Role of Dietary Immunomodulatory Factors in the Development of Immune Tolerance

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Abstract

The development of oral tolerance occurs during critical early stages of immune development. Rising rates of food allergy and other immune-mediated food reactions are an indication that oral tolerance is highly susceptible to environmental change. There is growing evidence that this may not be due to food allergens per se, but rather to changing exposure to other key immunomodulatory exposures in this critical period. Successful tolerance appears to depend on many concurrent environmental influences during the period of first allergen encounter, including favorable gut colonization, and the presence of key immunomodulatory factors in breast milk and the infant diet. This review explores the potential effects of early dietary and nutritional factors in tolerogenic immune processes that are normally initiated during initial food allergen encounter.

Introduction

The gastrointestinal tract arguably provides the most critical interface between the infant and its new environment. It is the site of highest exposure to microbial products, potential allergens and a broad range of dietary nutrients with immunomodulatory properties. These complex enteric exposures play a major role in the maturation of the mucosal immune system and have major implications for the success or failure of subsequent tolerance to foods. There is intense interest in how exposure to these oral exposures can be optimized to promote tolerance and prevent allergic disease.
With rising rates of allergic disease there was initial focus on the role of allergen exposure, with attempts to prevent allergy by delaying exposure to allergenic foods. This has not been successful. It is now increasingly evident that allergen exposure is not the primary cause of the allergy epidemic, and that allergen avoidance may even be detrimental in preventing allergy. Indeed, rising rates of immune disease are likely to reflect a combination of many environmental changes which compromise tolerance. This early period coincides with the establishment of healthy gut colonization, which has been shown to be essential in promoting tolerance to both allergens and self antigens [1–3]. Thus, delays in either colonization [1] or antigen/allergen exposure [4, 5] can lead to failure of oral tolerance. Other conducive dietary exposures during this period, such as breast milk and/or other immunomodulatory factors, are also likely to play a role in promoting successful oral tolerance.

While this review focuses on the role of specific factors, this ‘single component’ approach does not fully address the ‘composite effects’ of diets and dietary changes (or interaction with other environmental factors). It remains essential to recognize the overall complexity of dietary changes in the wider context. Ingested dietary material represents a highly complex array of predominantly organic materials with broad ranging properties that provide essential nutrition and promote colonization, immunity and gut integrity. Just as it appears that no single gene is responsible for allergy or asthma, it appears equally unlikely that a single environmental factor is responsible for the recent allergy crisis.

**The Immunomodulatory Role of Food Allergens**

There is growing evidence that tolerance depends on regular exposure to foods and other antigens during critical early stages of development. The timing of a possible ‘critical window’ for oral allergen exposure is not clear in humans, but preliminary evidence suggests that this may be between 4 and 6 months of life. A number of recent studies suggest that exposure to specific foods in the 4- to 6-month age range may reduce the risk of food allergies [4] and autoimmunity [5, 6] compared with children whose first exposed is either before or after this ‘window’. This remains to be confirmed.

Delays in complementary feeding can lead to failure of oral tolerance, with increases in allergic disease (food allergy, eczema, asthma) [4, 7, 8] and celiac disease [7] with avoidance until after 6 months of age. This has raised concerns over recommendations, by the WHO [9] and other bodies, for delaying introductions of complementary foods until after 6 months of age, especially in industrialized countries where the incidence of allergy is high [10–12]. Conversely, allergen exposure too early (before 3–4 months), when gut colonization and local immune networks are less established, may increase the risk of allergic or autoimmune disease (possibly through increased gut perme-
ability) [13]. As a result, many opinion leaders now recommending that guidelines for complementary feeding are revised to around 4–6 months [10–12].

The timing, dosage and pattern of allergen exposure are likely to play some role in the success of oral tolerance, however these factors are unlikely to have changed as much as other environmental factors. Thus, while the role of changing allergen exposure in the allergy epidemic remains unclear, it is increasingly likely that other environmental changes are playing a major role. Many of these changes could mediate immunological effects through the diet, either indirectly by altering colonization or more directly as immunomodulatory nutrients. This highlights the complex inter-relationships between exposure to allergens, colonizing flora and other immunomodulatory factors during this early period.

