

Hypogammaglobulinaemia secondary to cow-milk allergy in children under 2 years of age

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Introduction

Hypogammaglobulinaemia is defined as diminished levels of one or more immunoglobulin isotypes to values 2 SD below the mean estimated for age. The condition can be congenital (due to a primary genetic deficiency), acquired (secondary to an underlying disease) or physiological (due to an immature immune system).^{1,2}

Physiological hypogammaglobulinaemia occurs between 3 and 6 months of age and is a consequence of the rapid catabolism of the transplacentally acquired maternal immunoglobulin G (IgG) during this period, not compensated by self-production of IgG by the lactating infant.

Summary

Symptomatic hypogammaglobulinaemia in children younger than 2 years of age was studied to rule out a primary immunodeficiency. Thirty-four patients were referred to the Immunology Service to study the hypogammaglobulinaemia-associated clinical picture. Food allergy was documented in 10 patients by personal and familial history, presence of specific immunoglobulin E (IgE) and elevated total serum IgE levels. Coeliac disease and human immunodeficiency virus infection were also ruled out. Protein loss through stools was assessed by clearance of α 1-antitrypsin (AAT). Serum immunoglobulin levels were determined by nephelometry and functional antibodies were studied by enzyme-linked immunosorbent assay. The cellular immune response was assessed by *in vitro* lymphocyte proliferation in response to mitogens and cell subsets were analysed by flow cytometry. In five patients of the 10 patients we suspected a protein loss through the mucosa. Four of these five patients showed an increased AAT and the other showed an extensive cutaneous lesion. Immunological studies revealed normal antibody function, *in vitro* lymphoproliferative responses and cell numbers in four of the 5 patients. One patient showed abnormally low numbers of CD4⁺ T cells as well as a defective proliferative response to mitogens. After diagnosis of cow milk allergy, milk was replaced with infant milk formula containing hydrolysed proteins. Recovery of immunoglobulin values and clinical resolution were achieved. Hypogammaglobulinaemia during early childhood in some children may be secondary to cow milk allergy, and immunoglobulins and cells may leak through the inflamed mucosa. Resolution of symptoms as well as normalization of immunoglobulin values may be easily achieved by avoidance of the offending allergen.

Keywords: antibodies; cows' milk; food allergy; immunodeficiency; immunoglobulin E

When this situation persists beyond 6 months of age, the condition is called transient hypogammaglobulinaemia of infancy. It is characterized by reduced IgG levels and it can be accompanied by diminished IgA levels and preserved functional antibody response. It can be asymptomatic or present with recurrent infections, bronchiolitis, bronchitis, acute median otitis, diarrhoea and, less frequently, pneumonia or invasive diseases.^{1,3} Usually, this clinical picture resolves spontaneously by 2 years of age, but in some children normalization of immunoglobulin serum levels may occur at 4–5 years of age. Treatment is symptomatic and rarely requires replacement therapy with intravenous gammaglobulin.^{1,2} Transient

hypogammaglobulinaemia has been linked to immaturity of the infant immune system associated with the mucosal barrier (low intestinal proteolytic activity, low acid secretion, immature microvillus membrane, absence of IgA and IgM from exocrine secretions, low concentration of IgA in intestine, reduced T helper type 1 lymphocyte function and diminished interferon- γ production, etc.).^{4,5}

The immature immune system associated with the gastrointestinal mucosa is confronted with foreign proteins such as bacteria and food antigens. The normal healthy gut exhibits tolerance both to beneficial dietary antigens and to innocuous antigens derived from commensal flora, but will mount active and protective immune responses against damaging antigens from invading gut pathogens. When such immune control mechanisms do not work properly, the consequences can be devastating. Loss of tolerance to food antigens can manifest as food allergies, while loss of tolerance to the luminal commensal flora contributes to the development of inflammatory bowel disease. In any case the gut epithelial barrier does not separate the luminal contents from the mucosal immune system and the passage of antigens is inappropriately regulated. Gut epithelial barrier dysfunction could allow luminal antigens to enter the lamina propria and plasma proteins and cells to leak into the lumen.⁶

