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Original Article

Study on screening potential allergenic proteins from infant milk powders based on human mast cell membrane chromatography and histamine release assays

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ABSTRACT

Cow's milk allergy is mainly observed in infants and young children. Most allergic reactions affect the skin, followed by the gastrointestinal and respiratory systems. Conventional diagnosis is based on positive allergy studies and evaluation of parameters including IgE and IgG1 levels, acute allergic skin response and anaphylactic shock reactions. We developed a cell membrane chromatographic (CMC) method based on human mast cells (HMC-1) for screening potential allergens in infant formula milk powders (IFMP). HMC-1 cell membranes were extracted and mixed with silica to prepare cell membrane chromatography columns (10 mm × 2 mm i.d., 5 μm). Under the conditions of 0.2 mL/min flow rate and 214 nm detection wavelength, human breast milk showed no retention. However, IFMP showed clear retention. The retained fractions were collected and analyzed through matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Four major milk proteins, i.e., α-casein, β-casein, α-lactalbumin, and β-lactoglobulin A, were identified. Furthermore, these proteins and β-lactoglobulin B showed clear retention on HMC-1/CMC columns. To test the degranulation effects of the five proteins, histamine and β-hexosaminidase release assays were carried out. All five proteins induced HMC-1 cells to release histamine and β-hexosaminidase. Also, we established a reversed phase liquid chromatographic (RPLC) method for the determination of the five proteins in IFMP and the results showed that 90% proteins in IFMP were α-casein and β-casein. We concluded that cow's milk proteins may be potential allergens and caseins cause more β-casein allergic risk than other proteins. This conclusion was consistent with other studies.

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1. Introduction

Cow's milk allergy (CMA) is the most common food allergy in early childhood, with an incidence of 2%–3% in infants worldwide [1]. CMA is considered to be a failed oral tolerance induction resulting from complex interactions of gut permeability, bacterial colonization, and timing of antigen exposure [2]. Cow's milk (CM) typically consists of 80% caseins (CN) and 20% whey proteins. The latter mainly consists of β-lactoglobulin (β-LgA, β-LgB) and α-lactalbumin (α-Lac) in a 3:1 (w:w) ratio [3]. The main causal allergens in CMA are the caseins and proteins in lactoserum (β-lactoglobulin, α-lactoalbumin). The clinical manifestations of CMA are highly variable in their presentation and severity. Most allergic reactions affect the skin, followed by the gastrointestinal and respiratory systems. Severe anaphylaxis may occur [4].

The diagnosis of CMA is based on a suggestive clinical history, a positive allergy study and controlled exposure testing, which constitutes the gold standard for conformation [5]. Animal models have been used for evaluating the allergenicity of CM hydrolysates by determining parameters such as IgE and IgG1 levels [6], acute allergic skin response and anaphylactic shock reactions [7]. In these studies, however, IgE antibodies are sometimes generated upon systemic instead of oral sensitization [8]. The results are questionable with regard to the extrapolation to the human situation [9]. Over diagnosis and under diagnosis of CMA will occur when using allergen elimination for diagnosis. This can lead to inappropriate diets, escalating medical expenses, growth interference growth, decreased quality of life, and an overall public misconception about food allergies [2].

Based on this background, we proposed cell membrane chromatography (CMC) to screen for allergenic proteins in milk

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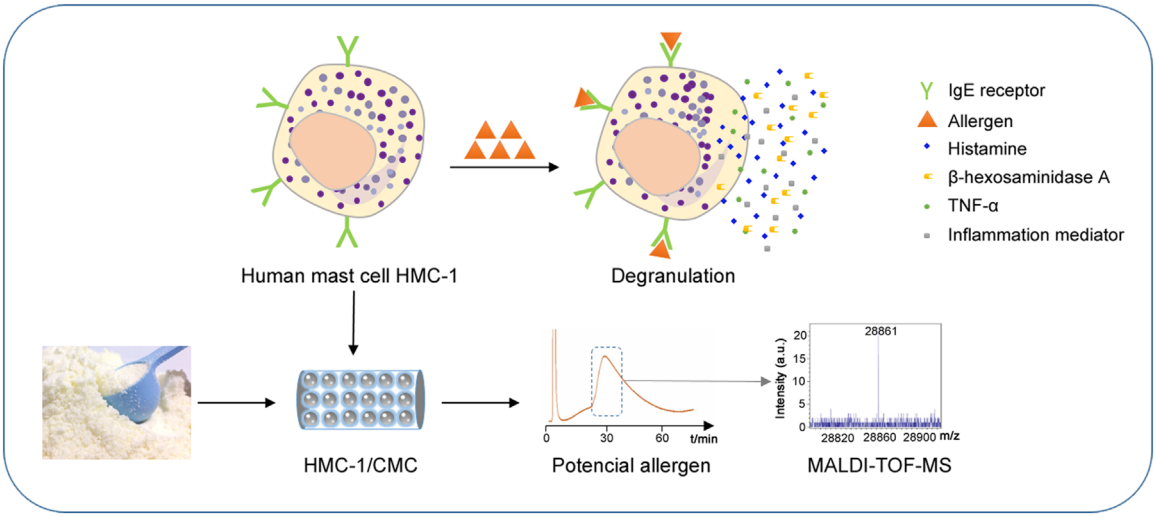