**Immunomodulatory Effects of Breast Milk**

Breast milk is the most important dietary exposure in infancy. In addition to the provision of essential nutrients, breastfeeding provides a wide range of other well-recognized benefits for both mother and infant. At this stage, the effects of the complex array of immunologically active compounds in breast milk are not fully understood. These include immunoglobulins, lactoferrin, lysozymes, oligosaccharides, long-change fatty acids, cytokines, nucleotides, hormones, antioxidants and maternal immune cells. In addition to protection from infection these factors are also likely to have other effects on immune development including oral tolerance. Animal studies demonstrate that maternal milk is more tolerogenic than formulae (promoting regulatory T cells in the developing gut). The addition of tolerogenic cytokines such as TGF-β to formula increases tolerance [14]. In a landmark study, Verhasselt et al. [15] recently demonstrated that tolerance to allergens present in breast milk is mediated by CD4+ regulatory T cells, and that this process is dependent on TGF-β present in the maternal milk.

Human milk is important for promoting intestinal integrity and immune function in infancy (via nucleotides, polyamines, immunoglobulins and soluble factors such as sCD14). These factors are also likely to play an important role in the development of oral tolerance. Although several studies have shown associations between the levels of these factors in breast milk and subsequent allergic disease [16, 17], the significance is not clear. Other factors may have immune effects through altered cellular composition and function, such as antioxidants and fatty acids (discussed below). Finally, some breast milk factors (such as oligosaccharides) have indirect immune effects by promoting favorable colonization (also discussed below).

Breastfeeding is strongly encouraged for many reasons although the specific role in allergy prevention is not clear. A systematic review of 12 prospective studies (8,183 infants) [18] found that exclusive breastfeeding in the first months of life was also associated with reduced rates of subsequent asthma
(OR 0.70, 95% CI 0.60–0.81), however a number of subsequent studies have failed to support this. There are inherent limitations in studies addressing the effects of breastfeeding on allergy, including recruitment and reporting biases, perceptions modifying feeding practices, confounding factors, and the inability to randomize and blind. Many of these studies do not examine the relationship between breastfeeding and the introduction of other foods. There is some evidence that continued breastfeeding during introduction of complementary foods is important for promoting tolerance [19]. Although more studies are needed, it seems logical to encourage continued breastfeeding as 'solid' foods are introduced.

**Dietary Factors that May Promote Tolerance through Optimal Colonization**

A wide range of factors in the early environment influence the microbial ingestion during feeding and subsequent patterns of intestinal colonization. These include maternal flora, delivery method, infant diet including whether or not the child is breastfed as well as the relative consumption of nondigestible, fermentable oligosaccharides in the subsequent diet. These oligosaccharides (now commonly referred to as ‘prebiotics’) are also nutrient substrates for bacteria and appear to favor so-called ‘beneficial’ or health-promoting bacterial species (including Bifidobacteria and Lactobacilli species) which are arguably the most abundant source of early immune stimulation and ‘microbial burden’ in early life.

There is good evidence from germ-free animal models that bacterial gut colonization is essential for maturation of immune function and induction of oral tolerance [1]. In humans, variations in early colonization in the first weeks of life have also been associated with the risk of subsequent allergic disease [for review see 20], suggesting that this may influence subsequent patterns of immune development. This has logically lead to environmental strategies that promote optimal colonization including supplementation with probiotic bacteria. Although there has been some early promise using probiotics in the prevention of allergic dermatitis [21], the evidence is not consistent enough for specific recommendations at this stage. More recently there has been growing interest in the role of prebiotics which could potentially have a more global effect on colonization than the addition of individual probiotic strains. The first trials for allergy prevention are only just emerging and also show some promising results with reduced atopic dermatitis in formula-fed infants at high risk of atopy who were supplemented with a mixture of galacto- and long-chain fructo-oligosaccharides for the first 6 months of life [22, 23]. Further studies are underway to determine the role of these and other dietary factors that may have immunomodulatory effects by promoting ‘optimal’ colonization.
The Role of Dietary Fatty Acids in Early Immunomodulation