Certain food and inhalatory allergens induce a sensitization state that can lead to a clinical picture of intolerance or allergy. Since the intestinal mucosa receives most of the antigen load, food allergy may present within a few weeks after the initial intake of cows' milk (CM) or maternal milk.⁷ The most affected organs are the skin and the intestinal and respiratory mucosae.⁸ A persistent involvement of these mucosae can be accompanied by protein loss through faeces or through extensive cutaneous lesions, leading to hypogammaglobulinaemia.^{2,9}

Thirty-four patients less than 2 years of age and with different clinical pictures associated with hypogammaglobulinaemia were studied during 2004–2005. Once a primary immunodeficiency (PID) was ruled out (32/34), a hypogammaglobulinaemia secondary to protein loss was considered, and cow milk allergy (CMA) was suspected based on the clinical picture. Ten patients showed clinical signs of atopy and positive specific IgE antibodies. In five of the 10 patients protein loss through the mucosa was suspected. Cow milk was removed from the diet as a therapy, leading to resolution of symptoms as well as normalization of immunoglobulin values.

Materials and methods

Subjects

During 2004–2005, 34 patients younger than 2 years of age with hypogammaglobulinaemia were studied. They

showed clinical signs of diarrhoea, infections and eczema and were referred to the Immunology Service to investigate a possible PID. In two of them PID was diagnosed, while in the remaining 32 subjects immunoglobulin levels were lower than normal according to age, and they showed a normal antibody function. Ten out of the 32 patients showed CM-specific IgE antibodies. Five patients (aged 4–18 months, two females and three males) with hypogammaglobulinaemia (serum immunoglobulin levels 1 SD or 2 SD below the mean value for age) suffered chronic diarrhoea (four of the five), bronchoconstriction (three of the five) and severe eczema (one of the five). These patients were thoroughly studied in this work because we hypothesized that there was likely protein loss occurring through the mucosa.

α 1-Antitrypsin clearance

Levels of α 1-antitrypsin were measured by radial immunodiffusion (DAKO Denmark, Glostrup, Denmark) in faecal samples, collected throughout 48 hr, and in serum samples. Values lower than 12.5 ml/24 hr were considered normal.

Immunoglobulin measurement

Total serum immunoglobulin levels were measured by nephelometry using commercially available kits (Beckman Immunochemistry Systems; IgM, IgG and IgA; Beckman Coulter Inc., Fullerton, CA). Specific antibody production was not evaluated. An Array 360 System (Beckman Coulter Inc., Fullerton, CA) was used.

Humoral immune response analysis

The capacity of the immune system to respond to specific stimuli was assessed through the administration of tetanus toxoid vaccine. The presence of specific antitetanus antibodies in serum was investigated 20 days later, using an indirect enzyme-linked immunosorbent assay (ELISA). A serum sample obtained at the moment of vaccination was assessed in parallel.

Analysis of peripheral cell subsets

Peripheral blood mononuclear cells from heparinized venous blood of patients were isolated on Ficoll–Hypaque gradients (Histopaque-1077, Sigma Chemical Co., St. Louis, MO), and different cell subsets were analysed by multiparametric flow cytometry in a FACScan (Becton Dickinson, San Jose, CA). Cells were stained for various surface markers by incubation with specific conjugated monoclonal antibodies (Becton Dickinson): anti-CD3 peridinin chlorophyll protein (PerCP; SK3), anti-CD4 fluorescein isothiocyanate (FITC; SK1), anti-CD8 phycoerythrin (PE; SK7), anti-CD20 PE (B73.1), anti-CD56

PE (NCAM 16.2). The respective isotype controls were performed.

In vitro lymphocyte proliferative responses to mitogens

Isolated peripheral blood mononuclear cells were cultured in microtitre plates with culture medium (RPMI-1640) supplemented with 10% fetal calf serum and antibiotics. Cells were grown at 37° with 5% CO₂. Cells (1 × 10⁵ per well) were stimulated for 72 hr with different mitogens: phytohaemagglutinin (PHA; 8 µg/ml), pokeweed mitogen (PWM; 2 µg/ml), phorbol 12-myristate 13-acetate (PMA)/Ionomycin (Io) (0.87 µg/ml), concanavalin A (Con A; 2 µg/ml), OKT3 (engineered monoclonal antibody to the T3 antigen of human T cells; 1 µg/ml). The cells were pulsed for the last 17 hr with 1 µCi/well [³H]thymidine. Radioactivity incorporated into cellular DNA was measured in a β scintillation counter.

