ALLEGERY TO COW’S MILK PROTEINS

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INTRODUCTION

Definition. Terminology. Cow’s milk protein allergy (CMPA) is defined as IgE-mediated or non-mediated immunologic reaction, towards one or more proteins from cow milk. Cow’s milk protein intolerance (CMPI) differs from CMPA as far as pathogenesis (non-immunologic reaction towards CMP) as well as evolution and prognosis are concerned.

Epidemiology. Most authors estimate a 5-15% prevalence of CMPI and a 2-7.5% prevalence of CMPA. The rates vary due to the action of several factors: age, nutrition, genetic factors, cultural factors, diagnosis criteria used. Sicherer et al.2 (Feb. 2010) report an CMPA- incidence of 1.9-2.8% during the first two years of age; the rate drops to 0.3% after age 3. After this age (sometimes after age 5), tolerance towards CMP sets in; the rare cases found in older children and adults are in fact multiple food allergies. Approximately 0.5% of breast-fed infants develop CMPA3; the low rate is due to the low concentration of CMP in breast-milk (100,000 times lower than in cow’s milk).

Etiopathogenesis. Food allergens are glycoproteins, 10-40 kDa in size, which are water-soluble, heat-, acid- and enzyme-resistant. The most important food allergens are: beta-lactoglobulin (from cow’s milk whey), 7S globulin-vicilin (from soy), Ara h1, Ara h2 and Ara h3 (from hazelnuts). In cow’s milk, more than 20 protein fractions have been identified. Whey (20%) is represented by beta-lactoglobulin and alpha-lactalbumin, and casein (80%), by the alfaS1, alfaS2, beta, gamma, kappa fractions. Beta-lactoglobulin is responsible for 60-80% of CMPA cases; casein proteins are low allergenic, due to their flexible (non-compact) structure.

CMPA is associated in 30-50% of cases with soy-protein allergy and secondary lactose intolerance.

Intestinal immunity. Physiopathological data.

The intestinal immune system is directly responsible for the onset of intestinal allergic reactions. The intestinal absorption of the food allergen depends on the immunatory state of the intestinal mucosa4. The maturity of the intestinal mucosa, the presence of the Peyer patches, the normal intestinal microflora and the absence of intestinal inflammation and infection maintain the immunocompetence of the intestinal mucosa.

The intestine has defense mechanisms that neutralize, disintegrate and inhibit the absorption of the food allergen. IgA and M and the lymphocytes of the intestinal epithelium and lamina propria act as the immunologic barrier, whereas gastric acid, intestinal mucus, lactoferrin, the normal microflora, the hepatic filter constitute the non-immunologic barrier6.

The regulatory T-lymphocytes insure the balance
between the Th1 lymphocytes (via the gamma-IFN and IL-2 which stimulate cell-mediated immunity and phagocytosis) and the Th2 lymphocytes (via IL-4, IL-6, IL-10, IL-13 that stimulate the humoral immune response). Studies show that the allergic reaction is the consequence of an inadequate regulatory response.

At the intestinal level, the food allergen can be absorbed in 3 ways: (4.8)
- the trans-cellular path (which represents the major means of absorption), either directly (maintaining the antigen intact) or indirectly (after prior degradation of the antigen);
- the para-cellular path and
- the direct path via the M-cells (minor path of absorption).

Once absorbed, the food allergen is taken over by the antigen-presenting cells (from the epithelium and the lamina propria) and transferred to the Peyer patches. Here, the antigen is presented to the GALT, which has the role of protecting the host against intestinal pathogens (by stimulating IgAs secretion) and of preventing intestinal allergic reactions (by initiating oral tolerance).

**Oral tolerance.** The new-born is confronted with a physiologically insufficient oral tolerance (OT), which is part of the normal maturation process. After the first month, the oral tolerance has the tendency to normalize due to the contribution of growth factors from colostrum (that contribute to the maturation of the intestinal mucosa and maintaining normal permeability) and of breast milk (that supplements the insufficient IgAs production from the intestine).

Under some pathological circumstances - cellular or humoral immunity suppression or intestinal inflammation (leading to increased permeability of the intestinal mucosa) – OT is blocked or becomes insufficient.

