

Analysis of Properties and Proinflammatory Functions of Cockroach Allergens Per a 1.01s

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Abstract

Cockroaches have been identified as one of the major indoor allergens inducing perennial rhinitis and asthma. Per a 1s are a group of the major allergens from American cockroach. Although Per a 1s are major allergens from American cockroach, factors contributing to the allergenicity of Per a 1s are still poorly defined. To investigate the effects of Per a 1s on the expression of PARs and the release of proinflammatory cytokines from mast cells. Per a 1.0101 and Per a 1.0104 were cloned from American cockroach and then expressed in *Escherichia coli*. The purified allergens were used to stimulate P815 mast cells, and the expression of protease-activated receptors (PARs) was determined by real-time RT-PCR and flow cytometry. The levels of IL-4 and IL-13 in culture media were detected with ELISA. Sera from 80 and 77.3% of cockroach allergy patients reacted to recombinant Per a (rPer a) 1.0101 and rPer a 1.0104, confirming they are major allergens. Both rPer a 1.0101 and rPer a 1.0104 had no enzymatic activity, but rPer a 1.0101 upregulated the expression of PAR-1 and PAR-2, and rPer a 1.0104 enhanced the expression of PAR-1 and PAR-4 proteins. Both recombinant allergens were able to increase the release of IL-4 and IL-13 from P815 mast cells. This is the first study aiming to investigate functions of group 1 allergens of American cockroach. rPer a 1.0101 and rPer a 1.0104 have the capacity to upregulate the expression of PARs and to enhance Th2 cytokine production in mast cells.

Introduction

Cockroach allergens have been identified as one of the major indoor allergens, which induce IgE-mediated allergic respiratory illness such as perennial rhinitis and asthma. Sensitization to cockroaches is well recognized in human beings throughout the world. The two most common domiciliary species associated with allergic diseases are the American cockroach (*Periplaneta americana*) and German cockroach (*Blattella germanica*) [1].

Three different types of major allergens have been identified from American cockroach, named Per a 1, Per a 3 and Per a 7 [2]. Per a 1 is a group of major allergens consisting of five members, Per a 1.0101, Per a 1.0102, Per a 1.0103, Per a 1.0104, Per a 1.0105 and Per a 1.02, known as isoallergens [3]. Among them, Per a 1.0101 showed 79.2% and 94% amino acid sequence identity with Per a 1.0104 and Per a 1.0102, respectively [4]. There is no cysteine and potential N-glycosylation site in Per a 1 molecules [3].

While functions of Per a 1 remains uninvestigated, the reports that development of anaphylactic cockroach sensitivity was dependent on the levels of cockroach allergen exposure during sensitization [5] and that cockroach allergens induced peribronchial inflammatory response and allergen-specific airway eosinophilia [6] may help us to understand how cockroach allergens cause allergic inflammation. It has been reported that German cockroach extract is capable of activating protease-activated receptor (PAR)-2 and provoking IL-8 secretion from bronchial epithelial cells [7], indicating that cockroach allergen may affect the expression of PARs and hypersecretion of cytokines. Indeed, we recently demonstrated that recombinant Per a (rPer a) seven can upregulate the expression of PARs and provoke Th2 cytokine, IL-4 and IL-13, production in P815 cells [8].

As Per a 1s are major allergens in American cockroach and their functions in provoking allergic reactions remain obscure and mast cells play a key role in allergic reactions, we generated rPer a 1.0101 and rPer a 1.0104 and

investigated their influence on the expression of PARs and cytokine production in P815 cells in the current study.

Material and methods

Patients and samples. A total of 21 allergic rhinitis patients with positive skin prick to allergen extracts and four healthy controls (HC) were recruited in the study. Among the allergic patients, 15 of them were allergic to American cockroach and six of them to ragweed. The informed consent from each volunteer according to the declaration of Helsinki and agreement with the ethical committee of the First Affiliated Hospital of Nanjing Medical University was obtained. Serum (2 ml) from peripheral venous blood was collected from each patient and HC for Western blot analysis.

Expression of Per a 1.0101 and 1.0104 proteins in *E. coli*. The procedures were mainly adopted from the one described previously for Per a 7 [8]. Briefly, pMD-Per a 1.0101 and pMD-Per a 1.0104 plasmids were digested and then ligated into unique *Nde* I and *Hind* III sites in a pET-28a expression vector, respectively. The resulting plasmids were transformed into *E. coli* BL21 (DE3) for the expression of proteins. The final expression condition, under which the proteins were expressed mostly in soluble form, was at 25 °C for 12 h in the presence of 0.6 mM of IPTG. rPer a 1.0101 and rPer a 1.0104 proteins were purified using BugBuster Ni-NTA His bind purification kit according to manufacturer's protocol as described previously [8]. Endotoxin contamination was examined with the LAL assay according to the manufacturer's instructions. The endotoxin levels detected with limulus amoebocyte lysate chromogenic endpoint assay for endotoxin (Hycult Biotech, Uden BV, The Netherlands) were very low, being <0.01 EU/mg in rPer a 1.0101 and rPer a 1.0104 proteins.