Fig. 1. Schematic illustration of HMC-1/CMC for screening potential allergic milk proteins.

powders. CMC is a bioaffinity chromatography technique, with a cell membrane stationary phase (CMSP) prepared by immobilizing cell membranes containing special receptors on a silica carrier [10]. For example, human mast cells (HMC-1) and rat basophilic cells (RBL-2H3) have a similar granular content to mast cells and are commonly used as prototypic convenient models in allergic studies [11]. In our previous work, RBL-2H3 cell membrane chromatography (RBL-2H3/CMC) was used successfully to screen allergic components from traditional Chinese injections [12]. In this study, we developed an HMC-1/CMC method for screening potential allergens in milk powders. The scheme is shown in Fig. 1. First, the potential allergens were screened through HMC-1/CMC and identified through matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Second, the potential allergens were incubated with HMC-1 cells. Third, histamine release assays were carried out through liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) and β-hexosaminidase assays by spectroscopy. Our method was simple, fast and inexpensive, and can predict potential allergens before allergic reactions occur.

2. Experimental

2.1. Chemicals and standard solutions

Bovine α_s-casein (α-CN), bovine β-casein (β-CN), bovine α-lactalbumin (α-Lac), bovine β-lactoglobulin A (β-LgA), bovine β-lactoglobulin B (β-LgB), histamine, urea, and n-octyl-β-D-glucopyranoside (OG) were from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA) and acetonitrile (ACN) were from Tedia Company (Fairfield, OH, USA). All other reagents were of analytical grade or higher grade. Silica gel (ZEX-II, 5 μm, 300 Å) was from Qingdao Meigao Chemical Co., Ltd. (Qingdao, China). All aqueous solutions were prepared using ultrapure water from an MK-459 Millipore Milli-Q Plus ultrapure water system. Commercial infant milk powder samples were from local shops.

Stock standard solutions of α-CN, α-Lac, β-CN, β-LgB, and β-LgA were prepared in a buffer consisting of 6 M urea and 0.2% OG (pH 3.3) [13], at concentrations of 10 mg/mL. Working solutions containing all analytes were % mM KCl, 2.54 mM $CaCl_2$, 1.19 mM KH_2PO_4, 10 mM HEPES, 5 mM glucose, 0.1% (w/v) BSA, pH 7.3). Stock internal standard solutions of histamine-d4 at 30 ng/mL were prepared in ACN.

2.2. Instrument configuration and chromatographic conditions

The analytical system consisted of a DGU-20A3 degasser, two LC-20AD pumps, an SIL-20A autosampler, a CTO-20AC column oven, an SPD-20A UV/Vis detector (UV), and an LC solution work station (Shimadzu Corporation, Kyoto, Japan). An HMC-1/CMC column (10 mm × 2 mm i.d., 5 μm) was packed using an RPL-10ZD column loading machine (Dalian Replete Science and Technology Co., Ltd., Dalian, China). The preparation of HMC-1/CMC columns is described in Section 2.3. The HMC-1/CMC column was thermostated at 37 °C and run with a mobile phase of water at 0.2 mL/min. Proteins were detected by UV absorption at 214 nm.

The LC-ESI–MS/MS system used for assays was a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of a binary pump (LC-20AD liquid chromatograph), an automatic solvent degasser (DGU-14A degasser), and an autosampler (SIL-20AD auto injector) coupled to a triple quadrupole LC-MS-8040 system equipped with an ESI interface. All MS data were measured in positive ion mode. Typically, source conditions were set as follows: nebulizing gas flow, 3 L/min; drying gas flow, 15 L/min; DL temperature, 250 °C; heat block temperature, 400 °C; and ESI voltage, 4.5 kV.

A Venusil HILIC column (2.1 mm × 150 mm, 3 μm; Agela, Tianjin, China) was used for LC-ESI–MS/MS. The mobile phase was 0.1% formic acid and 20 mM ammonium formate in water (solvent A) and 80% ACN (solvent B) at a flow rate of 0.3 mL/min.

2.3. Cell culture and preparation of CMC column

HMC-1 cell line was from American Type Culture Collection (USA). Cell cultures and HMC-1/CMC column preparation were performed as previously described [12,14,15]. HMC-1 cells were cultured in Dulbecco's modified Eagle medium (Invitrogen, Grand Island, USA) supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, and 100 U/mL streptomycin. Cells were cultured at 37 °C and 5% CO_2 in a humidified CF EasyFill-2 cell factory (Nunc, Denmark). Cells (7×10^6) were harvested at 80%–90% confluency and washed 3 times with physiological saline (pH 7.4) by centrifugation at 3000 *g*, 4 °C for 10 min. Cells were resuspended in 50 mM Tris–HCl (pH 7.4), ruptured by 30 min ultrasonication, homogenized for 3 min, and centrifuged at 1000 *g*, 4 °C for 10 min. Pellets were discarded and supernatants were centrifuged at 12,000 *g*, 4 °C for 20 min. Crude membrane precipitations were resuspended in 10 mL physiological saline and centrifuged at 12,000 *g*, 4 °C for 20 min. Precipitates were resuspended in 5 mL physiological saline and used for CMC column

