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The assessment of allergenicity of recombinant human lactoferrin (rhLF) using an animal model for its extensive use. In this study, 32 Brown Norway (BN) rats were divided into four groups that were exposed to ovalbumin (OVA) (positive control), rhLF, bovine lactoferrin (bLF) and saline (negative control), respectively, with daily intragastric administration for 42 days. The level of specific antibody (IgE, IgG and IgG2a), blood cell counts, blood pressure and pathology of important organs were measured and compared among the groups. OVA stimulated significant higher levels of specific-IgE and eosinophil (EO) counts ($p < 0.05$), lower blood pressure ($p < 0.05$) and more serious lesion in the lung of BN rats compared to the other groups. And the animals did not show obvious humeral, cell-mediated and local allergic response to rhLF and bLF. The results demonstrate a weak allergenicity of rhLF on the animal and reveal its potential value for further identified study.

Keywords: recombinant human lactoferrin; BN rat; allergenicity

1. Introduction

Recombinant human lactoferrin (rhLF) is a novel protein which is derived from cloned cows that are developed with transgenic technology (Yang et al., 2008). Previous studies indicated that the iron-binding and -releasing properties and antibacterial effect of this rhLF were identical to the native hLF (Yang et al., 2008). Those differences reflect a better iron absorption and higher resistance to decay in performances, which make rhLF an important novel protein that is applied to improve health conditions of human body and for the growth of infant babies. Thus, it can be added to milk powder as a nutritional supplement to increase iron absorption by infants. The incidence of milk allergy in children is about 1–5% or even 7.5% (Blanc et al., 2008). It is essential to evaluate the allergenicity of rhLF before adding it to milk powder as a nutritional supplement.

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According to the regulatory guidelines published by a related organisation, the evaluation of potential allergenicity of novel transgenic foods mainly contains the identification of the source of the gene, bioinformatics (sequence homology to known allergens), and stability in pepsin (Codex Alimentarius Commission, 2003; FAO/WHO, 2001; Goodman, Hefle, Taylor, & van-Ree, 2005; Goodman et al., 2008; Talor, 2006). Besides, because allergy may result in various circumstances, every factor should be considered for a complete evaluation. It is necessary to develop new methods for evaluating the risk of novel protein becoming allergens *de novo*.

Many researches demonstrated that an animal model can help to study the mechanism of allergenic response and provide useful information to assess the allergenicity of proteins (Griffiths, Dearman, Cumberbatch, & Kimber, 2005; Hausding, Sauer, Maxeiner, & Finotto, 2008; Knippels & Penninks, 2003; Knippels, Penninks, & Houben, 1998; Knippels, Penninks, Smit, & Houben, 1999; Knippels, vander-Kleij, Koppelman, Houben, & Penninks, 2000). An ideal animal model should mimic the clinical symptom and mechanism of human with respect to same route of exposure. Most importantly, it should discriminate a high percentage of known allergic proteins from non-allergenic proteins and predict their allergenicity (Aldemir, Bars, & Herouet-Guicheney, 2009). And to date several attempts have been made to this end (Akiyama et al., 2001; Bϕgh et al., 2009; de Jonge et al., 2008; Dearman & Kimber, 2001; Dearman et al., 2003; Feng & Collins, 1999; Gaudry et al., 2004; Hilton, Dearman, Sattar, Basketter, & Kimber, 1997; Huang, Zhong, Cai, & Zhang, 2009; Kimber, Betts, & Dearman, 2003; Knippels, Houben, Spanhaak, & Penninks, 1999; Knippels, Penninks, Spanhaak, & Houben, 1998; Navuluri et al., 2006; Vinje, Larsen, & Lϕvik, 2009, 2011). Though there is no animal model validated to be complete ideal so far, many studies have shown that the Brown Norway (BN) rat is a high responder strain (Knippels, Penninks et al., 1999; Knippels, Houben et al., 1999) and its recognition of induced antibodies to specific protein is comparable with that observed in sera from allergic patients (Knippels et al., 2000). Besides, the individual BN rat is larger than a mouse, so it is easier to operate and measure its blood pressure, and to get enough samples and sera compared to a mouse, and this can ensure a higher accuracy rate. Based on these advantages, BN rat is a better choice than a mouse for conducting an animal experiment on allergenicity evaluation.