Evaluation of solubility of American cockroach allergens. In order to express American cockroach allergens in a soluble form in *E. coli*, a statistical model for prediction of solubility of protein expression in *E. coli* was used [9]. A composite parameter canonical variable (CV), which is dependent on the contribution of each of the individual amino acid, was calculated as follows: $CV = 15.43 \{(N + G + P + S)/n\} - 29.56 \{(R + K) - (D + E)/n\} - 0.03$ where N, G, P, S, R, K, D and E represent the absolute number of asparagine, glycine, proline, serine, arginine, lysine, aspartic acid and glutamic acid residues, respectively. n is the total number of residues in the whole sequence. A threshold discriminate $CV' = 1.71$ [10] is introduced to distinguish soluble proteins from insoluble ones. A protein is predicted to be soluble if the difference between CV and CV' is negative.

Mass spectrometry (MS) analysis. Silver-stained protein bands on SDS-PAGE gels were removed to tubes for in-

gel digestion with modified trypsin solution [11]. Liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) identification of proteins was performed with a nanoflow liquid chromatography system and a LCQ-DECA ion trap spectrometer (Thermo Finnigan, CA, USA). The extracted peptide samples were loaded on an analytical column (RP-C18) of high-performance liquid chromatography and were eluted directly into the ESI source of a LCQ-Deca ion trap mass spectrometer. Peptide ions were analysed by using the data-dependent 'triple-play' method. Protein identification was performed using SEQUEST software against Per a 1.0101 or Per a 1.0104 cDNA sequences with default parameters.

Determination of enzymatic activities of rPer a 1.0101 and rPer a 1.0104. Serine proteinase activity of purified rPer a 1.0101 and rPer a 1.0104 was determined by their abilities to cleave a synthetic substrate BAPNA for tryptic activity or SAAPP for chymotryptic activity [12]. Trypsin and chymotrypsin were used as positive controls. Metalloproteinase and aspartic proteinase activities of purified rPer a 1.0101 and rPer a 1.0104 were determined by its ability to cleave casein and haemoglobin, respectively [13]. The carboxypeptidase A and pepsin were used as positive controls, respectively.

Western blot analysis of Per a 1 allergens in the serum from cockroach allergy patients. Purified rPer a 1.0101 and rPer a 1.0104 proteins were separated by 12% SDS-PAGE and then transferred onto polyvinylidene fluoride membrane. The membranes were incubated with human serum from cockroach or ragweed-positive allergic patients. After incubating with peroxidase-conjugated goat anti-human IgE antibody, the membranes were developed with DakoCytomation Liquid DAB + Substrate. Sera from 4 non-allergic subjects were used as negative control. The images were analysed on a VersaDoc Gel Imaging System (Bio-Rad, Hercules, CA, USA).

Cell culture and challenge. P815 cells were cultured as described previously [8]. Cultured P815 cells at a density of 1×10^6 cells/ml were incubated with the serum-free basal medium before challenge. For challenge experiments, cells were exposed to various concentrations of rPer a 1.0101 and rPer a 1.0104 (0.001–1.0 µg/ml) with or without their blocking antibody for 2, 6 or 16 h. The culture plates were centrifuged, and culture supernatants (2 ml per well) were collected. The cells were resuspended for immunofluorescence and real-time PCR analysis. Concentrations of IL-4 (R&D Systems, Minneapolis, MN, USA), IL-10 (Pierce Biotech Inc., Rockford, IL, USA), IL-12 (Pierce Biotech Inc.) and IL-13 (R&D Systems) in the supernatants were quantified using commercial ELISA kits according to the manufacturer's instructions.

Quantitation of mRNAs of PARs by real-time PCR. Expression of PAR mRNAs in P815 cells was

determined by real-time PCR as described previously [8]. Briefly, real-time PCR was performed by using SYBR[®] *Premix Ex Taq*[™] on the ABI Prism 7700 Sequence Detection System (Perkin Elmer Applied Systems, Foster City, CA, USA). The sequences of the primers are summarized in Table 1. PAR-1, PAR-2, PAR-3 and PAR-4 mRNA expression in each sample was finally determined after correction with β -actin expression.

