Early nutrition and future atopic diseases in children

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ABBREVIATIONS

AD - Atopic Dermatitis

CPGs - CpG islands

ESPGHAN - European Society for Pediatric Gastroenterology, Hepatology, and Nutrition

FA - Food Allergy

FLG - filaggrin

GALT - gut-associated lymphoid tissue

HMOs - human milk oligosaccharides

IFNG - interferon gamma

IgE - immunoglobulin E

LEAP - Learning about peanut allergy

LPS - lipopolysaccharides

OOM - Old Order Mennonites

RSV - respiratory syncytial virus

sIgA - secretory immunoglobulin A

TCR - T cell receptor

TGF- ß - Transforming growth factor beta

TLR4 - toll-like receptor 4

CONTENT

SUMMARY

Title: Early Nutrition and Future Atopic Diseases in Children

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The increase in the prevalence of atopic diseases in the developed world is becoming an increasing problem. Exclusive breastfeeding in early life has been associated with a reduction of incidence of atopic disorders, largely because breastmilk has the ability to shape the composition of the infant gut microbiome and the development of the immune system. The mother's breastmilk transmits elements of her own microbiome and immune responses, and also provides specific prebiotics to support growth of beneficial bacteria. It is important to evaluate the role of genetic, environmental and lifestyle factors that contribute to disease development. Exposure to allergens through a disrupted skin barrier in combination with nutritional factors in individuals with a genetic predisposition may lead to the development of allergic diseases.

Early environmental, microbial and dietary exposures may influence the development of the immune system by epigenetic mechanisms. The ability of the microbiome to act as an epigenetic modulator has been recently demonstrated by the observation of epigenetic DNA modifications after exposure to microbial antigens in immature human cells from intestinal epithelia. The interactions between early nutrition, microbiome and the epigenome during the early phases of human development are very complex and incompletely understood. Better elucidation of the protective bioactive components of breastmilk and the impact of intestinal microbial composition on the epigenome could lead to new targets for the treatment and prevention of allergic disease.

SAŽETAK

Naslov: Povezanost prehrane u ranoj dobi s kasnim razvojem atopijskih bolesti u djece

Povećavanje prevalencije atopijskih bolesti u razvijenom svijetu postaje sve veći problem. Isključivo dojenje u ranom životu, povezano je sa smanjenjem učestalosti atopijskih poremećaja, uglavnom zato što majčino mlijeko ima sposobnost oblikovanja sastava mikrobioma crijeva novorođenčadi i razvoja imunološkog sustava. Majčino mlijeko prenosi elemente vlastitog mikrobioma i imunoloških odgovora, a također pruža posebne prebiotike kao potporu rastu korisnih bakterija. Važno je procijeniti ulogu načina života te genetskih i okolišnih čimbenika koji doprinose razvoju bolesti. Izloženost alergenima kroz poremećenu kožnu barijeru u kombinaciji s prehrambenim čimbenicima kod osoba s genetskom predispozicijom može dovesti do razvoja alergijskih bolesti.

Rana izloženost okolišu, mikrobima i prehrani može utjecati na razvoj imunološkog sustava epigenetskim mehanizmima. Sposobnost mikrobioma da djeluje kao epigenetski modulator nedavno je prikazana promatranjem epigenetskih modifikacija DNA nakon izlaganja mikrobnim antigenima u nezrelim ljudskim stanicama iz crijevnog epitela. Interakcije između rane prehrane, mikrobioma i epigenoma tijekom ranih faza ljudskog razvoja vrlo su složene i ne u potpunosti razumljive. Bolje objašnjenje zaštitnih bioaktivnih sastojaka majčinog mlijeka i utjecaj crijevnog mikrobiološkog sastava na epigenome, moglo bi dovesti do novih ciljeva u liječenju i sprječavanju alergijskih bolesti.

1. INTRODUCTION

1.1 DEFINITIONS

Allergic diseases have a wide variety of clinical manifestations and conditions. Allergic diseases are characterized by immunoglobulin E (IgE) production in response to environmental triggers and are commonly referred to as *atopic disorders* (29). These include allergic bronchial asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis (AD), and food allergies (FA) (29). Individuals display a unique spectrum of these conditions during their life span, although certain patterns are well recognized (9). The concept of atopic diseases includes the *atopic (allergic) march*, which describes the prototypical progression throughout life, starting with atopic eczema or food allergies, shifting then later to allergic rhinitis and asthma, during which compromised barrier function at one surface will lead to other dysfunctional epithelial barriers (29, 37). Furthermore, several of the conditions may present simultaneously such as eczema together with food allergies or eczema together with asthma (37).

