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Expression and Rapid Purification of an *Aedes aegypti* Salivary Allergen by a Baculovirus System

Key Words

Recombinant allergen/antigen

Aed a 1

Baculovirus

Purification

Aedes aegypti

Mosquito allergy

Abstract

Mosquito salivary proteins cause allergic reactions in humans. Recombinant salivary allergens will facilitate both the diagnosis and immunotherapy of mosquito allergy. The Aed a 1, a 68-kD apyrase in the saliva of *Aedes aegypti*, has been demonstrated to be an allergen which binds to the IgE of mosquito-allergic subjects. The baculovirus expression vector pBlueBacHis C equipped with an N-terminal histidine tag was used to express the Aed a 1 protein. The cDNA coding for the 3' significant portion of Aed a 1 (Aed a 1 3') (150–562 amino acid residues) was cloned into pBlueBacHis C. The rAed a 1 3' protein expressed by recombinant baculovirus was verified by immunoblot using anti-Aed a 1 and anti-histidine antibodies, respectively. The histidine-tagged fusion protein was purified to apparent homogeneity from infected Sf9 cells by Ni²⁺ resin affinity chromatography. Both immunoblot and ELISA showed that the purified rAed a 1 3' binds to the IgE and IgG in mosquito allergic sera, indicating that the antigenicity of the rAed a 1 3' is identical to the native Aed a 1 of *Aedes aegypti* saliva.

Introduction

Reactions to mosquito bites are caused by mosquito saliva [1] with the involvement of IgE-mediated and lymphocyte-mediated hypersensitivities [2, 3]. Immunotherapy with injections of mosquito whole-body extract has been reported to relieve reactions to subsequent bites [4–6]. The major drawback of currently available mosquito whole body extracts lies in the presence of many non-salivary proteins [7] which may cause side effects without conferring benefit. Collection of mosquito saliva for use in immunotherapy is not feasible. During the past few years a number of important allergens have been expressed using recombinant DNA technology [8–10]. Utilization of this technique to produce pure mosquito allergens in large quantities will be an important advance in clinical immunology.

Saliva from adult *Aedes aegypti* contains as many as 20 peptides [11]. Among them, a 68-kD protein was identi-

fied as apyrase [12], an ATP diphosphohydrolase, which functions as a platelet anti-aggregator by destroying adenosine di- and tri-phosphates released from injured cells or by activated platelets [12]. In our experiments, the same 68-kD protein in *Aedes aegypti* saliva was found to react with an anti-apyrase antibody and to bind to IgE in 11/12 (92%) of the subjects allergic to mosquito bites [13]. This indicates that the 68-kD protein in the saliva is an apyrase and is also an allergen. According to the allergen nomenclature [14], we have now named the 68-kD apyrase as Aed a 1. The cDNA coding the 3' significant portion of Aed a 1 (Aed a 1 3') containing 150–562 amino acid residues has been cloned [15].

A baculovirus/insect cell system, which performs many of the post-translational modifications found in eukaryotic cells, has been used extensively for the production of large amounts of recombinant proteins with biological activity. pBlueBacHis vectors are designed for efficient expression

and purification of these recombinant proteins. These vectors contain six histidine residues near the N-terminus which have a high affinity for the Ni²⁺-resin column. The expressed fusion proteins with the six histidines can be easily purified by affinity chromatography with the Ni²⁺-resin column [16–18].

In this study, we expressed and purified the mosquito allergen Aed a 1 3' using the baculovirus expression vector pBlueBacHis C, and analyzed the immunological properties of the recombinant allergen.

