elements for their survival and replication in the host.

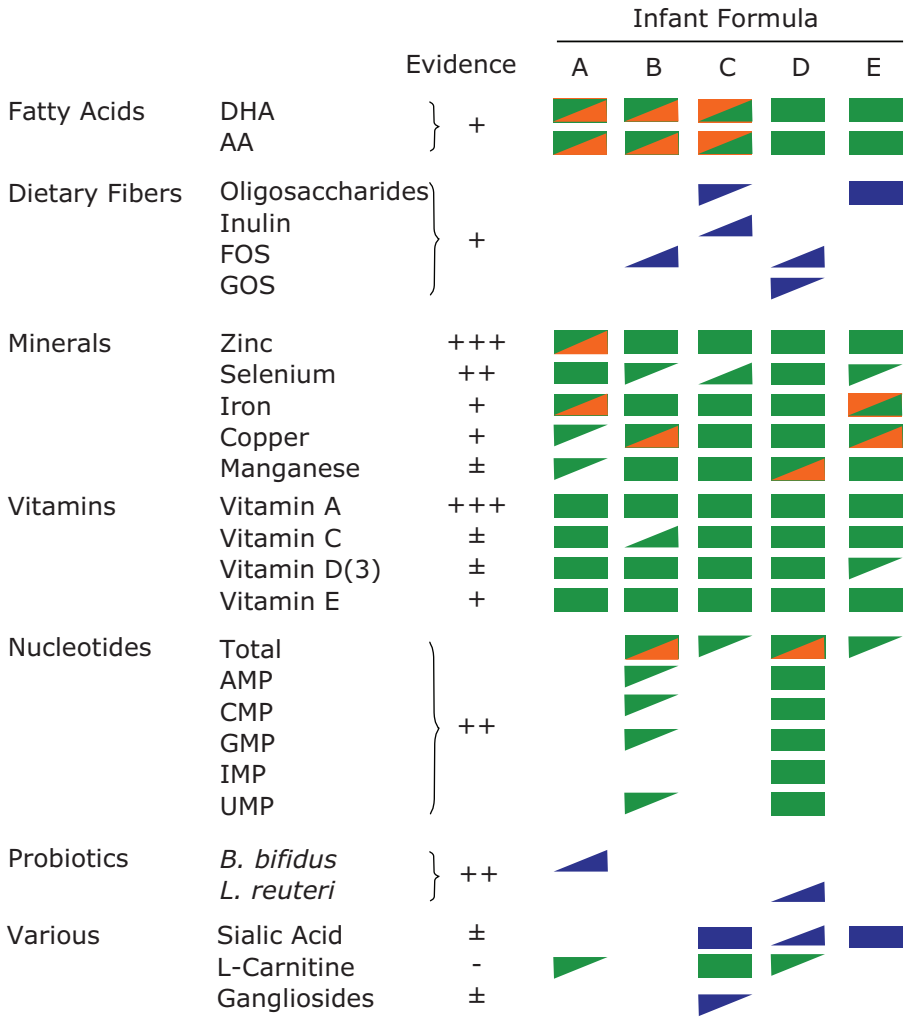

Figure 2. Comparison of immunomodulating ingredients in infant formula. Composition in comparison with requirements, taking into account the strength of evidence for immune-stimulating effects. Green rectangles ( ) indicate the compound is present within the recommended range (see Table 1); Orange ( ) indicates the concentration is above the maximal recommended level or the DHA/AA ratio is outside the range. Blue rectangles ( ): indicate the presence of compounds for which no recommendations exist. Open spaces indicate the absence of the particular compound (i.e. not listed on the label) or a concentration below the minimal recommended concentration. Upper-half rectangles ( ) represent formula for infants aged 0–6 months; lower-half rectangles ( ) represent formula for toddlers aged 1–3 years. Columns A–E represent infant formulas from the Asian market based on cow's milk. The trade names and manufacturers of formulas for 0–6-month-old infants (with the trade names of formulas for 1–3-year-old toddlers in parentheses) are as follows: **A**: Nan 1 (Neslac 1+), Nestlé, Singapore; **B**: Similac (Gain plus advance), Abbott, Singapore; **C**: Mamex Gold (Mamil Gold), Dumex, Malaysia; **D**: Friso 1 (Friso 3) Friesland Foods, Hong Kong; **E**: Enfalac A+ (Enfagrow A+), Mead Johnson, Thailand. Strength of evidence was graded as follows: ±, circumstantial evidence (i.e., data from in vitro/animal studies only or data from a single study); +, some supportive evidence, including data from a small number of human trials; ++, fairly supportive evidence (i.e., data from different type of studies such as adult subjects or preterm infants); +++, largely supportive evidence (i.e., consistent results from different type of studies, including RCTs in [malnourished] infants).

Zinc deficiency, which is frequently a component of protein calorie malnutrition (PCM), is clearly associated with a state of immunodeficiency: lymphopenia, thymic atrophy, and altered T-cell subsets and cytokine response.[19,25,26,27] Clinical signs include increased susceptibility to infections, skin lesions, and diarrhea.[19,24] Zn is an essential cofactor for many enzymes, including thymic hormone, and Zn depletion decreases the functional capacity of a variety of cells in the immune system. Zn supplementation has been found to reverse impaired immune functions, including cytokine production, and to reduce the incidence of diarrhea and pneumonia in both adults and children.[24,25, 26,28] However, care should be taken not to overdose Zn, since this can have negative effects on the immune status.[26]

Se deficiency, which can be associated with PCM, like Zn, and with vitamin E deficiency, impairs antioxidant defense systems and leads to the rapid progression