The development of atopic diseases is complex and characterized by chronic inflammation at body-environment interfaces such as the skin and reflects abnormal pro-inflammatory responses to otherwise harmless environmental antigens (29). Atopy is a critical component linking AD, asthma, and FA (37). The risk of developing atopic diseases is based on environmental, immunological, and genetic factors (18) While atopic diseases share risk factors, the features of disease can vary among individuals. There are a variety of genes that encode for molecules involved in the immune responses underlying atopic diseases. However, genes alone cannot explain the dramatic increase in incidence of atopic diseases throughout the past 70 years. It is now accepted that interactions between the environmental, the immune

system and intestinal microbiota play an important role in initiating and maintaining abnormal responses in predisposed individuals (4).

1.2 ATOPIC DERMATITIS

AD is a chronic pruritic inflammatory skin disease that occurs most frequently in children and affects characteristic sites (12). AD presents with dry skin, severe pruritus, and eczematous lesions which lead to itchiness, difficultly sleeping and low self-esteem (21). Almost half of cases begin in the first 6 months of life with 95% occurring before the age of 5 (12). The incidence is on the rise in industrialized countries and overall affects 20% of children (21). As stated previously, AD is the first step of the atopic march and is a major risk factor for the development of asthma (12). Studies have shown that children with eczema have 3-fold increased odds of developing asthma compared to children without eczema (37). The etiology is unknown but is thought to be related to an unbalanced immune response and skin barrier dysfunction (37). Recent studies have linked an abnormal epidermal barrier to mutations in the filaggrin (FLG) gene on chromosome 1 (37). Abnormal epidermal barrier function allows easier entrance of allergens and bacteria, stimulating an immune reaction (37). Further studies have identified more than 30 genetic loci that are linked to AD across different populations (21). This includes genes encoding IL4, the IL4 receptor, and IL13 which all lie on the T_H2 cytokine cluster (21). Despite these developments, the majority of the genetic risk underlying AD is unknown.

1.3 FOOD ALLERGY

FA is a hypersensitivity reaction caused by certain foods (10). Sensitized individuals have pathological immune reactions after ingestion of food protein allergens that lead to a range of clinical symptoms ranging from mild itching to severe anaphylaxis (24). The prevalence of IgE mediated food allergy has increased

over the past few decades and is now considered a major public health concern, affecting 2-10% of infants (10, 23). The most common FA are cow's milk, egg, peanut, and wheat (35). FA develops from a failure in the immunologic development of tolerance to foods (10). There are many factors that may contribute to the development of FA including atopic family history, ethnicity, atopic dermatitis, environmental and epigenetic factors (23).

1.4 ASTHMA

Asthma is defined as a chronic, reversible inflammatory airway disease characterized by recurrent attacks of breathlessness and wheezing (5). Asthma is one of the most common chronic diseases of childhood and affects an estimated 300 million people globally (27). The prevalence of asthma is increasing from 8.7% in 2001 to 9.3% of children in 2010 (30). The median age of onset is 4 years, and it affects boys more than girls in childhood. Over half of children who wheeze in early childhood stop wheezing by the age of 6.

Airway inflammation in asthma begins with mast cell activation. An immediate immunoglobulin E (IgE) response to environmental triggers occurs within 15 to 30 minutes which leads to vasodilation, increased vascular permeability, smooth-muscle construction and mucus secretion. These changes lead to symptoms of wheezing, cough, difficulty breathing, or chest tightness. Risk factors which favor the development of these symptoms include dust mites, animal and airborne allergens, viral infections or exercise. A delayed response occurs 2-4 hours later and is characterized by infiltration of inflammatory cells into the airway parenchyma, mucus hypersecretion, smooth muscle hypertrophy, angiogenesis that leads to intermittent airflow obstruction, and bronchial hyperresponsiveness.