Serum IgE measurement

Total IgE and CM-specific IgE were measured using capture ELISA and enzyme allegro sorbent test, respectively.¹⁰

Skin test

In vivo specific IgE was evaluated by skin prick tests on the anterior surface of the forearm using commercial extracts of whole cow milk (IgCenter, Buenos Aires, Argentina), in parallel with control solutions of buffered saline and histamine chlorhydrate (10 mg/ml). The wheals were measured after 15 min. Patients with skin reactions at least 3 mm greater than the negative control were defined as positive.

Coeliac disease serology

Screening methods for coeliac disease were used. IgG and IgA antibodies against tissue transglutaminase were measured by an indirect ELISA using recombinant human

transglutaminase (Biosystems SA, Barcelona, Spain). Anti-endomysial IgG and IgA antibodies were assessed by indirect immunofluorescence assay using commercial slides of monkey oesophagus sections (INOVA Diagnostic Inc., San Diego, CA).

Tissue specimens

Duodenal mucosal samples were obtained by biopsy during endoscopic examination, and specimens were processed for routine histological examination with standard formalin fixation and paraffin embedding. Sections were stained with haematoxylin & eosin to establish the villus : crypt ratio and the presence of cellular infiltrates.

Results

The age of symptom onset ranged between 2 days and 18 months. All the patients were seen in the Immunology Service with a presumptive diagnosis of PID based on the presence of different clinical signs associated with a clinical picture of hypogammaglobulinaemia.

Patient 1 (GV, aged 11 months) presented with chronic diarrhoea and treatment-resistant recurrent bronchiolytic syndrome leading to several hospitalizations. Both mal-absorption syndrome and cystic fibrosis were ruled out. A family history of atopy was recorded. At the first visit the patient had reduced IgG levels (Table 1), increased levels of total IgE and CM-specific IgE, and a positive prick test (Table 2). α1-Antitrypsin clearance was 15.6 ml/24 hr. Seven months after elimination of CM from the diet, serum immunoglobulin levels were normal (Table 1) and the patient had a favourable clinical course with resolution of diarrhoea and bronchoconstriction.

Patient 2 (CF, 18 months) presented with chronic diarrhoea secondary to a viral gastrointestinal infection. This patient required a prolonged hospitalization because of a severe hypoalbuminaemia accompanied by generalized oedema. Serum immunoglobulin levels were measured, and a severe hypogammaglobulinaemia involving the

Table 1. Serum immunoglobulin levels (mg/dl) before and after the exclusion diet

Patient	Age (months)	Immunoglobulin level before treatment			Immunoglobulin level after treatment		
		IgG ¹	IgA	IgM	IgG	IgA	IgM
GV	11	504 ² (935 ± 346)	27 (47 ± 29)	78 (120 ± 44)	804 (1094 ± 358)	67 (86 ± 48)	124 (119 ± 36)
CF	18	102 ³ (1094 ± 358)	12 ² (86 ± 48)	30 ³ (119 ± 36)	450 ³ (1089 ± 259)	27 ² (86 ± 48)	59 ² (105 ± 26)
CL	3	139 ³ (555 ± 132)	10 (22 ± 15)	46 (59 ± 41)	489 ² (935 ± 346)	13 ² (47 ± 29)	76 (120 ± 44)
RI	8	538 ² (935 ± 346)	57 (47 ± 29)	86 (120 ± 44)	766 (935 ± 346)	42 (47 ± 29)	66 ² (120 ± 44)
MF	10	486 ² (935 ± 346)	7 ² (47 ± 29)	115 (120 ± 44)	564 ² (1094 ± 358)	9 ² (86 ± 48)	101 (119 ± 36)

¹NV normal value.

²Immunoglobulin level 1SD lower than mean for age.

³Immunoglobulin level 2 SD lower than mean for age.

AAT, α1-antitrypsin; normal value <12 ml/24 hr.

