WHAT LIES BEHIND CHILDHOOD LIPOID NEPHROSIS?

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Abstract

Lipoid nephrosis or idiopathic nephrotic syndrome (INS) is a syndrome characterized by an increase in glomerular permeability followed by massive proteinuria, hypoproteinemia, hypogammaglobulinemia and dyslipidemia. Clinical expression of these biological changes is the installation of massive edema and oliguria. INS etiology is currently unknown but numerous studies are pointing out the connection between INS and atopic diatheses. Based on this finding, some authors have tried a diet of exclusion which resulted in decreased proteinuria but not significantly reduced the number of relapses. The link between diet and altered intestinal permeability and the decisive role of diet in the regulation of intestinal microflora and the modulation of intestinal and systemic inflammatory response is currently demonstrated by many authors. The presence in serum of patients with INS of antibodies to food, either IgE, or IgG4 may be considered a marker of increased intestinal permeability, local inflammation and intestinal microbiota disturbance. In our experience, exclusion from the diet of patients with INS of gluten, dairy products and of food for which there is an immune response (sIgE or sIgG4) resulted in a rapid decrease of proteinuria, allowing us to reduce the total length of corticosteroid therapy and prevented relapse of the illness over a period of one year. Changing the treatment protocol from administration of anti-inflammatory medication (glucocorticoid drugs) to restoration of a balanced intestinal microbiota, improvement of intestinal permeability and modulation of the gut inflammatory response must be the primary objective in the management of the child INS.

Keywords: atopy, gut microbiota, idiopathic nephrotic syndrome, IgG4-related disease, food allergy, podocyte

Introduction

Lipoid nephrosis or idiopathic nephrotic syndrome (INS) is a syndrome of unknown etiology characterized by an increase in glomerular permeability followed by massive proteinuria, hypoproteinemia, hypogammaglobulinemia and dyslipidemia. Clinical expression of these biological changes is the onset of massive edema and oliguria.

The link between nephrotic syndrome and atopy. INS pathogenesis is not currently known but is believed to be an immune-mediated process1,2 with the involvement of LT dependent circulating factors that cause podocyte dysfunction and massive proteinuria.3 Over time many authors have noticed the connection between INS and atopic diatheses (asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, etc.) which led to assumptions about involvement of IgE allergy in the pathogenesis of INS.4,5,6,7,8,9,10 Many reports describe INS relapses after exposure to allergens (pollen, mold, bee stings, administration of vaccines, etc).4,5,6,7,8,9,10 The main arguments that associate INS with atopic diathesis can be summarized as follows:

2. IgE serum levels are much higher in patients with INS than in those with other glomerular diseases.23 At the same time INS patients may have elevated levels of IgE in the absence of clinical signs of atopy.21,15
3. Many reports describe INS relapses after exposure to allergens (pollen, mold, bee stings, administration of vaccines, etc).24,25,26,27
4. There is an increase in serum levels of IgG4 during relapses of INS and a decrease in periods of remission.28

Food allergens and nephrotic syndrome. The potential role of food allergens in triggering INS and its relapses is frequently discussed in the literature. Cow’s milk proteins, gliadin, ovalbumin, fish, chicken or pork meat are the most frequent allergens linked to INS. The published results are difficult to interpret because they include: patients with steroid-resistant INS,8,29,30,31 patients with multiple relapsing INS,4 patients under 1 year old where the diagnosis of INS is questionable,2 patients in whom renal biopsies revealed other forms of nephrotic syndrome2. A number of exclusion diets have been used in patients with INS and food allergies such as elemental diets4,29 oligo-antigenic diets32 or diets that exclude food allergens (cow’s milk products, gluten)7,8,32,33. The reduction of proteinuria after the implementation of a restrictive diet has been reported in most cases,
sometimes even without adding prednisone therapy, but long-term effects and efficiency of diet in preventing relapses are much less consistent. However, some patients with INS have not relapsed after removal of food allergens from the diet.

**Common immunological aspects in INS and Atopy.** The mechanism of IgE immune reaction is triggered by two main signals: the release of IL-4 and IL-13 by TH2 lymphocytes and the expression of the surface antigens (CD40) by B lymphocytes that are fixed by a ligand on activated T cells (CD28). Kimata,54 Garin55 and Abdel-Hafa2 highlighted the increased production of IL-13, but not IL-4 in INS. Thus IL-13 levels are elevated in serum and urine of patients during INS decompensation and return to normal after remission occurs. Also serum levels of IL-13 correlates directly with serum IgE levels56. Thus the podocytes possess the ability to express receptors for IL-13 and furthermore, to express a binding membrane protein (CD80) with a role in T cell costimulation.5,6,37,38 Reiser et al. highlights that the increase of proteinuria in mice correlated with the expression of CD80 membrane protein associated with lining of podocytes suggest that CD80 expression by the podocytes could be a possible mechanism in INS.38,39 Such research shows that atopy does not play a direct role in the pathogenesis of INS but rather there is a pattern that predispose to both allergic and nephrological disorders. The increase of IL-13 on the one hand induces a shift in production of immunoglobulins from IgM to IgE from B lymphocytes, and on the other hand, induces the expression of CD 80 membrane proteins in the podocytes that is associated with proteinuria.

