International Immunopharmacology 14 (2012) 94–98

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Characteristics of patients suffering from cow milk allergy

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ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 5 June 2012
Accepted 6 June 2012
Available online 18 June 2012

Keywords:
Cow milk allergy
Cow milk-specific IgG
Cow milk-specific IgE
Cow milk protein

ABSTRACT

The most frequent symptoms among the manifestations of cow milk allergy (CMA) are gastrointestinal. CMA pathogenesis involves immunological mechanisms with participation of immunocompetent cells, production of immunoglobulin E (IgE) and immunoglobulin G (IgG). We aim to determine whether cow milk-specific IgE antibodies coexist with cow milk-specific IgG antibodies in CMA patients with diarrhea symptom, and if there is any relationship between both antibody types. 65 CMA patients (average age of 17 years, ranging from 2 to 74 years), all of who had diarrhea symptom of CMA, were enrolled in this study. The total cow IgE and IgG subclass in serum were measured by electrochemiluminescence immunoassay and rate immune scatter turbidimetry, respectively. And also the cow milk-specific IgE was determined by enzyme-linked immunosorbent assay. The number of eosinophils in serum was calculated by Sysmex XE-2100 Hematology Analyzer. Our data showed that both cow milk-specific IgG and IgE levels were significantly elevated in CMA patients compared to those of age-matched control subjects. Out of the 65 CMA patients, 40 showed elevated cow milk-specific IgE antibody level, among which, 28 cases presented highly sensitive reaction to cow milk-specific IgG, along with each six of moderate and mild sensitive reaction to cow milk-specific IgG; while 20 showed elevated total IgG levels. The IgG3 positive rate was 16.9%, which was the highest. A moderate correlation between cow milk-specific IgE and cow milk-specific IgG was found in the CMA patients ($r = 0.415$, $P = 0.001$). The results indicated that cow milk-specific IgE antibodies could coexist with cow milk-specific IgG antibodies in patients suffering from CMA. The aberrant changes in the concentration of cow milk-specific IgE antibodies were associated with cow milk-specific IgG antibodies.

1. Introduction

Human milk is the optimal food for human infants, but cow milk usually substitutes for human milk when breast-feeding is not available. This substitution can lead to nutritional and immunological problems, such as cow milk protein allergy. The milk proteins act as antigens in the body, and promote the production of milk-specific antibodies, which consequently lead to the inflammation response in the corresponding tissues. Finally signs and symptoms will appear in the body [1]. Milk hypersensitivity in early childhood is mostly mediated by milk protein immunoglobulin E (IgE) [2] and dependent on the activation of mast cells in specific tissues, including the skin, respiratory tract, gastrointestinal, mucosal, and cardiovascular system [3–5,12]. Milk allergy has also been involved in type I allergies. The impact of other types of immune reactions to cow milk is presently controversial, such as the association of antibodies of immunoglobulin G (IgG) and immunoglobulin A (IgA) isotypes with cow milk-induced adverse gastrointestinal symptoms in adults [3,4]. A new scientific publication by Jönnson et al. [5] proved previous scientific findings [6,7] that IgG could also be implicated in anaphylaxis. Another study showed similar levels of IgA and IgG antibodies against cow milk proteins in healthy individuals and in CMA patients [8]. IgG-mediated CMA can take several days before its appearance. Immune complexes are then formed, causing partial or systemic type III or type IV allergies [9–12].

The objective of current research is to study whether cow milk-specific IgE antibodies coexist with cow milk-specific IgG antibodies in CMA patients with diarrhea symptom, and if there is any relationship between both antibody types.

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doi:10.1016/j.intimp.2012.06.008

2. Materials and methods

2.1. Materials

In all, 65 CMA patients (average age of 17 years, ranging from 2 to 74 years, including 44 males and 21 females), who sought medical advice for the reason of diarrhea from November 2009 to September 2010, were screened at the Department of Digestive System of Xiamen Zhongshan Hospital. The clinical diagnosis of cow milk allergy was based on World Allergy Organization Diagnosis and Rationale for Action against Cow Milk Allergy guidelines [13,14]. The detailed characteristics of the investigated study groups were shown in Table 3. 45 healthy subjects (average age of 18 years, ranging from three to 70 years, including 24 males and 21 females) were also recruited. For infant's selection, all those breast-fed infants were excluded, and only formula cow milk-fed ones were involved. 6 mL blood samples were draw from all subjects followed by the completion of questionnaires on gastrointestinal symptoms and dairy consumption. This research was subjected to the approval of local ethics committee, and full written informed consent was obtained from the subjects.