Materials and Methods

Materials

Restriction enzymes and T4 DNA ligase were purchased from Promega (Madison, Wisc., USA). The DNA sequence kit was obtained from United States Biochemical (Cleveland, Ohio, USA). The PCR kit came from Boehringer Mannheim Canada (Quebec, Canada). pBluescriptII SK⁻ plasmid was obtained from Stratagene (La Jolla, Calif., USA). pBlueBacHis C expression vector, *S. frugiperda* insect cell line (Sf9), Grace's insect media, ProBondTM Ni²⁺-resin affinity column, and monoclonal antibody to histidine tag (Anti-XpressTM antibody) were purchased from Invitrogen (San Diego, Calif., USA). cDNA clone coding for 150–562 amino acid residues of Aed a 1 and rabbit IgG anti-rAed a 1 were kindly provided by Dr. A. James, University of California, Irvine. Monoclonal mouse anti-human IgG was purchased from Pharmingen (San Diego, Calif., USA). Monoclonal anti-human IgE (clone 7.12) was provided by Dr. A. Saxon, University of California, Los Angeles. Goat anti-human IgE was a gift from Dr. N.F. Adkinson, Jr., The Johns Hopkins Allergy and Asthma Centre (Baltimore, Md., USA). Alkaline phosphatase-conjugated goat anti-rabbit IgG, peroxidase-conjugated goat anti-mouse IgG, goat anti-human IgG and alkaline phosphatase-conjugated rabbit anti-goat IgG were obtained from Jackson ImmunoResearch Lab. Inc. (West Grove., Pa., USA).

Mosquito-Allergic and -non-allergic Sera

Three subjects with severe skin reactions to *A. aegypti* bites and 1 subject with negative reactions to the bites selected from the 41 subjects studied [3]. Umbilical cord serum was used as a negative control.

Plasmid Construction

The construction of the pBlueBacHis C/Aed a 1 3' expression vector is shown schematically in figure 1. In brief, the Aed a 1 3' cDNA fragment (1.2 kb) carried on the Lambda SGG12 clone [15] was excised by *Eco*RI digestion. This fragment was then cloned into the *Eco*RI site of pBluescriptII SK⁻, and the clones carrying the insert with *Bam*HI at the 5' end and *Hind*III at the 3' end were selected and identified by Csp451 analysis. The *Bam*HI-*Hind*III fragment derived from pBluescriptII SK⁻/Aed a 1 3' was cloned into the *Bam*HI/*Hind*III site of pBlueBacHis C, resulting in a ligation of the insert cDNA in frame with the 6-histidine cassette at the 5' end. The recombinant plasmids were identified by restriction enzyme analysis, and the reading frame of the Aed a 1 3' cDNA was further confirmed by DNA sequence analysis. The resultant plasmid was purified by CsCl gradient centrifuging and used for transfection experiments.

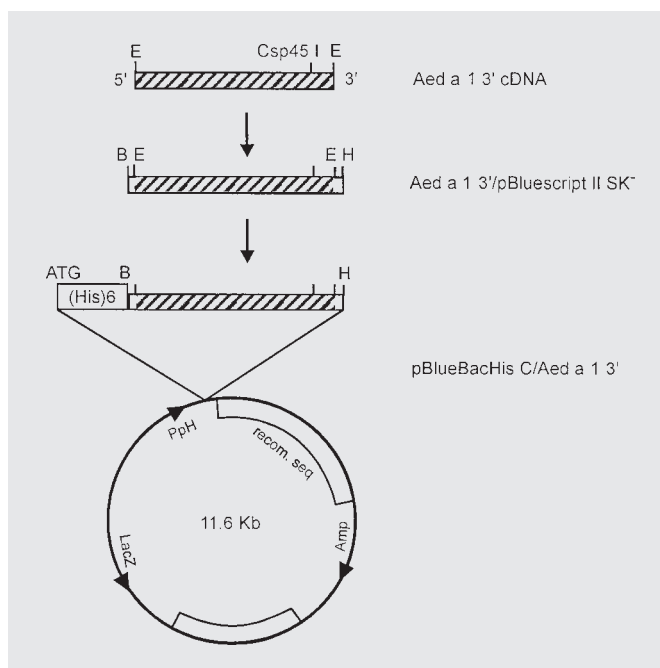


Fig. 1. Construction of pBlueBacHis C/Aed a 1 3' expression vector. Aed a 1 3' cDNA was introduced first into an intermediate vector pBluescript II SK⁻, then subsequently transferred into the *Bam*HI/*Hind*III restriction site of pBlueBacHis C. pBlueBacHis C/Aed a 1 3' encodes an (His)6 fusion protein containing aa 150–562 of Aed a 1 under the control of the polyhedron promoter.

