

Development of oral tolerance to allergens via breastmilk

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Immune responses induced in early life to environmental and dietary antigens will be decisive for children and their adult response to these antigens, and they will condition development of immune-mediated diseases such as allergies and autoimmunity. Maternal influence on neonatal tolerance induction through breast-feeding is probably of great importance because of dietary and environmental antigen transfer through breast milk and the pleiotropic effects of breast-feeding on gut and immune system maturation. In addition, maternal history and maternal sensitization to common environmental and food antigens will probably affect antigen transfer to the breastfed child along with tolerance induction. (Verhasselt, 2010, p. 331)

What is oral tolerance?

Oral tolerance is a state of immune unresponsiveness induced by the oral administration of an innocuous antigen, or potential allergen, such as food proteins (Pabst & Mowat, 2012). Oral tolerance is the physiological induction of tolerance, it occurs in the gut-associated lymphoid tissue (GALT) and at other mucosal services including the respiratory tract (nasal tolerance). It is important to remember that 'tolerance induction is the default immune pathway in the gut'. (Weiner, da Cunha, Quintana & Wu, 2011, p. 241). The oral administration of potential allergens may be medical or non-physiological and form part of a desensitisation program to a food allergen or it may be functional or physiological, that is, it occurs as a result of ingesting breastmilk and/or food.

The mucosal immune system forms part of the adaptive immune system and is located near the surfaces where most pathogens invade the body. It is made up of the gastrointestinal tract, upper and lower respiratory tracts, the urogenital tracts and, in the case of lactating women, the mammary gland. The role of the GALT in the development of oral tolerance has been thoroughly reviewed by Weiner et al. (2011):

The physiological role of the GALT is the ingestion of dietary antigens in a manner that does not result in untoward immune reactions and protection of the organism from pathogens. As such, the GALT is primarily a tolerogenic environment and a complex interplay of factors creates the environment. (Weiner et al., 2011, p. 241-242)

Induction of oral tolerance through exposure to allergens: the research/medical model

Desensitisation to peanut allergens

Peanut allergy is a common disease that causes severe and fatal food allergic reactions. Currently, the best treatment is avoidance as repeated reactions can occur. However, quality of life is reduced by fear of severe reactions and social limitations. Oral immunotherapy to desensitise children to peanut allergens is a novel treatment, currently being investigated experimentally, that may be an effective treatment for peanut allergy.

Two recent studies have used slightly different methods of oral immunotherapy to desensitise children, who are allergic to peanuts, to peanut allergens. The first study was undertaken in the UK with children aged 7–16 years being exposed to increasing amounts of peanut flour over 6 months (Anagnostou et al., 2014). The second study was undertaken in Australia with children aged 1–10 years being exposed to increasing amounts of peanut flour and a probiotic over a period of 18 months (Tang et al., 2015).

In the UK study (Anagnostou et al., 2014) children were randomly assigned to one of two groups: the active group or the control group. In the active group, daily immunotherapy (2 mg, 5 mg, 12.5 mg, 25 mg, 50 mg, 100 mg, 200 mg, 400 mg and 800 mg of peanut protein) was administered as peanut flour. Doses were increased at 2-weekly intervals to a maintenance dose of 800 mg of protein. The control group underwent peanut avoidance for 6 months during phase 1. A double-

blind, placebo-controlled food challenge to peanut (up to 1400 mg of peanut protein) was performed in both groups at 6 months. Desensitisation was observed in 62% (24/39) of the active group and none (0/46) of the control group [95% confidence interval (CI) 45% to 78% vs. 0% to 9%; $p < 0.001$]. At the end of 6 months, 84% (95% CI 70% to 93%) of the active group tolerated daily ingestion of 800 mg of protein ($\approx$ five peanuts). In children with peanut allergy of any severity, oral immunotherapy successfully induced desensitisation in the majority, with a clinically meaningful increase in peanut threshold. Quality of life measures also improved after intervention.

In the Australian study (Tang et al., 2015) children were randomly assigned to one of two groups: treatment or placebo. In the treatment group, children received increasing amounts of peanut flour and a probiotic (*Lactobacilli rhamnosus* CGMCC 1.3724) over a period of 18 months and the placebo group received ingredients that imitated the peanut flour and the probiotic. The researchers found that 89.7% of the children in the treatment group and 7.1% of children in the placebo group were desensitised to peanuts when challenged 2 to 5 weeks after the end of the trial.

The success of both of these experimental trials in substantially reducing the response of allergic children to peanuts is a promising result and was found to improve the quality of life for the children and their families.

