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Cow’s Milk Protein Allergy: Review of Literature on signaling pathways in CMPA and a Clinical Case Study

A thesis submitted in partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE, BIOCHEMISTRY AND MOLECULAR BIOLOGY

by

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May, 2015
We recommend that the thesis prepared under our supervision by

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be accepted in partial fulfillment of the requirements for the degree of

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Abstract

Cow’s milk protein allergy (CMPA) is a common allergic response in infants and young children. This report presents the key aspects of CMPA: clinical features, pathogenesis, symptoms, epidemiology, genetic and environmental factors, diagnosis, and treatment. Current research of methods for modulating allergic responses is also presented. One avenue being studied is the effect of omega-3 polyunsaturated fatty acids (PUFAs) on CD4+ T cell differentiation and regulation. Omega-3 PUFAs have been found to modify the plasma membrane organization of CD4+ T cells, thus affecting key signaling pathways involved in the manifestation of allergic diseases. The specific pathways and molecules in CD4+ T cells that are downregulated via omega-3 PUFA incorporation are presented. A clinical case study detailing the manifestations of this disease is also offered.
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Characteristics of CMPA

Introduction

Cow’s milk protein allergy (CMPA) is an immunological adverse reaction to cow’s milk protein and usually presents as the first food allergy in children, ages newborn to 4 years old. The proteins in cow’s milk that usually elicit a reaction are: α-lactalbumin, β-lactoglobulin, and casein (Solinas, Corpino, Maccioni, & Pelosi, 2010). The allergy can present as IgE or non-IgE mediated and depending on either characteristic, symptoms like anaphylaxis, respiratory reactions, or gastrointestinal reactions can manifest in affected children (Solinas et al., 2010). The proteins in cow’s milk initiate a cascade of reactions that affect a number of different types of cells in the immune system. The allergy involves antigen specific T cells, T regulatory cells, cytokines from different kinds of T cells, B lymphocytes, and mast cells (Coco, Andrea Domenico, Vitaliti, Cimino, & Lionetti, 2012). There are a number of methods utilized in diagnosing CMPA, most commonly an oral food challenge, a skin test, or an immunoassay (Jarvinen-Seppo MD PhD, 2013). Current treatments for CMPA require changing of the child’s diet to eliminate milk proteins until they overcome the allergy. There is ongoing research for other treatments of CMPA, including modulation of the immune system via nutritional intervention with polyunsaturated fatty acids (PUFAs) (Thang, Boye, Shi, & Zhao, 2013).

I. Clinical Features

Cow’s milk protein allergy usually manifests during the first few months of life. After the introduction of a cow’s milk-based formula or the ingestion of breast milk from a mother who consumes cow’s milk, the infant presents with CMPA within
days or weeks (Jarvinen-Seppo MD PhD, 2013). CMPA can be categorized as an Immunoglobulin E (IgE) mediated syndrome or non-IgE mediated. Some cases of CMPA can also present both characteristics of IgE and non-IgE, called mixed IgE and non-IgE CMPA. The IgE mediated reaction represents a classic allergic reaction and yields immediate clinical reactions (Solinas et al., 2010). The infant can present with reactions in the skin, oropharyngeal tract, upper and lower respiratory tracts, gastrointestinal tracts, and the cardiovascular system within hours after exposure to cow’s milk antigens (Jarvinen-Seppo MD PhD, 2013). Non-IgE mediated reactions also affect the same systems of the body as IgE-mediated reactions; however, non-IgE mediated reactions are delayed, and infants can present with symptoms within days or weeks after the initial exposure.

II. Pathogenesis

Ig-E mediated CMPA consists of a sensitization phase and an activation phase (Coco et al., 2012). In sensitization, IgE antibodies against cow’s milk proteins are secreted and bind to the surface of mast cells and basophils awaiting the next exposure to cow’s milk. In subsequent exposures, the activation phase starts when the IgE antibodies that were already joined to mast cells and basophils bind to the allergenic epitopes of α-lactalbumin, β-lactoglobulin, or casein (Coco et al., 2012). This causes a release of inflammatory mediators that then manifest in the clinical symptoms of CMPA.

There is less understanding of the mechanisms regarding non-IgE mediated CMPA, but has a number of different proposed theories. One reaction involves the binding of the cow’s milk antigen to the cell surface, which induces different
immunoglobulin classified antibodies (IgG, IgM, IgA) to disturb the cell membrane
and cause cell death (Solinas et al., 2010). Another reaction involves IgG, IgM, and
IgA antibodies becoming trapped in small blood vessels, while another reaction may
be mediated by sensitized T-lymphocytes (Solinas et al., 2010). Other general ideas
of non-IgE mediated CMPA include: reactions facilitated by helper T cells (Th1) and
interactions between T cells, mast cells, and neurons (Coco et al., 2012).

As a part of the pathogenesis of CMPA, the different kinds of T cells and their
roles in allergies are currently being studied. Naïve T cells can be sensitized and
differentiate into Th2 lymphocytes that then secrete IL-4, IL5, IL10, and IL13,
cytokines that promote the production of IgE and amplify the inflammatory response
(Coco et al., 2012). A dysfunction of Treg cells (regulatory T cells) could also explain
the lack of tolerance for milk proteins as these cells produce cytokines (TGF-β and
IL-10) that regulate the immune system and dampen T cell responses (Coco et al.,
2012). Studies are also focusing on CD4+, CD25+, and Foxp3+ T cells, as they are
important in suppressing T cell proliferation (Coco et al., 2012).