Several studies on the research of animal model of protein's allergenicity took place with daily oral administration to BN rat for 42 days. The results estimated that this exposure route aroused an evident immunology and allergy reaction in BN rats successfully (Akiyama et al., 2001; de Jonge et al., 2007; Dearman, Caddick, Stone, Basketter, & Kimber, 2001; Dearman et al., 2003; Knippels, Houben et al., 1999; Knippels, Penninks et al., 1999). Referring these articles, daily oral administration was adopted to manage animals in this research.

In previous research, bioinformatics study was conducted to predict the allergenic potential of rhLF, and the result indicated that it has strong potential allergenicity. Then, the *in vitro* stimulated gastrointestinal digestion experiment was carried out to determine its stability in gastric fluid and intestinal fluid. Besides, BN rats were also used for conducting animal experiments to evaluate allergenicity of rhLF directly. And it showed a low allergenicity in the animal experiment. This study provided reference data for comprehensive evaluation of allergenicity of the novel protein. The observation from this study may be treated as reference for further allergenicity study of transgenic food.

2. Materials and methods

2.1. Test samples

rhLF (purity >95%) and bovine lactoferrin (bLF, purity >95%) were obtained from the State Key Laboratory for AgroBiotechnology of China Agricultural University. They were purified from the milk of transgenic cows and native cows, respectively, with protocol method described by Yang et al. Ovalbumin (OVA, No. A5503) as a potent and representative food allergen was purchased from Sigma-Aldrich Inc., Shanghai, China.

2.2. Animals

Thirty-two female specific pathogen free (SPF) Brown Norway (BN) rats were obtained from Weitong Lihua Inc. (Beijing, China) and this experiment was approved by the Animal Ethics Review Committee of The Supervision, Inspection and Testing Center of Genetically Modified Organisms (Beijing). The rats' weights were 40–60g at the beginning of treatment. Following 5 days of acclimatisation, they were divided into four groups using stratified-by-weight randomisation methods. There were eight rats in each group and all of the groups were gavaged with different samples. During the experiment, two rats of the same group were housed in one stainless steel wire cage. Fresh water and diet without milk and egg were provided *ad libitum*. The cages were placed in an animal room maintained at 23 ± 2 °C, $50 \pm 10\%$ relative humidity, 15 air changes per hour and 12 h light/dark cycle.

2.3. Experimental design

Rats in all of the four groups were exposed to OVA, rhLF, bLF and saline by daily gavage, respectively, for 42 days without the use of adjuvant. The recommended intakes of egg and milk are 60 g/day and 400 mL/day, respectively, in China; the content of OVA in egg and LF in milk is 3 g/100g and 0.3 g/L. In general, the average weight of human body in China is about 60 kg. Through calculation, the recommended intakes of OVA and LF by human are 3 mg/100g bw/day and 0.2mg /100g bw/day, respectively. The treatment dose for animal is set at 5 times the human recommended intake. So in this experiment, the doses of both rhLF and bLF were 1 mg/mL, 1 mL/day/animal, and the dose of OVA was 15 mg/mL, 1 mL/day/animal. All the proteins were dissolved in saline and these doses were determined based on their recommended intake in human. On day 41, the blood pressure of all rats was detected as a baseline. On day 42, all rats were orally challenged by intragastric intubation with specific protein (1 mL of 0.15 g OVA/mL, or 1 mL of 10 mg rhLF or bLF/mL solution, or 1 mL saline).

Blood samples were collected from orbital venous plexus of rats' left eyes at 2-week intervals (on day 14, 28 and 42 after starting of the experiment) for the detection of cell counts. And at day 0, all rats were bled and their sera and plasma were pooled as negative control. Besides, blood samples for IgG, IgG2a and IgE were centrifuged for 10 min at 3000*g* and 4°C following coagulation for 2 h at room temperature to get the sera. Within 30 min after treatment, animals' blood samples were collected in centrifugal tubes, which contained heparin, for obtaining plasma histamine. The sera and plasma were stored at −20°C before analysing for

antigen-specific IgE, IgG, IgG2a and histamine levels by enzyme-linked immunosorbent assay (ELISA).