Induced oral tolerance (self tolerance) is known to be part of the strategy of preventing allergic reactions. In 1829, Dakin demonstrated the existence of self tolerance in mice that had been repeatedly exposed to egg proteins.

In 2001, Bennet et al. demonstrated the existence of two mechanisms of inducing oral tolerance, based on the amount of the food allergen. For a small dose of allergen, the regulatory cells are suppressed. The mutant gene FOXP3 blocks the Th1, Th2 response and is responsible for the IPEX syndrome, with X-linked inheritance and whose clinical signs are enteropathy, atopic dermatitis and food allergies. In the case of high doses, anergy and clonal deletion by inhibition of IL2 takes place.

A series of factors can influence the development of oral tolerance: allergen properties, the means of exposure, and genetic factors.

It is known that soluble food allergens are more tolerogenic and, although most food allergens are soluble, it seems that the way that they are processed changes their solubility.

Oral exposure to food allergens stimulates OT, as opposed to cutaneous exposure, which inhibits it. Strid et al. in 2005, have demonstrated that the epicutaneous and epidermal exposure to proteins from hazelnuts inhibit OT for hazelnuts, in mice with existing OT, by stimulating Th1 and a consecutive rise of IL-4 and IgE.

Li X(15), in 1999, carried out a study on the mice species C3H/HeSn, AKR/j and BALB/c which had been injected with Arach DNA for 3-5 weeks. The C3H/HeSn species developed anaphylaxis, whilst AKR/j and BALB/c hadn’t developed anaphylaxis, due to the high level of IgG2a (but not IgG1 and IgE). Another study (Morafo, 2003), carried out on the C3H/HeJ and BALB/c species, showed that the intragastric exposure to cow’s milk and hazelnuts induced anaphylaxis only in the C3H/HeJ species (87% to cow’s milk and 100% to hazelnuts), because high levels of IL-4 and IL-10 were found in the splenocytes of this species (whereas in the BALB/c species, only high levels of gamma-INF were found).

In 2001, Chatteate discovered the existence of the epitopes bound to kappa-casein (8 bound to IgE and 4 to IgG) and beta casein (9 bound to IgE and IgG). The author showed that epitopes increase the capacity of allergenic awareness in patients that had been systematically exposed to allergens. The presence of epitopes plays a predictive role in persistent allergy and is the base of oral and sublingual short-term immunotherapy.

**Physiopathology**

Three types of hypersensitivity reactions are involved in CMPA:
- type I, immediate hypersensitivity, IgE-mediated, which is responsible for high-risk symptoms (anaphylactic shock, urticaria, angioedema etc.);
- type III, hypersensitivity by circulating immune complexes, non-IgE-mediated, which is responsible for the symptoms that occur 2-3 hours after the allergenic exposure;
- type IV, cell-mediated, late hypersensitivity; the prototype of this kind of reaction is the coeliac disease.

**Clinical signs and symptoms**

The symptoms can occur within minutes from the ingestion of a small amount of milk (a few drops).

There is not even a single patognomonic symptom for CMPA. Often, various symptoms (with no apparent cause) are associated with CMPA.

Given these circumstances, early diagnosis and adequate treatment become indispensable to later normal weight development.

Given the diversity of symptoms, the clinical picture of CMPA can be classified in 4 categories:
- gastro-intestinal symptoms are the most frequent (50-80%); in order of frequency of occurrence there are regurgitations (16-42% of patients with CMPA suffer from associated gastro-esophageal reflux disease), vomiting, diarrhea, constipation (perianal erythema), stools with blood-strings (with consecutive iron-deficiency anemia);
- cutaneous symptoms (20-40%), atopic dermatitis, angioedema, urticaria etc.);
- type II, hypersensitivity by circulating immune complexes, non-IgE-mediated, which is responsible for the symptoms that occur 2-3 hours after the allergenic exposure; the presence of dermatitis increases the risk of sensibility to CMP 4 times and to egg-proteins 8 times;
- type IV, cell-mediated, late hypersensitivity; the prototype of this kind of reaction is the coeliac disease.