The anti-inflammatory effects of n-3 polyunsaturated fatty acids (PUFAs) have lead to interest in the role of these essential nutrients in the prevention of allergy and other inflammatory disease states. This notion has also been supported by protective associations between dietary n-3 PUFA (fish intake) during both pregnancy [24–26] and early childhood [27–29] and subsequent allergic outcomes. This suggests immunological effects during early immune development, which could be mediated through a number of pathways. Dietary changes in n-3/n-6 PUFA alter cell membrane fatty acid composition which in turn influence a number of aspects of cellular function. Effects in antigen-presenting cell (APC) and T-cell function are well described, together with effects on the production of inflammatory products (prostaglandins and leukotrienes) by many cells. Diets rich in n-6 PUFA provide increased substrate for the production of more inflammatory eicosanoids (prostaglandin-E2 and leukotriene B4). These products also synergize with IL-4 and promote allergic differentiation and IgE production. Conversely, the effects of n-3 PUFA-derived eicosanoids are significantly less inflammatory. Higher levels of n-3 PUFA also dampen T-cell responses, including lymphoproliferation and cytokine production. Although the mechanisms are not clear, proposed pathways include effects on membrane fluidity, intracellular signaling and gene transcription. Effects of n-3 PUFA on APC function include reduced capacity to present antigen to T cells by inhibiting the upregulation of MHC class II receptors, cytokine production and expression of co-stimulatory molecules. All of these effects collectively reduce the inflammatory response, providing a basis for the therapeutic properties of the n-3 fatty acid in a range of inflammatory conditions.

The decline in n-3 PUFA in modern diets (with parallel changes in breast milk levels over the last 20 years) has highlighted the potential role of fish oil supplementation during pregnancy and/or early childhood for allergy prevention. The first intervention study using fish oil for allergy prevention commenced the fish oil intervention (500 mg tuna fish oil/day or a placebo) around 6 months of age [30]. Although there was a reduction in respiratory symptoms (wheeze, coughing) at 18 months and 3 years, there was no reduction in the development of atopy, asthma or other allergic disease by 5 years of age [30]. As many infants show evidence of pre-symptomatic commitment to allergic ‘Th2 dominant’ immune responses by 6 months of age, it has been argued that interventions aimed at this age may be too late and that immunomodulatory effects are likely to be greater earlier in development. This notion has been supported by effects of maternal fish oil supplementation (3.7 g n-3 PUFA/day) on neonatal immune function [31] including effects on T-cell signaling [32]. In addition to the immunomodulatory effects noted in this population, there was also a 3-fold reduction in the clinical detection of egg sensitization (OR = 0.34, p = 0.055) by 1 year of age [31]. However, this study was too small
to be conclusive. Currently there are several larger pregnancy intervention studies in progress (in Europe and Australia) that are specifically designed to assess the clinical effects of fish oil on allergy prevention, and the results are awaited with great interest. Until this has been addressed more definitively, fish oil supplementation cannot be recommended specifically for allergy prevention.

**Potential Immunomodulatory Role of Antioxidants**

Antioxidants also have immunomodulatory effects by favorably altering cellular ‘redox’ status. Antioxidants can influence APC cytokine production which can in turn favor the development of type 1 T-helper (Th1) cells [33] and regulatory T cells [34] (with inhibition of allergic Th2 responses). Thus, with declining intakes of antioxidants (such as vitamin C, vitamin E, β-carotene, zinc and selenium) in Western diets these dietary factors have also been logical candidates for the rising rates of asthma and allergic disease [35, 36]. Epidemiological studies suggest a protective association between antioxidant-rich foods (such as fresh fruits and vegetables) and pulmonary function [37], with reduced risk of wheeze in both adults [38] and children [39, 40]. As with other environmental factors, any potential effects of these factors are likely to be greatest during early development. So far there are only observational (rather than interventional) studies to examine the relationship between maternal antioxidant status in pregnancy and immune function and/or allergy outcomes in the infants. One of the first groups to examine this noted that maternal intake of vitamin E intake during pregnancy was associated with lower cord blood mononuclear cell responses [41] and a reduced risk of developing childhood wheeze, asthma, and eczema by 2 and 5 years of age [35, 42]. More recently, they have also shown that maternal vitamin D intake during pregnancy may have the potential to reduce early childhood wheezing [43]. A Boston group also reported that maternal intakes of vitamin E and zinc were negatively associated with wheezing at 2 years of age, although they did not observe any correlations between antioxidant intake and the risks of eczema in the same children [44]. Relationships between allergic disease and other antioxidant levels, such as vitamin C, have been inconsistent. Some studies have suggested negative associations (increased risk of wheezing) with increased vitamin C intake [42] while others have demonstrated beneficial decreases in the risk of wheeze [39, 45]. One group has raised theoretical concern that antioxidant supplementation could increase the probability of Th2 differentiation (by inhibiting oxidative stress) and favor the development of asthma and allergic disease [46]. Until these concepts are better understood, there is currently no place for specific antioxidant vitamin supplementation specifically for allergy prevention. Even if conclusive benefits are ultimately demonstrated, these effects may not be strong and
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it is likely that any dietary recommendations would only form one part of multifaceted prevention approaches. It is also likely that fresh foods (such as fruits and vegetables) may have additional ‘protective’ properties that cannot be replicated by specific vitamin supplements, many of which may be synthetic. Thus, while the potential role of antioxidants in immune regulation is unclear, it is best to advocate a healthy balanced diet rather than specific vitamin supplementation.