2. EARLY NUTRITIONAL INTERVENTIONS

2.1 MATERNAL EXLCUSION DIET

Maternal diet allergen exclusion during pregnancy was thought to have an influence on atopic disease (18). However, available studies have not supported a protective effect of altering the prenatal diet (exclusion of cow's milk, eggs, and peanuts) (8). The lack of evidence to support maternal dietary restrictions during pregnancy and lactation is affirmed in 2 new systematic reviews (18). Furthermore, exclusion diets pose the additional risk of inadequate supply of certain nutrients (17). Due to the lack of evidence, maternal exclusion diets during pregnancy and lactation for the prevention of atopic disease are not recommended (8). However, a systematic review showed associations between maternal diets rich in fruits and vegetables, fish, and food containing vitamin D and a lower risk for allergic disease in their children (8). This review also found maternal diets with higher levels of vegetable oils and margarine, nuts, and fast food and a higher risk for allergic disease (8).

2.2 TIMING OF INTRODUCTION OF SOLID FOODS

Numerous studies have explored the role of infant feeding and the timing of introduction of food allergens and the development of FA (6). It is now thought that controlled exposure to allergenic foods throughout infancy is needed to prevent FA development (35). A cross-sectional study comparing children from the UK and Israel found a 10-fold higher incidence of FA in the UK than in Israel (6). This study showed that Israeli children had earlier and more frequent peanut consumption than in the UK (6). Israeli infants from 8-14 months of age had an average of 7.1g of peanut consumption compared to 0g in the UK (6). Furthermore, the Learning Early About Peanut Allergy (LEAP) study confirmed that early and sustained peanut

consumption in the first 11 months of life resulted in a significant reduction in the incidence of FA at 60 months of age when compared to children who avoided peanut (6). In a follow-up study to examine whether children achieved transient desensitization or long-term tolerance, researchers found that the lower levels of FA in the group with early peanut introduction persisted at the age of 72 months, even after 12 months of peanut avoidance (6). These studies shed light on the dual-allergen exposure hypothesis, which suggests that oral exposure to food allergen induces tolerance, whereas exposure to allergens through a disrupted skin barrier leads to allergic skin sensitization (6, 35). Therefore, infant exposure to allergens in the environment can increase the risk of developing FA if there is avoidance of specific food (such as egg or peanut) consumption (35).

While the optimal timing of introduction of solid foods is unknown, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) feeding guidelines now recommend the introduction of complementary foods beginning at 4 months of age and no later than 6 months of age (7). There is no current evidence that delaying the introduction of allergenic foods reduces the risk of allergy development in both low-risk and high-risk infants (7).

2.3 BREASTFEEDING

Multiple studies have attempted to evaluate the impact of breastfeeding on the development of atopy (31). While many experts agree that breastfeeding is the optimal form of feeding for children in the first months of life, there is still no clear consensus on its effects on allergic disease (19). The American Academy of Pediatrics stated that there was "evidence that exclusive breastfeeding for 3 to 4 months decreases the risk of eczema in the first 2 years of life" (8). This consensus statement is supported by various other studies, including a recent metanalysis

examining the development of atopic dermatitis in childhood in children who were exclusively breast-fed for at least 3 months (14). This study revealed that breast-feeding was associated with a decreased risk of atopic dermatitis (14). Interestingly, children who were exposed to mixed modes of feeding (formula or solid food feeding) had a 57% increased risk for food allergy symptoms at the age of 6 when compared to infants who were exclusively breastfed for 3 months (24).

Breastmilk is the most effective and appropriate solution for infants due to its nutritional and immunological benefits (17). Breast milk is a complex solution, containing multiple compounds that support infant growth and facilitate development of host defense mechanisms (20). However, the immunomodulatory composition of human milk has not been fully elucidated and varies between mothers (30). Infant exposure to breastmilk in this critical time during immune development has an impact on oral tolerance induction and development of allergy (30).

Neonates undergo extensive development of the immune system at birth. Exposure to bacterial antigens after birth play a very significant role in the development and maturation of gut-associated lymphoid tissue (GALT) (13). Antigen exposure primes and establishes a functional immune response in a healthy neonate. Germ-free animals that are exposed to dietary antigens, but not bacterial antigens have an underdeveloped immune system (16). Microbial colonization after birth influences the phenotype and function of lymphoid cells associated with GALT by largely unknown mechanisms (16). Different microbes can also directly influence intestinal cytokine profiles that are important variables in the immune responses involved in atopic disease.