of viral and other infections as well as to cardiomyopathy.[19,29] Model studies show that Se influences many components of the immune system,[29] because of its critical role for the function of selenoproteins. It has been demonstrated that fortification of infant formulas with Se improves the Se status of infants.[30] Attention must be paid to the risk of subclinical Se deficiency, especially in premature infants, and to the small interval between inadequate and excessive Se intake.[31]

Iron has diverse and partly opposing roles in defending the host against infectious diseases. Within the gut lumen, bacterial growth depends on Fe, and lactoferrin has potent antibacterial effects because of its Fe-sequestering properties. On the other hand, Fe deficiency causes T-lymphocyte dysfunction, which manifests in the form of impaired delayed-type hypersensitivity reactions as well as a number of other immune defects (i.e., decreased IgG levels and phagocytic activity).[19,32,33] Different components of the immune system are affected in different ways depending on the degree of Fe deficiency and concomitant infections. Clinically, Fe-deficiency anemia is frequently associated with infections. Oral Fe supplementation can have different effects in malarious regions than nonmalarious regions; morbidity and mortality may be worsened by Fe supplementation during infection with Fe-dependent organisms. Excess Fe intake should be avoided since it can induce free radical-mediated damage and may have negative effects on the Cu and Zn status.

Cu (and Zn) deficiency are fairly common in children with hypoproteinemia and anemia. Cu deficiency may cause lymphopenia and a decreased IL-2 response. Clinical signs of Cu deficiency are anemia, neutropenia, depressed growth, and abnormal bone development.[19,24] Cu is an essential cofactor of a number of antioxidant enzymes. Cu intake should be carefully controlled because excess Cu can induce free radical-mediated damage. Supraoptimal Zn and Fe intake, however, can cause a reduction in Cu status.[34,35]

Mn is needed for normal immune function because mitochondria and a number of cellular enzymes are Mn dependent.[36] The direct clinical consequence of Mn deficiency, however, is not well known, and no recent RCTs have been published. It should be noted that excessive Mn may induce neurotoxicity in neonates receiving parenteral nutrition.

Ca, while being an obvious essential nutrient, probably does not have a specific role in the function of the immune system. In adults, Ca supplementation has been shown to reduce the severity of enterotoxigenic *Escherichia coli*-induced diarrhea.[37] It has been speculated that low Ca intake may impair host resistance to food-borne intestinal infections. RCTs in infants have not been published.