On day 42, blood pressure of all rats in every group was detected for over a period of 7.0 h after challenge treatment.

2.4. Antigen-specific IgE analysis

The allergen-specific IgE concentration in animals' sera of different groups was detected using an ELISA method following the protocol described previously (Li, Serebrisky, & Lee, 2000), with some modification. Solution of OVA, bLF or rhLF (10 μg protein/mL carbonate–bicarbonate buffer, pH 9.6) was bound to 96-well microtiter plates with 100 μL/well overnight at 4°C. Then, 200 μL/well of a postcoat solution (PBS containing 1% bovine serum albumin) was added and the plate was incubated for 1 h at 37°C, followed by washing three times. The plates were washed and serial solutions of rat serum (test sera and negative control sera) were added to the wells (100 μL/well); the plates were incubated for 1 h at 37°C. After washing, 100 μL/well of horse radish peroxidase conjugated Goat anti-Rat IgE (ICL, Inc., USA) dilution (10,000 × diluted) was added. After incubating for 1 h at 37°C, the plates were washed again and 150 μL/well of TMB substrate solution was added. The plates were developed for 10 min at 37°C, and finally 50 μL/well of 2 M H_2SO_4 was added to stop the colour reaction. Optical densities (OD) were read with ELISA reader (MULTISKAN ASCENT, Thermo, USA) at 450 nm. Each serum sample was performed with three parallel tests and titrated starting at a 1:4 dilution. The reciprocal of the greatest serum dilution giving an OD higher than the reference value was read as the titre. The reference value is the total value of average OD of negative control samples and their 2SD.

2.5. Antigen-specific IgG and IgG2a assay

For the detection of antigen-specific IgG and IgG2a, 96-well microtiter plates were coated with 100 μL/well of 10μg /mL solution of OVA, bLF or rhLF in carbonate–bicarbonate buffer (pH 9.6); the procedure was similar to that described for IgE ELISA, with the expectation that the horse radish peroxidase conjugated Goat anti-Rat IgE (ICL, Inc., USA) in IgE ELISA was replaced by horse radish peroxidase conjugated Goat anti-Rat IgG or IgG2a (AbD Serotec, UK) with 2000 × dilutability. The method to determine titre was the same as described in IgE analysis.

2.6. Detection of blood cell counts

On day 0, 14, 28 and 42 of the experiment, rats in different groups were anesthetised and blood samples were collected from the orbital venous plexus of their left eyes at 30 min after treatment, respectively. And then the blood samples were stabilised using ethylenediaminetetraacetic acid. The animals were fasted overnight before collecting samples. The following characteristics were measured with a HEMAVET 950FS animal blood cell counter (Drew Scientific, Inc., Dallas, Texas, USA): white blood cell count (WBC), lymphocyte (LY), basophile (BA), eosinophils (EO) and neutrophils (NE).

2.7. Measurement of blood pressure

Before challenge, baseline blood pressure of all rats was determined using an Intelligent non-invasive blood pressure monitor (BP 98A, Softron Incorporated, Beijing, China). Prior to detecting blood pressure, animals were placed under a heating box (37°C) for about 20 min. Thereafter, an inflatable pressure cuff was put around the tail and a distal sensor was used to record the systolic blood pressure (SBP). After oral challenge, the blood pressure of OVA-, bLF- and rhLF-sensitised and control animals were recorded at 0.5, 1, 2, 3, 5 and 7 h.

2.8. Pathology

At termination of the experiment, all animals were anaesthetised and killed by cervical dislocation for gross and histopathological examination. A thorough necropsy of major organs was performed by visual inspection and pathological examination, with the spleen, thymus, heart, liver, lung and kidney excised, examined and weighed. The relative weight of each organ was normalised based on final individual body weights.

Samples from these organs were fixed in 4% buffered formaldehyde and embedded in paraffin, and then sections of 5-μm thick were affixed to slides and stained with haematoxylin and eosin for microscopic examination. Histopathological examination of tissue sections was conducted at the Experimental Animal Research Center, China Agricultural University.

2.9. Statistics

Statistical treatment of experimental results was conducted with SPSS Version 13.0 (SPSS Inc., Chicago, Illinois, USA). The concentrations of plasma histamine, the titres of antigen specific IgE and IgG2a, the various blood cell counts and organs' indexes among groups were compared using one-way ANOVA.