**Clinical forms**

Acute forms of CMPA are IgE dependant, whilst chronic forms are non-IgE dependant. Mild and moderate forms show as atopic dermatitis, generally in breast-fed infants, and severe forms manifest either acutely, with life-threatening symptoms or late symptoms (failure to thrive, growth faltering).

**Positive diagnosis** is based on data from personal and family history, symptomatology (even uncharacteristic), and is the base of oral and sublingual short-term immunotherapy.
elimination diet (good clinical evolution after a diet), provoking test (recurrence of the symptoms after re-exposure to CMP), determination of total and specific cutaneous and seric IgE levels (there is a 58.8% concordance between these two for commercial products and 91.7% for fresh foods).

Family history yields very useful information: a parent with atopia increases the risk by 20-40%, a sibling with atopia, by 25-35% and both parents with atopia increase it by 60%35.

The provoking test
It is carried out under medical supervision, initially, in the hospital. The test is carried out after 2-4 (6) weeks of elimination diet, under the condition that the symptoms are remitted.

In certain situations (acute and severe onset, untreatable diarrhea and MPC), the test is only carried out after the age of 12-18 months. Delactosed formulas are used, due to the 50% rate of association with secondary lactose intolerance. Thus, the first day 1 ml (according to some authors even one drop, the dose varying according to the severity of the disease) is applied on the hand or the lips. If there is no reaction (if a cutaneous or seric tests were negative develop tolerance much faster than those with positive tests).

The failure of treatment with extensively hydrolyzed formulas is due to residual allergens contained in them. There is no evidence of failure of treatment with amino acid-based formulas (if the symptoms persist during treatment, the diagnosis should be reconsidered).

Prophylaxis of CMPA
Natural food intake represents the gold standard in CMPA prevention, under the following conditions: a mixed diet should contain hypoallergenic formulas and the mother must avoid foods with allergenic potential during breastfeeding.

An artificial diet with cow’s milk is recommended only after age 1, and diet diversification should be done under special circumstances (avoiding too early diversification, before the age of 4 months).

The CMPA patient must be monitored periodically (at 2 months, 4 months, 1 year, 2 years, 3 years); during these check-ups, clinical evaluation and cutaneous and seric allergic testing should be carried out.

Treatment
If CMPA is suspected, the patient undergoes elimination diet for 2-4 weeks (with the exception of severe cases that require emergency medication). Elimination diet is suited for breast-fed, mixed-fed, artificially-fed infants as well as those who receive diversified food33.

For breast-fed infants, the diet actually addresses the mother, who should avoid allergenic foods (milk, eggs, hazelnuts, fish, wheat) during breastfeeding. We specify that complete elimination of these foods may result in an important imbalance of the mother’s diet. If the symptoms disappear, the mother will resume the intake of one of these foods every week; if the symptoms reoccur, the mother will avoid the respective food during the whole period of breastfeeding. A calcium-supplement (1000 mg/day) is also necessary for the mother, during breastfeeding.

In the case of severe atopic dermatitis and important growth faltering, natural food intake can be excluded, and the infant receives therapeutic formula.

Mixed-, artificially- and diversified-fed infants will receive therapeutic formula (which is tolerated by 90% of CMPA patients). The first choice formula is extensively hydrolyzed and contains omega 3 fatty acids, oligopeptides (smaller than 2 kDa in size); it does not contain lactose.

There are situations (rejection due to its unpleasant taste, persistency of symptoms, MPC), when use of the extensively hydrolyzed formula is discontinued, and an amino acid-based formula is introduced.

Diversification should be undertaken using the following guidelines:
- the food first introduced is rice and only after the age of 6 months;
- at the age of 7 months, vegetables are introduced (carrots, potatoes) and at the age of 8-10 months, fruits are brought in (apples, pears, bananas, peaches, plums, apricots);
- at the age of 10 months cereals are introduced (corn, rye, oats, wheat) and at the age of 12 months, meat is introduced (lamb, pork, turkey, beef);
- cow’s milk is introduced only after 12 months;
- eggs are introduced after age 2-3 and hazelnuts, walnuts, fish after 3-4 years of age.

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