Vitamins

Largely supportive evidence exists for indicating a number of vitamins play an important role in the development and function of the immune system. Inadequate intake of antioxidant vitamins can lead to clinically significant immune deficiency and infections in children.[19] It has thus been demonstrated that vitamin A deficiency leads to the impaired activity of TH2 cells, phagocytes, and NK cells. Clinical signs of vitamin A deficiency include night blindness, mucosal damage and dry skin (both of which contribute to the loss of barrier function that prevents entry of pathogens), and hyperkeratosis.[19,54] Vitamin A supplementation has been shown to reduce the risk of mortality and morbidity from some forms of diarrhea, measles, malaria, and HIV.[38,39] One RCT conducted in infants aged 5–15 months demonstrated that regulation of the mucosal immune response depends on the type of enteric pathogen. Furthermore, the effect of supplementation in infected children was significantly different from the effect in uninfected children,[40] which may explain the inconsistent effects on the incidence of diarrheal disease. Overdosing vitamin A can lead to loss of appetite, dermal dryness, and loss of hair, among other things.[54]

In animal experiments, vitamin E deficiency has been associated with specific defects in immune function and increased susceptibility to infection. In humans, severe vitamin E deficiency is associated with impaired T lymphocyte function.[41] Correction of the deficient state may reverse these abnormalities.[41] Clinical signs of milder vitamin E deficiency in humans include atopic diseases as well as neurological symptoms.[19] Increasing the intake of vitamin E can have an immune-stimulating effect.[42,43]

Poor vitamin D status has been reported to be associated with chronic mycobacterial disease, and vitamin D may augment the function of regulatory T cells, thereby preventing the development of autoimmunity.[45] Vitamin D appears to be a selective regulator of the immune system and the outcome of vitamin D treatment or of vitamin D deficiency (receptor) depends on the nature of the immune response (e.g., infectious disease, asthma, or autoimmune disease). An additional factor that determines the effect of vitamin D status on immune function is dietary calcium.[44]

Vitamin C is a good example of a nutrient for which animal experiments have shown that deficiencies consistently increase susceptibility to infection; yet, in human studies, evidence of such a disadvantage from deficiency or a benefit from supplementation is contradictory at best.[23]

Overdosing vitamins appears to have no negative effects on the function of the immune system, with the

exception of vitamin E; at high doses, vitamin E may depress phagocytosis and intracellular killing of bacteria.[43] Previous reports on the relationship between excess vitamin D supplementation and increased risk of food allergy and asthma in later life have had methodological flaws, including the presence of confounding factors, the use of relatively high doses, and an extremely long period between supplementation and outcome assessment.[46,47]

Long-Chain Polyunsaturated Fatty Acids

Long-chain polyunsaturated fatty acids (LC-PUFA) are primarily added to infant formulas in order to improve growth, visual acuity, and neurodevelopmental performance. Docosahexanoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are the main LC-PUFA in breast milk. LC-PUFA of the n-3 and n-6 series are metabolic competitors with differential effects on eicosanoid metabolism, membrane physiology, and immune function, to name a few examples. Eicosapentanoic acid (EPA, 20:5n-3) is found in only minor concentrations in breast milk and infant tissues and is a direct metabolic competitor of AA.