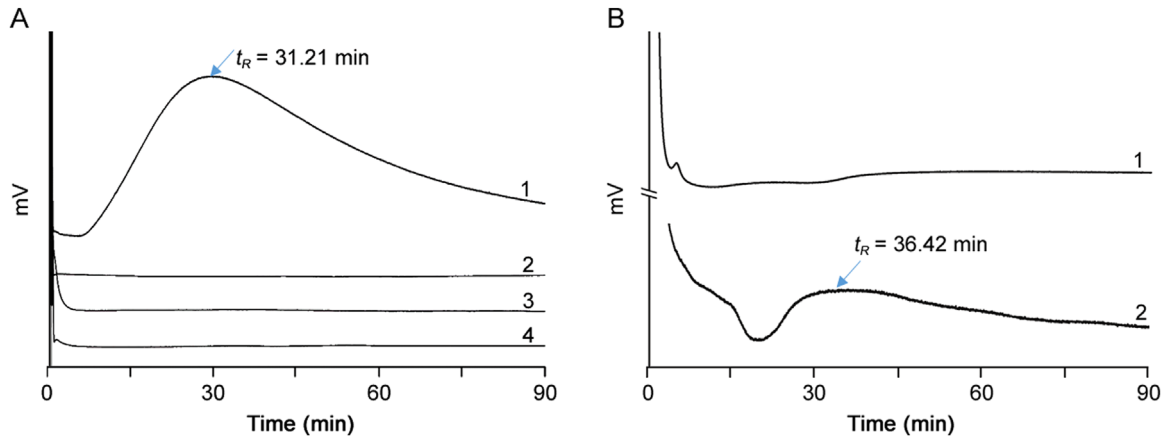

Fig. 2. Validation and application of the HMC-1/CMC model. (A) Retention of four drugs on HMC-1/CMC. 1.quercetin, 2. metoprolol, 3. captopril, 4. gefitinib. (B) Retention of breast milk (1) and IFMP (2) on the HMC-1/CMC.

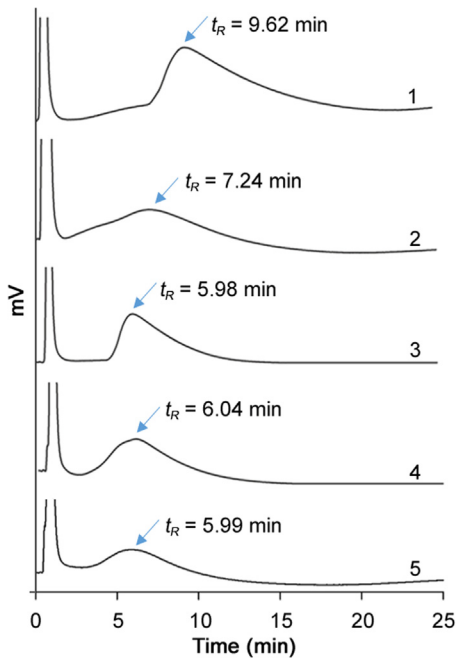

Fig. 3. Retention of five bovine milk proteins on HMC-1/CMC. 1.α-CN, 2. β-LgA, 3. β-CN, 4. α-Lac, 5. β-LgB.

preparation. To prepare CMC columns, 50 mg silica was activated at 105 °C for 30 min. Cell membrane suspensions prepared as above-mentioned were slowly added to the activated silica under a vacuum at 4 °C with agitation. Mixtures were agitated for 30 min with a magnetic stirrer and left to stand for 14 h. HMC-1/CMC columns were produced by packing the stationary phase as above-mentioned into a column (10 mm × 2.0 mm i.d.) following a wet packing procedure on a column loading machine. The well-prepared columns were stored at 4 °C until use. The life-span of this HMC-1/CMC column was about 3 days under a continuous usage.

2.4. Assays for screening potential allergenic proteins

Prepared HMC-1/CMC columns took approximately 2 h to establish equilibrium with the chromatographic system before injection. Standard solutions or sample solutions (10 μL) were injected. All assays were carried out within the lifespan of the columns. The retained fractions were collected and freeze dried.

Table 1
Retention time and capacity factors for five major proteins on HMC-1/CMC.

Proteins	t_R (min)	t_0 (min)	k[a]	Allergic[b]	Reference
α-CN	9.623	0.849	10.3	+	[29]
β-LgA	7.242	0.833	7.7	+	[31]
β-CN	5.985	0.846	6.1	+	[32]
α-Lac	6.045	0.848	6.1	+	[29]
β-LgB	5.994	0.853	6.0	+	[31]

[a] Retention factors were calculated from $k = (t_R\text{-}t_0)/\ t_0$, where t_R is retention time of proteins and t_0 is retention time of solvent.
[b] + represents protein reported as an allergen in the reference.