Generation of Recombinant Baculovirus

Recombinant baculoviruses carrying the Aed a 1 3' cDNA were generated by cotransfection of Sf9 cells with the recombinant transfer vector together with linearized baculovirus AcMNPV DNA using the cationic liposome method. Briefly, Sf9 cells (2×10^6) were seeded onto each 60-mm tissue culture plate and allowed to attach for 30 min. The transfection mix was prepared by mixing 1 µg of linearized AcMNPV DNA and 3 µg of purified pBlueBacHis C/Aed a 1 3' DNA with 20 µl of cationic liposome solution, and incubating for 15 min at room temperature. After washing the culture plate once with 2 ml Grace's media without supplements or FBS, 1 ml of transfection mix was added. Then the tissue culture plate was incubated at room temperature for 4 h. 1 ml of complete TNM-FH media was added to each 60-mm plate and incubated at 27°C for a further 48 h. The transfection media were then harvested for purification of the recombinant viruses.

Pure recombinant viral stocks were isolated by 3 rounds of end-point plaque assay followed by PCR identification. Viral DNAs were extracted from the medium supernatant of the 12-well plate 3 days after infection and analyzed by PCR following the PCR Core kit instruction. The PCR products were further verified by analyzing the unique Csp451 site within Aed a 1 3' cDNA sequence.

Expression of rAed a 2 3'

To compare the expression level of different clones of recombinant viruses, 1.0×10^6 Sf9 cells/well in 12-well plate were infected with each recombinant virus at a multiplicity of infection (MOI) of about 10 and incubated at 27°C. Forty-eight hours after infection, the cell ly-

sates were analysed by SDS-PAGE and immunoblot using rabbit anti-Aed a 1 and monoclonal antibody to the histidine tag of the fusion protein, respectively. To assess the time course of protein expression, suspension culture (30 ml) of Sf9 cells at 2.0×10^6 cells/ml was infected with the Aed a 1 3' recombinant baculovirus, at an MOI of 10 in a 125-ml spinner flask. At various times after infection, 1 ml of suspension culture was collected and centrifuged at 2,000 *g* for 5 min. The cell pellets were lysed by 3 cycles of freezing in liquid nitrogen and thawing at 42°C, and the lysates were assayed for recombinant Aed a 1 3' by immunoblotting as described later.

For protein production, 200 ml of suspension culture of Sf9 cells were infected with the Aed a 1 3' recombinant baculovirus in a 500 ml spinner flask under the same conditions as described in the assay for time course of expression. Cells were harvested at the time of post-infection at which maximum expression occurred, by centrifuging at 800 *g* for 20 min (Beckman GPR centrifuge), then washed in ice-cold PBS (20 mM sodium phosphate and 500 mM NaCl at pH 7.8), re-centrifuged and stored at -70°C for future use.

Purification of rAed a 1 3'

The rAed a 1 3' fusion protein contained at its N-terminal a 6 histidine tag which has high affinity to Ni²⁺-resin. Purification of the protein was performed by immobilized metal affinity chromatography on an Ni²⁺-resin column under mild non-denaturing conditions as described in the manufacturer's instructions. The cell pellet with the accumulated recombinant protein derived from the infected 4×10^8 Sf9 cells was resuspended in 20 ml binding buffer consisting of 20 mM sodium phosphate and 500 mM NaCl at pH 7.8 supplemented with 0.5 µg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride. The suspension was subjected to 2 cycles of freezing in liquid nitrogen and subsequent thawing at 42°C. The DNA in the sample was sheared by passing the preparation 4 times through an 18-gauge needle, followed by centrifuging at 30,000 *g* for 30 min. 20 ml of the supernatant from four 5-ml aliquots were loaded onto a Ni²⁺-resin column (2 ml) pre-equilibrated with 20 ml of binding buffer. The column was sequentially washed with 20 ml of binding buffer and then 40 ml of washing buffer (the same as binding buffer but at pH 6.3). The bound proteins were subsequently eluted by applying consecutively 10 ml of 0.05 M imidazole washing buffer, 8 ml of 0.1 M imidazole washing buffer, and 8 ml of 0.2 M imidazole washing buffer. The fractions were monitored by the absorbance at OD₂₈₀. The fractions with high absorbance were pooled, dialysed against binding buffer, and concentrated using a 10,000-kD centrifugal concentrator (Filtron Technology Corp., Northborough, Mass., USA).