The immune system functions to protect against infectious agents and to minimize the damage they cause (16). However, the immune system also has to

process dietary antigens and provide a pathway to discriminate between dangerous bacterial antigens and harmless ones. The inability of the immune system to develop proper immune responses to these diverse stimuli can lead to disease such as food-related allergy, autoimmunity, and inflammatory bowel disease (16). The innate immune system is an important first line defense against pathogens. Immune cells such as macrophages, neutrophils and natural killer cells are able to identify molecular patterns that are shared among groups of pathogens (16). Receptors important for the innate immune response include C-type lectins, toll-like receptors and CD14 (16).

The adaptive immune system is activated after antigen uptake in Peyer's Patches and presentation to T and B cells in GALT. Antigen recognition is a specific process that also requires a co-stimulatory signal. Without co-stimulation, a partial T-cell response or tolerance induction will occur. After antigen exposure and priming, T and B lymphocytes migrate to the mucosa to protect against environmental pathogens and allergens (16). B lymphocytes provide protection by the secretion of specific secretory IgA (16). In the neonate, the adaptive immune system is naive and not fully developed. T-cell function in neonates is inadequate and the ability of neonates to clear foreign antigens is dependent of the innate immune system.

The immunological advantages of maternal milk on the neonate have been studied using antibody responses to vaccines as probes of immunity (16). A recent study comparing cell-mediated immune responses in breast-fed vs. bottle fed infants have found important immunophenotypic differences in lymphocyte populations in infants who were breastfed (16). Specifically, breastfed infants displayed lower levels of CD4+:CD8+ cells, an increase in interferon-gamma levels. This highlights that maternal feeding can facilitate the development and function of the neonatal immune

system (13). However, results from numerous studies describe many immunosuppressive effects of breastfeeding. The immunosuppressive effects are most likely seen due to the reduced uptake of dietary and bacterial antigens due to secretory IgA. Other immunosuppressive factors seen in breastmilk that include IL10 and transforming growth factor-beta (TGF- ß). These immunosuppressive factors found in maternal milk may promote tolerance induction to harmless food antigens and antigens associated with commensal bacteria (28).

Maternal milk plays an important role in modulating neonatal immune responses. However, more research needs to be done to understand the complex interplay between the numerous immunostimulatory and immunosuppressive factors found in breast milk and their effect on the neonatal immune response.

3.0 BIOACTIVE COMPONENTS IN MILK

3.1 CYTOKINES

Many bioactive components of human milk have been postulated as conferring a protective effect against the development of allergies in the infant (11). Many of these immunologically active components in breast milk are missing in processed cow's milk and infant formulas (11). Human milk contains factors that interact with the infant immune system and intestinal milleu such as immune cells, immunoglobulins, cytokines, chemokines, growth factors, lactoferrin, oligosaccharides, enzymes, secretory components, and hormones, along with foreign food antigens, bacteria and viruses (30).

Cytokines, which include chemokines, interleukins, interferons, and growth factors, are small soluble glycoproteins that are involved in cell signaling and play an integral role in cellular communication (28). Cytokines operate in networks and orchestrate immune system development and function (20). The relative composition of immunostimulatory and immunoregulatory cytokines in human milk varies among mothers (28). The variation in overall concentrations of cytokines among mothers may be due to varying maternal (microbial) exposures (30).

Human milk contains many cytokines that have clinical significance due to their role in the development of allergic disease, namely the T_H2 cytokines like IL4, IL5, and IL13. Tolerogenic cytokines, like TGF- ß and IL-10 are involved in immunoregulation and are prominent breast milk cytokines (14). Recent studies have shown that TGF- ß plays an important role in the development and maturation of the mucosal immune system in neonatal mice and has been associated with the protection against allergic asthma (30).

3.2 TGF- ß

TGFß is an important regulatory cytokine involved in IgA production and the induction of oral tolerance (28). TGFß also suppresses the T_H1 and T_H2 pathways (30). TGFß is the most abundant cytokine in human milk and has three isoforms. Studies have confirmed that TGFß is immunologically active and plays a role in oral tolerance (30). A recent review examining 12 human studies by Oddy and Rosales (28) determined that over 60% of the studies showed a positive association of TGFß-1 or TGFß-2 in preventing atopic outcomes in infancy and early childhood (30). Furthermore, a recent study showed that human milk TGFß was associated with increased diversity of the infant gut microbiome composition (30). TGFß was also found to increase the infant's ability to produce IgA against b-lactoglobulin, casein, gliadin, and ovalbumin (28). These studies show that milk TGFß modulates the development and maintenance of the immune system and may influence the development of allergic disease and asthma.