**Dietary Contaminants with Immunomodulatory Effects**

Increasing levels of previously nonexistent industrial and agricultural byproducts have been among the most dramatic environmental changes with progressive industrialization. The effects of these dietary contaminants on the developing fetus are poorly understood. Many classes of persistent organic compounds, such as the polychlorinated biphenyl compounds, dioxins, furans, and organochlorines (OCs), contaminate food and water sources and are detectable in breast milk and cord blood. Many also have immunological effects. Although high doses are immunosuppressive, lower levels of contamination have been shown to more selectively inhibit type 1 immune responses [47] leading to speculation that this could possibly favor allergic (type 2) immune responses. Although no causal pathways have been identified, these more subtle pro-allergic effects could be mediated by the ‘estrogenic’ hormonal activity of many of these compounds. In our recent studies we noted that although levels of these pollutants had dropped significantly in the last 20 years, OCs were still detectable in 94% of maternal adipose tissue (collected at cesarean section) and 62% of breast milk samples [48]. Moreover, OC contamination was also associated with lower maternal (but not fetal) Th1 IFN-γ responses [48]. At this stage there are no data to directly link these and other dietary contaminants to the epidemic rise in allergy and many other immune diseases over the last half century. As research in humans is limited to observational studies, this will remain difficult. However, it remains important not to overlook the potential role of these factors, which have well-documented adverse effects in animals.

**Conclusion – Current Dietary Approaches to Promote Tolerance**

Diet remains a logical and highly feasible approach to promote favorable conditions for normal tolerance during immune development. As indicated above, successful tolerance is likely to depend on many conducive exposures (such as favorable gut colonization [1], breast milk [19] and/or other nutritional immunomodulatory factors [31]). Thus, general approaches can be considered broadly in 4 areas: (1) using diet to promote optimal colonization
and gut maturation; (2) promoting optimal allergen exposure (timing, dose, interval and route); (3) exploring the role of immunomodulatory factors such as n-3 fatty acids and breast milk that may promote tolerogenic conditions during allergen encounter and processing, and (4) understanding the role of modern dietary contaminants that could be altering normal immune development.

Currently, there are very few formal recommendations on these points as good evidence is still not available. Challenging many long-held concepts, there are now studies that will examine the hypothesis that earlier introduction and regular exposure to ‘allergenic’ foods (rather than avoidance) may reduce the risk of specific allergies to these foods. Although there is little doubt that optimal colonization plays a major role in promoting tolerance, the best way to achieve this is not clear and more studies on prebiotics and probiotics are needed before recommendations can be made. Similarly, while exposure to fish oil in early life may have some beneficial effects, the role in allergy prevention is still unclear and large-scale trials are still in progress. The specific role of breastfeeding in allergy prevention is unclear, but it is still recommended for other reasons and there is some evidence that continued breastfeeding during introduction of complementary foods promotes tolerance [19].

While the rise in allergic disease is multifactorial, dietary changes remain likely contributing factors. The immunomodulatory properties of many dietary nutrients also make them potential adjunctive strategies in managing this health crisis through preventing the development of allergic immune responses. Again, it must be emphasized that isolated supplementation does not reproduce the complexity of a healthy diet, which is likely to have ‘protective’ properties that cannot be replicated by specific supplementation. Given the multifactorial nature of allergic disease, it is likely that any benefits of dietary intervention will not be strong enough to overcome the allergy epidemic, but may serve as safe noninvasive adjunct strategies to other more definitive measures.