Flow cytometry and immunofluorescent microscopy analyses of PARs. The staining procedures were mainly adopted

from the one described previously for Per a 7 [8]. Cells were then analysed on a FACS Calibur flow cytometer with CellQuest software (BD Biosciences, San Jose, CA, USA) or on a Nikon EZ-C1 confocal laser-scanning microscope (Japan).

Statistical analysis. Data were expressed as mean $\pm$ SEM for four independent experiments. Where analysis of variance indicated significant differences between groups with ANOVA, for the preplanned comparisons of interest, Student's *t* test was applied utilizing the SPSS 13.0 version (SPSS Inc., Chicago, IL, USA). *P* < 0.05 was taken as statistically significant difference.

Table 1 Primer sequences of PARs and β -actin for real-time PCR.

Primer	Sequence	Size of product (bp)
PAR-1 Forward	5'-GTTGATCGTTTCCACGGTCT-3'	225
PAR-1 Reverse	5'-ACGCAGAGGAGGTAAGCAAA-3'	
PAR-2 Forward	5'-CACCTGGCAAGAAGGCTAAG-3'	298
PAR-2 Reverse	5'-CCCAGGGTTACTGACGCTAA-3'	
PAR-3 Forward	5'-TCAATGGCAACAACCTGGGTA-3'	205
PAR-3 Reverse	5'-AAACCATGACCCACACCAT-3'	
PAR-4 Forward	5'-GCAGACCTTCCGATTAGCTG-3'	292
PAR-4 Reverse	5'-AGGGCTCGGGTTTGAATAGT-3'	
β -actin Forward	5'-GCTACAGCTTCACCACACAG-3'	288
β -actin Reverse	5'-GGTCTTTACGGATGTCAACGTC-3'	

Results

Expression and purification of recombinant Per a 1.01 proteins

In order to investigate the functions of Per a 1.01, we prepared rPer a 1.0101 and rPer a 1.0104. The *E. coli* generated approximately 82 and 23 mg/l culture mixture rPer a 1.0101 and rPer a 1.0104 proteins respectively, which consisted of approximately 24% of total soluble bacterial proteins (Fig. 1A). After purification, the recombinant proteins with apparent molecular weights 28 and

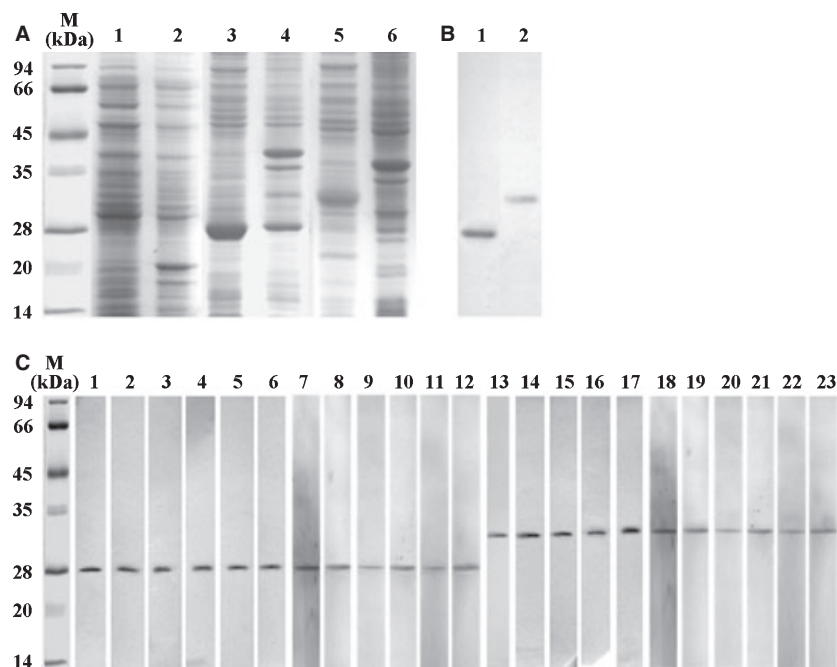


Figure 1 SDS-PAGE and Western blot analysis of recombinant Per a 1 proteins. (A), SDS-PAGE examination of expression of the target proteins stained with Coomassie brilliant blue. Lane 1, bacterial culture supernatant without induction; lane 2, bacterial deposit without induction; lane 3, rPer a 1.0101 in the supernatant; lane 4, rPer a 1.0101 in the deposit; lane 5, rPer a 1.0104 in the supernatant; and lane 6, rPer a 1.0104 in the deposit; lane M represents protein markers. (B), purified rPer a 1.0101 (lane 1) and rPer a 1.0104 (lane 2) were exhibited on SDS-PAGE. (C), Western blot analysis of Per a 1.0101 and Per a 1.0104 allergens in the sera from cockroach allergic patients. Lane 1-12, rPer a 1.0101 was detected with serum (dilution 1:4) from 12 different cockroach allergy individuals. Lane 13-23, rPer a 1.0104 was detected with serum from 11 different cockroach allergy individuals. Lane M represents protein markers.

