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1 **Hypoallergenic chimeric virus-like particles for the development of shrimp tropomyosin**
2 **allergen Pen m 1-specific blocking antibodies.**

3 **Supapich Chanasit[1], Elecia Johnston[2], Chanatip Thanasarnthungcharoen[1], Sandip D**
4 **Kamath[2,3], Barbara Bohle[3], Andreas L Lopata[2,4], Alain Jacquet[1].**

5 1. Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok,
6 Thailand
7 2. Australian Institute of Tropical Health and Medicine, James Cook University, Townsville,
8 Australia.
9 3. Institute of Pathophysiology and Allergy Research, Center of Pathophysiology,
10 Infectiology and Immunology, Medical University of Vienna, Vienna, Austria
11 4. Tropical Futures Institute, James Cook University, Singapore.

12 Author for correspondence: Assoc. Prof. Dr. Alain Jacquet
13 Department of Biochemistry, Faculty of Medicine,
14 Chulalongkorn University,
15 1873 Rama IV Road, Pathumwan,
16 Bangkok 10330, Thailand.
17 Tel: (+66) 02 2563659
18 Fax: (+66) 02 6523100
19 alain.j@chula.ac.th
20 ORCID ID: 0000-0002-0980-9741

21 **595 words**

22 **Short Title:** Allergenicity and Immunogenicity of VLP displaying Pen m 1

23

To the Editor,

Sustained unresponsiveness to peanut by oral immunotherapy (OIT) was associated with the blocking capacity of induced peanut allergen-specific IgG/IgG4[1]. Currently, approved immunotherapy for shrimp allergy is lacking. Shrimp sensitizations are only managed by strict avoidance and treatment with adrenaline.

Virus-like particles (VLPs) displaying single allergen were shown to be hypoallergenic and to elicit blocking IgG antibody responses[2] To develop a new treatment of shrimp allergy, we designed a (AP205 capsid protein-based VLP) decorated with Pen m 1, the major tropomyosin allergen from the black tiger shrimp (*Penaeus monodon*), and using the SpyTag (ST)/SpyCatcher (SC) conjugation system[3]. Pen m 1 is a rod-shaped parallel coiled-coil dimeric protein which contains multiple mostly linear IgE-binding epitopes[4].

Purified dimeric (ST)Pen m 1-His_6 was successfully conjugated to (SC)AP205 VLPs under physiological conditions (Fig.1.A, B; Fig.S1)[3]. The excess of unconjugated Pen m 1 was removed from the VLP-Pen m 1 preparation by Captocore700 beads (Fig.1.B). The absence of unconjugated SC-VLP subunit indicated that each VLP-Pen m 1 particle carries 90 copies of dimeric Pen m 1. Dynamic light scattering (DLS) measurements confirmed the monodispersity of VLP-Pen m 1 particles, with a hydrodynamic radius of 53 nm (Fig.S2.A).

Indirect and competitive ELISA IgE assays, using sera from shrimp-allergic patients (Table S1), showed that VLP-Pen m 1 overall shares the IgE reactivity to natural Pen m 1 or ST-Pen m 1-His_6 (Fig. 1. C, D). Pearson correlation analysis evidenced a highly significant correlation between particulate and soluble forms of Pen m 1 (Fig.1.C). As previously shown for other VLP displaying allergens[4,2], functional RBL-SX38 assays revealed that VLP-Pen m 1 exhibited a 41 to 100-fold

46 reduced capacity to degranulate effector cells compared to equimolar amounts of ST-Pen m 1-His$_6$
47 (Fig. 1.E). In comparison with VLP decorated with other allergens, VLP-Pen m 1 displayed a
48 weaker hypoallergenicity which could result from the non-globular Pen m 1 tropomyosin
49 structure[2,4].

50 In naïve BALB/C mice (Fig.2.A), two intramuscular immunizations with unadjuvanted VLP-Pen
51 m 1 triggered much higher specific IgG1 and IgG2a levels than equimolar amounts of ST-Pen m
52 1-His$_6$ mixed with untagged AP205 VLPs ($P<0.05$) (Fig.2.B). A second VLP-Pen m 1 booster did
53 not significantly increase the antibody response ($P>0.05$). This high specific IgG2a production
54 together with the absence of detectable Pen m 1-specific IgE (data not shown) and the highly
55 significant IFNγ induction by restimulated splenocytes (Fig.2.C) confirmed that VLP-Pen m 1
56 induced a Th1-biased immune response. The Th1 polarization is at least mediated by bacterial
57 RNA packaged in the particles (Fig. S2.B), which serves as TLR7/8 ligand.