3. Results

3.1. IgE response to antigens

As indicated in Table 1, during the whole study, the production of antigen-specific IgE of animals in three treatment groups was detected on day 14, 28 and 42. At day 14, the level of rhLF-specific IgE in rhLF treatment group was significantly lower

Table 1. Comparison of BN rats' IgE level in different groups.

	14th day	28th day	42nd day
OVA	1.00 ± 0.20	1.02 ± 0.29	1.01 ± 0.13
bLF	0.69 ± 0.56	0.79 ± 0.35	0.50 ± 0.17*
rhLF	0.31 ± 0.20*	ND	ND

Notes: Data are displayed as ln(reciprocal of titre of antigen specific IgE), average $\pm$SD, $n = 8$.
*Statistically significant ($p < 0.05$) compared to OVA group.

than that in other two groups (OVA group and bLF group). And after the 14th day, the rhLF-specific IgE level in treatment group was not detected.

3.2. IgG response to antigen

To determine the immunological reaction of BN rats to the three different proteins, antigen-specific IgG level of animals in different treatment groups was detected on day 14, 28 and 42. As shown in Figure 1, the antigen-specific IgG levels in three treatment groups increased after the 14th day, reached the highest level on day 28 (titre of antigen-specific IgG in OVA group: 7.31 ± 1.02; bLF group: 9.57 ± 0.58; rhLF group: 8.22 ± 0.45) and remained at this level until the end of this trial (on day 42, titre of antigen-specific IgG in OVA group: 7.34 ± 0.62; bLF group: 9.21 ± 0.52; rhLF group: 7.90 ± 0.51). BN rats exhibited a certain immune response to all the three proteins OVA, rhLF and bLF.

3.3. IgG2a response to antigen

Figure 2 shows the detection result of IgG2a level in different groups. The production of antigen-specific IgG2a was observed on days 14, 28 and 42. In summary, the variation tendency of IgG2a level in different groups was similar to that of IgG level. On the 14th day of the experiment, the IgG2a of animals in rhLF treatment group (2.35 ± 0.34) was significantly lower than that in OVA group (3.37 ± 0.17). Besides, on the 28th and 42nd days, the rhLF treatment animals' IgG2a level (on day 28, it was 8.16 ± 0.25; on day 42, it was 7.64 ± 0.25) was significantly lower than that of OVA (on day 28, it was 9.04 ± 0.28; on day 42, it was 8.89 ± 0.34) and bLF (on day 28, it was 8.92 ± 0.68; on day 42, it was 8.41 ± 0.27) treatment animals. And the IgG2a level of bLF group was lower than that of OVA group on day 42, the termination of the experiment. OVA as a strong allergen induced a fierce specific immune response, whereas the oral administration of rhLF and bLF induced a weak immune response and the rhLF was weaker than bLF.

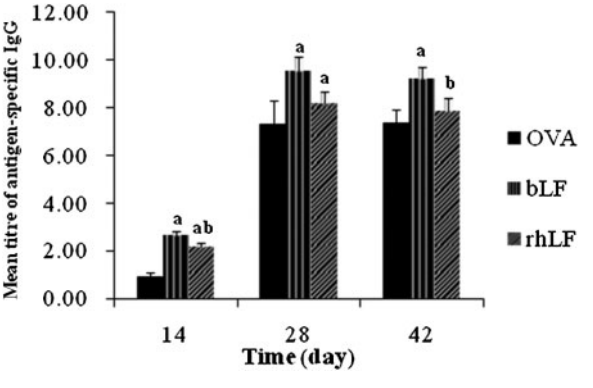

Figure 1. Antigen-specific IgG responses to antigens exposed to different proteins. Data are displayed as ln(reciprocal of titre of antigen-specific IgG), average $\pm$SD, $n = 8$. (a) Statistically significant ($p < 0.05$) compared to OVA group; (b) statistically significant ($p < 0.05$) compared to bLF treatment group.