Table 2 Identification of Per a 1.0101 (Per 01) and Per a 1.0104 (Per 04) peptide fragments digested by trypsin by using LC-ESI-MS/MS analysis.

Identified peptide sequence	Position in sequence	MH ⁺
R.DKGVDVDHYIELIRALFGLTR.A	23–44 (Per 01)	2431.78
K.GVDVDHYIELIRALFGLTRAAR.N	25–47 (Per 01)	2486.86
K.IITTIEGLPEFANFYNFLK.E	93–112 (Per 01)	2231.58
K.EHGLDVADFLNEIHSIIGLPPFVPSRRHAR.R	112–143 (Per 01)	3491.95
R.GVGINGLIDDVIAILPVDELKALFQEK.L	144–171 (Per 01)	2881.36
K.LETSPDFKALYDAIR.S	171–186 (Per 01)	1739.95
R.DKGVDVDHFIELIRSLFGLP.-	211–231 (Per 01)	2271.60
K.ALFAQELETSLDFKAFYDAVR.S	20–41 (Per 04)	2492.81
K.LETSLDFKAFYDAVR.S	26–41 (Per 04)	1775.98
K.TLFQEKLETSPDFKALYDAIR.S	208–229 (Per 04)	2486.81
K.TLFQEKLETSPDFK.A	208–222 (Per 04)	1683.88

33 kDa were observed on a SDS–PAGE (Fig. 1B). Solubility analysis showed that Per a 1.0101 and Per a 1.0104 possessed very high probabilities (>90%) of being soluble when expressed in *E. coli*.

MS analysis of rPer a 1.0101 and rPer a 1.0104

In order to ensure our recombinant proteins are Per a 1.0101 and Per a 1.0104 molecules, we examined the proteins by LC-ESI-MS/MS analysis. Following trypsin digestion, seven peptide fragments from Per a 1.0101 and 4 peptide fragments from Per a 1.0104 (Table 2) were obtained. They matched well with Per a 1.0101 and Per a 1.0104 protein sequences.

Enzymatic activities of rPer a 1.0101 and rPer a 1.0104

As large numbers of allergens possess enzymatic activities [14, 15], we examined tryptic, chymotryptic, metalloproteinase and aspartic proteinase activities of purified rPer a 1.0101 and rPer a 1.0104. At the concentrations of 0.5, 5.0 and 50 µg/ml, rPer a 1.0101 and rPer a 1.0104 failed to show any tryptic or chymotryptic metalloproteinase and aspartic proteinase activities towards substrates BAPNA, SAAPP, casein and haemoglobin, respectively.

IgE reactivity of the rPer a 1.01 proteins

To confirm Per a 1.0101 and Per a 1.0104 are major allergens of American cockroach, IgE reactivity of them was determined with sera from 15 cockroach allergy patients. As shown in Fig. 1C, rPer a 1.0101 protein reacted to 80% (12 of 15) of the sera from cockroach allergy patients, while rPer a 1.0104 reacted to 73.3% (11 of 15) of the sera. Among the cockroach allergy patients, eight reacted to both rPer a 1.0101 and rPer a 1.0104. Both allergens did not react to the sera from 6 ragweed allergic patients and four HC. Other proteins of *E. coli* BL21 (DE3) did not react to the sera from cockroach allergic patients (data not shown).

Effects of rPer a 1.0101 and rPer a 1.0104 on mRNA expression of PARs

It has been reported that German cockroach extract can activate PAR-2 [7] and that rPer a 7 can upregulate the expression of PARs on P815 cells [8]. We therefore anticipate that rPer a 1.01 may also affect the expression of PARs on P815 cells. As expected, real-time PCR showed that rPer a 1.0101 and rPer a 1.0104 upregulated mRNA expression of PAR-1 in P815 cells at 6 h following incubation (Fig. 2A). rPer a 1.0101 and rPer a 1.0104 induced also an upregulated expression of PAR-2 (Fig. 2B) and PAR-3 (Fig. 2C) mRNAs in P815 cells. Similarly, both rPer a 1.0101 and rPer a 1.0104 elicited concentration-dependent increase in PAR-4 mRNA expression, which started at 2 h and reached the peak value at 6 h following incubation (Fig. 2D). Specific antibody against rPer a 1.01 blocked the rPer a 1.0101- and rPer a 1.0104-induced expression of PAR mRNAs by approximately up to 78.4% and 82.1%.