III. Symptoms

CMPA generates a variety of symptoms in infants afflicted with the disease.
Immediately after the consumption of milk proteins, if the child presents with
symptoms such as: frequent regurgitation, vomiting, hives, swelling of the lips or eye
lids, wheezing, and a dry cough, then the child can be suspected of having CMPA.
Symptoms from CMPA do not always come about immediately. Late onset symptoms
include: diarrhea, blood in the stools, iron deficient anemia, gastroesophageal reflux,
and colic (episodes of crying for more than 3 hours a day) over a three-week period
The classification of CMPA (IgE, non-IgE, and mixed IgE and non-IgE) can also determine a more specific list of symptoms. IgE-mediated CMPA causes anaphylaxis, angioedema, as well as oropharyngeal and gastrointestinal reactions. Non-IgE mediated CMPA causes enterocolitis where the child presents with severe vomiting and diarrhea. The child can also have gastroesophageal reflux and constipation. Non-IgE mediated CMPA may also cause Heiner syndrome, a pulmonary disease that presents the child with cough, fever, wheezing, and nasal congestion. Mixed IgE and non-IgE reactions can cause atopic dermatitis (eczema), asthma, or eosinophilic gastrointestinal disorders (inflammation of the esophagus by eosinophils) (Jarvinen-Seppo MD PhD, 2013).

There are rare symptoms associated with severe cases of CMPA. These include: severe atopic dermatitis, hypoalbuminemia (low blood albumin levels), failure to thrive, and anemia due to rectal bleeding and loss of protein.

IV. Epidemiology

Cow’s milk protein allergy is the most common food allergy in young children. It makes up about 9% of total reported cases of food allergies (Gaudin et al., 2008). It affects about 2% of children under four years of age (Jarvinen-Seppo MD PhD, 2013). In one-year old children, the prevalence of the disease is higher with a 2.2-2.8% initial occurrence compared to a 0.6% occurrence in three-year old children (Jarvinen-Seppo MD PhD, 2013). The prevalence of CMPA in adults is much less common with 0.1-0.3% of adults with the confirmed allergy. Cow’s milk is one of the most common foods responsible for anaphylactic reactions. A pediatric allergy clinic
that focused on food allergies reported a 40% incidence of CMPA in their patients (Jarvinen-Seppo MD PhD, 2013).

Children usually outgrow Cow’s milk protein allergy. 51% of infants develop a tolerance within the first two years of life and if it persists for longer, 80% of children will develop a tolerance within 3-4 years of age (Coco et al., 2012). Non-IgE mediated CMPA is usually resolved more quickly than IgE-mediated CMPA. IgE-mediated CMPA can persist until late childhood or adolescence, but 64% of such children outgrow the allergy by 12 years old (Jarvinen-Seppo MD PhD, 2013).

V. Genetic and Environmental Factors

It has been reported that individuals genetically predisposed to developing CMPA are more likely to sensitize T cells to the Th2 response that releases inflammatory cytokines (Beyer et al., 2002). Such cytokines are responsible for much of the clinical manifestations of CMPA. For individuals that have been diagnosed with CMPA, they can have genetic predispositions to developing a tolerance to the allergy more easily. Such factors include: naturally low initial levels of milk-specific IgE, faster rate of decline of milk IgE, and lack of allergic rhinitis (inflammation of the mucous membrane of the nose) or asthma (Coco et al., 2012).

Environmental factors associated with CMPA include the initial exposures of children to the cow’s milk proteins, which can come from ingestion of milk proteins in formula or in breast milk. It is theorized that the industrialization of societies has decreased the microbial exposure infants receive, which plays a factor in Treg cell dysfunction and thus promotes allergic disorders (Rook & Brunet, 2005). No other genetic or environmental factors are appreciably associated with the disease.
VI. Diagnosis

To diagnose CMPA, the clinical history of the child as well as laboratory tests are used with a timetable or an outlined process of diagnosis also being followed (Figure 1). An oral food challenge is mostly utilized for diagnoses, specifically a double-blind, placebo-controlled food challenge, but an open food challenge is used when the child is younger (Solinas et al., 2010). The suspected food is eliminated, then used to challenge the child, and then re-eliminated. In breastfed infants, the mother avoids ingesting cow’s milk while formula fed infants are given formula without milk proteins. If the CMPA is suspected to be mild to moderate, the elimination phase starts with a therapeutic formula with no milk proteins for 2-4 weeks. If symptoms disappear after this period, then an open challenge with formula containing milk proteins is performed (Solinas et al., 2010). If the child does not develop symptoms after the repeated challenge, a one-week follow-up is done which then determines if the child can resume a normal diet.