58 To measure the avidity of the specific IgG induced by both Pen m 1 immunogens, we measured
59 the strength of the IgG-Pen m 1 binding following a treatment with several urea concentrations.
60 VLP-Pen m 1 elicited specific IgG with higher avidity than soluble ST-Pen m 1-His$_6$ ($P<0.05$)
61 (Fig.2.D). Following the second booster with chimeric VLP, a time-dependent affinity maturation
62 of Pen m 1-specific IgG was observed ($Avidity_{day\ 56} > Avidity_{day\ 42}$, $P<0.05$). The blocking capacity
63 of affinity-purified Pen m 1-specific IgG from pooled sera collected at day 56 was tested in ELISA
64 IgE assays (Fig.2.E). Der p 1-specific purified polyclonal antibodies were used as control. Purified
65 Pen m 1-specific IgG inhibited the IgE binding to Pen m 1 in a dose-dependent manner reaching
66 70% of inhibition at an IgG/allergen molar ratio of 200. Such level of inhibition was in line with
67 those previously obtained with monoclonal IgG4 antibodies to allergen used at the same range of
68 molar IgG/allergen ratio[5].

In conclusion, our data show that the unidirectional display of Pen m 1 on VLP surfaces drastically reduced its allergenicity and optimized the induction of TM-specific IgG blocking antibodies.

Future studies will aim to investigate the reactogenicity of VLP-Pen m 1 as well as the therapeutic capacity to prevent shrimp-induced anaphylaxis in animal models of shrimp allergy, notably using human FcεRI transgenic mice preloaded with sera from Pen m 1-sensitized patients or Pen m 1-specific monoclonal IgE[6].

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100 encoding SpyTag and SpyCatcher-AP205 VLP.

101

102 **Conflict of interest.**

103 The authors declare they have no conflict of interest.

104 **Author contributions.**

105 A.J. designed the study; A.J., S.K. and E.J. designed the genetic construction for SpyTag-Pen m 1
106 production in bacteria; S.C. and E.J. conducted the experiments on allergenicity; S.C. and C.T.
107 performed the immunogenicity experiments; A.J., A.L. and B.B. provided supervision and
108 analyzed the data. A.J., and B.B. drafted the manuscript. All authors contributed to and approved
109 the final version of the manuscript.

110

111 **Figure Legends**

112 **Fig. 1. Production, IgE binding properties and allergenicity of VLP-Pen m 1**

113 (A) Schematic representation of SpyTag (ST)-Pen m 1-His_6 and SpyCatcher (SC)-AP205 VLP;
114 (B) SDS-PAGE analysis: Lane 1: molecular weight markers, Lane 2: purified SC-VLP, Lane 3:
115 purified ST-Pen m 1-His_6, Lane 4: conjugation of ST-Pen m 1 to SC-VLP, Lane 5: purified VLP-
116 Pen m 1 after incubation with Captocore beads to bind unconjugated ST-Pen m 1. (C) IgE reactivity
117 to coated VLP-Pen m 1, natural and recombinant Pen m 1 or untagged AP205 VLP (naked VLP)
118 in eight sera from shrimp allergic patients. The dashed line represents the cut-off of the assay
119 (mean + 2 SD of two sera from non-allergic individuals). ** P<0.01 for significant difference
120 between proteins. Correlation between specific IgE levels to different forms of Pen m 1 (n = 8; r
121 = Spearman coefficient. (D) Inhibition (%) of IgE binding to nPen m 1 by preincubation of sera
122 (pool of 10 Pen m 1-positive sera) with 2 or 20 µg/ml of VLP-Pen m 1, nPen m 1 or ST-Pen m 1.
123 (E) Allergenicity of VLP-Pen m 1 and ST-Pen m 1 measured with RBL-SX38 cells preloaded with
124 four sera positive to Pen m 1 (three sera from HDM-allergic patients and positive to Der p 10 and
125 Pen m 1 (Table S2, Fig. S4) and one serum from a shrimp-allergic subject (Patient #4)). One serum
126 from non-allergic subject was used as negative control. Amounts of VLP-Pen m 1 were normalized
127 to Pen m 1 content. ∗P <0.05 for significant difference between VLP-Pen m 1 and ST-Pen m 1.
128 Pen m 1 and VLP-Pen m 1 concentrations eliciting half-maximal basophil activation (EC50) are
129 shown. The fold-reduced capacity of VLP-Pen m 1 to degranulate basophils was calculated as
130 EC50 VLP-Pen m 1/EC50 Pen m 1 ratio.