References

Prescott


Discussion

Dr. Isolauri: I didn't quite understand the dietary intake of your birth cohort. You mentioned that they were advised not to consume cow's milk before 12 months but cow's milk was introduced to the diet at the age of 10 months. So what kind of formula were they taking?

Dr. Prescott: There wasn’t a lot of time to talk about it. These were children who had been given no specific feeding advice, and were involved in other studies. We
really wanted to see if they asked about feeding. I referred to the national guidelines and then it was up to them to decide what to do. So this was an analysis of a prospective cohort that was recruited for other reasons and we allowed for reversed causation, maternal allergy, early appearance of symptoms, and all the other confounding factors. We wanted to know when they feed, and was there a relationship between how they were fed and their subsequent allergic outcomes. To reiterate we saw a protective effect of the duration of breastfeeding but we didn't see any clear relationship between when solids, including cow's milk formula, were first introduced and subsequent allergy either to the specific solid or in general.

**Dr. Isolauri:** If cow's milk was introduced at 10 months, do you mean unadapted cow's milk?

**Dr. Prescott:** Yes. At 4.1 months they received the cow's milk in cow's milk formula. About 96% were breastfed, but about 76–79% also used formula at some stage. The average age at starting normal cow's milk formula was from about 4 months. Interestingly enough the rate of cow's milk protein sensitization was only about 3 or 4% in this population whereas egg was 20%. Obviously egg was introduced later than cow's milk. That's just circumstantial of course but it was a quite interesting observation that cow's milk protein allergy was unusually low in a high-risk population.

**Dr. Salminen:** It was very interesting indeed and I was delighted to see that you also brought up two factors that are often forgotten, the contaminants and stress. We know that stress influences intestinal microbes and even that is a pathway to influence the infant. The other thing is contaminants. In today's society one should really consider the constant long-term exposure to contaminants, even though low but potentially having an immune effect, and ways to counteract that. You mentioned some components, aflatoxins in breast milk in Australia and PCBs in breast milk in Finland. So we all have our own local contaminants and it's certainly something we should pay more attention to; perhaps use bacteria to decontaminate them?

**Dr. Prescott:** It's very difficult indeed trying to study pesticide levels, it's not a popular thing to do, and when you detect it, what can you do about it, these do accumulate. It was good to see that the levels have fallen substantially since the 1970s but you are quite right, they are still there and we really don't understand what effect they are having. To further emphasize your comment about stress, we certainly also found that the mood in pregnancy, again not profoundly depressed mood but subtly reduced mood scores in pregnancy, was associated with increased inflammatory cytokine production in cord blood.

**Dr. Tobin:** Could you give us a little bit more detail about the association of DDT and dieldrins with lower Th1 cytokines? What cytokine profile did you actually look at?

**Dr. Prescott:** We looked at IL-10, IL-13 and IFN-\(\gamma\) production in response to, in that instance, mitogens and PHA, and we didn't see any relationship with Th2 or IL-10, it was really IFN-\(\gamma\). Of course these are correlational studies. You can't do anything else unless you get into an animal model and then it is hard to extrapolate that to humans. I think we need to acknowledge the limitations. Of course the more you look at things, the more you are going to find. I think a cautionary note but interesting observations.

**Dr. Hernell:** I was just thinking when we discussed this window that no one really knows exactly where the window is. Do you think it might actually be dynamic rather than static depending on what we actually feed the babies? During weaning the microbiota changes to become much more diversified than during breastfeeding. But it also seems as if weaning turns on the exocrine pancreas from being relatively downregulated as long as the child is breastfed. In other words, when formula or other foods are introduced, secretion of enzymes from the pancreas is induced, which might of course also affect the development of tolerance or intolerance.
**Dr. Prescott:** I probably didn’t make the point I intended and I want to reemphasize that that window is probably quite plastic, and it may vary between individuals depending on the environment and genetics. There are probably some individuals who need a stronger allergen hit to tolerize them, but that’s going to depend on a number of environmental factors. I also want to stress that the guidelines, or the advice as we call them, that we are endorsing at the moment are really just going back to basics. We are actually trying to remove the restrictive guidelines. We are not saying prescriptively, 4–6 months; we are trying to say when the child is developmentally ready, some time between 4 and 6 months, feed the child a new food every 2–3 days and take all the usual precautions, etc. This is really just going back to the way grandma used to do it. We are actually not trying to enforce this by saying that there is evidence to support this; we are just saying there is no evidence to support what we used to do, if that makes sense.