A skin test can also be used to diagnose CMPA. A portion of the infant’s skin is exposed to commercial cow’s milk extract. A wheal diameter of 8mm in children over 2 years old and 6mm in children under 2 years old indicates CMPA (Jarvinen-Seppo MD PhD, 2013). More specific guidelines for wheal diameters in infants report different measurements for the different milk proteins: 12mm for α-lactalbumin, 10mm for β-lactoglobulin, and 9mm for casein (Solinas et al., 2010). Immunoassays measuring the amount of specific IgE antibodies can also be used to diagnose children with the cut-off level of 0.35 U/ml (Solinas et al., 2010).
VII. Treatment

To treat cow’s milk protein allergy, removal of milk and milk products from the diet will prevent exposure to the antigenic proteins. There are three types of infant formula that can be used to replace a diet containing milk: soy, extensively hydrolyzed, and amino acid. Soy formula is usually the first alternative to milk but around 10% of children with IgE-mediated CMPA and 60% of children with non IgE-
mediated CMPA have been shown to have an allergy to soy (Solinas et al., 2010). Extensively hydrolyzed formula is the first choice for infants under 6 months of age. It is also used to treat infants who have presented with non-anaphylactic symptoms, enterocolitis (inflammation of the digestive tract), atopic eczema, and gastrointestinal symptoms. If there is a poor response to extensively hydrolyzed formula, amino acid formula is then chosen. Amino acid formula is also used for infants who have severe CMPA or have been diagnosed with anaphylaxis or eosinophilic oesophagitis (inflammation of the esophagus) (Solinas et al., 2010). If the allergy persists past infancy, any food that contains traces of milk must be avoided.

For infants that maintain breast-feeding, the mother will have to avoid milk, milk products, and eggs until the infant is weaned. The mother is usually advised to take calcium supplements during this time (Solinas et al., 2010).

**Body of Review**

Current research on the modulation of the allergic response in CMPA exploits CD4+ T cells and their mechanism of action in allergies. CD4+ T cells can differentiate into various kinds of T cells. Notable for CMPA, is the differentiation of CD4+ T cells into type 1 helper T cells (Th1) and type 2 helper T cells (Th2). It has been noted that the Th2 response is elevated in food allergies and asthma (Thang et al., 2013). Th2 cells release the inflammatory cytokines: interleukin (IL)-4, IL-5, and IL-13, which are responsible for the activation of eosinophils and mast cells as well as the production of IgE (Jang, Lim, Lee, & Park, 2013). Such characteristics are responsible for the clinical manifestations of CMPA. Thang et al also noted that a shift from Th2 responses to Th1 responses helps modulate allergic responses. Thang and his team attributed this outcome
to the role of Th1 cytokines. Th1 cells produce an anti-inflammatory cytokine, IL-10, as well as a cytokine essential for down-regulation of airway hyper-responsiveness, IL-12p40 (Thang et al., 2013). Th1 cells also produce the chemokine MCP-1, which was reported to be found at higher levels in children who had overcome CMPA (Glez, Franco, & Matheu, 2012). A shift to Th1 responses seems to modulate allergic responses and one factor responsible is the intervention of omega-3 polyunsaturated fatty acids (Thang et al., 2013).

Polyunsaturated fatty acids (PUFAs) are fatty acids with at least one double bond in their backbone. There has been much research on omega-3 and omega-6 PUFAS and their effects on different areas of the human body and of human diseases. Omega-3 PUFAs have been found to have anti-inflammatory and anti-proliferative effects while omega-6 PUFAs have shown the opposite (Thang et al., 2013). PUFAs are under research for their effects on allergic diseases. It has been theorized that PUFAs can affect allergies, such as CMPA, through the disruption of CD4+ T cells, thus affecting the mechanism and differentiation explained above that play a large role in CMPA.

Conclusions

Though a rather simple disease that is mostly outgrown, Cow’s milk protein allergy remains one of the most common food allergies as well as the first allergy infants acquire. CMPA presents with many different symptoms that at first, can be difficult to diagnose as a simple food allergy. Research for new, better diagnostic tools as well as therapeutics for the modulation of CMPA and all allergies remains an important area of study.
Literature Review: Omega-3 PUFAs alter the plasma membrane molecular organization of CD4+ T cells, affecting downstream signaling pathways responsible for Cow’s Milk Protein Allergy.

Introduction

Mostly diagnosed in infants and young children, Cow’s Milk Protein Allergy is an immunological reaction to the proteins found in cow’s milk: α-lactalbumin, β-lactoglobulin, and casein (Solinas, Corpino, Maccioni, & Pelosi, 2010). One pathway that characterizes milk protein allergy, and most other food allergies, involves CD4+ T cells. Activation of CD4+ T cells is essential to the development of immune responses and they are reliant on signal cascades through the plasma membrane in order to complete the activation steps of the T cell (Brix et al., 2010). In order to target T cell activation, research has looked to the tampering of the cells’ plasma membranes. Numerous studies have proposed that omega-3 polyunsaturated fatty acids alter the plasma membrane organization of a number of cells, notably CD4+ T cells (Brix et al., 2010)(Shaikh, Jolly, & Chapkin, 2012)(Stulnig et al., 2001)(Jang, Lim, Lee, & Park, 2013). Omega-3 polyunsaturated fatty acids are a class of PUFAs that have a double bond three carbons away from the terminal end of their backbone. The altering of the plasma membrane of CD4+ T cells interrupts the signaling needed for activation of the cells as well as the production of cytokines responsible for allergic responses such as CMPA (Brix et al., 2010). Figure 2 shows the mechanism in which omega-3 PUFAs affect CD4+ T cell activation. First, a CD4+ T cell synapses with an antigen-presenting cell, which then induces activation of the T cell. It is proposed that omega-3 PUFAs increase the molecular order of lipid rafts in the plasma membrane, which subdues the recruitment of
signaling proteins for T cell activation (Shaikh et al., 2012). Lipid rafts are “transient sphingolipid/cholesterol assemblies…that compartmentalize signaling proteins” (Shaikh et al., 2012). The second messengers: PIP₂ and DAG, as well as the proteins: PLC-1, PKCθ, F-actin, and NF-κB, are all involved in CD4+ T cell activation and are suppressed by the incorporation of omega-3 PUFAs into the plasma membrane (Shaikh et al., 2012).