131

132 **Fig.2. Immunogenicity of VLP-Pen m 1.**

133 (A) Immunization and bleeding schedule; (B) Pen m 1-specific IgG1 and IgG2a titers
134 measured two weeks after booster #1 (day 28) as well as two and four weeks after booster
135 #2 (day 42 and 56) (C) IFNγ and IL-5 levels produced by splenocytes from immunized
136 mice restimulated with ST-Pen m 1. One representative of 2 similar experiments is shown.
137 * $P<0.05$, ** $P<0.01$, for statistically significant difference; (D) Avidity of purified Pen m
138 1-specific polyclonal antibodies from immunized mice to Pen m 1. Data are expressed as
139 residual binding percentage of purified Pen m 1-specific IgG from immunized mice to
140 coated ST-Pen m 1 following a wash with 2, 4 or 7 M urea solutions. One representative
141 of 2 similar experiments is shown. * $P<0.05$, ** $P<0.01$ for statistically significant
142 difference; (E) Blocking capacity of purified Pen m 1-specific IgG triggered by VLP-Pen
143 m 1 four weeks post-booster #2 (day 56). Purified Der p 1-specific IgG was used as
144 negative control. Several IgG/Pen m 1 molar ratio were tested to inhibit the binding of Pen
145 m 1-specific IgE from a pool of fourteen Der p 10/Pen m 1-positive sera. The percentage
146 of inhibition was calculated as follows: $(1-(OD450S/OD450U)) \times 100\%$. OD450S and
147 OD450U represent optical density values in the presence or absence of purified IgG
148 respectively. * $P<0.05$, ** $P<0.01$ for statistically significant difference.
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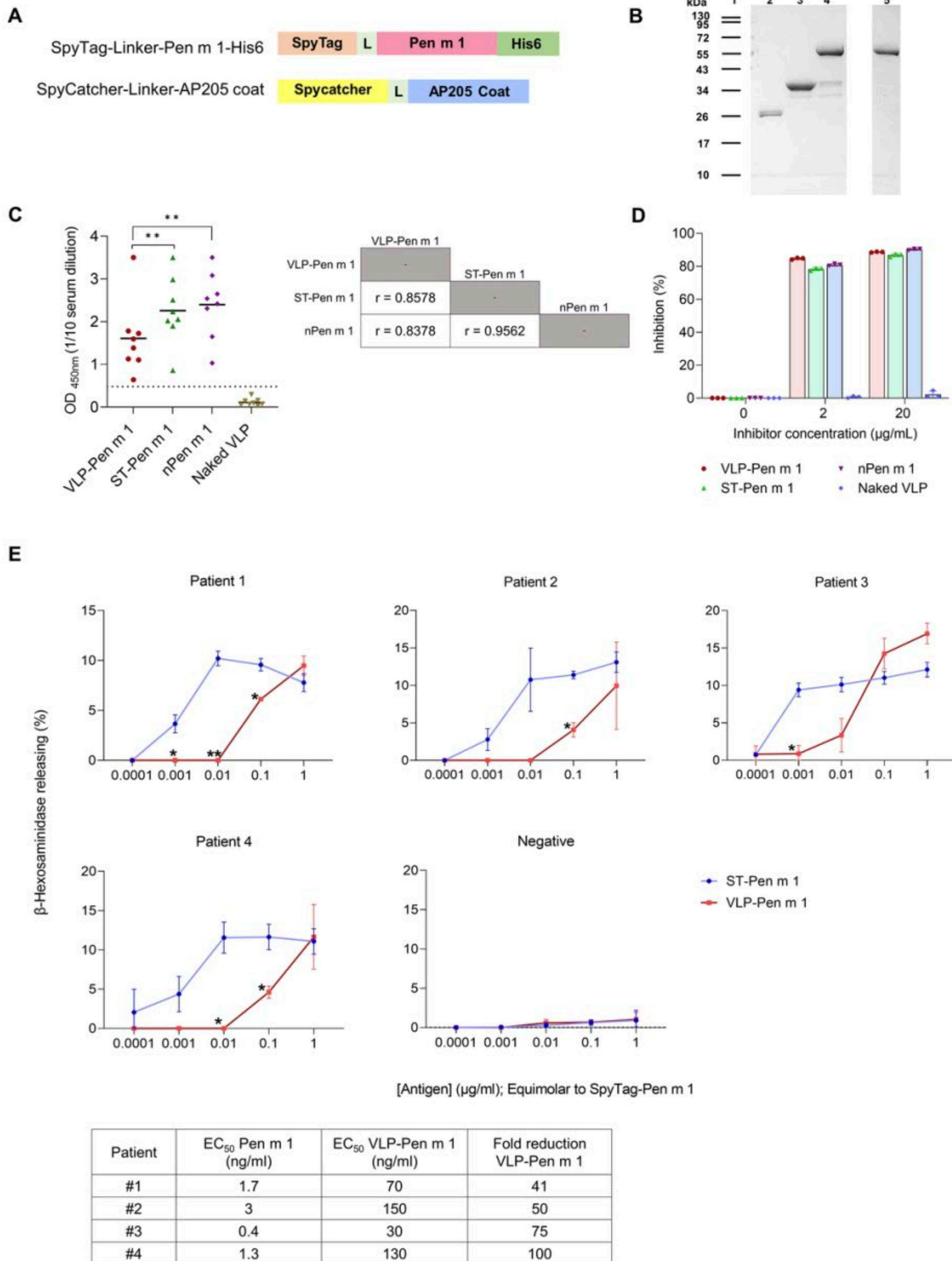