SDS-PAGE and Immunoblotting

Various recombinant protein preparations were dissolved in SDS-PAGE sample buffer with 5% 2-mercapto-ethanol and heated at 100°C for 5 min. 10 µl of each preparation were loaded per well and SDS-PAGE was then performed using the Bio-Rad Mini-Protein II apparatus (Bio-Rad, Hercules, Calif., USA) with 10% polyacrylamide gel. Sf9 cells infected with wild-type virus and uninfected cells were used as negative controls. After electrophoresis, gels were stained with Coomassie brilliant blue. For immunoblotting with rabbit anti-rAed a 1, electrophoretic transfer of proteins from gels to nitrocellulose membranes was performed at 100 V for 1.5 h at 4°C in a Trans-Blot cell (Bio-Rad). The transferred nitrocellulose membranes were blocked overnight with 3% BSA in PBS-T (0.02 M PBS, pH 7.2, supplemented with 0.05% Tween-20). The membranes were incubated for 1 h at room temperature with rabbit IgG anti-rAed a 1 diluted 1:5,000 in

incubation buffer (PBS-T, 0.02 M PBS, pH 7.2, supplemented with 0.05% Tween-20). After three washes with PBS-T, colour was developed by incubation with 1:2,000 diluted enzyme-conjugated goat anti-rabbit IgG (1 h) followed by incubation with the substrate (4-chloro-3-indoylphosphate *p*-toluidine salt) and NiCl₂·6H₂O (0.1% in 0.1 M sodium azolium chloride). Pre-stained SDS-PAGE standards were used to determine the relative molecular weights of the separated components.

The same method, with minor modifications, was used in the study of the binding of rAed a 1 3' to human IgG. The blocked membranes were incubated with 1:100 diluted human sera at 4°C overnight, and then washed with monoclonal anti-human IgE or IgG (1:10,000 for 1 h at 4°C followed by 5 h at room temperature) followed by incubation with peroxidase-conjugated goat anti-mouse IgG (1:5,000) for 1.5 h at room temperature. The membranes were finally incubated with ECL detecting reagent (Amersham, Arlington Heights, Ill., USA) and then exposed to the X-Omat film (Kodak, Rochester, N.Y., USA).

Enzyme-Linked Immunosorbent Assay (ELISA) and Inhibition Tests

ELISA was developed to detect Aed a 1 3'-specific IgE and IgG in human sera. Optimal conditions were chosen by checkerboard titration. Microtiter plates were coated overnight at 4°C with 0.05 µg/well of purified rAed a 1 3' protein or 0.02 µg/well of *A. aegypti* saliva [7] in 0.05 M bicarbonate buffer, pH 9.6. The plates were washed three times with PBS-T and blocked with 2% BSA in PBS-T for 1 hour at room temperature. After washing, 100 µl of 10-fold serially diluted serum samples were added to each well. The plates were incubated overnight at 4°C. After washing, the bound specific IgE or IgG were detected by adding 100 µl of 1:2,000 diluted goat anti-human IgE or anti-human IgG. After incubation at 37°C for 1.5 h and a further washing, 100 µl of 1:2,000 diluted alkaline phosphatase conjugated rabbit anti-goat IgG were added and incubated at 37°C for 1.5 h. Following a final wash, the enzyme substrate solution (1 mg/ml of *p*-nitrophenylphosphate in diethanolamine buffer, pH 9.8) was added. The reaction was stopped by adding 100 µl of 1 N NaOH. Optical absorbance at 410 nm was read using a THERMO-max microplate reader (Molecular Devices, Calif., USA).

An inhibition test was performed to examine the inhibiting of the binding of rAed a 1 3' to human IgE by the native allergen. Due to the difficulty in obtaining the large amount of mosquito saliva required to perform inhibition tests, an *A. aegypti* head and thorax extract was used. The mosquito extract was 10-fold diluted. Each dilution was mixed with an equal volume of a 1:100 diluted mosquito-allergic serum and incubated at 4°C overnight. Incubation of PBS-T with the diluted serum served as a positive control. These incubations were then measured for rAed a 1 3'-specific IgE and *A. aegypti* saliva-specific IgE by ELISA as described above.