There is emerging data that other human milk cytokines and chemokines may play a role on allergic disease development. Cytokines involved in IgE production and eosinophil levels are IL-4, IL-5, and IL-13 and were found to exist in higher concentrations in breast milk of atopic mothers compared to nonatopic mothers (28).

3.3 SOLUBLE CD14/TLR

Soluble CD14 may protect against allergy development due to its high concentrations in breast milk and its importance in the T_H1 induction response to bacteria (30). CD14 is the soluble component of the Toll-like receptor 4 (TLR4), which plays a role in intestinal homeostasis (30). It facilitates the interaction of intestinal epithelial cells with lipopolysaccharides (LPS) from Gram-negative bacteria (30). Researchers hypothesize that early exposure to LPS decreases the risk of

developing allergic disease (14). One theory is that early exposure to LPS from commensal bacteria may increase the T_H1 response and thereby diminish T_H2 allergy promoting responses (14). Developing immune responses is critical during early postnatal life, when antigen exposure first occurs, because the response that develops is likely to determine life-long reactivity to that antigen. Studies examining soluble CD14 levels in colostrum and human milk have found lower concentrations of soluble CD14 in mothers with children who develop atopy or eczema (30). It is important to note that colostrum is rich in soluble CD14 and has decreasing levels over time. Meanwhile, neonates lack CD14 and do not attain significant plasma levels of CD14 until 4 months of age. The neonate is therefore unequipped in the early postnatal period to respond to microbial products and relies on exogenous factors such as CD14 to facilitate the maturation and function of the neonatal immune system.

3.4 IMMUNOGLOBULIN A

The most abundant immunoglobulin in human milk is IgA, most of which is in the form of secretory IgA (sIgA) (33). Human milk IgA is produced as a result of prior maternal exposure to bacterial and protein antigens. This occurs via the "enteromammary link" whereby mammary gland cells responsible for IgA production have migrated from the mother's intestine (30). Thus, infant feedings are rich in sIgA antibodies to a broad range of bacterial and protein antigens to which the mother has had exposure. Infants are unable to produce their own levels of IgA until one month after birth (33). As stated previously, breast milk composition changes over time (30). Accordingly, sIgA concentrations are highest right after delivery, during the colostral phase (30). Studies examining the colostrum concentrations of sIgA found lower

levels in allergic mothers compared to nonallergic mothers (14). Low levels of sIgA in breast milk was also associated with an increased risk of cow's milk allergy (28).

3.5 PROBIOTICS/PREBIOTICS

Prebiotics are nondigestible food products that can promote the growth and activity of colonic bacteria and help maintain beneficial microflora (36). Human milk oligosaccharides (HMOs) are prebiotics that promote *bifidobacteria* and *bacteriodes* during exclusive breastfeeding (33). HMO composition does vary significantly from different mothers (33). Studies examining mothers with mutations preventing HMO secretion into breast milk found a delayed establishment of bifidobacteria-laden microbiotia and higher risk for IgE-associated eczema in infants delivered via c-section (30).

Probiotics are live microorganisms that have beneficial properties on human health when given in appropriate amounts (36). The role of probiotics as a protective factor against development of atopic diseases has gained much attention (9). Lower levels of infections and frequent use of antibiotics in infancy may predispose to allergic responses. The mechanisms underlying this process have not been discovered. However, studies have shown that the microbiome of infants with AD vary significantly when compared to nonallergic children (14). Probiotics may provide prophylactic and therapeutic benefits in atopic disease (9). A recent study of the probiotic bifidobacterium showed an increase in IgA secretion and an indirect increase in T regulatory cell responses, thus promoting mucosal tolerance and preventing allergic responses (14).

Recent systematic reviews have concluded that probiotics failed to demonstrate clear benefits for the treatment for atopic dermatitis. However, a number of randomized controlled trials have shown a beneficial effect of probiotic

supplementation on AD development and severity (25). Other studies have shown that supplementation with specific probiotics are effective in AD prevention. A recent study examining probiotic supplementation with Lactobacillus GG saw a protective effect against AD when given to mothers during pregnancy and while breastfeeding, and to the infants after 3 months of age (25). Probiotic treatment to mothers may increase breast milk levels of TGFß-2, which can suppress the infant inflammatory intestinal response. Further investigations are needed to determine whether prebiotics and probiotics can effectively prevent or reduce allergic disease in childhood.