2.5. MALDI-TOF-MS identification

Identification of the retained fractions by MALDI-TOF-MS was performed on a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with FlexControl (version 3.0) software (Bruker Daltonics) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2–20 kDa, according to the instructions of the manufacturer. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation (version 2.0) software (Bruker Daltonics) and the default settings. The lyophilized powder of retention fraction was overlaid with 1 μL of HCCA matrix (a saturated solution of α -cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid) and air dried at room temperature to allow cocrystallization with the experimental sample. The spectra were then acquired by the mass spectrometer.

2.6. Histamine release assays

Histamine release from HMC-1 cells was evaluated using a method described by Tachibana et al. [16]. HMC-1 cells (3×10^5 cells/mL) were washed and resuspended in 1640 medium. Cells were grown in 96-well plates (3×10^4 cells/well) for 24 h at 37 °C and treated with various concentrations of α-CN, α-Lac, β-CN, β-LgB, and β-LgA (1, 5, and 10 mg/mL) for 30 min. After centrifuging at 1500 r/min, supernatants were collected and treated with a double volume of internal standard solution (5 ng/mL). After centrifuging at 12,000 *g*, supernatants were collected and injected into LC-ESI–MS/MS prepared for histamine analysis. Supernatants from unstimulated cells were used as negative controls, and supernatants from cells stimulated with A23187 and PMA were used as positive controls.

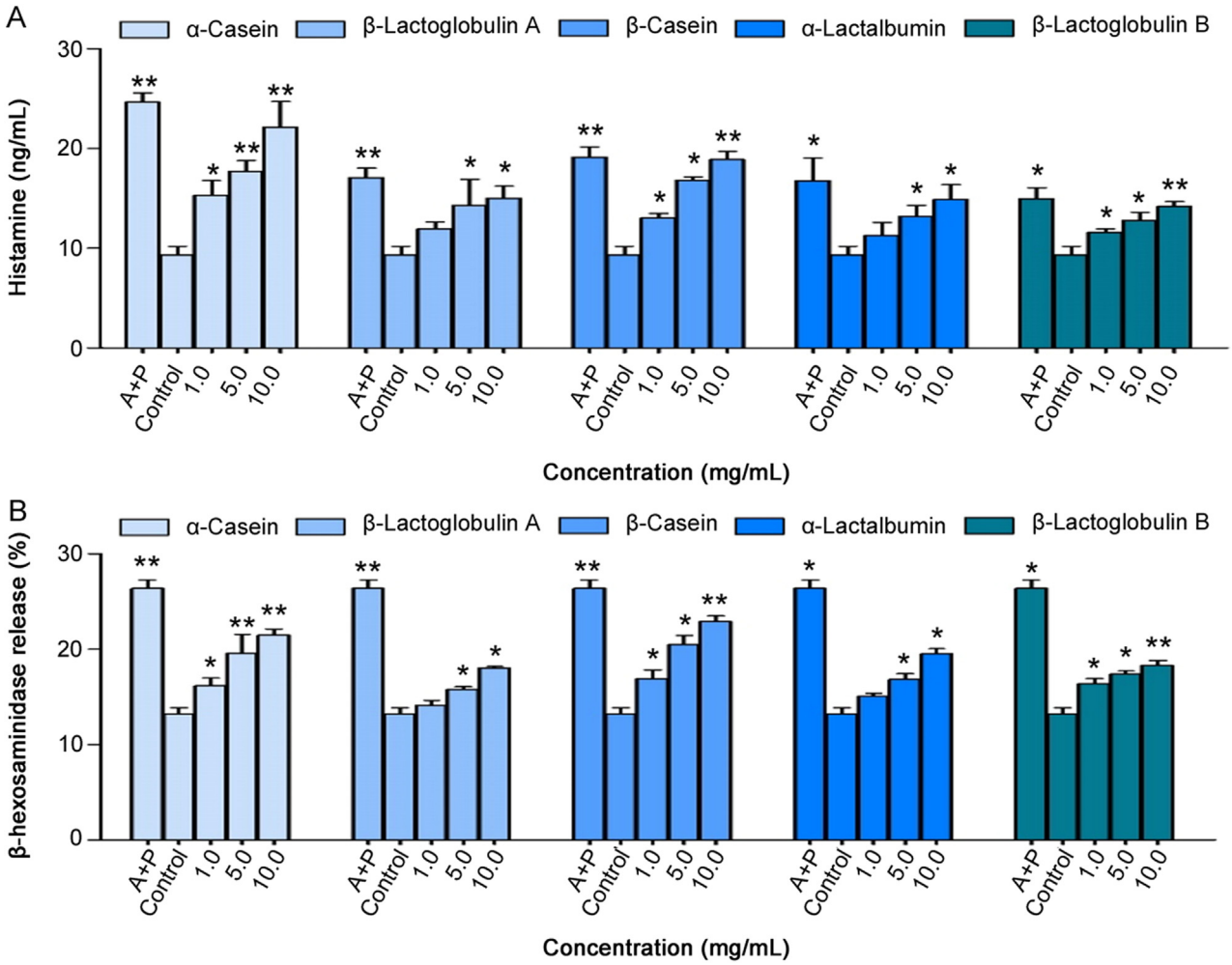

Fig. 4. Histamine and β-hexosaminidase release after HMC-1 cells were stimulated by five proteins. (A) histamine release; (B) β-hexosaminidase release. Bars are mean ± S.D. of four independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control.