Results

Expression of rAed a 1 3'

The resultant recombinant baculovirus expression vector (fig. 1) was cotransfected with wild-type AcMNPV DNA into the Sf9 insect cells. Seven putative recombinant virus clones were selected, plaque-purified, and evaluated by PCR analysis. All 7 clones were found to contain the rAed a 1 3' cDNA inserts of the appropriate size (data not shown).

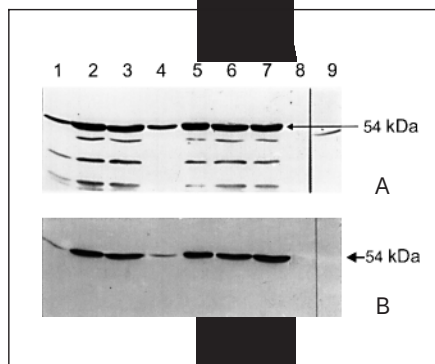


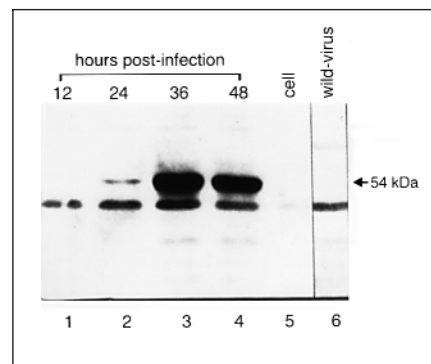
Fig. 2. Immunoblotting of the histidine-tagged rAed a 1 3' protein expressed by 7 recombinant baculovirus clones. Sf9 cell lysates 48 h after infection with 7 baculovirus clones (lanes 3–7) were subjected to immunoblot analysis with anti-rAed a 1 (A) and anti-histidine tag (B) antibodies as described in 'Methods'. The same uninfected cells (lane 8) and wild-type virus-infected cells (lane 9) were used as negative controls.

The Sf9 cell lysates 48 h after infection with the 7 clones of recombinant baculoviruses were analyzed by immunoblotting with anti-rAed a 1 and anti-histidine tag antibodies, respectively. All 7 of the clones of recombinant viruses were found to express a 54-kD molecule (fig. 2). The same molecular size as theoretically estimated. This molecule was recognized by both the anti-rAed a 1 (fig. 2A) and anti-histidine tags (fig. 2B) antibodies. No 54-kD immunoreactive protein was detected in the lysates of wild-type baculovirus-infected cells or uninfected cells (fig. 2, lanes 8 and 9). The results indicate that the expressed product is a fusion protein with Aed a 1 antigenicity and a histidine tag. As shown in figure 2, the intensity of the expressed rAed a 1 3' varied among the clones of the recombinant viruses. The clone which expressed the highest level of rAed a 1 3' was selected for subsequent studies.

Figure 3 shows the time-course of the rAed a 1 3' expression in baculovirus infected Sf9 cells. The maximum production of the recombinant protein was detected at 36 h post-infection. It appeared that the amount of recombinant protein produced at 48 h post-infection was slightly less than that at 36 h post-infection. Therefore, in order to maximize the expression product, infected Sf9 cells should be processed between 36 and 48 h post-infection. The lower faint band found in cell lysates (lanes 1 to 4) was due to the cross-reactivity of the anti-rAed a 1 with the wild-virus, because this band was also presented in the wild-virus control.

Purification of rAed a 1 3'

Figure 4 shows that the histidine tagged rAed a 1 3' protein is tightly bound to the Ni^{2+} -resin and can be eluted only



Time-course of rAed a 1 3' expression in baculovirus-infected Sf9 cells. Sf9 cell lysates 12–60 h after infection (lanes 1–4) were analyzed by immunoblotting with anti-rAed a 1 antibodies as described in 'Methods'. Uninfected cells (lane 5) and cells infected with wild-type virus (lane 6) were used as negative controls.

with imidazole (lane 6). The small amount of rAed a 1 3' found in the effluent (lane 2) may be due to sample loss. The proteins which did not bind to the column were those which eluted with the washing buffer (lanes 1 and 2) and those which eluted with the 0.05 M (lane 4) and 0.1 M (lane 5) imidazole. The proteins which were removed prior to the elution with the 0.2 M imidazole by which the rAed a 1 3' was eluted as a homogeneous protein (lane 6).