**The role of gut and gut microbiota in modulating the host immune response.** The human gut, with a total area of approximately 300m² in adults, is the place where more than 100 trillion organisms coexist, including bacteria, viruses, fungi, bacteriophages and archaea. Had been established a mutually beneficial relationship between the human body and the enteric microbial flora. Enteric environment supplies intestinal microbiota with nutrients. Furthermore, it contributes to functional balance and body health through degradation of some food components (complex carbohydrates, xenobiotic substances, etc), production of short chain fatty acids, synthesis of vitamins, and by influencing the metabolic profile. Above all gut microbiota plays a crucial role in the development, maturation and modulation the host immune response.40,41,42,43,44 Microbial colonization of the digestive tract occurs during and immediately after birth with germ in early conditions revealed that the intestinal microbiota can be restored by repopulating the gut with commensal bacteria.44,45. Through the evolutionary process host organisms have developed a complex system of immune tolerance related to intestinal microbiota. In parallel they also developed improved methods of keeping microbial population in the lumen of the gut and gut microbiota. Involvement of commensal bacteria in modulating the immune response is extremely complex.41,42,43

Current knowledge can be summarized as follows, without any pretense of completeness: role in the induction of immune tolerance phenomenon40, role in the regulation of number of protective CD4+ T lymphocytes45, Bifidobacterium spp. enhance the maturation of the mucosal slgA system32. Segmented filamentous bacteria are required for induction of intestinal TH17. TH17 play an important role in stimulating the production of mucus and antibacterial proteins (at the level of both intestinal mucosa and mucosa of the respiratory tract), enhance cell epithelial tight junctions and contribute to antifungal and antibacterial protective mechanism45,54. Early colonization with Bacteroides fragilis down-regulates lipopolysaccharide responsiveness in infancy. Bacteroides fragilis activate CD4+ T helper cells and promote TH1/TH2 balance through polysaccharide A. Bacteroides fragilis and some Clostridium spp. promote T reg cells and increase the releasing of IL-10, an anti-inflammatory cytokine that protects against chemically induced colitis.57. The presence of Clostridium spp. during the early life may play a significant role in resistance to allergy and autoimmune diseases56. Clostridium coccoides and C. leptum have a protective action against inflammatory bowel disease (IBD)59 and are major producers of butyrate, a short chain fatty acid that represent a source of energy for colonocytes and a protection against damaging during local inflammatory response59,60.

**Diet, intestinal microbiota and human pathology.** Intestinal microbiota is influenced in proportion of 57% by the diet while genetic factors account for only 12%.61 If a balanced diet is the promoter of a healthy gut and a balanced microbiota, nutritional imbalances can cause a number of disorders by affecting intestinal homeostasis.60. A large number of studies highlights diet-induced changes on intestinal microbiota60.
- High fat diet decreases the population of *Bifidobacterium*.
- Diet high in fat and sugar increases population of *Clostridium difficile*, *C. perfringens* and *Enterococcus spp.* and while decreasing the population of *Bacteroidetes spp.*.
- Vegetarian diet prevents the growth of pathogenic bacteria such as *E. coli* and other members of the *Enterobacteriaceae* family.
- Eating complex carbohydrates increases the number of beneficial *Bifidobacteria spp.*
- Caloric-restriction diets prevent the growth of *Clostridium cocoides*, *Lactobacillus spp.* and *Bifidobacteria spp.* - the main butyrate producers that play an essential role in the nutrition and homeostasis of enterocytes.

It is now known that “Western diet” (high in fat and sugars) induces intestinal dysbiosis with impaired gastrointestinal cell metabolism and disruption of immune homeostasis. Also, gut microbiota in European children is lower in *Bacteroidetes* and higher in *Enterobacteriaceae* compared with African children, due to a low intake of fiber.