2.7. LC-ESI–MS/MS for histamine analysis

MRM mode was used in the quantitative analysis for precursor/product ions of histamine (*m/z* 111.90–95.05) and histamine-d4 (*m/z* 116.00–99.10), according to the literature [17,18]. Standard histamine solutions (2.5, 5, 12.5, 25, and 50 ng/mL) were used for calibration curves. Standard solution contained 5 ng/mL internal standard solution. The ratio of standard to internal standard peak area was used to calculate histamine content by linear regression analysis.

2.8. β-Hexosaminidase release assays

Exponentially growing HMC-1 cells were harvested and plated in 96-well plates at 30,000 cells per well with 100 μL 1640 medium. After 24 h at 37 °C, cells were treated with α-CN, α-Lac, β-CN, β-LgB, or β-LgA (1, 5, and 10 mg/mL) for 30 min. For degranulation assays, culture supernatant samples (50 μL) were incubated with an equal volume of substrate solution (0.2 M citrate, 1 mM 4-methylumbelliferyl N-acetyl-β-D-glucosaminide, pH 4.5) for 90 min at 37 °C. Reaction was terminated with 150 μL of 0.2 mol/L sodium carbonate buffer (pH 10.5). Release of 4-methylumbelliferyl in the medium was measured with a 96-well plate reader at a wavelength of 405 nm. To determine the total amount of β-hexosaminidase released, remaining cells were lysed with assay buffer containing 0.1% (v/v) Triton X-100 before incubation with substrate, by the same procedure used to determine activity in supernatants. Percent β-hexosaminidase release was calculated as the ratio of absorbance of supernatant to cell lysate. Effects of treatments on β-hexosaminidase release were reported as percentage of control.

3. Results and discussion

3.1. Evaluation and application of the HMC-1/CMC model

CMC is a bio-chromatography method, which is made from cell membrane and can reflect the interaction between the analyte and receptor on the cell membrane [10]. This technique has been used for screening active components or potential allergic components from traditional Chinese medicines [12,19].

The human mast cell line HMC-1 is an effector cell of immediate hypersensitivity reactions, which has many feature characteristics and functional properties, e.g. the expression of the high-affinity receptors for IgE, and the release of histamine upon activation [11]. According to the literature, HMC-1 cells highly express with γ-chain of IgE receptors [20]. Therefore, we chose HMC-1 cells for CMC stationary. Quercetin has been commonly used as a positive-control drug in allergy research [21]. The receptors of metoprolol, gefitinib and captopril are β1-receptor [22], EGFR receptor [23], and Angiotensin II receptor [24], respectively. Therefore, metoprolol, gefitinib and captopril were chosen as negative control drugs. Quercetin was clearly retained