IgE and IgG Binding Detected by Immunoblotting

As shown in figure 5, the three mosquito allergic subjects (lanes 2 to 4) have both IgE (A) and IgG (B) antibodies specific to rAed a 1 3'. No apparent rAed a 1 3'-specific IgE and IgG was found in either the mosquito non-allergic serum or the cord serum.

IgE and IgG Binding Measured by ELISA

An rAed a 1 3' capture-ELISA was developed to detect rAed a 1 3'-specific IgE and IgG. As shown in figure 6, rAed a 1 3'-specific IgE and IgG levels were significantly higher in the 3 subjects with severe skin reactions to *A. aegypti* bites compared to the non-allergic serum and the cord serum. The subject without skin reaction to the bites had very low levels of specific IgE and IgG. No IgE and low IgG levels were found in the cord serum.

ELISA Inhibition Tests

As shown in figure 7, the binding of the IgE in the mosquito-allergic serum to both rAed a 1 3' and saliva was successfully inhibited by the mosquito head and thorax extract in a dose-dependent manner. This indicates that the recom-

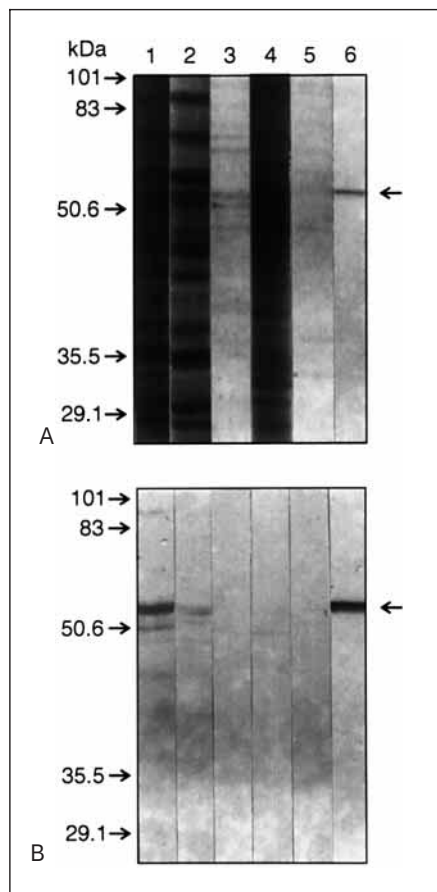


Fig. 4. SDS-PAGE and Coomassie blue stain (A) and immunoblot analysis (B) of fractions obtained during purification of rAed a 1 3' on immobilized metal affinity chromatography. The cell lysate supernatant (lane 1) was loaded onto a Ni^{2+} -resin column equilibrated with binding buffer. The column was washed with binding buffer (lane 2) followed by washing buffer (lane 3), and then sequentially eluted by 0.05 M imidazole (lane 4), 0.1 M imidazole (lane 5), and 0.2 M imidazole (lane 6) as described in 'Methods'. The effluent and elutions were analyzed by SDS-PAGE with Coomassie blue stain (A) and with immunoblotting using anti-rAed a 1 as described in 'Methods'. The rAed a 1 3' was purified as a homogeneous protein as indicated by the arrow.

binant Aed a 1 3' allergen and the natural allergen in the head and thorax extract have identical antigenicity and that the rAed a 1 3' capture-ELISA was specific for the detection of Aed a 1-specific IgE.

Discussion

The native Aed a 1 presented in *A. aegypti* saliva is an apyrase which functions as a platelet anti-aggregation component and may play a significant role in blood feeding [12].

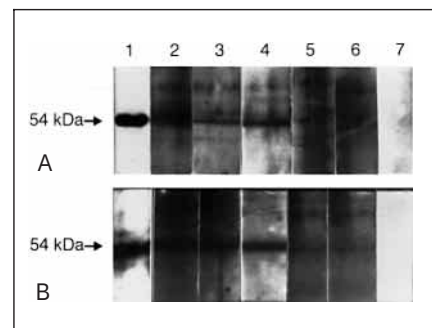


Fig. 5. Binding of rAed a 1 3' to human IgE and IgG in immunoblot. rAed a 1 3' was separated by SDS-PAGE and transferred to nitrocellulose membranes. Lane 1 was incubated with anti-Aed a 1 followed by incubation with goat anti-rabbit IgG conjugate as a positive control. Lanes 2–7 were incubated with 3 mosquito allergic sera (lanes 2–4), a mosquito non-allergic serum (lane 5), a cord serum (lane 6), and dilution buffer (lane 7). This was followed by sequential incubations with monoclonal anti-human IgE (A) or anti-human IgG (B) and with enzyme-conjugated goat anti-mouse IgG.