Unbalanced diet cause disruption of intestinal microbial flora and induce: an increased production of mucus, alteration of intestinal motility, enterocyte dysfunction (by reducing the intake of nutrients) and local inflammatory reactions. This results in the appearance of a leaky gut syndrome: an alteration of intestinal permeability and loss of functional barrier of the gut to intraluminal environment and triggers local and systemic inflammatory reactions. Altering of the TH1/TH2 ratio in allergies and associated disorders and mucosal TH17 overstimulation with increased mucosal permeability and podocytes dysfunction are examples of pathogenic mechanisms with intestinal starting point. Asthma and allergic inflammation, Type 1 diabetes, metabolic syndrome, IBD, celiac disease, acute pancreatitis, psoriasis, rheumatoid arthritis, ankylosing spondylitis, ADHD, multiple sclerosis, schizophrenia and mood disorders, some forms of autoimmune encephalitis or cancer (glioma, human lung squamous cell carcinoma, pancreatic carcinoma, hepatocellular carcinoma) are examples of diseases presently connected with microbiota disruption. And the range of diseases is growing. The whole concept of the pathogenesis of autoimmune and inflammatory diseases is currently under review by identifying the crucial role of tight junctions in regulating intestinal antigens traffic along the intestinal barrier, in the process of immune tolerance and by revealing the interaction between intestinal epithelium - neuroendocrine system – immune system - intestinal microbiota.

**Immune reactions to food.** Immune reactions to food are of several types. They can be both immune and non-immune related. IgE immune reactions to food are well known and studied, however, regarding non-IgE mediated immune reactions. The latter may be associated with a large variety of glandular, digestive, renal, respiratory, skin and vascular symptoms. Sometimes the two types of immune responses may coexist, in conditions such as atopic dermatitis.

IgG4 immune reactions to food (also called food intolerance) is a controversial topic nowadays, most allergists considering that the presence of IgG4 represents no more than a marker for the installation of immune tolerance to a particular food. Therefore, The European Academy of Allergy and Clinical Immunology drew the following conclusion: "IgG4 against foods indicates that the organism has been repeatedly exposed to food components, recognized as foreign proteins by the immune system. Its presence should not be considered as a factor which induces hypersensitivity, but rather a "IgG4 against foods indicates that the organism has been repeatedly exposed to food components, recognized as foreign proteins by the immune system. Its presence should not be considered as a factor which induces hypersensitivity, but rather a "IgG4 immune response indicator for immunological tolerance, linked to the activity of regulatory T cells". A series of data however come to contradict this belief:

1. Results from IgG4 panels made in patients with various symptomatology highlight a growth in IgG4 antibodies titers only for a limited number of foods. In general these foods are about the same for all patients and are well-known for their potential of inducing food allergy. In our patients with INS the top foods that cause IgG4 type immune reactions are: bananas, cow’s milk, wheat, rye and kiwi.
2. In some situations, the same patient shows an increase in both IgE and IgG4 antibodies.
3. Despite conventional opinion which considers IgG4 molecules as anti-inflammatory, this Ig is currently found to be involved in a number of immune-mediated disorders.
4. The production of both IgE and IgG4 is controlled by TH2 lymphocytes. IL-4 and IL-13 amplify the synthesis of both types of Ig, whereas IL-10 mediate the shift in the synthesis from IgE to IgG4.

It has been already recognized the involvement of IgG4 in diseases such as: atopic dermatitis, thrombotic thrombocytopenic purpura, pemphigus, glandular pathology (salivary, lacrimal, and thyroid), chronic inflammatory processes of the aorta and great vessels, respiratory tract, orbit, mediastinum, retroperitoneum, kidney, etc. In some circumstances IgG4 has also rheumatoid factor activity. In many pathologic circumstances IgG4 and IgE growth occurs simultaneously.

**IgG4 and renal pathology.** Although still in its infancy, several research already established a role of IgG4 in immune-related pathology. Regarding renal pathology it is currently known the involvement of IgG4 autoantibodies in membranous glomerulonephritis (MGN), where they act like auto-antibodies against phospholipase A2 M-type receptor found on the podocytes. Tubulointerstitial nephritis (IgG4-TIN) with increased IgG4-positive plasma cells and fibrosis is another feature of IgG4 related kidney disease and may cause acute or chronic renal dysfunction. In some patients IgG4-TIN might present concurrently with MGN or with other fibroinflammatory conditions. It is also known that the production of IgG4 is mediated by TH2 lymphocytes as well as the production of IgE, with IL-13 playing an essential role. Meanwhile IL-13 is involved in the pathogenesis of INS in children through binding to a podocytes membrane protein (CD80), followed by the appearance of...
proteinuria\textsuperscript{36,37,38,39}. The pathogenic triangle: IgE – IgG4 – podocytes dysfunction takes on this way new meanings. It remains to be determined whether there is a causal relationship between these conditions or they are based on a common cause.