Table 2. Results from Prick test, serum IgE levels and α 1-antitrypsin clearance

Patient	Prick test ¹ (mm)	Total IgE ² (IU/ml)	CM-specific IgE ³ (classes)	AAT clearance (ml/24 hr)
GV	3	167	2	15.6
CF	0	104	1	18.5
CL	ND	551	2	3.3
RI	4	106	2	18.2
MF	ND	118	3	18.2

¹Positive prick test: papule-erythema diameter > 3 mm. ND, not done.

²Normal values for total IgE: lower than 60 IU/ml.

³CM-specific IgE: class 0, negative; classes 1–4, positive.

AAT clearance, α 1-antitrypsin clearance. Normal value: < 12 ml/24 hr.

three isotypes (IgG, IgA, IgM) was detected. The study of peripheral cell subpopulations revealed a CD4⁺ T-cell lymphopenia and diminished cellular function. Histological examination of intestinal biopsies revealed a grade II enteropathy (widened villi in the duodenal mucosa, abundant enterocytes, preserved microvilli, lamina propria oedema, plasma cell infiltrate, and occasional eosinophil infiltrate). Coeliac disease serology was negative. The α 1-antitrypsin clearance was 18.5 ml/24 hr. Parenteral feeding led to clinical improvement. A subsequent immunological evaluation revealed a marked improvement of serum levels of immunoglobulins, and normalization of lymphocyte subpopulations and of functional humoral and cellular immune responses, which led us to rule out a primary immunodeficiency. A second intestinal biopsy revealed mild oesophagitis, moderate to severe enteropathy, unspecific colitis (oesophagitis in the lower third, pale and oedematous gastric mucosa, duodenitis, vascular pattern, and pale, oedematous and friable colonic mucosa). Addition of CM to the diet worsened the diarrhoea and the generalized oedema, leading to re-hospitalization. Faecal protein loss was demonstrated by an increased α 1-antitrypsin clearance. Since intolerance to cow milk proteins (CMP) was suspected, CM-specific IgE antibodies were measured and prick tests were performed (Table 2). Avoidance of CM was indicated because specific IgE antibodies as well as high levels of serum total IgE were detected. Complete restriction of CM allowed the patient to recover. Immunoglobulin levels after 10 months of diet exclusion are shown in Table 1.

Patient 3 (CL, 3 months) was referred with severe eczema involving 100% of the body surface. Immunoglobulin levels were measured to rule out a combined immunodeficiency, and reduced levels of IgG were found (more than 2 SD below the mean for age) (Table 1). The patient had been fed with infant formula since birth, and had presented with seborrheic eczema in the scalp within the first 24 hr of life, which evolved to a generalized

severe atopic dermatitis at 20 days. Weight and height showed adequate progress and the patient did not suffer severe infections. Immunological studies revealed functional antibodies, normal leucocyte count, and lymphocyte populations within normal percentages for age. The generalized skin involvement and the early age led to suspicions of an alimentary allergy to CMP. Therefore, total IgE and CM-specific IgE were measured (Table 2). The generalized cutaneous involvement precluded the execution of skin tests. The clearance of α 1-antitrypsin was normal (3.3 ml/24 hr). Taking into account the presence of serum-specific IgE and the clinical picture consistent with CMA, CM was eliminated from the patient's diet and was replaced with infant milk formula containing a hydrolysed protein. This led to a marked improvement of symptoms and serum immunoglobulins. At the 1 year follow-up, the clinical picture of the patient showed continued favourable progress. In Table 1 immunoglobulin levels before and after 5 months of CM exclusion diet are shown.

Patient 4 (RI, 12 months) had recurrent respiratory infections and chronic diarrhoea. As shown in Table 1, serum immunoglobulin measurement revealed diminished IgG levels, a good functional antibody response, and lymphocyte subsets within normal percentages for age. Total IgE levels were increased for age, and CM-specific IgE antibodies were detected. In addition, the skin test was positive for CMP (Table 2). The clearance of α 1-antitrypsin was 18.2 ml/24 hr. This patient was usually breastfed, and the clinical signs resolved when his mother started a CMP-free diet. He was also indicated to avoid CM ingestion and after a 3-month period of diet exclusion the serum IgG level was normalized.