Effects of rPer a 1.0101 or rPer a 1.0104 on expression of PARs in P815 cells

To confirm influence of rPer a 1.0101 or rPer a 1.0104 on the expression of PAR proteins, immunofluorescent microscopy and flow cytometry analyses were applied. Immunofluorescent microscopy showed that rPer a 1.0101 induced an upregulated expression of PAR-1 and PAR-2, whereas rPer a 1.0104 provoked an enhanced expression of PAR-1 and PAR-4 in P815 cells (Fig. 3A). The more detailed study with flow cytometry analysis (Fig. 3B) revealed that minimum of 1.0 µg/ml of rPer a 1.0101 or rPer a 1.0104 was required to induce significantly enhanced expression of PAR-1 or PAR-4 proteins, respectively. rPer a 1.0101 at 0.1 and 1.0 µg/ml provoked also enhanced PAR-2 expression by up to 2.5-fold (Fig. 3C). The time course study showed that rPer a 1.0101 and rPer a 1.0104 induced upregulation of expression of PARs initiated at 2 h and continuously increased until 16 h following incubation (Fig. 3D).

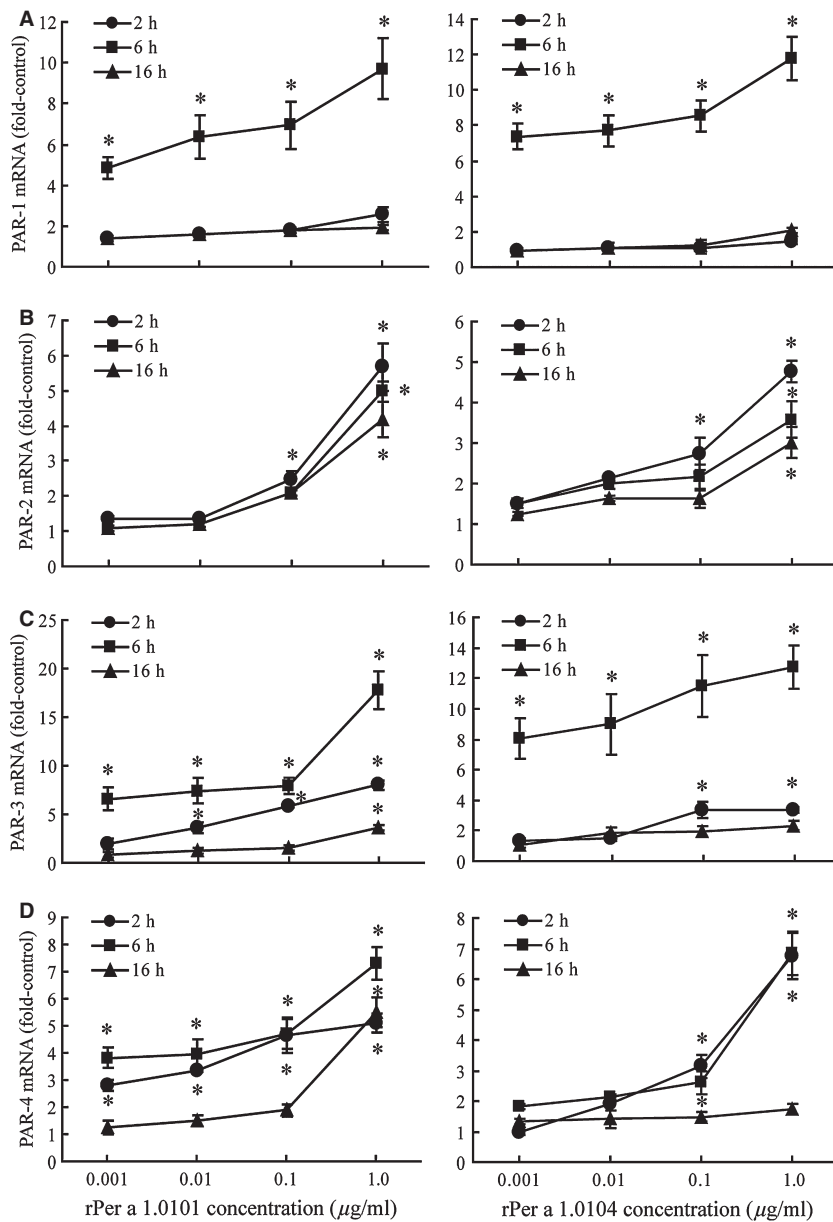


Figure 2 Effects of rPer a 1.0101 and rPer a 1.0104 on the expression of PAR-1 (A), PAR-2 (B), PAR-3 (C) and PAR-4 (D) mRNAs in P815 cells. P815 cells were incubated with various concentrations of rPer a 1.0101 or rPer a 1.0104 at 37 °C for 2, 6 and 16 h before expression of PAR mRNAs was determined by real-time quantitative RT-PCR. The data were expressed as mean $\pm$ SEM for four separate experiments performed in duplicate after normalized to housekeeping gene β -actin. * P < 0.05 compared with the medium alone control.