Allergens in breastmilk

Various food allergens have been detected in breastmilk. A study of 20 Danish mothers found β -lactoglobulin in the breastmilk of 95% (19/20) of study participants after they drank 500 mL of milk (Høst, Husby, Hansen & Østerballe, 1990). In a South Australian study, ovalbumin was detected in the breastmilk of 68% (28/41) of study participants who ate a cooked egg (Palmer, Gold & Makrides, 2005). Peanut protein (including both major peanut allergens *Ara h 1* and *Ara h 2*) has also been detected in the breastmilk of 43% (11 of 23) of study participants in a Canadian study (Vadas et al., 2001).

Exposure to gluten in the diet is the trigger for coeliac disease and gliadin is a component of gluten. Gliadin has also been detected in 67.5% of breastmilk samples after women consumed 20 g of gluten. Importantly, in the same group of women, gliadin could not be detected in serum. The researchers speculated that 'the transfer of gliadin from mother to infant might be critical for the development of an appropriate specific immune response to gliadin later in life'. (Troncone et al., 1987, p. 453).

Only a few potential food allergens had been detected in breastmilk until recently. It was thought that rather than food allergens not being in breastmilk, the techniques

used to detect them were not sensitive enough. So, Spanish researchers (Pastor-Varga et al., 2015) created a homemade microarray and used antibodies produced against major food allergens, in order to increase the sensitivity to detect the presence of the food allergens in breastmilk. Women's breastmilk samples varied in the range and quantity of allergens found, but across the participant women each of the following food allergens were found: fish, egg, wheat, gliadin, mustard, peach and watermelon.

Allergens in breastmilk: possible impact on immune system education

In addition to its impact on child growth and prevention of infectious disease, breastmilk may help to educate the immune system towards acceptance of non-dangerous antigens, including possible allergens. That the transfer of allergens is 'intentional' or 'active' and not just a passive process has led to the development of a working hypothesis on the role of breastmilk in presenting allergens to the infant in a 'supportive' environment, that is, in an immune-modulating environment that develops oral tolerance. Verhasselt (2010) describes how the induction of oral tolerance by breastmilk-mediated transfer of allergens may depend on the presence of breastmilk immunomodulatory factors including tolerogenic factors (TGF- β and other anti-inflammatory cytokines) and probiotics, including *Lactobacilli*.

Importantly, the medical model to induce oral tolerance to peanut allergens used by Tang et al. (2015) can be considered to have mimicked the way in which infants receive small amounts of allergens through breastmilk, which naturally contains probiotic bacteria, including *Lactobacilli*.

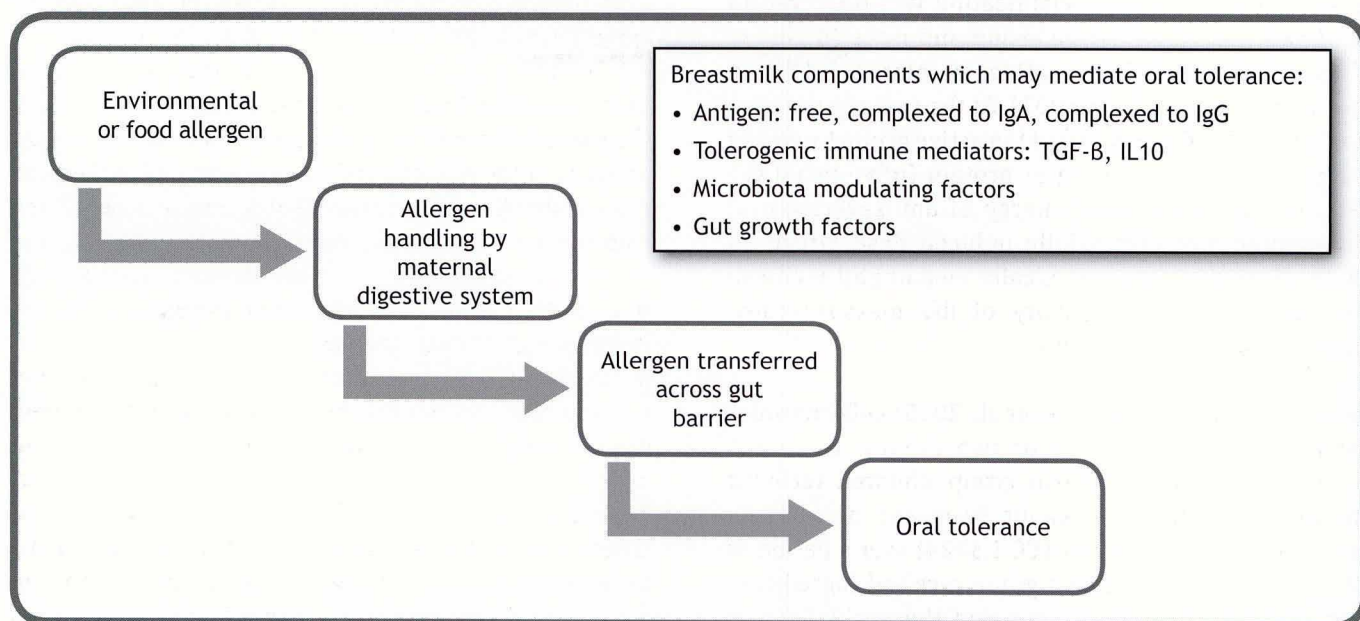
Induction of oral tolerance through exposure to allergens: the breastmilk model