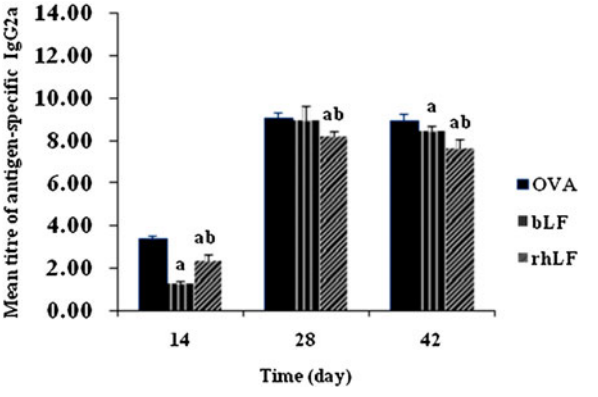

Figure 2. Antigen-specific IgG2a responses to antigens exposed to different proteins. Data are displayed as ln(reciprocal of titre of antigen-specific IgG), average $\pm$SD, $n = 8$. (a) Statistically significant ($p < 0.05$) compared to OVA group; (b) statistically significant ($p < 0.05$) compared to bLF treatment group.

3.4. Blood cell counts

To detect the influence of oral administration of different proteins on BN rats' immune system, the related counts of white blood cell (WBC), lymphocyte (LY), basophile (BA), eosinophil (EO) and neutrophils (NE) were measured at different times during the trial. Figure 3 indicated that the eosinophil level of animals in OVA group was increased throughout the experiment, while the other three groups did not display the same tendency.

3.5. Blood pressure

To further detect the effect of rhLF on animals' immune system and the body reaction resulting from it, animals' blood pressure was measured before and after the

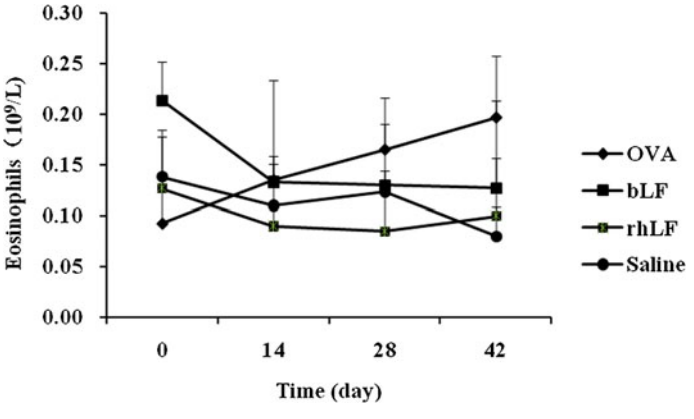

Figure 3. The variation tendency of eosinophils in different treatment groups. Data are shown as average $\pm$SD, $n = 6$. The eosinophil level of OVA group was always increased during the experiment.

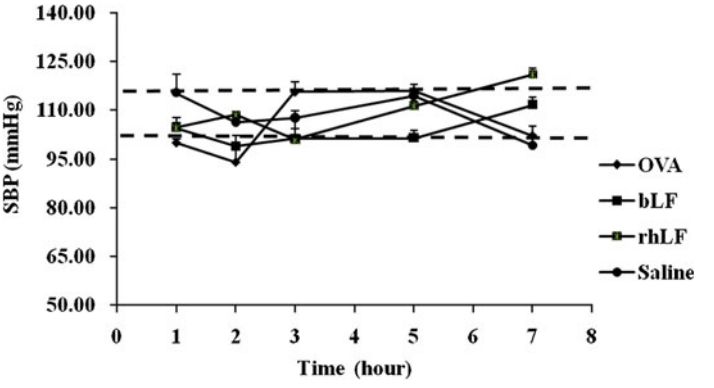

Figure 4. Variation of systolic blood pressure (SBP) in different groups after challenge. The range indicated by dotted line was 95% confidence interval of systolic blood pressure (SBP) baseline 102.88–114.39mmHg; this range was measured before challenge and as a normal range of SBP. The SBP of almost all the animals was in the normal range besides that animals' SBP in OVA group was decreased below 102.88mmHg and then recovered to normal soon.

challenge. As shown in Figure 4, the 95% confidence interval of systolic blood pressure (SBP) baseline was 102.88mmHg–114.39mmHg. The SBP of animals in OVA group was decreased at the 2nd hour after challenge and returned to normal level soon. Besides, the SBP of animals in the other three groups (rhLF, bLF and saline) remained in the normal range.