Specific antibody against rPer a 1.01 blocked the rPer a 1.0101 induced expression of PAR-1 and PAR-2 by approximately 74.6% and 77.2%, and rPer a 1.0104 induced the expression of PAR-1 and PAR-4 by approximately up to 72.5% and 80.1%, respectively. Calcium ionophore A23187 (100 ng/ml) had little effect on the expression of PARs on P815 cells following 2-, 6- and 16-h incubation (data not shown).

Induction of IL-4 and IL-13 release from P815 cells by rPer a 1.0101 and rPer a 1.0104

It has been recognized that cytokines such as Th2 cytokines play a key role in the pathogenesis of allergic

inflammation and that mast cells are one of major sources of cytokines. As mast cells can release IL-4, IL-10, IL-12 and IL-13 [16, 17], and American cockroach allergen Per a 7 has been found to induce IL-4 and IL-13 secretion [8], we investigated the influence of rPer a 1.0101 and rPer a 1.0104 on these cytokine secretion from P815 cells. The results showed that rPer a 1.0101 and rPer a 1.0104 provoked a dose-dependent increase in IL-4 and IL-13 release following 16-h incubation period. They also induced increase in IL-4 (Fig. 4A) or IL-13 (Fig. 4B) release at 6 h following incubation. rPer a 1.0101- and rPer a 1.0104-induced IL-4 and IL-13 release were significantly blocked by specific antibody against rPer a 1.01. At the concentrations tested, rPer a 1.0101 and rPer a