**Dr. Wiedermann:** You said that oral tolerance induction is probably most effective. Do you think this is also true for the prevention of respiratory allergy? In particular if we think of prevention with probiotics, effects on dermatitis are most often seen, but less on bronchitis. There are new studies showing that there can be an increase in wheezing bronchitis. I am wondering what it is, and are there perhaps different windows of opportunity for respiratory tolerance and oral tolerance induction?

**Dr. Prescott:** I think so, and cutaneous exposure as well. We know that animal models can be sensitized to inhalant allergens through the skin, so I am sure the route is very important; we probably ate a lot of dust mite as well. I think this is a very important question.

**Dr. Björkstén:** I have a slightly philosophical question regarding how we use the word stress. We tend to talk about stress, but one would perhaps think that famine or war in traditional societies would be more stressful, and I don’t know precisely what model stress means. One stress factor of course is all the advice resulting in guilt feelings put on mothers. Would you like to expand a little bit on what stress is in this context?

**Dr. Prescott:** You are quite right, modern stress is different from the overwhelming situation of war or famine; you deal with it, you get on. We are under different kind of stress in our society, time pressure and constant anxiety pressure. Again it is a very different kind of stress, and it is also quite difficult to study effects, relationships between the HPA axis, because of the difficulties in measuring cortisol levels reliably. In pregnancy it is quite difficult, we have to get things at exactly the right time of day. There is certainly good evidence in animal models and human studies looking at depression and stress. There are clear relationships with immune function, and it is very important to highlight this as a potential factor contributing to the general immune decay of regulatory function in modern society. There are so many different associations. I can’t comment further than to say that war and famine are very different to the kind of chronic time pressure stress that we are under today.

**Dr. von Berg:** What is your definition of stress? We looked at the impact of early stressful life events on allergic diseases in our study on the influence of lifestyle on the development of the immune system and allergy (LISA) [1]. We found that divorce/separation of the parents in the first 2 years was associated with a significantly increased and, interestingly, severe disease of a first-degree family member with a significantly decreased incidence of atopic eczema in the subsequent 2 years of life.

**Dr. Prescott:** We looked at both perceived stress on questionnaires and also the more objective measures of stress such as income, educational levels, etc., and we tried to look at the effects of those factors. We wanted to look at salivary levels of cortisol and various other things, and found it incredibly difficult to get pregnant women to come in at the right time of day, in a fasting state, and do all the things needed to
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Dr. von Berg: The difference is that you looked at stress in the mothers, while we looked at stress in the offspring.

Dr. Prescott: That’s fascinating; of course the work of Wright et al. [2] in Boston showed that chronic caregiver stress in the immediate postnatal period is associated with differences in infant immune function, and particularly IFN-γ. So again there is growing circumstantial evidence if you like, but it is an area that we need to investigate further.

Dr. Heine: I would like to come back to the model of a ‘tolerogenic window of opportunity’. We have already heard about the protective effects of breast milk on celiac disease where the introduction of a weaning food under breast milk cover proved to be long-term protective. Could you speculate on whether this effect is preserved in formula-fed babies who start formula feeding early and who don’t get the full benefit of breastfeeding. Is it mainly the timing or is it something specific in breast milk? Which do you think is the predominant factor?

Dr. Prescott: We need proper randomized control trials to answer that question; we also need good animal studies of which there are some. We know that breast milk is tolerogenic in animal models and there is some evidence that if it’s co-administered with allergen it may be more tolerogenic than just giving the milk alone. In other words you need to see them together. But we don’t have proper human studies that look specifically at these. Most of the breastfeeding studies were either retrospective or just weren’t looking at the timing of both of those things together.

Dr. Heine: Do the recommendations on complementary feeding include formula-fed babies?