It has been demonstrated in in vitro studies and animal experiments that DHA and AA can alter immune function in several ways. Thus, fatty acids can affect T-cell function by increasing or decreasing the production of eicosanoids from AA. Production of these mediators is decreased by n-3 LC-PUFA.[48] Furthermore, EPA is a substrate for cyclooxygenase and lipoxygenase, enzymes that alter the structure of eicosanoids and reduce biological potency. A novel family of EPA-derived eicosanoid-like mediators, termed E- and D-resolvins, have been shown to be anti-inflammatory and inflammation resolving in cell culture and animal models, respectively.[49] Fatty acids can also alter gene expression through modification of transcription factor activity as well as incorporation of n-3LC-PUFA into membrane phospholipids and subsequent modulation of membrane structure and function. n-3 LC-PUFAs are potentially potent anti-inflammatory agents and they may be of therapeutic use in acute and chronic inflammatory diseases.[48] From a different perspective, it has been hypothesized that increased n-6 PUFA and decreased n-3 PUFA dietary intakes have contributed to the recently observed increases in asthma and other allergic diseases.[50] Associations with n-6 and n-3 PUFA appear to be very complex and might be different in asthma compared with atopic dermatitis. In atopic dermatitis a mild enzyme deficiency has been proposed, resulting in an altered PUFA metabolism that compromises epithelial structure and function.[51]

Data on the effects of PUFA supplementation in infants is limited. In one study of pre-term babies, the addition of LC-PUFA to infant formula resulted in patterns of lymphocyte populations, phospholipid composition, and cytokine production more consistent with that observed in infants fed with breast milk.[52]

Although large amounts of LC-PUFA may increase lipid peroxidation and oxidative stress, there is no evidence that concentrations within the range found in human milk are harmful. For instance, one recent RCT involving preterm infants demonstrated that LC-PUFA-supplemented formula can achieve LC-PUFA levels in plasma that are similar to those of breast-fed babies without evidence of adverse effects.[53]

In 1998 the expert committee of the Life Science Research Office (LSRO) did not set minimum and maximum values for the addition of LC-PUFA to infant formulas, but later, expert reports from Europe supported the optional addition of DHA and AA. At this time, there is insufficient documentation of the benefits and safety of adding DHA to infant formula at levels above 0.5% of total fat content, or of adding DHA without the concomitant addition of AA. If LC-PUFA are added, the proper balance between n-6 and n-3 should be obtained. Note that the requirements established by the Scientific Committee on Food are slightly different from those of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[54,55]

Clearly, there is a need for larger, properly designed and controlled studies with longer follow-up periods to investigate functional outcomes in relation to intake levels of LC-PUFA in healthy, full-term infants.[55] Also, more studies on the potential preventive effect of n-3 fatty acid supplementation on the development of asthma are needed.[51,56]

Prebiotics

As indicated above, the intestinal flora plays an important role in the postnatal development of the immune system. The lower incidence of (gastrointestinal) infections found in breast-fed infants may be related, in part, to the early pattern of microbial colonization. The colonizing bifidobacteria and lactobacilli may inhibit the growth of pathogenic microorganisms through the production of lactic, acetic, and other organic acids, with a consequent decrease of intraluminal pH that inhibits the growth of some bacterial pathogens. In contrast, formula feeding tends to favor a flora associated with a near-neutral pH of the feces. Moreover, bifidobacteria and lactobacilli compete with potentially pathogenic bacteria for nutrients and epithelial adhesion sites. Accumulating evidence also indicates that the gut flora modulates mucosal physiology, barrier function, and systemic immunologic and inflammatory responses.[57]

Two different approaches towards modifying the development and balance of intestinal microflora can be taken: one is the addition of live lactic acid bacteria and bifidobacteria (probiotics) and the other is the addition of oligosaccharides that survive passage through the small intestine and are used by colonic bacteria (prebiotics).

Breast milk contains many different oligosaccharides that may have prebiotic activity and thus have an effect on the composition of the intestinal flora. Because of the variety, variability, complexity, and polymorphism of their structure, it is currently not feasible to add a similar oligosaccharide composition, such as that contained in human milk to infant and follow-on formulas. Alternatively, the addition of a more simple mixture of galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) to infant formulas and to follow-on formulas has been proposed.[58,59]