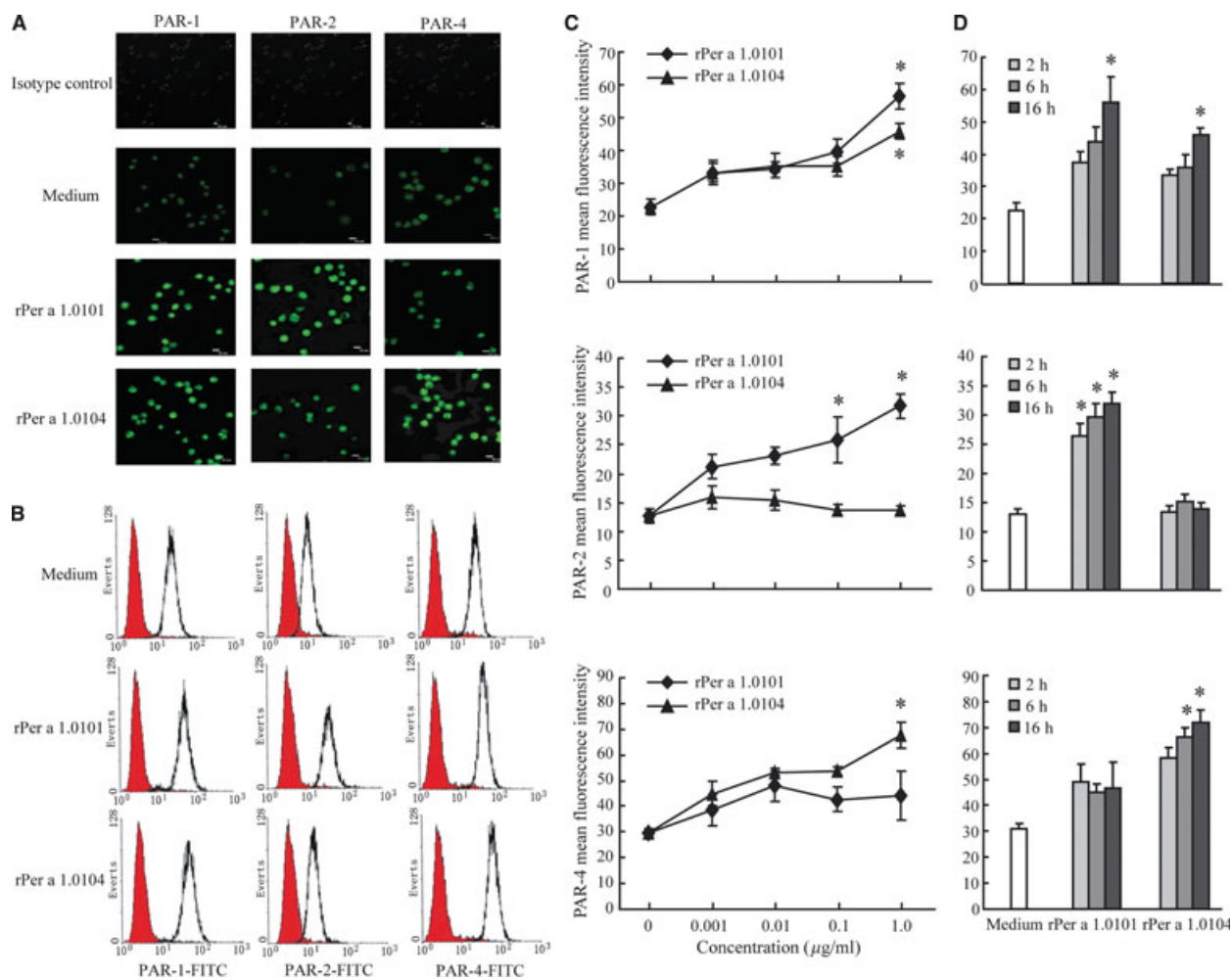


Figure 3 Effects of rPer a 1.0101 and rPer a 1.0104 on the expression of PARs on P815 cells. (A), P815 cells were incubated with 1.0 $\mu\text{g/ml}$ of rPer a 1.0101 or rPer a 1.0104 for 16 h, and immunofluorescent microscopy was performed with a laser scanning confocal microscope. (B), P815 cells were incubated with 1.0 $\mu\text{g/ml}$ of rPer a 1.0101 or rPer a 1.0104 for 16 h, and expression of PARs was determined by flow cytometry analysis. (C), P815 cells were incubated with various concentrations of rPer a 1.0101 or rPer a 1.0104 for 16 h at 37 °C before expression of PARs was determined by flow cytometry analysis. (D), P815 cells were incubated with 1.0 $\mu\text{g/ml}$ of rPer a 1.0101 or rPer a 1.0104 for 2, 6 and 16 h before expression of PARs was determined by flow cytometry analysis. The data were expressed as mean $\pm$ SE for four separate experiments performed in duplicate. * $P < 0.05$ compared with the medium alone control.

1.0104 failed to induce IL-10 and IL-12 release from P815 cells following 6-h and 16-h incubation periods (data not shown).

Discussions

ET-28a is a powerful prokaryotic expression vector capable of producing reasonable quantities of foreign proteins in *E. coli* when induced by IPTG [18]. The BugBuster Protein Extraction Reagent can solubilize cell components, thereby release cellular proteins without denaturation and remain greater activity. Our results showed that the target proteins retain their unique molecule sequences and immunological activity as assessed by LC-ESI-MS/MS and Western blot analyses. Using the same

expression system, Wu *et al* [3] showed recombinant Per a 1.0104 can react with specific IgE from serum of allergic patients, indicating the protein possesses biological activity. Lack of cysteine residue and potential N-glycosylation site in Per a 1 molecule also supports that the *E. coli* expression system employed in the present study is suitable for producing functional Per a 1 proteins.

To our surprise, sera from 80% of cockroach allergy patients react to rPer a 1.0101 protein, which is a much higher incident rate than that reported by Wu *et al* [3] (~50%). It is rather difficult to explain the reason for the discrimination, but nevertheless, it confirms that Per a 1.0101 is a major allergen of American cockroach. Sera from 73.3% of cockroach allergy patients react to rPer a 1.0104 protein is agreed well with a previous report