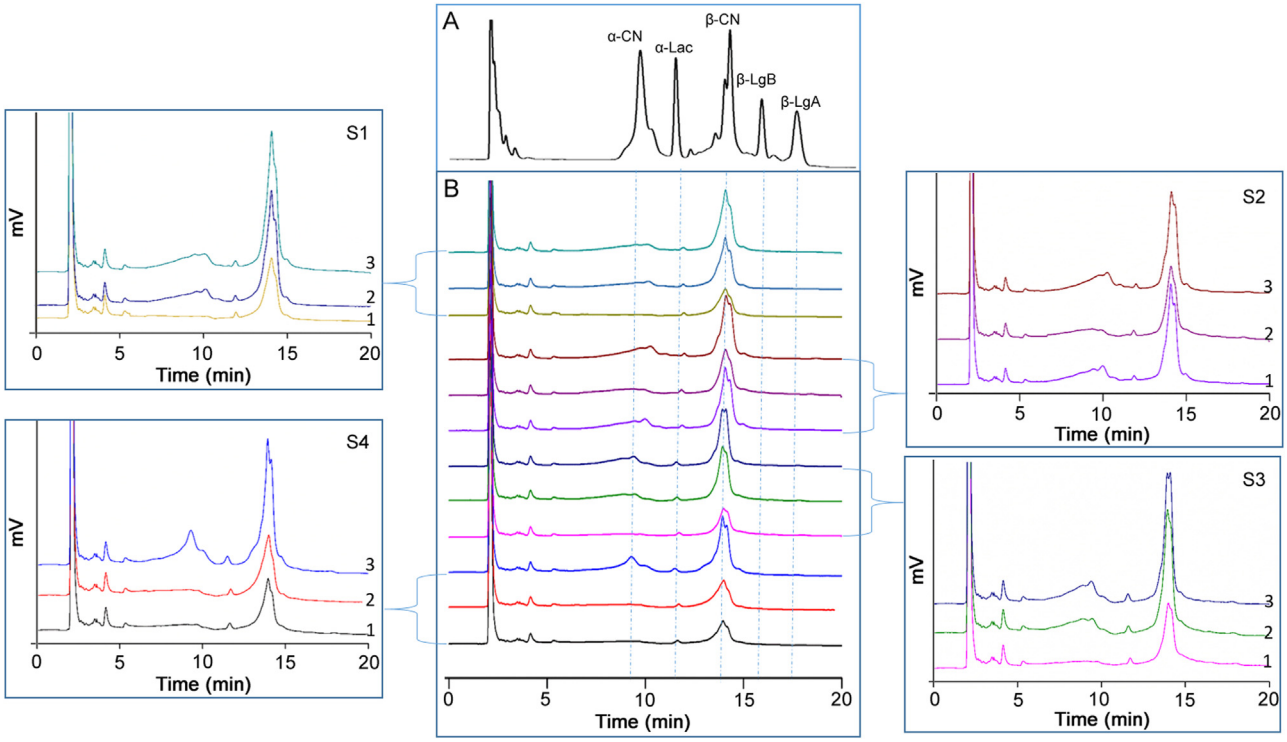

Fig. 5. RPLC chromatograms of major proteins of commercial samples. (A) Chromatogram of standard mixtures of five major proteins; (B) Chromatograms of 12 commercial infant formula milk powders (four brands containing three stages). S1-S4, amplified figures of four brand samples. 1. first stage, 2. second stage, 3. third stage.

Table 2
Method validation data for quantitative determination of five proteins.

Protein	Linear range (μg/mL)	Regression equation[a] y = ax + b	R^{2}[b]	LOD (μg)[c]	LOQ (μg)[d]	Repeatability[e](%)	Reproducibility[f](%)
α-CN	60–960	y = 2412.2x + 16060	0.9990	0.10	0.33	4.47	4.97
α-Lac	20–1500	y = 5834.1x − 15760	0.9998	0.02	0.08	2.97	4.43
β-CN	60–1300	y = 4294.1x − 51288	0.9987	0.10	0.33	3.66	4.17
β-LgB	60–600	y = 1915x − 76610	0.9995	0.10	0.33	3.10	3.38
β-LgA	75–1000	y = 2551x − 104357	0.9999	0.13	0.42	2.60	5.84

[a] y is the average peak area of the protein ($n = 3$), x is the mass concentration of the protein in mg/mL.
[b] Regression coefficient (R^2).
[c] Detection limit (LOD). Computed as LOD= 10 × (3 × SD) where SD is the standard deviation of the background noise.
[d] Quantification limit (LOQ). Computed as LOQ= 10 × (3 × SD) where SD is the standard deviation of the background noise.
[e] Six aliquots of the same standard mixture of five proteins were injected consecutively.
[f] Six aliquots of the standard mixture of five proteins were injected over 6 days.

and metoprolol, gefitinib and captopril were not (Fig. 2). The reproducibility of the different HMC-1/CMC columns was tested by the quercetin standard solutions. The results showed that the RSD (%) of retention time (t_R) of quercetin peak was 15.50% when changing HMC-1/CMC columns ($n = 5$). The precision between the columns met the assay requirements. These results indicated that HMC-1/CMC could be used as a tool for screening potential allergenic substances. We applied HMC-1/CMC into breast milk and infant formula milk powder (IFMP) to analyze their retention behavior.

HMC-1/CMC chromatograms of breast milk and IFMP are shown in Fig. 2B. Breast milk was not retained on the HMC-1/CMC model, while IFMP was. This result indicated that human breast milk may not cause allergic phenomena in humans, but IFMP might. This conclusion is consistent with previous findings [2]. The prevalence of CMA is increasing and this increase may be explained by a decrease in breast feeding and an increase in feeding with CM-based formulas [25]. To identify the retained components, the freeze-drying fractions were analyzed through MALDI-TOF-MS (Fig. S1). We found four major milk proteins, i.e., β-LgA (MW: 18 479 Da), β-CN (MW: 24 092 Da), α_{s2}-CN (MW: 25 390 Da) and α-Lac (MW: 28 861 Da) [26]. The molecular weight of α-Lac is different from that reported (MW: 14 200 Da) [27], which may be because of the formation of dimer.

3.2. HMC-1/CMC chromatography of five proteins

From Fig. 3, we can see that all five bovine milk proteins were retained on the HMC-1/CMC model. These results indicate that these bovine milk proteins may cause allergic phenomena on humans, consistent with conclusions in the literature [28,29]. Many milk-fed infants have positive associations with allergic diseases such as infantile eczema [30].

In the CMC method, retention time can reflect binding affinity between proteins and receptor on membranes. Retention time and retention factors of the five major proteins on the HMC-1/CMC model are shown in Table 1. The protein α-CN had the strongest binding affinity of the five proteins, whereas β-LgB was the weakest. These results are consistent with those reported in the literature [31,32]. Goldman et al. [31] found allergic reactivity to casein in 40%, to α-lactalbumin in 26% and to β-lactoglobulin in 25%. Docena et al. [32] studied the purified proteins in CMA

Table 3
Recovery of five proteins in spiked milk samples ($n = 3$).