Patient 5 (MF, 10 months) had ponderal growth retardation and a history of chronic diarrhoea and recurrent bronchial obstruction associated with hypochromic microcytic anaemia. Serum immunoglobulin measurements (Table 1) revealed diminished levels of IgG and IgA. Total IgE level was increased and CM-specific IgE was detected. The clearance of α 1-antitrypsin was 18.2 ml/24 hr. Since CMA was suspected, an exclusion diet was indicated, which led to a marked clinical improvement. As depicted in Table 1 immunoglobulin levels showed a slight recovery after 6 months of CM exclusion.

The response to tetanus toxoid was normal in all patients (normal value 0.1 IU/ml). Lymphocyte proliferation assays with mitogens and cell subset analyses were normal in four patients. Only one patient (Patient 2) had a low number of CD4⁺ T cells shown by flow cytometry, and also had decreased lymphoproliferative response to PHA, Con A, PWM and PMA/Io. Serological tests for coeliac disease were negative in all patients (anti-endomysial and anti-transglutaminase IgG and IgA). Duodenal biopsy was performed in patients FC and RI because they showed more severe clinical manifestations. Histological

analyses revealed non-specific alterations, and coeliac disease was ruled out. No patient had human immunodeficiency virus-specific IgG antibodies.

Discussion

Cow milk allergy is one of the most common food allergies in childhood and constitutes a pathological condition in which the recognition of CMP in the intestinal mucosa is altered. About half of the immunological responses to food are IgE-mediated^{8,11} and it has become evident that allergen-specific T helper type 2 cells play a central role in the genesis and maintenance of the allergic inflammatory reactions in both human subjects and mouse models.^{12,13} Factors responsible for the polarization of the specific immune response to a predominant T helper type 2 response in atopic subjects and laboratory animals remain undefined. Both environmental and genetic factors may influence the differentiation of T cells from a common precursor. Functional and phenotypic differences in subclasses of dendritic cells^{14,15} and epithelial cells^{16,17} between allergic and non-allergic donors have been reported. These cells may be involved in the abnormal activation of T cells against innocuous antigens.

The diagnosis of CMA is based on the family and personal history, complementary *in vivo* and *in vitro* tests and the double-blind placebo-controlled food challenge (DBPCFC), which is considered the confirmatory standard test.¹⁸ Although the DBPCFC is 100% specific, it is time-consuming and may elicit undesirable reactions in positive challenges. In addition, life-threatening anaphylactic reactions may occur in patients with a previous history of severe reactions.¹⁹ The DBPCFC is not performed in Argentina; the screening includes only an elimination diet.

Immunoglobulin synthesis begins immediately after birth in response to colonization of the gastrointestinal tract, infections and other antigenic stimulation. This reciprocal interaction between the intestinal microbiota, mainly commensal microbes, and the mucosal immune system may contribute to immune disorders, such as allergies and inflammatory bowel disease.²⁰ In this context, the intestinal microbiota and the integrity of the intestinal barrier are relevant for the appropriate development of the B-lymphocyte repertoire. PID arises as a consequence of failures in some of the immunological mechanisms involved in these complex processes. In the last 10 years, as a result of greater knowledge of their pathogenesis and with the implementation of more modern and accurate laboratory tests, different clinical conditions have been identified.^{1,3,21} Although numerous pathogenic mechanisms have been proposed, the cause of the transient hypogammaglobulinaemia of infancy remains unknown.²² A delayed maturation of B-cell function²³ defects in T helper cell maturation²⁴ or cytokine

imbalance^{25,26} may underlie the impaired synthesis of antibodies. On the other hand, secondary immunodeficiencies exhibit similar clinical pictures and can be caused by very diverse processes (onco-haematological, metabolic or infectious diseases, malnutrition, etc.).^{9,27}

The Argentinean Society for Paediatrics (SAP) published the PID registry for the 1994–1999 period.²⁸ It included 652 immunodeficiency cases, of which 489 (75%) were humoral deficiencies. In 137 cases (28%) the diagnosis was established before the age of 2 years. Transient hypogammaglobulinaemia of infancy was diagnosed in 26 patients. In 2004 an internal SAP bulletin reported 1240 cases of PID, of which 871 (70%) corresponded to humoral immunodeficiencies (unpublished data).