3.6. Pathology

At the end of this trial, for estimating the influence of proteins on animals' related organs, the relative weights of spleen, thymus, heart, liver, kidney and lung were calculated and the histopathological examination of these organs was conducted. No gross adverse anatomical differences were found in organs at necropsy among the four groups. Statistical differences were presented between rhLF group and the other three groups, as revealed in Table 2. The relative weight of lung in the OVA group was significantly higher than that in bLF and saline groups, and for the rhLF group, this result was not obtained. Figure 5 shows the results of histopathological examination of organs. In the OVA administrated group, eosinophil infiltration or

Table 2. Comparison of organs' relative weight in different groups.

	OVA	bLF	rhLF	Saline
Heart	0.414 ± 0.040	0.439 ± 0.067	0.421 ± 0.052	0.402 ± 0.039
Liver	3.413 ± 0.560	3.426 ± 0.174	3.233 ± 0.262	3.494 ± 0.428
Spleen	0.220 ± 0.024	0.240 ± 0.051	0.212 ± 0.022	0.203 ± 0.063
Lung	0.881 ± 0.096	0.776 ± 0.045*	0.841 ± 0.069	0.765 ± 0.033*
Thymus	0.261 ± 0.031	0.205 ± 0.032	0.243 ± 0.022	0.213 ± 0.068
Kidney	0.766 ± 0.034	0.753 ± 0.050	0.827 ± 0.084	0.780 ± 0.051

*Statistical difference ($p < 0.05$) compared to OVA group.