Protein	Added (μg)	Found (μg)	Recovery[a] (%)	RSD (%)	Average (%)
α-CN	0	136.7	–	1.2	96.9
	50.0	186.5	99.5	3.0	
	200.0	342.4	102.9	1.4	
	600.0	667.0	88.4	3.1	
α-Lac	0	0	–		95.6
	50.0	47.9	95.9	1.2	
	200.0	196.4	98.2	1.3	
	1000.0	928.4	92.8	1.8	
β-CN	0	136.1	–	1.1	98.3
	50.0	186.0	99.8	3.5	
	250.0	388.4	100.9	3.1	
	850.0	936.9	94.2	2.3	
β-LgB	0	0	–		100.5
	80.0	84.6	105.8	2.7	
	200.0	201.7	100.8	2.2	
	500.0	474.3	94.9	3.3	
β-LgA	0	0	–		98.1
	90.0	91.1	101.3	4.4	
	250.0	246.5	98.6	3.8	
	750.0	707.8	94.4	4.7	

[a] Mean values of three measurements.

children, which suggested that casein might be more allergenic than the other proteins.

Regardless of the most allergenic protein, these results indicate that the HMC-1/CMC model could be used for screening potential allergic proteins from milk products.

3.3. Histamine and β-hexosaminidase release

Histamine and β-hexosaminidase release occurs when a causative antigen binds to the specific IgE on the surface of mast or basophil [33]. To test the allergneic effect of the five proteins, HMC-1 cells were incubated with different concentrations of proteins in 96 wells. After 30 min, histamine and β-hexosaminidase release was determined by LC-ESI–MS/MS [18] and a spectrophotometric method [34], respectively.

Fig. 4 shows the results of the histamine and β-hexosaminidase release. A23187 and PMA (A+P) are commonly used as positive control drugs in allergy research [35]. HMC-1 cells treated with A+P were used as the positive controls and cells without drugs or proteins were used as blank controls.

From Fig. 4, we find that the five proteins induced histamine and β-hexosaminidase release in a dose-dependent manner from 1.0 to 10.0 mg/mL in HMC-1 cells, although the release effect was less than the positive drug A+P. Histamine and β-hexosaminidase had the same release trend. Compared with blank controls, α-CN, β-CN and β-LgB at 1.0 mg/mL were significantly different ($^*p < 0.05$), and α-Lac and β-LgA at 5.0 mg/mL. These results indicate that α-CN, β-CN and β-LgB result in higher allergy risk than α-Lac and β-LgA. These findings were consistent with those reported in the literature [32].

3.4. Establishment of RPLC method for quantification of five proteins in IFMP

We established a rapid RPLC method for determination of five proteins from IFMP. Sample preparation was as the described previously [36]. Five proteins were separated completely within 20 min as shown in Fig. 5. After optimization of all experimental parameters, the method was validated for linearity, detection limits (LODs), quantification limits (LOQs), mid-precision and accuracy, and the results are shown in Tables 2 and 3. Linear calibration curves were found to have a regression coefficient $R^2 > 0.9987$. LODs of the proteins were 0.02–0.13 μg (signal-to-noise ratio, S/N = 3). The mid-precision was less than 5% and recoveries of the five proteins were 95.6%–100.5%. The data indicate that this method could be used for determination of real samples.

Fig. 5 shows the RPLC chromatogram of the five proteins in the 12 IFMP samples, including four brands of three stages. The contents of the five proteins in 12 commercial milk power samples are shown in Table 4. We found that 90% proteins in IFMP were α-casein and β-casein, and these proteins are the cause of allergies in infants.

Management of CMA requires a balance between avoiding allergens and promoting acquisition of tolerance [2]. Therefore, a suitable content of proteins in IFMP should be considered from two perspectives, allergic and trophism, especially for allergic infants. By contrast, increasing the content of α-Lac can decrease the risk of CMA.

4. Conclusions

Firstly, breast milk and IFMP can be differentiated by the HMC-1/CMC method. Secondly, α-CN, α-Lac, β-CN, β-LgB, or β-LgA were screened as potential allergens from IFMP. Thirdly, these proteins could lead to histamine and β-hexosaminidase release. Fourthly,

Table 4
Contents of five proteins in 12 commercial milk powder samples.