In the current study patients showed a symptomatic hypogammaglobulinaemia and were referred to the Immunology Service with clinical signs consistent with an early manifestation of a primary immunodeficiency. Once PID was ruled out, the hypogammaglobulinaemia was considered to be secondary to other conditions. Food allergy was documented by history and elevated serum IgE levels. We suspected that in five patients the hypogammaglobulinaemia was the consequence of protein loss through the skin or the gut.

Patient 1 (GV), while having a clinical picture suggestive of CMA, had a strong family history of allergic disease. Her parents had atopic disease and she had a 4-year-old-sister with CMA diagnosed at the age of 10 months. Her sister had been treated with an exclusion diet up to the age of 2 years, and two years later she was diagnosed a coeliac disease. The coexistence of coeliac disease and CMA has been previously reported.²⁹ Patient 2 (CF) was the only one presenting with lymphopenia, which was probably associated with the hypogammaglobulinaemia. This deficiency was severe and included the three immunoglobulin isotypes measured. The damaged gut mucosa and the augmented clearance of α 1-antitrypsin led us to consider that the diminished serum levels of immunoglobulins and albumin could result from protein loss through stools. The loss of IgM would reflect a severe intestinal involvement, because this protein has a high molecular weight. The presence of acute malnutrition during the initial phase of the disease masked the diagnosis. The diminished functional cellular response in the first assessment was linked to the acute loss of lymphocytes and nutrients.^{2,9,27} After 10 months of CM-free diet, the patient exhibited a marked improvement in the serum level of immunoglobulins. Patient 3 (CL) had an important cutaneous reaction, possibly as a result of her atopic condition. It is usually assumed that IgG loss occurs mainly through skin. After the initiation of treatment the eczema improved and the serum IgG level recovered thereafter. It is widely described that some of the most common clinical manifestations of CMA are the cutaneous reactions, mainly atopic dermatitis.^{30,31} Patient 4 (RI)

had a strong family history of systemic diseases (atopy, rheumatoid arthritis, coeliac disease). Family history disclosed an atopic mother affected by rheumatoid arthritis, a cousin with coeliac disease and an aunt affected by systemic lupus erythematosus. Latham *et al.* reported familial history of autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, thyroiditis, etc.) in 30% of children suffering CMA.³² A relationship with food allergy was clear in Patient 5 (MF) because she presented with progressive reduction of weight and insignificant increase in height, and had clinical gastrointestinal and pulmonary signs. A CM exclusion diet also led to clinical improvement and serum immunoglobulin recovery. Regarding IgE antibodies, all the patients studied exhibited high levels of total and specific IgE. Skin prick tests could only be performed in three patients, two of whom had positive results. The absence of a skin response to allergens could relate to the low age of the patients.^{33,34} Although specific IgE to CMP was detected in all our patients, it must be kept in mind that the absence of these antibodies does not rule out an allergic process. A marked improvement in clinical and laboratory parameters after allergen restriction is a strong indicator of this entity, which is further confirmed by an immediate reappearance of the clinical picture upon a new inclusion of the offending allergen. The complete recovery of the IgA level after treatment may be delayed. The effect of the exclusion diet treatment may lie on the retrieval of the mucosal integrity. IgA antibodies are mainly produced by B cells from the lamina propria and the commensal flora should be restored to achieve the homeostasis of the gut mucosa.

Fineman *et al.* have reported that hypogammaglobulinaemia associated with elevated IgE level and food allergy may be secondary to a decreased production of antibodies. This study included four patients younger than 2 years of age and gastrointestinal protein loss was not detected. The authors hypothesized that T-cell-dependent B-cell activation was impaired. All patients were treated with intramuscular gammaglobulin and had a diet free of the suspected allergen. Both recovery of immunoglobulin values and clinical resolution were achieved.²³ Walker *et al.* found that a significant proportion of children with hypogammaglobulinaemia suffered food allergy. They suggested that the allergen-induced gastrointestinal inflammation might be a contributing factor to the protein loss from the bowel. They found normal number and function of lymphocytes, thus ruling out a deficiency of T helper cells.³⁵ Kilic *et al.* also described hypogammaglobulinaemic patients with allergic symptoms (mainly bronchial asthma and atopic dermatitis), with a normal capacity to synthesize specific antibodies in response to immunization. Patients needed replacement therapy with intravenous immunoglobulin to recover the immunoglobulin levels.³⁶ Kidon *et al.* also reported a high prevalence