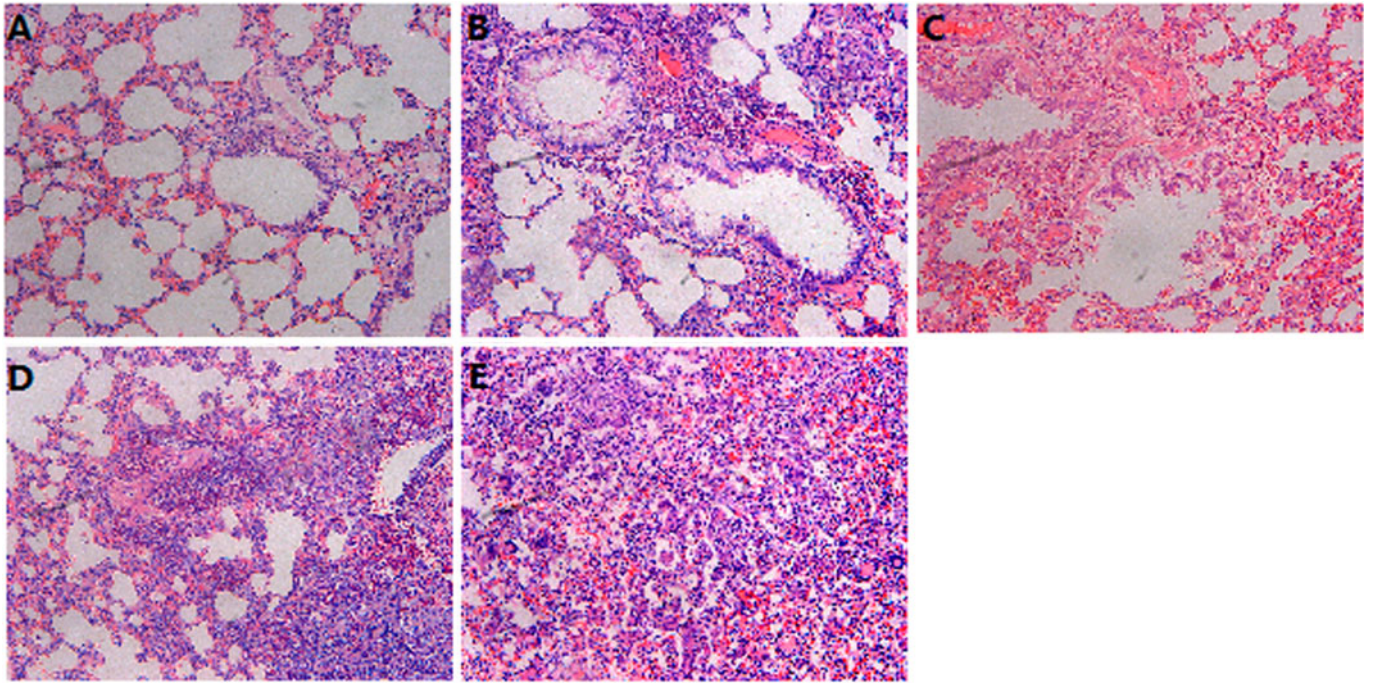

Figure 5. Results of histological observation of rats' lungs in different treatment groups. (A) The structure of animals' lungs in the saline group was normal. (B) Eosinophil infiltration was observed in one rat (No. 67) in bLF group. (C) Eosinophil infiltration was observed in one rat (No. 28) in rhLF group. (D and E) Eosinophil infiltration and inflammatory granuloma lesion were discovered in more than 50% animals of OVA group.

granulomatous lesions in the lung appeared in four of five animals. However, the lesion appeared in only one animal in the rhLF group.

4. Discussion

Food allergy is a worldwide prevalent disease and the interest in the development and evaluation of approaches for the allergenicity assessment of novel foods is increasing rapidly. Recombinant human lactoferrin is a novel protein, expressed in dairy cows, was transferred to the hLF gene by a biotechnological procedure. As the human has long history of exposure to hLF, it is not considered as an allergen, but the allergenicity of rhLF is in suspension. So, as a novel protein, potential allergenicity of rhLF is comprehensively researched with BN rats in this study. The results from current research indicate that the allergenicity of rhLF is weaker than bLF. And because of the access to enough human lactoferrin is difficult and the consideration of ethics, bLF was used as a sample to compare with rhLF.

In previous studies, acute toxicity and sub-chronic toxicity of rhLF have been measured according to the safety evaluation standard (Chinese Standard GB/T10765, 1997; Zhou et al., 2011) and the results did not indicate either kind of toxicity (data not shown). Studies about its allergenicity were then conducted following the toxicology study. Bioinformatics (amino acid sequence comparison) and *in vitro* digestion assay were included in the "Assessment of the Allergenic Potential of Foods Derived from Biotechnology Decision Tree" formulated by FAO/WHO in 2001 and then adopted by the Codex Alimentarius in 2003 (Hausding et al., 2008). One of the purposes of bioinformatics is to evaluate whether the new protein is sufficiently similar to a known allergen to evoke a cross-reaction (Codex Alimentarius Commission, 2003; FAO/WHO, 2001; Metcalfe et al., 1996). The amino acid sequence comparison for rhLF was conducted with databases widely used in the world at present (http://www.allergenonline.com; http://fermi.utmb.edu/SDAP/sdap_src.html; http://allergen.nihs.go.jp/ADFS/). The methods of "search for 80 amino acid alignments by FASTA" and "search for 8 amino acid exact matches" were used. It appeared that rhLF shared great similarity with bovine lactoferrin, which made this new protein likely allergenic according to the decision tree (FAO/WHO, 2001). The potential allergenicity of rhLF was researched using a BN rat animal model on the basis of previous research results and relevant guidelines. Because the bLF was reported as an allergen of milk (Gaudin et al., 2008) and because of the great similarity between rhLF, which is a recombinant, and bLF, allergenicity comparison between rhLF and bLF was conducted.

Most of the food allergies are IgE-mediated Th2-type sensitisation, so the specific IgE level in the blood is often used as an indicator to assess a food's allergenicity. Besides, the other indicators, IgG2a, immunocyte, blood pressure and so on are also considered as auxiliary pointers to evaluate allergenicity. As a surrogate of IgE in the rat, IgG2a was regulated in a manner similar to IgE regulation; hence, it is sometimes used as a marker of allergy (Dearman & Kimber, 2009), although some authors suspect this (Gracie & Bradley, 1996; Knippels & Penninks, 2003). Antigen-specific IgG2a levels produced in both rhLF and bLF groups indicated that both bLF and rhLF made the animals produce a similar immune response. But the level of antigen-specific IgE was just increased at a low level at day 14 and was not detected at days 28 and 42, whereas animals in bLF group produced a certain amount of

antigen-specific IgE. So, it is presumed that the ability to evoke humoral-mediated allergic reaction of rhLF was weaker than that to evoke humoral-mediated allergic reaction of bLF.