2.2. Laboratory test

2.2.1. Measurement of milk-specific IgG antibody in serum

Blood samples were allowed to stand for 20–30 min before centrifugation at 3000 RPM for 15 min. The serum was aspirated and frozen at −20° for subsequent analysis. Samples were processed in the central laboratory (Xiamen Zhongshan Hospital, China) using a commercially available enzyme-linked immunosorbent KIT (ELISA, Biomerica, Inc., Newport Beach, CA, USA, Kit number: 7145-HA). All manipulations were according to the instructions of the ELISA Kit, and the following are the brief procedures.

Prepare 50, 100, 200 and 400 U/mL of milk IgG calibrator and add into the microplate; set up blanks and positive controls. This was the calibration curve to be used in the assay. Dilute serum samples by 100 fold with serum dilute regents. Then add 100 μL of samples into the microwells precoated with milk antigens. Cover plate with plate cover and incubate for 60 min at room temperature. Thoroughly decant any liquid from wells and wash wells 4 times. Add 100 μL of milk IgG-HRP into each well; incubate for 30 min at room temperature. Thoroughly decant any liquid from wells and wash wells 4 times. Add 100 μL of working substrate mix (TMB and H_2O_2) into each well. Incubate for 10 min at room temperature in the dark. Add 50 μL of 1 N sulfuric acid per well to stop the reaction. Read the absorbance of each well at 450 nm using a BIO-RAD 550 ELISA plate reader (Bio-Rad Laboratories, Inc, USA). The IgG concentration against milk antigen was expressed as units per milliliter. And the following Table 1 defined the sensitive ranks according to IgG concentration.

2.2.2. Measurement of milk-specific IgE antibody in serum

Milk-specific IgE antibody in serum was measured using an ELISA kit (DR. FOOKE Laboratorien GmbH, Germany, Kit number: K980818). And the following Table 2 defined the sensitive ranks according to IgE concentration.

Table 1
The sensitive ranks according to IgG concentration.

Cow milk-specific IgG concentration (U/mL)	Rank
<50	Negative
50–100	Mild
100–200	Moderate
>200	High

Table 2
The sensitive ranks according to IgE concentration.

Cow milk-specific IgE concentration (U/mL)	Rank
<0.35	Negative
0.35–0.70	Low
0.70–3.50	Moderate
>3.50	High

2.2.3. Measurements of total serum IgG, IgG subclass antibody, total IgE and eosinophils

Total IgG and IgG subclass were measured using a full automation specific protein analyzer (Siemens, Germany, Total IgG, IgG1, IgG2, IgG3, IgG4 kit numbers 153063, 090145, 090249, 086145, 086046). The total IgE concentration was measured using an electrochemiluminescence immunoassay analyzer (Roche Modular Analytics E170; Roche Diagnostics, Mannheim, Germany, Kit number: 162012). The number of eosinophils was calculated with a Sysmex XE-2100 Hematology Analyzer (Sysmex, Kobe, Japan). All tests were performed following the manufacturer's specifications.

2.3. Statistical analysis

All statistical analysis was conducted using SPSS for Windows version 13. The data were expressed as mean ± standard deviation. Means of two independent groups were compared with an unpaired Student's t-test. Chi-square tests were employed to determine the significant differences across groups. The correlations between cow milk-specific IgG and cow milk-specific IgE were assessed using Pearson's correlation test. $P<0.05$ (2-tailed) was considered significant.

3. Results

3.1. Cow milk-specific IgG, cow milk-specific IgE and total IgE levels in CMA patients

Serum from CMA patients and controls were analyzed by ELISA and electrochemiluminescence immunoassay regarding their IgG and IgE reactivity to cow milk. Cow milk-specific IgG and IgE levels were significantly higher in CMA patients with diarrhea predominant symptom than those in healthy subjects (Table 4).

3.2. Serum IgG level

Serum from 65 CMA patients suffering from diarrhea were subjected to ELISA for measurement of cow milk-specific IgG. The cow milk-specific IgG concentration was 267.8 ± 195.1 U/mL which was positive. Among the 65 patients, 15 (23.1%) showed mildly sensitive reactivity, 17 (26.2%) were moderately sensitive reactivity, and 33 (50.8%) were highly sensitive reactivity. The concentrations of cow

Table 3
Characterization of study groups.

Number of patients with cow's milk allergy in diagnosis	65
Average age[a]	17 (2–74)
Males/females	44/21
Mean introduction time of cow's milk protein[b]	1–7
Presenting symptoms	
Gastrointestinal	65
Number of controls	45
Average age[a]	18 (3–70)
Males/females	24/21
Mean introduction time of cow' s milk protein	–

[a] Years. Results are expressed as mean value (scope).
[b] Days. Results are expressed as range.