4. EPIGENETICS

4.1 CLASSICAL EPIGENETIC MECHANISMS

Epigenetic modifications are one of the most important mechanisms by which environmental factors modulate early cellular differentiation (15). Epigenetic modifications play a key role in the development of new phenotypic traits during pregnancy and within the neonatal period (26). It is now clear that this period in early life is a key window of vulnerability where environmental exposures can alter lifelong health and disease by modifying inflammatory molecular pathways (26).

Epigenetic modifications are biochemical changes in DNA or histones that do not affect the nucleotide sequence of the genome (1). These modifications allow certain genomic loci to be accessible to transcription enzymes which then allows for gene expression (29). A number of factors, such as environmental pollutants, maternal and neonatal nutrition, and reduced exposure to infectious and symbiotic microbiota may lead to epigenetic modifications (26).

DNA methylation, the first epigenetic mechanism recognized, involves the covalent addition of a methyl group to cytosine residues (1). Methylation occurs in response to various cellular stressors and signals by methyltransferases (1). Methylation is usually restricted to cytosines of CpG dinucleotides, which are abundant throughout the genome (1). The CpG dinucleotides cluster into CpG islands (CPGs), which are stretches of DNA characterized by high CG content, which are found in the majority of human gene promoters (32). The CPGs in promoter regions are typically unmethylated, and the lack of methylation assists transcription (32). High methylation of the CPGs is usually associated with lower gene expression or full gene silencing (29). The exact mechanism by which DNA methylation plays in gene silencing is not fully understood.

Histone proteins can be modified by various posttranslational modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation (1). Histone methylation refers to the addition of a methyl group to lysine residues of the N-terminal tail of the histone molecules (1). This modification is catalyzed by histone methyltransferases and can alter the chromatin configuration, either making it more open or tightly packed (1). Histone methylation can be associated with either gene repression or activation (32). The influence of histone methylation on gene activity depends on the location of the amino acid residue and on the number of methyl-groups added (29). Acetylation of histone proteins is a process where acetyl groups are added to lysine residues in histone proteins. Acetylation results in an open chromatin configuration, which favors active transcription. Histone phosphorylation, ubiquitination, and sumoylation are other modifications whose effects are not completely characterized. Lastly, noncoding RNAs that bind to DNA can have an impact on transcriptional activity also (1).

4.2 EPIGENETIC REGULATION OF IMMUNE CELLS

Epigenetic changes in genes specific for T helper cells have been implicated in the pathogenesis of asthma and allergic diseases (32). There are two major subsets of helper T cells, T_H1 and T_H2 cells (2). T_H1 cells produce IFNG and are particularly important for the defense against intracellular bacterial and viral infections (2). T_H1 cells also secrete IL-2 and/or TNF-alpha. T_H2 cells have been found in airways of patients with asthma and are therefore closely associated with asthma pathophysiology. The main function of T_H2 cells is to produce the cytokines IL-4, IL-5, and IL-13 to recruit eosinophils to control extracellular parasites. IL-4 is important for allergic sensitization and IgE production, IL-5 stimulates growth and differentiation of eosinophils, and IL-13 has pleiotropic effects in the lungs (2).

T helper cells arise from a common precursor, the naive CD4+ T cell (2). Naive CD4+ T cells, are immature and undergo differentiation in the periphery after thymic development (2). Differentiation occurs after activation through the T cell receptor (TCR) in the presence of appropriate cytokines (IL-12 for T_H1 and IL-4 for T_H2) (32). Numerous epigenetic mechanisms have been identified that are involved in differentiation of T helper cells (32). For example, when antigen and cytokine stimulation by IL-4 occur, the Il4, Il5, Il13 genes of the T_H2 cluster are epigenetically activated and the set of genes for T_H1 cells (Ifng) are silenced (2). Signals from cytokines initiate reciprocal activation and silencing of INFG and IL4 cytokine gene loci by epigenetic mechanisms in order to maintain differentiation (2).