Sample	α-CN (mg/g)	α-Lac (mg/g)	β-CN (mg/g)	β-LgA (mg/g)	β-LgB (mg/g)
Sample 1					
Stage 1	16.13 ± 0.26[b]	0.94 ± 0.02	31.99 ± 0.52	ND[a]	ND
Stage 2	36.69 ± 0.59	0.89 ± 0.02	43.21 ± 0.70	ND	ND
Stage 3	59.85 ± 1.05	1.18 ± 0.02	66.39 ± 1.16	1.09 ± 0.02	ND
Sample 2					
Stage 1	15.29 ± 0.26	1.03 ± 0.02	33.58 ± 0.56	0.69 ± 0.01	ND
Stage 2	52.68 ± 0.92	1.07 ± 0.02	59.11 ± 1.04	1.24 ± 0.02	ND
Stage 3	65.05 ± 1.03	1.26 ± 0.02	66.79 ± 1.05	1.26 ± 0.02	ND
Sample 3					
Stage 1	55.24 ± 0.89	0.77 ± 0.01	74.61 ± 1.20	0.77 ± 0.01	ND
Stage 2	43.10 ± 0.69	0.87 ± 0.01	56.38 ± 0.91	1.50 ± 0.02	ND
Stage 3	59.89 ± 0.95	0.73 ± 0.01	76.62 ± 1.21	1.00 ± 0.02	ND
Sample 4					
Stage 1	18.04 ± 0.28	0.73 ± 0.01	39.18 ± 0.63	ND	ND
Stage 2	43.34 ± 0.68	0.72 ± 0.01	66.75 ± 1.07	ND	ND
Stage 3	41.88 ± 0.67	0.65 ± 0.01	67.52 ± 1.08	ND	ND

[a] ND: not detected.
[b] Values of concentrations are means ± RSD ($n = 3$).

90% proteins in IFMP were α-casein and β-casein, and these proteins are the cause of allergies in infants.

The results above show that the HMC-1/CMC method can screen potential allergenic components in milk or milk powders. The study also verified the importance of breast-feeding. In the future, we will combine the HMC-1/CMC method with MS/MS to screen and study more potential allergen candidates from other foods.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.jpha.2018.08.004.

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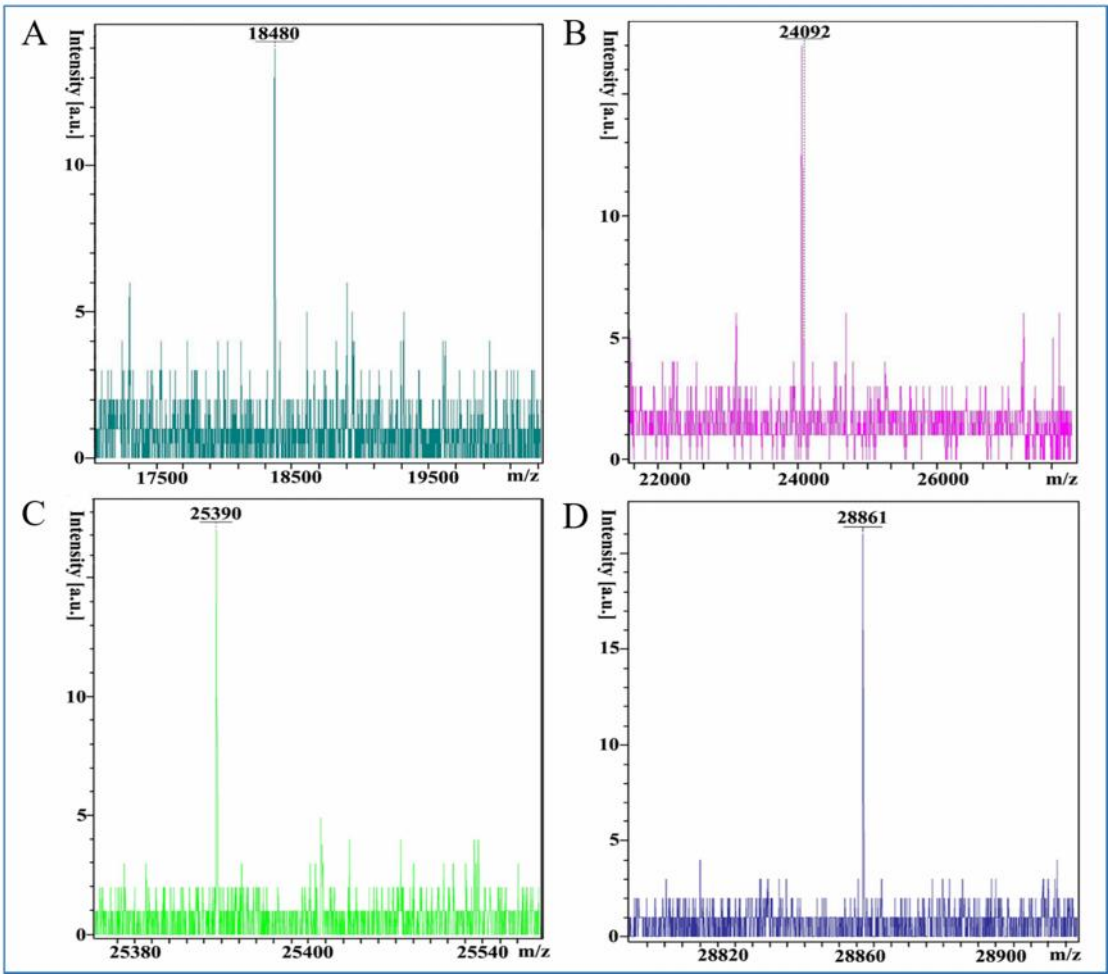

Fig. 1. MALDI-TOF-MS spectra of retained fractions from HMC-1/CMC.

A: β-LgA, B:β-CN, C:α-CN, D:α-Lac.