Nutritional intervention with PUFAs holds promise as a method to modulate CMPA and possibly other allergies that rely on plasma membrane organizations of lymphocytes.

![Image](image_url)

**Figure 2. Omega-3 PUFAs target the plasma membrane organization of lipid rafts of CD4+ T cells.** Red circles represent omega-3 PUFAs targeting the cell. Figure taken from Shaikh et al (Shaikh et al., 2012).

**Methods and Results**

*Signaling proteins of T cells are displaced upon treatment with Omega-3 PUFA*

While it was previously observed that omega-3 PUFAs affect T cell activity, the method of disruption needed further confirmation. Stulnig et al studied the in vitro effects
of omega-3 PUFAs on T cells’ signaling proteins by observing Jurkat T cells, immortalized human T cells, treated with the omega-3 PUFA, eicosapentanoic acid (20:5), and the control, stearic acid (18:0). Figure 3 shows a quantitative analysis of immunoblots done to measure the levels of proteins, Lck and LAT. The signaling proteins Lck and the transmembrane adaptor LAT, are members of the Src family kinases, which are tyrosine kinases that phosphorylate the tyrosine residues of other proteins (Stulnig et al., 2001). Compared to the control, the signaling proteins, Lck and Fyn (Fyn not shown in figure) as well as LAT, were displaced at a higher percentage from T cell membranes when treated with eicosapentanoic acid and the displacement of such kinases downregulates T cell differentiation (Stulnig et al., 2001).

Stulnig et al also investigated whether the displacement of these proteins results from if omega-3 PUFAs alter the lipid raft environment or through S-acylation of proteins by omega-3 PUFAs. S-acylation is the targeting of transmembrane and intracellular proteins to membrane domains by acylation with fatty acyl moieties, such as palmitoyl residues (Stulnig et al., 2001). Stulnig et al reasoned that if omega-3 PUFAs displace proteins via S-acylation, then proteins that are already acylated with palmitate should not be affected by eicospentanoic acid. A group of Jurkat T cells were labeled with $[^3]H$ palmitate and Figure 3 shows that both non-labeled and $[^3]H$ palmitate-labeled proteins were displaced from the membrane; suggesting that omega-3 PUFAs alter the membrane environment by incorporating into the lipid rafts (Stulnig et al., 2001).
Figure 3. Percent displacement of Lck and LAT in Jurkat T cells. Immortalized human T cells (Jurkat T cells) were treated with stearic acid (18:0) and eicosapentanoic acid (20:5) and immunoblotted for protein levels. Black bars represent unlabeled proteins while gray bars represent $[^3]H$ palmitate labeled proteins. Figure taken from Stulnig, et al. (Stulnig et al., 2001).

Activation and maturation of T cells are downregulated with omega-3 PUFAs

To corroborate other studies’ in vitro findings of omega-3 PUFAs affecting T cells, Jang et al studied the effects of omega-3 PUFAs on CD4+ T cells of mice. Jang et al used Fat-1 transgenic mice, which have higher levels of omega-3 PUFAs in their organs and tissues. They prepared cultures of CD4+ T cells from the spleens and lymph nodes of the Fat-1 mice for evaluation. Figure 4 A and B shows a quantitative analysis of flow cytometry experiments, which measured the activation of T cells in mice. After a 48 hour culture, cells were permeabilized and stained for flow cytometry, which then analyzed the number of stained cells activated with anti-CD69 and anti-CD25 antibodies to measure the percentage of CD69+ and CD25+ cells in Fat-1 transgenic mice (Jang et al., 2013). The cells showed lower levels of CD69+ and CD25+ cells, which are markers of T cell activation (Jang et al., 2013). The decreased levels of CD69+ and CD25+ in
Figure 4 highlight a decrease in CD4+ T cells’ ability to proliferate. CD69+ is a glycoprotein involved in activation of CD4+ T cells and CD25+ is an important receptor for IL2, a growth factor for CD4+ T cells (Jang et al., 2013).

The decrease in activation resulting from the high omega-3 levels in Fat-1 transgenic mice would in turn affect cytokines produced by a normal mature CD4+ T cell. Figure 4 C shows levels of cytokines produced from wild type CD4+ T cells compared to Fat-1 transgenic mice’s CD4+ T cells. The expression of cytokines: IL-2, IL-4, and IFN-γ decreased with higher levels of omega-3 PUFAs as shown with Fat-1 mice (Jang et al., 2013).

Figure 4. Measurement of T cell activity in Fat-1 transgenic mice. A and B: Cells were left unstimulated with anti-CD3 antibody or stimulated with anti-CD3/anti-CD28 antibodies. Cells were stained and analyzed via flow cytometry for percentage of CD69+ and CD25+. C: Cytokine levels of T cells were measured via ELISA. Figure taken from Jang, et al. (Jang et al., 2013)
T cell signaling is disrupted by omega-3 PUFAs

Jang et al confirmed that omega-3 PUFAs reduce the activation and maturation of CD4+ T cells (Figure 4) and sought to explore the mechanisms of T cell activation that omega-3 PUFAs acted on. Figure 5 A and B shows Western Blots for levels of tyrosine phosphorylation and levels of phosphorylated signaling molecules. At certain time periods after stimulation of T cells with anti-CD3/anti-CD28 antibodies, levels of tyrosine phosphorylation and levels of phosphorylated Lck and Zap70 (signaling molecules) are decreased in Fat-1 transgenic mice compared to wild type (Jang et al., 2013).