In our previous experiments, 11 of 12 mosquito-allergic subjects had Aed a 1-specific IgE as detected by immunoblotting [13], indicating that this protein also elicits an IgE response and therefore is an allergen. The Aed a 1 is composed of 562 amino acid residues, with a molecular weight of 68 kD based on immunoblotting [15]. Two segments in the cDNA library comprise the full-length apyrase cDNA, the 5' segment (1–149 amino acid residues, lambda SGG12-5) and the 3' significant portion (150–562 amino acid residues, lambda SGG12) [15]. The 3' portion was expressed by a baculovirus system in the present study.

The recombinant baculovirus expression system, which performs many of the posttranslational modifications found in mammalian cells, is an excellent system for the production of large amounts of biologically active proteins. The efficiency of expression of baculovirus system differs from gene to gene by approximately 1,000-fold [19]. Most heterologous proteins are produced at levels ranging from 1 to 100 mg/10⁹ cells (i.e. a 1-litre culture) [19–22]. The varieties of expression vectors may also play a critical role in the expression levels of baculovirus system.

The expression was under the control of the very late promoter, *polh*, which exhibits high activity beginning about 18–48 h post-infection. Cell lysis usually begins about 60 h after infection, and by 72 h after infection, most cells are no longer synthesizing proteins. This was supported by our study, in which the maximum expression of the recombinant protein in infected Sf9 cells occurred at 36 h after infection. Furthermore, the decline in host cell functions may result in a decline in the efficiency of post-trans-

Fig. 6. Detection of rAed a 1 3'-specific human IgE and IgG by ELISA. Microplates coated with purified rAed a 1 3' were incubated with 3 mosquito-allergic human sera (●, ○, △), one mosquito non-allergic serum (□), and a cord serum (▲). The plates were then incubated with monoclonal anti-human IgE (A) or anti-human IgG (B) followed by incubation with enzyme-conjugated goat anti-mouse IgG.

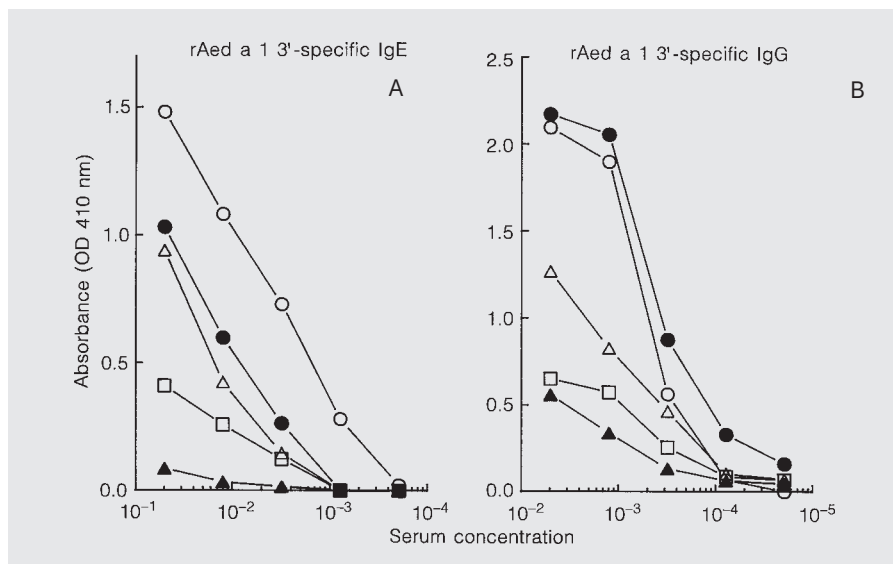
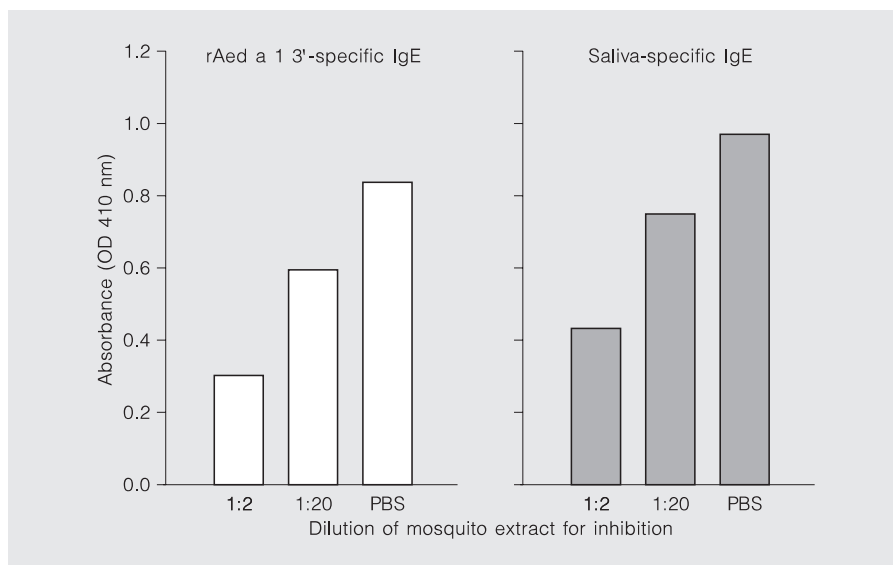