of atopic manifestations in patients with symptomatic hypogammaglobulinaemia.³⁷

In summary, CMA, which can be easily treated, must be suspected in children with clinical signs of atopy and a clinical picture and laboratory findings consistent with PID, once this latter condition and other underlying diseases have been ruled out. On the other hand, transient hypogammaglobulinaemia should lead the physician to suspect a secondary condition associated with protein loss in children with diarrhoea. The α 1-antitrypsin clearance is a significant marker of mucosal integrity and constitutes the first evidence of protein loss.^{2,9}

Although we have found a 14.7% association between hypogammaglobulinaemia and protein loss as a consequence of CMA, more patients will be included in the study to estimate the prevalence of this condition.

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References

- 1 Simonte SJ, Cunningham-Rundles C. Update on primary immunodeficiency: defects of lymphocytes. *Clin Immunol* 2003; **109**:109–18.
- 2 Ochs H, Stiehm E, Winkelstein J. Antibody deficiencies. In: Stiehm RE, Ochs H, Winkelstein J, eds. *Immunologic Disorders in Infants and Children*, 5th edn. Philadelphia: Elsevier Saunders, 2004:356–426.
- 3 IUIS. Scientific committee Primary Immunodeficiency Diseases. *Clin Exp Immunol*; 1999; **118** (Suppl. 1):1–28.
- 4 Burgio GR, Lanzavecchia A, Plebani A, Jayakar S, Ugazio AG. Ontogeny of secretory immunity: levels of secretory IgA and natural antibodies in saliva. *Pediatr Res* 1980; **14**:1111–14.
- 5 Hyman PE, Clarke DD, Everett SL, Sonne B, Stewart D, Harada T, Walsh JH, Taylor IL. Gastric acid secretory function in preterm infants. *J Pediatr* 1985; **106**:467–71.
- 6 Unno N, Fink MP. Intestinal epithelial hyperpermeability. Mechanisms and relevance to disease. *Gastroenterol Clin North Am* 1998; **27**:289–307.
- 7 Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG. Transplacental priming of the human immune system to environmental allergens. Universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; **160**:4730–7.
- 8 Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999; **103**:717–28.
- 9 Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986; **109**:270–6.
- 10 Docena GH, Fernandez R, Chirido FG, Fossati CA. Identification of casein as the major allergenic and antigenic protein of cow's milk. *Allergy* 1996; **51**:412–16.
- 11 Powell GK. Enterocolitis in low-birth-weight infants associated with milk and soy protein intolerance. *J Pediatr* 1976; **88**:840–4.