Jang et al also investigated the NF-κB pathway, which is known to play a key role in T cell activation, cytokine production, and of survival. NF-κB expression involves the phosphorylation of the p65 subunit as well as degradation of IκB-α which are first triggered by T-cell receptor engagement (Jang et al., 2013). Figure 5C shows a Western Blot of the intermediates, p65 and IκB-α. The phosphorylation of the p65 subunit and the degradation of IκBα were both inhibited in the mice’s CD4+ T cells, which suggests an inhibition of the NF-κB pathway (Jang et al., 2013).
Costimulatory molecules in T cell activation are reduced with omega-3 PUFAs

Brix et al sought to investigate how CD4+ T cells’ signal transduction was affected with high levels of omega-3 PUFAs. They analyzed spleen cells from mice fed a high omega-3 diet. One focus of their study was the costimulatory molecules of CD4+ T cells. Brix et al postulate that the “type and level of costimulatory molecule expression on activated CD4+ T cells are considered as key elements for the activation of adaptive immune responses.” Figure 6 shows an inverse linear correlation of CD4+ T cell division and CD28 expression. CD4+ T cell division was measured via flow cytometry where cells were stained with a PKH dye and cultured for 14 days with a measurement of the dilution of PKH left after the 14 days (Brix et al., 2010). CD28 expression was also measured via flow cytometry. CD28 is an important costimulatory molecule that is constitutively expressed in naïve T cells and helps stimulate the production of IL-2 (Brix et al., 2010).
et al., 2010). Figure 6 shows that the lower CD28 expression in high omega-3 PUFA spleen cells correlated with lower T cell division.

Brix et al also measured levels of the costimulatory molecule, inducible costimulator (ICOS), which is dependent on CD28 signaling. Flow cytometry analysis in Figure 7 showed consistently lower levels of ICOS compared to mice fed a high saturated fat diet (Brix et al., 2010).

Collectively, the results from the aforementioned experiments suggest that omega-3 PUFAs negatively affect mechanisms involved in CD4+ T cell growth and maturation. Through alteration of the plasma membrane organization of CD4+ T cells, omega-3 PUFAs can modulate allergic responses that involve these T cells such as CMPA.

**Figure 6. Correlation of T cell division and CD28 expression.** T cell division and CD28 expression on viable spleen CD4+ T cells were measured through flow cytometry. Mice were fed an omega-3 PUFA diet or a saturated fat diet. Figure taken from Brix, et al (Brix et al., 2010).
Discussion

Interruption of the plasma membrane organization of cells can have numerous effects on cell function. Researchers describe omega-3 PUFAs as modifying “the compressibility, phospholipid flip-flop, acyl chain packing, elasticity, and lipid domain formation” of the plasma membrane of cells (Shaikh et al., 2012). Such changes take place in the immunological synapse of a CD4+ T cell and an antigen-presenting cell as omega-3 PUFAs incorporate into the lipid rafts of T cells and disrupt the molecular organization, thus affecting signaling within the cell (Shaikh et al., 2012). CD4+ T cells are good targets for modulating allergic diseases like Cow’s Milk Protein Allergy as they produce the inflammatory cytokines IL4, IL5, and IL13, as well as mast cells and IgE (Jang et al., 2013).

Omega-3 PUFAs, through the modification of lipid rafts, affect different
components of CD4+ T cells. Altering lipid rafts in the plasma membrane affects signal
cascades downstream from the membrane and this was shown in the decrease of tyrosine
phosphorylation as well as the downregulation of the NF-κB pathway (Jang et al., 2013).

Costimulatory molecules expressed on the surface of CD4+ T cells were also
affected by omega-3 PUFAs. Brix et al state that CD28 and T-cell receptor signaling is
dependent on a number of signaling proteins, including LAT, which is known to be
decreased in the omega-3 disruption of plasma membranes (see Figure 3). They also state
that CD28 is targeted to the lipid raft during T cell activation, which makes it susceptible
to omega-3 incorporation. Decreases in CD28 and T-cell receptor signaling reduce the
responsiveness of CD4+ T cells and affect other costimulatory molecules (such as ICOS)
that are important for the regulation of T cells (Brix et al., 2010). It has been shown in a
previous study that a decrease in ICOS decreases inflammation as well as reduces serum
IgE production (Brix et al., 2010). Inflammation and IgE levels are both important
characteristics in allergic diseases, especially in CMPA.

Modulation of the immune system response in allergic diseases, such as CMPA,
by nutritional intervention holds much promise. Incorporation of omega-3 PUFAs into
the plasma membrane affects key signaling pathways in CD4+ T cells. The use of omega-
3 PUFAs has already been tested in clinical trials. Such trials include: the observance of
maternal omega-3 supplementation on allergic responses of neonates and the study of fish
oil supplementation on 6 month old infants with high risks for allergic hypersensitivity
(Klemens, Berman, & Mozurkewich, 2011)(D’Vaz et al., 2012). Such studies show
promising results that reflect those seen in in vitro and animal studies. Further studies of
omega-3 PUFAs may lead to treatments for many allergic diseases, not just CMPA.
Clinical Case Study

Chief Complaint

Kevin Westbrook is a 3 week old, African American male infant brought in by his parents to the physician’s office after three days of diarrhea with small streaks of blood.