A role for breastmilk in the induction of oral tolerance (the tolerogenic potential of breastmilk) depends on maternal exposure to common environmental and potential dietary allergens and the efficiency of allergen transfer across mammary epithelium. Induction of tolerance upon breastmilk-mediated allergen transfer will also depend on the presence of immunomodulatory factors in breastmilk and its impact on neonatal gut and immune system maturation (Figure 1).

Induction of oral tolerance through breastmilk exposure to allergens: the evidence

The induction of tolerance through the mucosal immune system, the gut and the respiratory tracts, has been described in young animals and there have been some promising results in young children in research trials (Anagnostou et al., 2014; Tang et al., 2015). However, documented development of oral tolerance through the ingestion of milk by neonates is mostly confined to

Figure 1. Simplified model of maternal role in the development of oral tolerance in infants through breastfeeding.
Source: Verhasselt (2010)



animal models (mice) and rare, case-study observations in humans which may form part of normal allergy prevention. Although mice are clearly different from people, and mouse models do not necessarily translate to the human situation, a recent review entitled *Food allergy: insights into etiology, prevention and treatment provided by murine models* concluded that ‘allergy is one field where the transcriptional analysis approach has shown a remarkable consistency between murine samples and human samples’. (Oyoshi, Oettgen, Chatila, Geha, & Bryce, 2014, p. 2).

Food allergens

The intestine has an unenviable task: to identify and respond to a constant barrage of environmental stimuli that can be both dangerous and beneficial. The proper execution of this task is central to the homeostasis of the host, and as a result the gastrointestinal tract contains more lymphocytes than any other tissue compartment in the body, as well as unique antigen presenting cells with specialized functions. When antigen is initially encountered through the gut, this system generates a robust T-cell mediated hyporesponsiveness called oral tolerance. (Vickery, Scurlock, Jones, & Burks, 2011, p. 576).

Oral tolerance to hen egg ovalbumin (ovalbumin), a recognised allergen, has been induced in mice pups from day 7 when administered via maternal milk. Suckling mothers were fed for 3 days with 200 mg of ovalbumin/animal/day at several time points between giving birth and 18 days of age, that is, before weaning. Oral tolerance to ovalbumin could be induced by direct administration (gavage) of mice pups but, importantly, the amount of

ovalbumin required to induce oral tolerance was 1000 times lower when administered via maternal milk (Strobel, 1996). In 2012, a group of Japanese researchers demonstrated that they could also induce oral tolerance to ovalbumin and suppress food allergy symptoms, in mouse pups, through the ingestion of maternal milk containing ovalbumin. Importantly, they also demonstrated that the effect was more marked in mice mothers allergic to ovalbumin (Yamamoto, Tsubota, Kodama, Kageyama-Yahara, & Kadowaki, 2012). Similarly, an induction of oral tolerance to peanut allergen has been reported in mice pups whose mothers were fed low doses of peanuts during pregnancy and lactation (López-Expósito, Song, Järvinen, Srivastava & Li, 2009).

The way in which the transfer of food allergens to mice pups is inducing oral tolerance has just begun to be investigated. Very recently, (Verhasselt, 2015) reported that the transfer of ovalbumin through maternal milk induced long-term oral tolerance, and prevention of allergy in mice, by the induction of T helper type 1 (Th1) cells. Also, Kizu, Matsunaga, Hirose, Kimura and Narita (2015) demonstrated that the induction of oral tolerance in mice pups occurred by the transfer of food allergens as IgA-immune complexes in maternal milk.

Airborne allergens

A group of French researchers have hypothesised that, in a similar manner to the induction of oral tolerance to food allergens, the induction of tolerance to airborne allergens can be induced orally through exposure via maternal milk (Verhasselt et al., 2008; Mosconi et al., 2010).