Accumulating evidence indicates that the addition of oligosaccharides to infant formula may induce a composition and metabolic activity of the intestinal flora that closely resembles that of breast-fed infants.[60] Most studies thus far have been performed with a combination of 90% short-chain GOS and 10% long-chain FOS. While the bifidogenic effect of prebiotics has been demonstrated convincingly, limited data exist on the direct effects on the infant immune system. There is evidence that prebiotics may improve the antibody response to vaccination and may reduce the incidence of atopic dermatitis.[61,62] The most recent reports of expert committees conclude that, at present, there is insufficient conclusive evidence to indicate that a flora dominated by bifidobacteria is related to health and well-being and offers protection against enteric infections.[55,57] On the other hand, SCF had no objection to adding a combination of 90% GOS and 10% FOS to infant formulas. An upper level of supplementation has been proposed (Table 1) based on the results of animal studies indicating that above a certain concentration threshold, oligosaccharides may have a negative effect on water balance.[55] Clearly, additional research is needed on the optimal composition, dosage, and combinations of different oligosaccharides.

Probiotics

Probiotics are live, microbial food ingredients that are considered to be beneficial to health. Bacterial strains with these properties most frequently belong to the genera *Bifidobacterium* and *Lactobacillus*. Probiotics can have a health-promoting effect related to their interaction with gut microbiota, i.e., they can help fortify the gut's barrier function and modulate the immune system. The net outcome of these combined activities is an increase in the level of host defense against infection. In this respect, several studies have shown that the addition of probiotics to infant formulas has beneficial effects, especially in reducing the severity of acute diarrhea.[63] Probiotics have also been demonstrated to be effective in preventing antibiotic-associated diarrhea, reducing the incidence and severity of necrotizing enterocolitis, and reducing recurrent *Clostridium difficile* infections.[64,65] The interaction of probiotics with cells of the immune system, particularly dendritic cells, results in improved function of regulatory T cells.[66] Therefore, probiotics may be effective in the prevention of atopic dermatitis, asthma, and other allergic and immune-mediated diseases. Clinical studies suggest select probiotics have a beneficial effect in the primary prevention of atopic diseases.[67,68] So far, no effects have been shown in the treatment or prevention of IgE-mediated allergic disease. If the efficacy of probiotics in the treatment of allergic symptoms can be confirmed in subsequent studies, it is reasonable to expect that this may be more obvious in, or even limited to infancy and early childhood, i.e., before the immune responses to allergens and immune regulatory networks have been fully developed and at a time when the gut microbiota are not yet established.

It should be noted that in most studies different probiotics (or combinations thereof) have been used. Not all probiotics are created equal; therefore, different strains may have different effects and more research into the mechanisms of action of individual probiotic strains is needed. Administration of probiotics to (even preterm) infants appears to be safe, and no serious adverse effects, including no cases of pathogenic infection caused by a probiotic organism, have been reported.[69,70,71] Yet, probiotics are not indicated for immunocompromised (malnourished, ill) neonates, and the long-term effects on the development and function of the immune system have not been studied in detail.[65] SCF has no objection to the addition of probiotics to follow-on formulas, provided that the requirements with respect to safety, viability, and labelling are fulfilled.[55]

Nucleotides

In breast milk, nucleotides (NT) are present in the form of nucleic acids, nucleosides, nucleotides, and related metabolic products. An exogenous supply of NT may be important during infancy, when NT requirements are elevated to facilitate nucleic acid synthesis. This may be especially important for infants born prematurely, since preterm birth is associated with limitations of many metabolic functions and with limited opportunities for breastfeeding. In addition to serving as nucleic-acid precursors, NT act as intercellular and intracellular biological mediators. Infant formulas supplemented with NT are currently being marketed globally. Animals fed on diets with or without NT supplements show enhanced indices of humoral

and cellular immunity, as well as enhanced survival rates following infection with pathogens.[72]

A number of randomised studies of pre-term as well as healthy full-term babies indicate the antibody response is improved after vaccination and lymphocyte maturation is enhanced by the addition of free NT to infant formulas.[73,74,75] However, the results of recently reported large RCTs were not always consistent. For instance, in one large RCT, supplementation of a cow's milk-based formula with NT at a low dosage level resulted in a modest improvement in certain antibody responses in healthy term infants, with no effect on other markers of immune status and growth.[76] The authors speculate that the greatest benefit of NT supplementation may be obtained by high-risk infants, such as those born prematurely and those from socially disadvantaged backgrounds.[76] Some studies of infants indicate that the addition of NT may protect against diarrheal disease.[72,76,77] However, data from well-controlled studies demonstrating that NT may protect against infections is currently limited.