History of Present Illness

Kevin’s mother reports that Kevin primarily breast feeds around every 3 hours, supplementing with Enfamil Premium for Newborns formula as needed, using the recommended ratio: 2 fl oz of water to one scoop of powder formula. Kevin has bowel movements around 5-8 times a day. In the last three days however, Kevin has had more frequent bowel movements with wet stools containing small streaks of blood. His mother is unsure of the number of wet diapers over the last two days. She reports frequent and multiple spit ups, but no vomiting. No change in appetite has been apparent. Kevin’s mother also reports seeing rashes on his chest, arms, and a little on the legs, but says she recently just tried a new brand of laundry detergent that may be causing his clothes to irritate his skin. Kevin has been healthy and has not been on any medications or antibiotics leading up to this visit. The mother denies any coughing or nasal congestion. Kevin has had no recent sick contacts.

Medical History

Birth History: Kevin’s mother had an uncomplicated pregnancy with a C-section due to failure to progress. Information obtained from his birth records indicate there were no complications during delivery. Kevin was born at full term, 40 weeks, weighing 7.45 lbs, with a height of 20.23 inches. Kevin received an APGAR score of 9 at one minute after birth and at five minutes after birth: he received a 2 for his breathing, a 2 for his
heart rate being greater than 100 beats per minute, a 2 for his muscle tone, a 1 for his
grimace response, and a 2 for having a pink color throughout his body.

*Immunizations*: Hepatitis B at birth

*Other*: Parents deny any significant medical issues for Kevin.

**Social History**

Kevin’s mother works from home as a Mary Kay consultant, managing her own
business and reports having low-stress. Kevin’s father works in the Accounting
Department at one of the local casinos. Kevin is the second of two boys, his older sibling
being 4 years old. Parents do not smoke and there are no pets in the house.

**Family Medical History**

Kevin’s family history includes heart disease and high cholesterol from his
paternal grandfather and breast cancer from his maternal grandmother. Kevin’s older
brother has no significant medical issues, is up to date on all immunizations, and is
currently being taken to a local day care twice a week.

**Physical Exam**

*Vital Signs and Measurements*  
Length: 21.73 inches  
Weight: 8.45 lbs  
Head Circumference: 15.66 inches  
BP: 65/45  
Temperature: 98.8°F  
RR: 30  
HR: 100

*General Appearance*  
Appears irritable but still conscious and alert. Patient is
fussy when examination is attempted.
**HEENT**  
Skull is symmetrical. Anterior fontanelle is open and flat. Pupils are equal and reactive to light. Red reflex bilaterally. Left and right TM and canal clear. Bony landmarks and light reflex present in both ears. No septal deviation or nasal polyps. MM slightly dry but throat is clear with no erythema or exudates.

**Neck**  
No neck masses, no enlarged nodes noted, no thyromegaly.

**Chest/Lungs**  
Clear to auscultation bilaterally. No wheezing and no retractions.

**Heart**  
Regular rate and rhythm with no murmurs. Normal S1 and S2.

**Abdomen**  
Soft with no masses and abdomen is non-distended. Umbilicus has detached with no swelling or redness in the area. Regular bowel sounds are present. No organomegaly noted.

**Genitourinary**  
Descended testes bilaterally with healing circumcision site.

**Extremities**  
Fingers and hands appear normal. Hips symmetrical with full range of movement. Palpable femoral and brachial pulses. Legs are symmetrical and feet appear normal.

**Spine and Back**  
Straight spine with no deformities.

**Neurologic**  

**Skin**  
Skin does not feel too warm or too cold to the touch but feels slightly dry. Skin color is normal but red, scaly rashes can be seen on the bends of the elbows, the upper chest, and some spots on the legs. Rash resembles what is pictured. Skin turgor is slightly reduced and a capillary refill time of less than 2 seconds was
observed.

Figure 8. Pediatric atopic dermatitis (eczema). Figure adapted from Paller et al.

Laboratory and Imaging Findings

**CBC**

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC Count</td>
<td>$4.5 \times 10^6/\mu l$</td>
<td>(3.6-6.2)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14 g/dL</td>
<td>(12.5-20)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>45%</td>
<td>(39-63)</td>
</tr>
<tr>
<td>MCV</td>
<td>90 fl</td>
<td>(86-124)</td>
</tr>
<tr>
<td>MCH</td>
<td>33 pg</td>
<td>(28-40)</td>
</tr>
<tr>
<td>MCHC</td>
<td>32 g/dL</td>
<td>(28-38)</td>
</tr>
<tr>
<td>WBC Count</td>
<td>10,000/\mu l</td>
<td>(5,000-20,000)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>36%</td>
<td>(25-37)</td>
</tr>
<tr>
<td>Monocyte</td>
<td>7%</td>
<td>(0-9)</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>2.5%</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.5%</td>
<td>(0-1)</td>
</tr>
<tr>
<td>Absolute Neutrophil</td>
<td>$19.2 \times 10^3/\mu l$</td>
<td>(6.0-23.5)</td>
</tr>
<tr>
<td>Platelets</td>
<td>$300 \times 10^9/L$</td>
<td>(150-350)</td>
</tr>
</tbody>
</table>
### Electrolytes