Dr. Prescott: The current recommendation is obviously breastfeed if you can. I should emphasize our feeding advice for Australian is for all Australians, it is not targeting high risks specifically. It says that you should breastfeed if you can, if you can’t breastfeed, and this is the only caveat, and you are high risk then feed the partially hydrolyzed formula. Then from 4 to 6 months, when you child is developmentally ready regardless of atopic risk, consider feeding and no specific avoidance of highly so-called allergenic feeds, regardless again of perceived allergy risk or perceived allergenicity of the food. So that is really where it stands at the moment. Because this is a general population statement, if you need to formula feed then a normal cow’s milk formula is recommended unless you are considered to be at high risk as in a first-degree family relative with allergic disease. Then beyond 4–6 months as you start to introduce solids, breast milk or formula should remain the main drinking milk, but according to our guidelines, we are very happy for them to have milk, cow’s milk in cooked foods. They are not avoiding it anymore, but it’s not their main drink.

Dr. Robledo: Is there a difference between foods prepared at home by mothers and processed foods that can be bought commercially?

Dr. Prescott: Certainly yes, because if you are preparing food yourself you know exactly what is in it. In terms of other contaminants it is most likely that there are more preservatives and other things in prepared foods. But our recommendations do not include this issue at all; we have not addressed that. So obviously that is more relevant when there is a known established food allergy to a certain food and you are trying to avoid it and you need to know what is in the food you are giving. We are talking about prevention and we are talking about feeding what the family is eating, and that will vary with what is culturally appropriate.

Dr. Venter: Do you think we can extrapolate the data that breastfeeding whilst introducing gluten could protect against celiac disease to other antigens? Then if so, do we tell mothers to wean earlier and introduce foods every 1–2 days whilst actually measure this. In our current studies we mainly rely on questionnaires which, I acknowledge, have their limitations.
breastfeeding, rather than weaning later and introducing foods more slowly by which time they may not be breastfeeding?

*Dr. Prescott:* The idea is to take away the anxiety about feeding, so I think it has to be less prescriptive, less obsessive than what we are doing. Starting new foods, they don’t have to be in massive amounts but just regular tastes; once something is introduced to the diet, give it regularly. I take all the points that you made, but we have fed children for centuries and somehow managed, I think we have just become over focused on the details. I think the celiac data certainly look that way but we need more studies; specifically we need studies in allergy. Certainly in our observational study we tried to look at that again, and in our cohort we didn’t see that it really mattered how long the overlap between the foods and breast milk was. I also emphasize that the protective effect of breastfeeding, while it’s there in many studies, is not huge, and some women can’t breastfeed for all sorts of reasons, and we need to take some of the stress out of that as well. So I haven’t answered your question because it’s going to depend on the individual and how they do it, but the idea again is to try not to be so focused on the whole thing.

*Dr. Brandtzaeg:* I would like to challenge you on something you said, and perhaps you could elaborate on this. Talking about the window of opportunity you said that optimal microbial colonization in the gut is needed before food antigens are introduced. But going back to Dr. Renz’s talk yesterday, we heard that there may also be some opportunity in utero with antigen transfer from the mother through the placenta and also the swallowing of antigens present in amniotic fluid. So there is a paradox in tolerance induction, as it can be induced in several ways.

*Dr. Prescott:* There are several windows. The in utero window is obviously where there are many factors that need to be assessed, and maternal colonization and microbial exposure in utero are probably very important as well. The window that we are talking about for feeding is just one factor. But it’s very clear from the animal study [3] that everybody quotes that if colonization is not optimal, then you are more likely to have failed tolerance. If the immune system is immature and, as you said yourself, if the mucosal barrier is leaky or integrity has in someway not been established, then there is reasonable evidence that problems with oral tolerance are more likely to develop.

*Dr. Brandtzaeg:* So we probably have to distinguish clearly between mucosally induced or so-called oral tolerance and other tolerance mechanisms.

*Dr. Prescott:* Just emphasizing again, I think that this is an ongoing evolving process. It may start before birth, and there may be different factors involved at different stages. The fetus is not exposed to large amounts of bacteria but potentially the mother is during pregnancy. Then you have postnatal colonization as another step, if you like, in the maturation process, and obviously that’s going to vary on many other environmental factors.

*Dr. Belli:* As a pediatric gastroenterologist I agree that allergy is important, but the whole development of the child is especially important, for example the main role of vitamin D to influence bone development and not oral tolerance. So my question is how do you deal with these two different interests in your program, especially for the window of opportunity and your advice?