Patient	EC_{50} Pen m 1 (ng/ml)	EC_{50} VLP-Pen m 1 (ng/ml)	Fold reduction VLP-Pen m 1
#1	1.7	70	41
#2	3	150	50
#3	0.4	30	75
#4	1.3	130	100

FIGURE 1

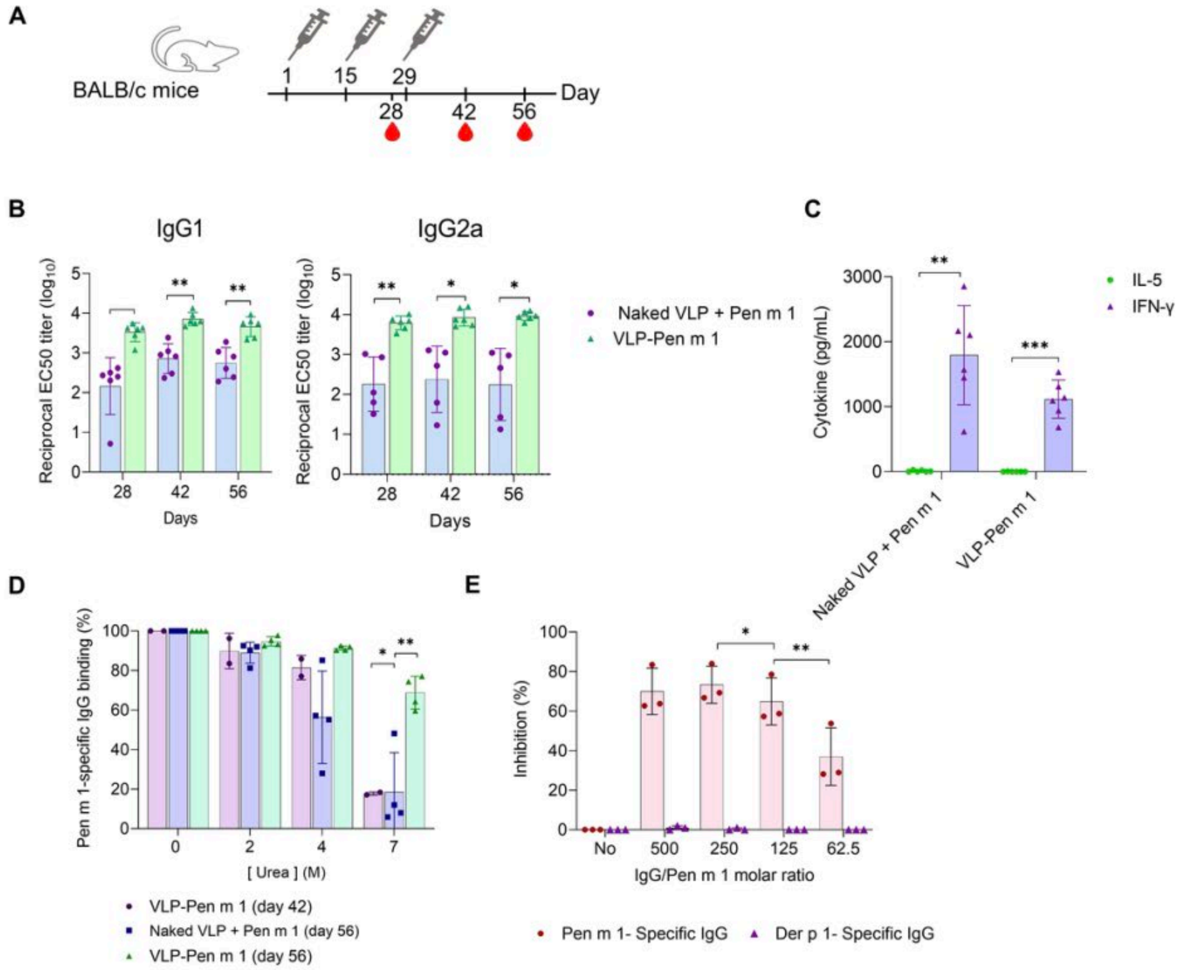

FIGURE 2