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Results</th>
<th>Normal Values</th>
<th>Electrolyte</th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>133 meq/L</td>
<td>(133-146)</td>
<td>pH</td>
<td>7.40</td>
<td>(7.35-7.45)</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.8 meq/L</td>
<td>(3.7-5.9)</td>
<td>pCO₂</td>
<td>38 mmHg</td>
<td>(34-46)</td>
</tr>
<tr>
<td>Chloride</td>
<td>98 meq/L</td>
<td>(98-106)</td>
<td>pO₂</td>
<td>92 mmHg</td>
<td>(80-100)</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>25 meq/L</td>
<td>(23-28)</td>
<td>O₂ Saturation</td>
<td>98%</td>
<td>(&gt;95%)</td>
</tr>
</tbody>
</table>

### Blood Gases

<table>
<thead>
<tr>
<th>Blood Gases</th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.40</td>
<td>(7.35-7.45)</td>
</tr>
<tr>
<td>pCO₂</td>
<td>38 mmHg</td>
<td>(34-46)</td>
</tr>
<tr>
<td>pO₂</td>
<td>92 mmHg</td>
<td>(80-100)</td>
</tr>
<tr>
<td>O₂ Saturation</td>
<td>98%</td>
<td>(&gt;95%)</td>
</tr>
</tbody>
</table>

### Urinalysis

<table>
<thead>
<tr>
<th>Urinalysis</th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.0</td>
<td>5.0-7.0</td>
</tr>
<tr>
<td>Urine osmolarity</td>
<td>600 mOsm/L</td>
<td>50-600 mOsM/L</td>
</tr>
<tr>
<td>Urine specific gravity</td>
<td>1.020</td>
<td>(1.005-1.030)</td>
</tr>
<tr>
<td>RBC</td>
<td>1/hpf</td>
<td>&lt;2/hpf</td>
</tr>
<tr>
<td>WBC</td>
<td>1/hpf</td>
<td>&lt;2/hpf</td>
</tr>
<tr>
<td>RBC Casts</td>
<td>0/hpf</td>
<td>0/hpf</td>
</tr>
<tr>
<td>Negative for bilirubin, blood, ketone, leukocytes, nitrites, proteins</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Blood Urea Nitrogen

<table>
<thead>
<tr>
<th>Blood Urea Nitrogen</th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 mg/dL</td>
<td></td>
<td>(8-20)</td>
</tr>
</tbody>
</table>
**Liver Function Tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>23 IU/L</td>
<td>0-60 IU/L</td>
</tr>
<tr>
<td>ALT</td>
<td>20 U/L</td>
<td>0-50 U/L</td>
</tr>
<tr>
<td>GGT-P</td>
<td>24 IU/L</td>
<td>0-50 IU/L</td>
</tr>
<tr>
<td>ALP</td>
<td>85 IU/L</td>
<td>75-375 IU/L</td>
</tr>
</tbody>
</table>

**Stool Culture**
- Rotavirus Assay- negative
- Microscopy results indicate presence of fecal leukocytes

**Lactose Hydrogen Breath Test**
- <5 ppm above basal values over 2 hour period

**Abdominal Ultrasound**
- No masses. Negative for intussusception.

*Figure 9. Normal abdominal ultrasound. Image adapted from Hayden et al.*
Skin Prick Test

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Diameter</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow milk</td>
<td>5 mm</td>
<td>(&lt;6 mm)</td>
</tr>
<tr>
<td>Egg</td>
<td>4 mm</td>
<td>(&lt;7 mm)</td>
</tr>
<tr>
<td>Peanut</td>
<td>4 mm</td>
<td>(&lt;8 mm)</td>
</tr>
</tbody>
</table>

Specific IgE RAST

- 0.32 U/ml (<0.35 U/ml)

Diagnosis

Kevin’s electrolyte levels are on the low normal end and his slightly dry MM, slightly dry skin, and reduced skin turgor suggests he has mild dehydration from his episodes of diarrhea. Results from stool cultures indicate Kevin is not suffering from a rotavirus but his stool does have some leukocytes. Lactose intolerance is eliminated after a normal result is obtained from a lactose hydrogen breath test. An abdominal ultrasound appeared normal, ruling out intussusception. A food allergy was now highly suspected but Kevin’s wheal diameters were within normal ranges for cow’s milk, egg, and peanut. A specific IgE RAST was also performed, which yielded normal results.

Since the mother reports multiple, frequent spit-ups, indicative of gastroesophageal reflux, as well as diarrhea with blood streaks in the stool, along with the observed rashes, indicative of atopic eczema, a tentative diagnosis of Cow’s Milk Protein Allergy (CMPA) was made. Since lab results were negative for milk and IgE levels, Non-IgE CMPA was considered. To confirm the diagnosis, Kevin’s mother was placed on a strict no milk diet for two weeks. Kevin was also given a hypoallergenic, extensively hydrolyzed formula to supplement breast-feeding as needed. Kevin’s symptoms improved in that time. To ensure the correct diagnosis was made, the mother was put back on a milk diet and Kevin’s previous formula was used for supplementing
breastfeeding for one week. In this week, Kevin’s diarrhea returned with the formation of rashes on his skin beginning again. Kevin’s diagnosis of Non-IgE mediated Cow’s Milk Protein Allergy was confirmed.