Fig. 7. Inhibition of rAed a 1 3' specific IgE ELISA reactions by mosquito extract. Serum from a mosquito allergic subjects was incubated with 10-fold diluted mosquito head and thorax extract and then assayed for mosquito specific IgE using both rAed a 1 3' capture-ELISA and saliva capture-ELISA.



lational modifications [19]. Therefore, the expression products should be harvested between 26 and 48 h after infection.

Purification of recombinant proteins is often extremely labour-intensive. Histidine tagging for purification of recombinant proteins by immobilized metal affinity chromatography has been used for a number of proteins both in prokaryotic [23] and eukaryotic [17, 24] expression systems. In our study, we used the baculovirus expression vector pBlue-BacHis C to efficiently purify recombinant Aed a 1 3'. The rAed a 1 3' fusion protein containing a 6-histidine tag bound to the column tightly and was eluted only by a high imidazole concentration (0.2 M). The contaminated proteins were

removed either in the effluent or by elutions with 0.05 and 0.1 M imidazole. While this one-step rapid purification by the baculovirus expression system is very useful for obtaining homogeneous pure recombinant proteins, it should not be used to express proteins with signal peptides, in which most of the expression product was found to be a non-fusion protein without the N-terminal histidine-tags [25].

The histidine-tagged rAed a 1 3', like the native Aed a 1 found in mosquito saliva, bound to the IgE and IgG in mosquito allergic sera as demonstrated by immunoblotting (fig. 5) and ELISA (fig. 6). Further, the binding of rAed a 1 3' to human IgE could be inhibited by the native Aed a 1 in the mosquito head and thorax extract (fig. 7). These data

indicate the following: (1) the allergic epitopes of native Aed a 1 are located in the significant portion of the 3' part (150–562 amino acid residues) and, thus, the rAed a 1 3' and native Aed a 1 have identical allergenicity; (2) the presence of 40 non-relevant residues at the N-terminus (including the histidine tag) does not significantly affect the binding of rAed a 1 3' to IgE and IgG. A similar result was reported when Der p 1 peptides expressed as fusion proteins were able to bind to human IgE and IgG [26].

Even though reactions to mosquito bites have been investigated since the early 1900s [27], and the clinically relevant allergens have been confirmed to be the saliva proteins [1], there is only one documented investigation of bite reac-

tions using a partially purified saliva fraction [28]. This report of recombinant Aed a 1 3' will further the characterization of native Aed a 1 and facilitate the study of mosquito allergy.

Acknowledgements

We thank Dr. Anthony James, University of California, Irvine, for kindly providing us with the Aed a 1 3' plasmid and the rabbit anti-rAed a 1. This study was supported by a grant and personnel awards (Dr. Z. Peng and Dr. F.E.R. Simons) from the Children's Hospital Foundation, Winnipeg, Manitoba, Canada.

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