At present, there are no studies that evaluate a dose-response relationship between the concentrations of NT in infant formula and relevant outcomes in infants. In different regions of the world, the recommended maximum doses of NT vary.[55,56,78] One recent RCT suggested NT supplementation had a negative effect on the incidence of upper respiratory tract infections throughout the first year of life.[77]

Whether the observed immunomodulating effects may be translated into clinical benefits in well-nourished infants requires further study. Also, more research into the relative contribution of individual nucleotides and into optimum dosage levels and working mechanisms is needed.

Miscellaneous Ingredients

Apart from the nutrition factors discussed above, there is circumstantial evidence to indicate that several other ingredients, such as vitamin B12, have immune-stimulating effects.[43] Furthermore, breast milk contains a number of proteins with antibacterial activity that could potentially be added to infant formula. It is technically feasible to add bovine lactoferrin or transgenic human transferrin, but bovine lactoferrin does not bind consistently to human lactoferrin receptors and does not increase Fe absorption. The efficacy and safety of human lactoferrin has not been adequately evaluated.[55,79]

Gangliosides and Sialic Acid

Gangliosides are glycosphingolipids that contain sialic acid (N-acetylneuraminic acid) as part of their carbohydrate moiety. GM, a ganglioside present in human milk, binds to *E. coli* and *Vibrio cholerae* toxins and may thus help protect the infant against infection by those enteropathogens.[80]

Breast milk is a rich source of sialic acid-containing oligosaccharides. Animal studies have documented that supplemental sialic acid has favorable effects on learning, even in well-nourished animals. In infants who died of sudden infant death syndrome, higher concentrations of ganglioside and glycoprotein sialic acid were found in the brain in infants who were fed breast milk; this suggests increased synaptogenesis and differences in neurodevelopment.[81] The same research group reported that the saliva of preterm breastfed infants contained twice the level of sialic acid as that of formula-fed infants. The higher sialic acid level may suggest greater viscosity and enhanced protection of the mucosal surfaces in breast-fed infants.[81,82] In the absence of sufficient data, no recommendations on the addition of sialic acid can be made, which, unless supplemented, is lower in infant formula than in human milk.[55]

L-carnitine is considered an indispensable nutrient for newborn infants because of their temporarily compromised synthesizing capacity. Its function is to transport across membranes carboxylic acids that have been activated to the co-enzyme A level, thereby delivering substrates for oxidation and removing toxic compounds. Infants receiving non-supplemented soy showed lower levels of carnitine in serum, higher levels of free fatty acids and increased excretion of medium-chain dicarboxylic acids. The minimal dietary carnitine requirement of a newborn infant has been estimated to be 1.7 mg/kg/day due to the almost absent endogenous synthesis. Because cow's milk is rich in carnitine compared with human milk, it is not necessary to add carnitine to infant formula based on cow's milk. Supply from appropriate complementary food and from endogenous synthesis should be sufficient in older infants.[55]

ESPGHAN experts set a minimum L-carnitine content of 1.2 mg/100 kcal. In the absence of indications of any untoward effects of higher L-carnitine intakes in infants, no maximum level needs to be set.[54]