In a 2008 study published in *Nature Medicine*, Verhasselt revealed that airborne allergens were

efficiently transferred from mouse mothers to their pups through their milk. After aerosol exposure, 2–4% of the allergens (ovalbumin and bovine serum albumin) were detected in the lungs and 65–80% were detected in the gut. It appeared that these airborne allergens reached maternal milk via the gut, despite being presented as an aerosol and initially breathed in by the mouse mothers. The researchers assessed the effect of airborne allergen (ovalbumin) exposure of lactating mice on the development of allergic asthma in their offspring. Compared with mice that had not been exposed to ovalbumin via maternal milk, mice that had been exposed to ovalbumin via maternal milk had:

- decreased airway hyper-reactivity
- reduced number of eosinophils in bronchioalveolar lavage
- milder peribronchial and perivascular cellular infiltration
- decreased mucus deposition in the airways.

In this model, maternal milk-induced tolerance required the presence of TGF- β , was mediated by regulatory CD4⁺ T lymphocytes and depended on TGF- β signalling in T cells (Verhasselt et al., 2008). It is important to note that the tolerance to the airborne allergen, which reduced the asthma symptomology, was induced orally.

Further to the 2008 study, in 2010 the French researchers investigated whether mice pups fed maternal milk by allergic (sensitised) mice mothers could induce a more significant level of protection from allergic airway inflammation in mice pups. They found that the protection from asthma acquired from the milk from allergen-exposed (ovalbumin aerosol), allergic mouse mothers was more profound and persistent than that from allergen-exposed, non-allergic mouse mothers. Maternal milk from allergen-exposed, allergic mouse mothers contained IgG-immune complexes that were transferred to the neonatal mice and induced allergen-specific regulatory T cells.

The latest research on breastfeeding and the development of asthma in humans shows a strong protective association at 0 to 2 years of age, which may diminish over time (Silvers et al., 2012; Dogaru, Nyffenegger, Pescatore, Spycher, & Kuehni, 2014). Even if there is no long-term effect of breastfeeding on asthma past early childhood, babies and infants are protected when asthma medications are ineffective: ‘Therapeutic benefit from asthma medications is poor for those 1–2 years of age and usually absent in the first year’. (Oo & Le Souef, 2015, p. 362).

Insect allergens

A case was reported where a 12-day-old breastfed infant developed urticaria after her mother was stung by a bee. The infant was breastfed one hour after the mother was stung. No other foods or medications were taken by either

the mother or the infant. This incident suggests that an allergen passed from the mother through the breastmilk to the infant, inducing a response which sensitised the infant (Kaya & Okur, 2012).

Gut microbiome and allergies

Evidence is accumulating that optimal gut development, function and microbiome colonisation is crucial to reduce allergies in infants. A 2015 review outlined the relationship between the roles of respiratory, gut, and environmental microbiomes in asthma and allergic disease development, manifestation, and attenuation and recognised that diet ‘represents a strong selective pressure on the gut microbiome, a factor that likely explains the increased risk of allergy associated with formula feeding’ (Fujimura & Lynch, 2015, p. 598).

Breastfeeding, in particular exclusive breastfeeding, is crucial for optimal gut development and function and for the establishment of the microbiome.

Initial colonization of the infant gut by microbes sets the stage for the lifelong, relatively stable adult microbiome. Infants rely on colonization to complete development of the immune system and gastrointestinal tract. The first days and weeks of life represent a crucial window of opportunity for shaping the development of the gastrointestinal tract and immune system, as well as the adult microbiome. The nature of infant environmental exposures, acting through the microbiome, affects the likelihood of developing childhood and adult diseases such as obesity, food allergy, and inflammatory bowel disease (IBD). (Houghteling & Walker, 2015, p. 294).

So, has a breastfeeding, gut microbiome, allergy connection been established? The evidence is emerging. A US study (WHEALS) has shown that 1-month-old infants who were being breastfed had a decreased risk of developing allergic-like response to pets. Both breastfeeding and allergic-like response to pets were significantly related to the composition of the gut microbiome (Sitarik, Havstad, Levin, Fujimura, Wegienka, Zoratti et al., 2015).

SUMMARY

- Oral tolerance is a state of immune unresponsiveness induced by the oral administration of an innocuous antigen, or potential allergen, such as food proteins.
- Tolerance induction is the default immune pathway in the gut.
- Oral administration of potential allergens may be medical or non-physiological and form part of a desensitisation program to a food allergen or it may be functional or physiological, that is, it occurs as a result of ingesting breastmilk and/or food.
- Food allergens have been detected in breastmilk: fish, egg, wheat, gliadin, mustard, peach and watermelon.

- A role for breastmilk in the induction of oral tolerance (the tolerogenic potential of breastmilk) has been hypothesised and depends on maternal exposure to common environmental and potential dietary allergens.
- The induction of oral tolerance via maternal milk, to both food and airborne allergens, has been reported in mice.
- Evidence is accumulating that optimal gut development, function and microbiome colonisation is crucial to reduce allergies in infants.

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