We allow in this moment to issue the following hypothesis: several genetic and epigenetic factors (individually or in association) lead to the disturbance of intestinal microbiota and alter the local and systemic immune response (among them a switch in the TH1/TH2 ratio and mucosal TH17 overstimulation). Alteration of the intestinal barrier creates conditions for entering into circulation of incompletely digested food structures that become antigens. These antigens stimulate TH2 production of pro-inflammatory cytokines IL-4, IL-13. They induce hyper IgE and IgG4 with related symptoms. In turn, IL-13 levels by binding to podocyte membrane receptor CD80 generate dysfunctions in the endothelial cells barrier and the appearance of proteinuria. INS may also represent not so much a dysfunction of podocytes, as an adaptive pathway to remove the excess of pro-inflammatory molecules.

Discussions

Atopy is unlikely to have a role in the pathogenesis of INS, first, because allergic immune response is a type of immune reaction limited to the contact surfaces of the body with the outside environment, where the body comes in direct contact with allergens, in order to limit the penetration of the allergen into the body. This type of immune response is pointless in the kidney whose function is to remove endogenous substances. However frequent association between atopy and INS and the proof of efficiency of exclusion diets (even partially) in decreasing proteinuria and induction of remission lead towards a common cause of both types of disorders.

Intestinal microbial flora represents a “virtual organ” whose role is crucial in modulating the immune response. Altered intestinal microbiota is able to induce a local and systemic inflammatory response and to create hyperpermeability of the intestinal mucosa. Remotely, there are evidences of increased permeability of the podocyte lining in the Bowman's capsule, in the airway epithelium and even alterations of the blood-brain barrier.

The enhanced systemic inflammatory response is responsible for altering the TH1/TH2 balance with the production of IL-4 and IL-13 and overstimulation of TH17 lymphocytes with increased production of IL-17, IL-21 and IL-22. From here to the impaired function of podocytes in the renal glomeruli is just a step. In fact, we ask ourselves if in the conditions of a systemic inflammatory response INS is a pathological process or a defense mechanism intended to eliminate excess of circulating pro-inflammatory mediators thus protecting the rest of the organs and systems. From this pathogenic perspective, the limited success of nutritional interventions implemented so far in INS is understandable. Removing food immune aggression is followed naturally by a decrease in inflammatory response and decrease in proteinuria. But in the absence of restoring the local microbiota and the intestinal permeability, nutritional intervention become only partial and ineffective. Moreover, a number of studies show that administration of cortisone type medication increases intestinal permeability and amplify the host allergic immune response and the production of IgE. This may explain the existence of numerous cases of multiple-relapsing, corticodependent or corticoresistant INS.

Current studies about INS focus on IgE allergic reactions to food, ignoring other type of immune-reactions like IgG4 antibody production. This may be another explanation why exclusion diets have failed in some published studies. In our patients with INS at onset we have achieved remission, early normalization of proteinuria and we have prevented relapse for a period exceeding one year by intervention especially on the digestive tract. Because we haven’t found any correlation between IgE and IgG4 reaction to food in the same patient the exclusion diet targeted both IgE and IgG4. In addition, we focused therapy on restoring gut microbiota in order to reduce inflammation and intestinal permeability.

The current trend to extend corticosteroid therapy in hopes of obtaining more prolonged remissions can be dangerous and may paradoxically increase the duration, severity and number of relapses because: corticosteroid medication increases IgE serum levels in patients with atopy\textsuperscript{72} induce production of IgE and IL-4 in purified B cells from patients without atopy\textsuperscript{73} increase the gut permeability by affecting the epithelial lining of the gut.

Conclusion

In conclusion, a shift from administration of steroid anti-inflammatory medication to restoration of intestinal microbiota, improvement of intestinal permeability and modulation of the inflammatory response at the intestinal level must be the primary objectives in the management of the child with INS. An initial diet of exclusion is mandatory but the emphasis should be on food for which there is food intolerance reactions (IgG4) and not IgE mediated ones. Exclusion of dairy and gluten should be part of the nutritional plan even if there are no allergies or food intolerances to these components. Both are irritants of the gut, stimulate excessive mucus production, favor the multiplication of pathogenic bacterial strains, maintain increased intestinal permeability, and reduce recovery of intestinal and systemic inflammatory processes. Adherence to a schedule of eating is also essential. Abundance and diversity of fresh and unprocessed natural products provides the necessary macro and micronutrients while the high content in prebiotic, fiber and antioxidants of fresh fruits and vegetables restore the intestinal microbiota, regulate intestinal motility and reduce local and systemic inflammatory processes.
References


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