It is important to understand the epigenetic mechanisms that underlie the differentiation and regulation of T cells due to their role in the development of asthma (34). Researchers have found that methylation of a highly conserved CpG islands in the INFG promoter region is associated with polarizing naive T cells into pro-allergic T_H2 cells (2). Furthermore, in children suffering from allergic asthma, demethylation of the promoter region for IL4 and methylation of IFNG promoter was observed (34). Another study observed methylation of CpG islands within the promoter region of the ADAM33 gene and found it to regulate gene expression. ADAM33 is a gene important in the development of asthma in humans. Recent research into the mechanism underlying allergen-specific immunotherapy elucidated the importance of epigenetic suppression of T_H2 cell growth in the treatment of asthma (34). Researchers analyzed changes in DNA methylation of cytokine genes in T helper cells in children suffering from allergic asthma and receiving house dust mite allergen-specific immunotherapy. In the treated group, children had lower levels of IL-4, IL-5 and IL-2 and an increase in IL4 promoter methylation (34). It was

demonstrated that immunotherapy inhibited synthesis of these cytokines and T cell proliferation and therefore induced antigen-specific T cell tolerance via epigenetic modifications. These studies show that DNA modifications of the IL4 locus in T_H2 cells is an integral epigenetic mechanism that is involved in the pathophysiology of asthma.

4.3 THE ROLE OF EARLY LIFE ENVIRONMENTAL EXPOSURES

There is increasing evidence that early life is an important time in which environmental exposures can lead to epigenetic changes that contribute to the development of atopic diseases. The contribution of environmental factors on the increasing prevalence of allergic diseases was confirmed in recent epidemiological studies (29). Studies have observed a dramatic rise in the incidence of allergies in the former German Democratic Republic (East Germany) over the past 20 years despite the genetic characteristics of the population remaining the same (29). The loss of protective factors or the increase in negative environmental factors could explain the increased incidence. In particular, the hygiene hypothesis, which emphasizes the importance of early life biodiversity, is thought to underlie this process (29). Children exposed in early life to diverse microbiota present in rural/farming environments develop less allergic conditions (29). Many studies have been done to uncover the mechanisms of this protective effect. Studies on a number of bacteria common in farming environments displayed a T_H1 polarizing effect in human dendritic cells *in vitro* (29). Another study in mice observed protective effects on neonates after maternal exposure to *Acinetobacter lwoffii* mediated by IFNG **(**29). Researchers found that the transmaternal protection by IFNG was partly mediated by preservation of histone acetylation at the IFNG promoter of CD4 T cells isolated from mice offspring (29).

The establishment of the intestinal microbiota during early life has a major impact on human physiology. It was commonly accepted that the development of the gut microbiota occurs postnatally. While controversy still exists about whether the fetus resides in a sterile environment, there is considerable evidence suggesting that the fetus is exposed to a microbial environment *in utero*. Quantitative culture techniques have found significant levels of bacteria in the amniotic fluid even without a ruptured amniotic sac (13). Immediately after birth, the infant is colonized with *ex utero* microbiota (13). Infants who were born vaginally develop microbiota originating from the mother's intestine, vagina, and skin while infants delivered by C-section have colonization from the mother's skin and hospital environment (30). An epidemiological study on 1.9 million people from Denmark observed a significantly higher incidence of immune-mediated diseases such as asthma in children born via C-section (13). This confirms the drastic impact aberrant gut microbiota can play in long-term health and disease.

The composition of the microbiome postnatally is influenced in a large part by diet. Numerous studies have observed enhanced growth of *Bifidobacteria* species in breastfed infants (13). Breastfeeding can shape the gut microbiota after birth, both directly by milk microbiota delivered to the neonate and indirectly, via the variety of components in breast milk that affect bacteria growth and metabolism (33). Recent studies have shown that more than one-fourth of infant gut microbiota is provided by human milk alone (30). Accumulating evidence suggests that maternal environmental exposures may affect levels of breast milk cytokine and IgA levels. The Old Order Mennonites (OOM) are a religious community that practice a lifestyle without modern technology and emphasize growing/raising their own food, consuming unpasteurized milk, home births, breastfeeding and infrequent use of

antibiotics. An analysis of an OOM community of western New York found a lower prevalence of atopic disease than the general population. Breastmilk collected from OOM mothers demonstrated higher levels of IgA and microbiome diversity when compared to urban mothers. Therefore, maternal environmental exposures in the OOM population is associated with beneficial changes in breast milk composition that supports the infants gut microbiome and immune system.

Viral infections have important immunomodulatory effects that contribute to the development of allergic disease. Certain airborne viruses like human rhinoviruses and respiratory syncytial virus (RSV) are associated with the development of asthma (3). Smoke exposure is another environmental factor known to increase the risk of allergic disorders, especially asthma (29). Active smoking has been clearly shown to influence methylation of DNA and *in utero* exposure of smoke affected methylation of DNA in the offspring (29). Two recently published meta-analyses sought to analyze the epigenetic effects of smoking during pregnancy by analyzing newborn blood DNA methylation profiles. These studies found more than 6000 CpGs with differential methylation patterns in relation to maternal smoking at the genome-wide statistical significance level (26). In the Northern European RHINE study, higher asthma risk was even found in persons whose paternal grandmother smoked, even when the mother did not smoke (26).