As an important immunocyte, basophil's functions and biochemical characteristics have been demonstrated in many reports. Many researchers' studies showed that the basophils play a critical role in Th2 immune responses, and have the ability to function as antigen-presenting cells (Mukai et al., 2005; Perrigoue et al., 2009; Sokol, Barton, Farr, & Medzhitov, 2008; Sokol et al., 2009; Yoshimoto et al., 2009). However, one publication indicated that basophils are not a key factor in inducing a Th2 immune response (Falcone, Knol, & Gibbs, 2010; Wada et al., 2010). Eosinophils, the main inflammatory cells of an allergic reaction, are essential for the allergen-dependent Th2 pulmonary immune response mediated by dendritic cells (DCs) (Jacobsen, Zellner, Colbert, Lee, & Lee, 2011). Some researchers found that the eosinophils can influence the activation of mast cells in inflammatory response progress (Fleminger & Schaffer, 2009; Piliponsky, Gleich, Bar, & Schaffer, 2001). In this study, there was no significant difference in the variation trend of basophil count in different groups, and this result was consistent with previous research (Falcone et al., 2010). At the aspect of eosinophils, the count showed a rising trend in animals of OVA group, the positive control group, whereas the eosinophil count in rhLF group and bLF group did not increase. These may indicate that the basophil count in BN rats' blood is not influenced by food allergy or the relation between allergy and basophil is not very obvious but the eosinophil. And also both of the two lactoferrins did not cause an evident cellular immune response.

Besides the humoral-mediated and cell-mediated allergy response, the possible occurrence of systemic effects was investigated by monitoring blood pressure and several organs' pathological change. After the challenge, most of mast cells in the sensitised body appeared degranulated and released histamine, cytokines and other substances into blood. These substances can cause vasodilation and inflammation in the local tissue, resulting in decreased blood pressure and other symptoms. So, the decrease in blood pressure is included in the clinical criteria for diagnosing anaphylaxis by WAO (Simons et al., 2011). Oral challenge with OVA did not induce a clear effect on blood pressure in the majority of animals in the OVA group, while some animals demonstrated a temporarily slight decrease in SBP, which is in accordance with previous studies (Knippels, Houben et al., 1999; Penninks & Knippels, 2001). However, SBP of animals in the other three groups (rhLF, bLF and saline group) was maintained at the normal level and did not generate large fluctuations. In addition to this, histopathologic changes of the respiratory organ lung in different groups were examined in this study. Compared with the other groups, a dramatic increase in relative weight of lung and eosinophil infiltration lesions were found in more than 50% of rats in the OVA-administrated group. This suggested an absence of local and systemic immune response in BN rats treated orally with rhLF and bLF. Both of them cannot evoke a clear clinical manifestation of allergy.

In recent years, a number of attempts have been made and researches done to develop an appropriate animal model. Some results indicated that the BN rat is likely to be a useful animal model to study the potential allergenicity of novel food (Knippels & Penninks, 2003). However, significant inconsistency appears in the production of IgE antibody responses in BN rats (Dearman et al., 2001; Gaudin

et al., 2008; Knippels et al., 1998; Pilegaard & Madsen, 2004). Besides, the mouse is more likely to be included in the development of a broadly accepted, standardised allergy model because it has numerous advantages (Ahuja et al., 2010; Bodinier et al., 2009; Hausding et al., 2008; Knippels & Penninks, 2003; Pilegaard & Madsen, 2004).

5. Conclusion

Theoretically, rhLF is a protein existing in the human milk and it cannot be allergic to human body. However, some modification can occur in the recombinant rhLF and its expression, so it is necessary to estimate its allergenicity using a series of methods. The results of this study indicate that the potential allergenicity of rhLF is not conspicuously revealed. However, the allergenic potential of rhLF is still unclear, and consequently, its allergenicity needs to be further explored using different methods. Lastly, the protein's structure analysis and human serological experiment are important to identify its allergenicity and should be developed.

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