**Treatment**

Kevin is put on a strict no milk diet. Kevin’s parents are given a list of extensively hydrolyzed and amino acid based formulas appropriate for his diet. Kevin’s mother is given a list of foods that contain milk that should be avoided if she chooses to continue breastfeeding. She is advised to see a dietitian to help her choose a well-balanced, nutritious diet that will keep her healthy while breastfeeding. Kevin’s parents are advised to reintroduce cow’s milk after 6 months of avoidance to see if tolerance has been achieved. The physician, dietitian, and family will undertake a course of action if, at that time, symptoms reoccur. Kevin’s parents will most likely be advised to reintroduce cow’s milk when Kevin is around 12 months old to check again for tolerance. For Kevin’s atopic eczema, he is prescribed a 2.5% hydrocortisone cream, to be applied once a day for two weeks. Kevin’s dehydration is mild enough that this does not warrant rehydration with IV fluids, but he will need to be monitored closely for signs that his dehydration is worsening (i.e. lack of urination, lack of tears, loss of skin turgor).

**Prognosis**

Over half of infants who are diagnosed with Cow’s Milk Protein Allergy develop a tolerance within the first two years of life. Kevin is expected to follow this trend. If he displays adverse reactions to cow’s milk after 6 months and after 12 months of age, he will be placed on a no milk diet again and will be tested for tolerance at 24 months of age. His mild dehydration and atopic eczema should disappear soon after elimination of
Plan Implementation

Kevin’s parents will be given a list of proper alternate formulas appropriate for his feeding (Figure 10). They will also be given a list of foods that contain milk (Figure 11), which he should avoid as he transitions to solid foods in the next several months. Kevin’s mother will be referred to a dietitian who will help her manage a no milk diet if she chooses to continue breastfeeding. Kevin’s mother is assured that Kevin’s development will not be negatively impacted if she does forego breastfeeding. Kevin’s growth and response to this diet will be monitored during his upcoming visits for his immunizations. Before his 6-month scheduled immunizations, he will be reintroduced to milk so his response can be observed at the time of his visit. This will be repeated at the 12-month mark if there is an adverse response.

Hypoallergenic formula options for infants with dietary protein-induced conditions

<table>
<thead>
<tr>
<th>Extensively hydrolyzed casein protein</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Similac Alimentum</td>
<td>(Abbott)</td>
</tr>
<tr>
<td>Enfamil Nutramigen</td>
<td>(Mead Johnson)</td>
</tr>
<tr>
<td>Enfamil Nutramigen with Enflora LGG</td>
<td>(Mead Johnson)</td>
</tr>
<tr>
<td>Pregestimil</td>
<td>(Mead Johnson)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amino acid-based</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecare Infant</td>
<td>(Abbot)</td>
</tr>
<tr>
<td>Neocate Infant DHA and ARA</td>
<td>(Nutricia)</td>
</tr>
<tr>
<td>Puramino (previously known as Nutramigen AA)</td>
<td>(Mead Johnson)</td>
</tr>
</tbody>
</table>

Courtesy of Alan Lake, MD.

Graphic 79377 Version 11.0

Figure 10. Formula options for patient. Figure adapted from UptoDate.com.
How to read an ingredient label for a milk-free diet

<table>
<thead>
<tr>
<th>Avoid foods that contain milk or any of these ingredients:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter, butter fat, butter oil, butter acid, butter ester(s)</td>
</tr>
<tr>
<td>Buttermilk</td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
</tr>
<tr>
<td>Caseinates (in all forms)</td>
</tr>
<tr>
<td>Cheese</td>
</tr>
<tr>
<td>Cottage cheese</td>
</tr>
<tr>
<td>Cream</td>
</tr>
<tr>
<td>Curls</td>
</tr>
<tr>
<td>Custard</td>
</tr>
<tr>
<td>Diacetyl</td>
</tr>
<tr>
<td>Ghee</td>
</tr>
<tr>
<td>Half-and-half</td>
</tr>
<tr>
<td>Lactalbumin, lactalbumin phosphate</td>
</tr>
<tr>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Lactulose</td>
</tr>
<tr>
<td>Milk (in all forms, including condensed, derivative, dry, evaporated, goat's milk and milk from other animals, low fat, malted, milkfat, nonfat, powder, protein, skimmed, solids, whole)</td>
</tr>
<tr>
<td>Milk protein hydrolysate</td>
</tr>
<tr>
<td>Pudding</td>
</tr>
<tr>
<td>Recaldent</td>
</tr>
<tr>
<td>Rennet casein</td>
</tr>
<tr>
<td>Sour cream, sour cream solids</td>
</tr>
<tr>
<td>Sour milk solids</td>
</tr>
<tr>
<td>Tagatose</td>
</tr>
<tr>
<td>Whey (in all forms)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Milk is sometimes found in the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial butter flavor</td>
</tr>
<tr>
<td>Baked goods</td>
</tr>
<tr>
<td>Caramel candies</td>
</tr>
<tr>
<td>Chocolate</td>
</tr>
<tr>
<td>Lactic acid starter culture and other bacterial cultures</td>
</tr>
<tr>
<td>Luncheon meat, hot dogs, sausages</td>
</tr>
<tr>
<td>Margarine</td>
</tr>
<tr>
<td>Nisin</td>
</tr>
<tr>
<td>Nondairy products</td>
</tr>
<tr>
<td>Nougat</td>
</tr>
</tbody>
</table>

All FDA-regulated manufactured food products that contain milk as an ingredient are required by US law to list the word “milk” on the product label.

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Graphic 63271 Version 6.0

Figure 11. Foods containing milk to avoid.

Figure adapted from UptoDate.com.
References