*Dr. Prescott:* Vitamin D is a very interesting issue, and it has already been discussed here in Australia where we have a lot of sunlight exposure, but we are actually becoming vitamin D-deficient because we don’t go outside and we cover up. So this is an issue that I think needs to be addressed. I am not a specialist on vitamin D metabolism and there are other people here who are and who may wish to comment on this, but the whole feeding story, as you pointed out, is highly complex.

*Dr. Tobin:* I can just say a little bit about the autoimmune story, and this is mostly from work looking specifically at that issue and the idea that our previously accepted
normal range for vitamin D may be appropriate for bone development but not for immune development. Looking at levels of around 75 mg to actually get good regulatory T-cell function is where the endocrinologists are now looking, particularly in relation to vitamin D levels. There was one study from Queensland recently [4] where we didn’t expect anybody would be vitamin D-deficient. They looked at vitamin D in relation to type 1 diabetes, and most of those children were vitamin D-deficient at diagnosis in sunny Queensland. It’s something to think about, and the story in the first year of life is still very unclear with vitamin D, but some have suggested work looking at vitamin D supplementation in pregnancy on potentially lowering type 1 diabetes and the allergic story as well.

Dr. Vaidya: Your data show that a lot of mothers are introducing cow’s milk at 10 months of age in Australia. In India it is even earlier because fresh cow’s milk is used in various kinds of porridge. Now considering the idea that the breast milk actually promotes tolerance, perhaps it would be a good to introduce small amounts of cow’s milk under the cover of breast milk thereby decreasing allergy. Probably this is the reason why in India cow’s milk allergy is not as common.

Dr. Prescott: That is essentially what we are saying. I just showed you what people did under the old guidelines, but in the new guidelines we are saying that from 4 to 6 months they can have cow’s milk in cooked foods, cereals, etc., but still definitely the main drinking milk should be formula or breast milk, which ever they happen to be on. I also want to just clarify one thing. Our guidelines are aimed at Australia and highly industrialized countries. In India and places where there may be increased gastrointestinal morbidity and other problems, what we are saying is a little in conflict with the WHO recommendations for exclusive breastfeeding for 6 months. We know that that recommendation was based on the Belarusian study, and it was made primarily because of the increased risk of gastrointestinal morbidity and mortality. I think the recommendations need to be appropriate for the setting, and what we are advising in Australia is really most appropriate for a country where we are seeing an epidemic of allergic disease and food allergy, particularly at the moment.

Dr. Du Toit: Do you believe that there is a window of immunological protection after birth against IgE allergy? Many children receive milk formula as a top up at birth while breastfeeding is being established and a small percentage will go on to breast milk formula, infant formula, cow’s milk formula feeding, but IgE allergic reactions 1 or 2 days or even in the first 2–3 weeks are not well described and anecdotally not often seen. Non-IgE reactions are common, colitis perhaps, colics, refluxes, that is a huge group, but these children are certainly primed. In cord blood we have seen evidence from Dr. Renz and you have shown us that these children are immune mature at birth; they are exposed to these allergens in utero, and there is some evidence of IgE in cord blood to egg and milk, but the reactions are not common place very early on in life.

Dr. Prescott: I must say that there is still debate about what perinatal IgE levels actually mean. They correlate more with maternal levels (including allergen-specific IgE) rather than subsequent infant levels and may not be of fetal origin. We also don’t know the meaning of the specific T-cell responses in cord blood, and there is evidence that these don’t affect exposure or true immune memory. At this stage we can’t answer that specifically.

Dr. Cerf-Bensussan: You speak about stress but sometimes I feel that the ability to cope with stress is perhaps more important than the nature of the stress. So when you do your studies, is there any scale to measure the coping more than the stress? You suggest that it is more depression which is associated with what you see than the nature of the stress of a given person.

Dr. Prescott: Absolutely, that is why perceived stress is important because someone may perceive the same stressors quite differently which is why we asked the
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persons to give an objective rating of their conception as well as collecting information about the so-called stressors.

Dr. Cerf-Bensussan: So when you do that do you have more correlation with the perceived stress?

Dr. Prescott: We are doing this in one of our big cohort studies but we haven’t yet got the results. The results I am referring to are in a smaller study of 80 women, and we are now looking at this in about 400 women, so I can’t answer that yet.

References