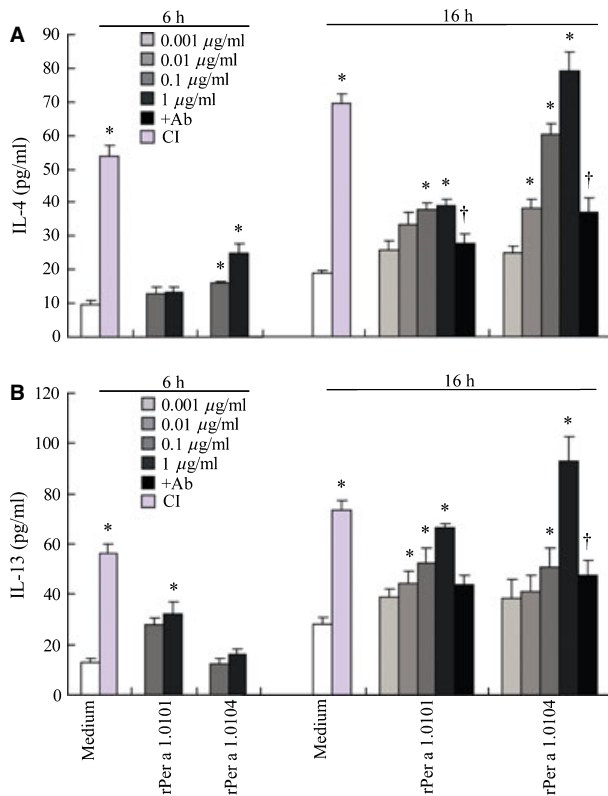


Figure 4 Effects of rPer a 1.0101 or rPer a 1.0104 on the release of IL-4 (A) and IL-13 (B) from P815 cells. P815 cells were incubated with rPer a 1.0101 or rPer a 1.0104 with or without their blocking antibody for 6 or 16 h, and the accumulated cytokines in supernatant were determined. Data are means $\pm$ SE of four separate experiments performed in duplicate. * P < 0.05 compared with the medium alone control. † P < 0.05 compared with rPer a 1.0101 or rPer a 1.0104 at 1.0 μ g/ml. +Ab = rPer a 1.0101 or rPer a 1.0104 at 1.0 μ g/ml + specific antibody against rPer a 1.01 at 3.0 μ g/ml. CI = calcium ionophore A23187 at 100 ng/ml.

which showed that 77.3% cockroach-sensitive atopic patients reacted to per a 1.0104 during skin prick [19]. The very similar reactivity of specific IgE to rPer a 1.0101 and rPer a 1.0104 implicates that allergenicity of these two molecules is similar, and the epitopes of allergenicity are likely located in the identical parts of the two molecules.

As little is known of functions of Per a 1 allergens, we demonstrate for the first time that recombinant Per a 1.0101 and Per a 1.0104 are able to induce enhanced expression of PAR-1 protein. Induction of upregulated PAR-2 and PAR-4 expression by rPer a 1.0101 and rPer a 1.0104, respectively, indicates that these two Per a 1 isoallergens can act differently on the expression of PARs even if they share nearly 80% identity in their protein sequence. Like rPer a 7 [8], as much as 1 μ g/ml of rPer a 1.0101 and rPer a 1.0104 are required to stimulate significant increase in PAR-1 protein expression, and minimum of 1 μ g/ml of rPer a 1.0104 is needed to induce

significant PAR-4 expression. As it is unlikely to accumulate such a high concentration of the allergens in the body, upregulated PAR-1 and PAR-4 expression should not play an important role in cockroach allergy. In contrast, Per a 1.0101-induced upregulation of expression of PAR-2 may be involved in cockroach allergy as only 100 ng/ml of Per a 1.0101 is required to induce significant increase in PAR-2 expression. Activation of PAR-2 has been recognized to play an important role in allergic diseases. Patients with asthma express an increased amount of PAR-2 on respiratory epithelial cells [20], and PAR-2 activation in human airways is associated with contraction of human airways and contributes to the hyperplasia and hyper-responsiveness evident in the asthmatic airway [21]. Furthermore, our results indicate that Per a 1.0101 and Per a 1.0104 are not proteases. Therefore, their actions on PARs should not depend on enzymatic activity.

Once again like rPer a 7, we observed the expression of certain mRNAs of PARs, but not corresponding proteins in P815 cells upon rPer a 1.0101 and rPer a 1.0104 challenge. This dissociation between gene and protein expression has been reported previously [22] and there are many complicated and varied post-transcriptional mechanisms involved in turning mRNA into protein [23], which may help to explain our earlier observations.

Like Per a 7, both rPer a 1.0101 and rPer a 1.0104 can induce secretion of Th2 cytokines IL-4 and IL-13 from P815 cells. As overexpression of IL-4 is predominantly found in the airways of asthmatics [24] and IL-4 is the key cytokine in development of Th2 cell responses [25], IL-13, which shares a receptor component with IL-4, is a critical cytokine for allergen-induced asthma [26], and the findings that rPer a 1.0101 and rPer a 1.0104 can induce IL-4 and IL-13 release from mast cells may be of importance for cockroach allergy. As much lower concentrations of rPer a 1.0101 and rPer a 1.0104 are required to induce IL-4 and IL-13 release than to upregulate expression of PARs, cytokine release may be an earlier event than altered expression of PAR expression when mast cells are challenged by Per a 1.01 allergens.

In conclusion, we have demonstrated for the first time that American cockroach allergens Per a 1.0101 and Per a 1.0104 have no enzymatic activity, but can modulate the expression of PARs in P815 cells. They can also provoke Th2 cytokines IL-4 and IL-13 secretion from the mast cells. Our results suggest that Per a 1.0101 and Per a 1.0104 are likely to contribute to the development of cockroach-related allergic disease through modulation of mast cell behaviour.

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