- 12 Morafo V, Srivastava K, Huang CK, Kleiner G, Lee SY, Sampson HA, Li XM. Genetic susceptibility to food allergy is linked to differential TH2-TH1 responses in C3H/HeJ and BALB/c mice. *J Allergy Clin Immunol* 2003; **111**:1122–8.
- 13 Bosco A, McKenna KL, Devitt CJ, Firth MJ, Sly PD, Holt PG. Identification of novel Th2-associated genes in T memory responses to allergens. *J Immunol* 2006; **176**:4766–77.
- 14 Maldonado-Lopez R, Maliszewski C, Urbain J, Moser M. Cytokines regulate the capacity of CD8alpha(+) and CD8alpha(-) dendritic cells to prime Th1/Th2 cells *in vivo*. *J Immunol* 2001; **167**:4345–50.
- 15 Hagendorens MM, Ebo DG, Schuerwegh AJ, Huybrechs A, Van Bever HP, Bridts CH, De Clerck LS, Stevens WJ. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy* 2003; **33**:633–9.
- 16 Soumelis V, Reche PA, Kanzler H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; **3**:673–80.
- 17 Roggen EL, Lindstedt M, Borrebaeck C, Verheyen GR. Interactions between dendritic cells and epithelial cells in allergic disease. *Toxicol Lett* 2006; **162**:71–82.
- 18 Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, Metcalfe DD. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988; **82**:986–97.
- 19 Sicherer SH, Morrow EH, Sampson HA. Dose–response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 2000; **105**:582–6.
- 20 Noverr MC, Huffnagle GB. The ‘microflora hypothesis’ of allergic diseases. *Clin Exp Allergy* 2005; **35**:1511–20.
- 21 Shearer WT, Cunningham-Rundles C, Ochs HD. Primary immunodeficiency: looking backwards, looking forwards. *J Allergy Clin Immunol* 2004; **113**:607–9.
- 22 Dalal I, Roifman CM. Hypogammaglobulinemia of infancy. *Immunol Allergy Clin North Am* 2001; **21**:129–39.
- 23 Fineman SM, Rosen FS, Geha RS. Transient hypogammaglobulinemia, elevated immunoglobulin E levels, and food allergy. *J Allergy Clin Immunol* 1979; **64**:216–22.
- 24 Siegel RL, Issekutz T, Schwaber J, Rosen FS, Geha RS. Deficiency of T helper cells in transient hypogammaglobulinemia of infancy. *N Engl J Med* 1981; **305**:1307–13.
- 25 Kowalczyk D, Mytar B, Zembala M. Cytokine production in transient hypogammaglobulinemia and isolated IgA deficiency. *J Allergy Clin Immunol* 1997 October; **100**:556–62.
- 26 Kowalczyk D, Baran J, Webster AD, Zembala M. Intracellular cytokine production by Th1/Th2 lymphocytes and monocytes of children with symptomatic transient hypogammaglobulinaemia of infancy (THI) and selective IgA deficiency (SIgAD). *Clin Exp Immunol* 2002; **127**:507–12.
- 27 Cunningham-Rundles C, Carr RI, Good RA. Dietary protein antigenemia in humoral immunodeficiency. Correlation with splenomegaly. *Am J Med* 1984; **76**:181–5.
- 28 Grupo de Trabajo de Inmunología comité SAP. Inmunodeficiencias primarias: informe del Registro Argentino. *Arch Argentino Pediatría* 2001; **99**:263–8.
- 29 Zauli D, Grassi A, Granito A, Foderaro S, De Franceschi L, Ballardini G, Bianchi FB, Volta U. Prevalence of silent coeliac disease in atopics. *Dig Liver Dis* 2000; **32**:775–9.
- 30 Sampson HA. The evaluation and management of food allergy in atopic dermatitis. *Clin Dermatol* 2003; **21**:183–92.
- 31 Nitta A, Suzumura H, Tsuboi Y, Yoshihara S, Arisaka O. Cow’s milk allergy with severe atopic dermatitis in a 605-g extremely low birth weight infant. *J Pediatr* 2006; **148**:282.
- 32 Latcham F, Merino F, Lang A *et al.* A consistent pattern of minor immunodeficiency and subtle enteropathy in children with multiple food allergy. *J Pediatr* 2003; **143**:39–47.
- 33 Hill DJ, Duke AM, Hosking CS, Hudson IL. Clinical manifestations of cows’ milk allergy in childhood. II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988; **18**:481–90.
- 34 Hsieh KY, Tsai CC, Wu CH, Lin RH. Epicutaneous exposure to protein antigen and food allergy. *Clin Exp Allergy* 2003; **33**:1067–75.
- 35 Walker AM, Kemp AS, Hill DJ, Shelton MJ. Features of transient hypogammaglobulinaemia in infants screened for immunological abnormalities. *Arch Dis Child* 1994; **70**:183–6.
- 36 Kilic SS, Tezcan I, Sanal O, Metin A, Ersoy F. Transient hypogammaglobulinemia of infancy: clinical and immunologic features of 40 new cases. *Pediatr Int* 2000; **42**:647–50.
- 37 Kidon MI, Handzel ZT, Schwartz R, Altboum I, Stein M, Zan-Bar I. Symptomatic hypogammaglobulinemia in infancy and childhood-clinical outcome and *in vitro* immune responses. *BMC Fam Pract* 2004; **October** **21**:5–23.