CONTRIBUTION OF INFANT FORMULA TO DEVELOPMENT AND FUNCTION OF THE IMMUNE SYSTEM

Compositional Requirements for Infant Formulas

Different scientific and regulatory bodies have established compositional requirements for infant formulas.[54,55,78] The first guiding principle is that human breast milk is the gold standard. However, the levels of the various (immunomodulatory) components in human milk are not easily translated into composition guidelines

for infant formula, because of possible differences in bioavailability and the fact that substances other than components found in human milk may need to be used to achieve the desired effects in infants. The second principle is that all infant formulas must be safe and nutritionally adequate, meeting the normal nutritional requirements for babies. The establishment of minimum and maximum values must also take into account differences in bioavailability and losses incurred during processing and shelf-life. Third, the maximum nutrient values are based on the available scientific data on infants' requirements and the absence of adverse effects. The immature organ system should not be charged with ingredients without reasonable evidence of their efficacy. Therefore, infant formulas should contain components only in such amounts that serve a nutritional purpose, provide another benefit, or are necessary for technological reasons.

As indicated above, the comparison of compositional data with requirements is hampered by differences in the recommendations of different expert committees. Furthermore, differences in national legislation may cause differences in the composition of products in many cases.

Assessment of the Composition of Five Asian Infant Formulas

In order to evaluate how the accumulating knowledge on immunomodulation by nutrition ingredients has been translated into the incorporation of these ingredients into currently available infant formula, we assessed the composition of five infant-formula products marketed in Asia. This specific geographic area and commercial market was chosen because children born in this region are at increased risk of infection due to climatic and socioeconomic circumstances. In Asia there is a high level of awareness among parents regarding the need to protect their children against infectious diseases. Because of this awareness and the concern of parents for their infants' health, a broad spectrum of infant formulas supplemented with ingredients to support an infant's immune system is commercially available. For each of the five brands we evaluated, the composition of both infant and toddler formulas, as listed on the product labels, was reviewed and the respective manufacturers were asked to confirm the nutrient content of each product.

The compositional data were evaluated, taking into consideration the estimated strength of evidence for the immunostimulating effects of individual nutrition factors and in comparison with the requirements for infant formulas (Figure 2). It should be noted that ingredients which may be important for growth and development from a nutritional perspective but have no impact on the immune system are not listed in Figure 2.

We used the nutrient requirements established by the most recent expert consultation by ESPGHAN,[54] except for specific values for individual nucleotides and oligosaccharides, which were taken from the earlier report of the SCF[55] (see Figure 2). Specific nutritional requirements for infant formulas designed for 1–3-year-old toddlers have not yet been established. Consequently, we extrapolated figures for these products from the SCF data on follow-on formulas, which are actually designed for infants aged 6–12 months.[55] It was taken into account that the daily doses of formula products consumed by this age group, in addition to the increasing amounts of complementary feeding, are highly variable. This approach allowed us to obtain a gross indication (at least) of the contribution made by these formula products to the immune system in toddlers.

The assessment revealed that all the leading brands investigated have added fatty acids, vitamins, and minerals, and four of five also added nucleotides (Figure 2). Prebiotics and probiotics are not yet widely used and, in most cases, these ingredients are not added to formula products for 0–6 month olds.

During the review we discovered that the clinical and experimental data available for a number of potentially important ingredients are limited. In such cases, we made educated guesses as to the importance of an individual nutritional factor for the infant's immune system. Still, this makes it difficult to make an overall judgment or to compare different formula products. Nevertheless, in view of the immature status of the immune system of neonates and the (long term) effects on health, it is extremely important to optimize the composition of infant formulas, taking into account the available evidence for immunostimulation.

FINAL CONSIDERATIONS

During the first 6 months of life, the infant's immune system develops gradually. Every primary infection the infant experiences induces a response that leads to elimination of the invading pathogen and to the generation of specific T and B memory lymphocytes, which protect against recurring infection with the same pathogen. As a general rule, the number of infectious episodes decreases with age. Especially during the first 6 months, but also thereafter, it is important to sustain the infant's immune system in order to afford protection against infection. In order to grow up and develop in a world teeming with microorganisms, the infant depends on an adequately functioning the immune system. Breastfeeding and infant formula supplemented with specific ingredients, as indicated, support the infant's immune system.

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