While many studies have shown the effect of environmental exposures on the development of allergic diseases, the simple presence of an allergic disease in animal models is enough to substantially increase the risk in offspring. Studies on peanut-allergic mothers showed a higher T_H2 response in offspring at low levels of sensitization with increased IgE and IL4 levels when compared with the controls. The

offspring of peanut-allergic mothers showed lower levels of DNA methylation at the IL4 promoter.

Another factor that can lead to perturbations in the infant gut microbiota that precede the development of atopy, atopic eczema, and food allergy/sensitization is the administration of pharmacologic agents, especially antibiotics (30). Observations from the Urban Environment and Childhood Asthma study, which analyzed allergen and bacterial exposures of children, suggest that early life antigen exposures may differ from allergen exposure throughout life and the combination of high-level allergen and bacterial exposure in early life may be protective against allergen sensitization and asthma (22). In animal asthma models, asthma severity was worse after treatment with vancomycin but not streptomycin (4). Changes in microbial composition and lower levels of regulatory T cells were observed in the mice treated with vancomycin (4). Furthermore, when antibiotic treatment was given to neonatal and adult mice, an increase in disease severity was seen in only neonate mice, suggesting that gut microbiota are susceptible in this crucial developmental period and that later alterations may not result in disease development (4). Evidence is accumulating that highlights the importance of early lifestyle and dietary changes and their ability to influence the microbiome and subsequent development of atopic disease.

5. CONCLUSION

The intrauterine period and postnatal period are both important for the development of the microbiome and immune development. Modern day society, with increased standards of hygiene, increases in cesarean sections and an increase of formula feeding have changed the gut flora of infants. The development of the immune system in early life may be dependent on the composition of intestinal microbiota, which can be inflammogenic or tolerant in nature (4).

Maternal milk contains a diverse variety of factors that interact with the infant immune system and intestinal milleu including allergens, cytokines, immunoglobulins, prebiotics and soluble CD14. TGF- ß is a prominent tolerogenic cytokine that plays a pivotal role in intestinal homeostasis, inflammation, and oral tolerance development. Furthermore, TGF- ß has been associated with an increased diversity of the infant gut microbiome composition. Increasing evidence has shown the role of soluble CD14 and IgA from breastmilk in immune system development and function in the neonate. These exogenous factors are excreted in high concentrations in colostrum and in the early postnatal period. This could represent a critical vulnerable period of the developing immune system as neonates are unable to produce significant levels of CD14 or IgA until 4 months of age (30).

It is important to keep in mind the importance of the skin as a gatekeeper for the development of allergic diseases. The dual-antigen exposure hypothesis describes how a disrupted skin barrier can lead to allergic skin sensitization in the setting of avoidance of specific foods. Based on this new understanding, recommendations have been made that highlight the importance of controlled exposure to allergenic foods throughout infancy to promote oral tolerance starting at 4 months of age (7).

The changes in diet, breastfeeding practices, antibiotic use, and environmental exposures in industrialized societies are implicated in the epigenetic changes seen in atopic individuals (4). Epigenetic programming takes place during fetal life and microbial contact can have an impact on disease risk even before birth. Epigenetic alterations of differentiation and regulation of T cells appear to be important epigenetic mechanisms underlying atopic disease development in future generations. Silencing of genes involved in the T_H1 pathway such as IFNG and epigenetic activation of IL4 genes have been observed in children suffering from allergic asthma.

The protective benefits of rural/farming environments on atopic disease development is related to the epigenetic modulations conferred by specific microorganisms. Breastfeeding induced microbiota has greater diversity and higher concentrations of *Bifidobacteria* species. Furthermore, breastmilk contains HMOs that can improve the gut microenvironment during early life to promote the development of a tolerogenic immune system. The interactions between breast milk, the intestinal milieu, and the developing immune system are extraordinarily complex and are just beginning to be uncovered. However, a thorough understanding of how specific gut microorganisms affect epigenetic programming in early life and future disease development is needed to develop new prevention strategies and novel treatments for atopic disorders.

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