

Epitope mapping and structural analysis of the anti-Der p 1 monoclonal antibody: insight into therapeutic potential

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Abstract Group 1 allergen from *Dermatophagoid pteronyssinus* (Der p 1) belongs to the papain-like cysteine protease family and is a major cause of allergic rhinitis and asthma. An anti-Der p 1 monoclonal antibody, mAb W108, was selected and isolated from Der p-specific IgG2b-producing hybridoma clones. Two-dimensional electropho-

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resis and immunoblotting showed that mAb W108 reacted with four components of Der p extracts with a molecular mass of 35 kDa and pI values varying from 4 to 6; it also reacted with IgE antibodies in the sera of Der p-sensitive patients. In the competitive assay and using azocasein as a substrate, we found that mAb W108 inhibited not only the binding of Der p 1, but also its cysteine protease activity in a dose-dependent manner. The two peptide segments of Der p 1 identified by mAb W108 (aa 151–197 and 286–320) were parts of inter-connecting loops located in the substrate-binding cleft and on the surface of the domain comprising mainly β -sheets. From the predicted interaction between the amino acid sequence in the CDR3 of mAb W108 and Der p 1-binding epitopes, the possible binding sites for mAb W108 to Der p 1 may sterically hinder the IgE epitope and the active site of cysteine protease activity. Administration of mAb W108 in the Der p-sensitized murine model of asthma alleviated allergen-induced airway inflammation and the Th2 cytokine immune response, suggesting its therapeutic potential. These findings can provide new insights into understanding IgE-mediated disease and the design of modified allergen vaccines for future allergen-specific immunotherapy.

Keywords Protease allergy · House dust mite · Der p 1 · Monoclonal antibodies · Cysteine protease

Abbreviations

aa	Amino acids
BALF	Bronchoalveolar lavage fluids
Der f	<i>Dermatophagoides farinae</i>
Der p	<i>Dermatophagoides pteronyssinus</i>
GM-CSF	Granulocyte macrophage-colony stimulating factor
mAbs	Monoclonal antibodies
PBS	Phosphate-buffered saline

RANTES	Regulated upon activation normal T cell expressed and secreted
2-DE	Two-dimensional electrophoresis

Introduction

House dust mites are a major source of allergens which contribute to the rising incidence of allergic diseases [1, 2]. In Taiwan, about 80% of asthmatic children are sensitive to *Dermatophagoides pteronyssinus* (Der p) and/or *Dermatophagoides farinae* (Der f), depending on their geographic location within Taiwan [3, 4]. At least 17 allergen groups have been identified in Der p and Der f [5]. Group 1 allergens (Der p 1 and Der f 1) display cysteine protease activity [5]. Although the patho-physiological role of the proteolytic activity of these mite allergens has not been fully elucidated in vivo, several reports have demonstrated that Der p 1 is capable of cleaving human proteins with potentially immune-modulatory effects, including A1-Pi (α 1-proteinase inhibitor) [6], CD23 [7], CD25 [8], and CD40 [9]. Moreover, the incubation of confluent airway epithelial cells with Der p 1 has been shown to cleave their tight junction adhesion proteins, causing an increase in epithelial permeability and allergen access to dendritic cells [10]. Moreover, several studies indicate that Der p 1 can also induce the release of pro-inflammatory mediators, such as IL-8, IL-6, eotaxin, GM-CSF, or RANTES, by airway epithelial cells [11]. These mediators trigger the accumulation of inflammatory cells, such as eosinophils and neutrophils, to perpetuate the chronic allergic inflammation of the airways [12].

Der p 1 is a 27–36-kDa cysteine protease produced in the mite as an enzymatically inactive pro-enzyme which becomes active after cleavage and detachment of the pro-peptide [5]. The gene encoding Der p 1 precursor (P 08176, 320 aa) has been cloned and sequenced [13] and displays an iso-allergenic variation [13, 14]. Apart from inhibiting the activity of the pro-enzyme, the pro-peptide may also act as a folding scaffold for mature Der p 1, as is suggested for other proteases [15]. A recombinant Der p 1 was produced in *Escherichia coli*, but the resulting protein had much reduced IgE-binding activity, indicating improper folding [16]. In *Pichia pastoris*, a recombinant pro-Der p 1 can be produced as a hyperglycosylated pro-enzyme with reduced enzymatic activity and IgE binding [17]. However, after in vitro maturation, enzymatic activity and IgE binding can be restored independently of glycosylation [18].

Recently, the structures of the pro-enzyme and the mature form of Der p 1 have been demonstrated by X-ray crystallography and this has mostly confirmed that the original models were constructed from the coordinates of

papain [19, 20]. However, the exact interactions between the residues within the folds and the epitopes responsible for binding to IgE, which may be important for the clinical therapeutic use of Der p 1, are not known. The majority of monoclonal antibodies (mAbs) raised against Der p 1 do not inhibit the binding of human IgE to Der p 1, indicating that they recognize different epitopes, or are of much lower affinity than human anti-Der p 1 IgE [21]. Previously, Furmonaviciene et al. [22] developed a mouse anti-Der p 1 mAb (mAb 2C7) which inhibited the binding of human IgE anti-Der p 1 up to 70% and predicted that mAb 2C7 recognizes a conformational epitope comprising the sequence Leu147 to Gln160 in Der p 1 [22]. However, the authors failed to show any biological activity of this mAb in vitro or in vivo.

Here, we report a mAb W108, which was selected and isolated from hybridoma clones producing Der p-specific IgG2b and which strongly inhibited the binding of human anti-Der p-specific IgE antibodies and the cysteine protease activity of Der p 1. The administration of mAb W108 in the Der p-sensitized murine model of asthma alleviated allergen-induced airway inflammation and the Th2 cytokine immune response, suggesting its benefit in the treatment of mite allergen-induced diseases and asthma.

Materials and methods

Preparation of Der p extracts

Der p extracts from Pharmacia Allergon, Inc. (Sweden) were prepared following the manufacturer's instructions and stored at -80°C before use. Native Der p 1 was purified from whole culture extracts of Der p (a generous gift from Dr. K. Y. Chua). The protease activity was measured as described previously [13].

Human sera

Sera from Der p-sensitive patients were collected in the Allergic Clinic of National Cheng Kung University Hospital. The allergic phenotype was confirmed by clinical history, skin prick tests, and a high level of IgE reactivity against Der p in UniCAP tests (Pharmacia Diagnostics, UK). A panel of 10 Der p extract-positive sera from asthmatic and/or atopic dermatitis patients was used for IgE enzyme-linked immunosorbent assay (ELISA), and 10 sera from individuals who were not sensitive to any inhalant allergens were used as negative controls. All sera were stored at -80°C until use.

Preparation of anti-Der p 1 mAb

Five 6-week-old female BALB/c mice were used for immunization with Der p extracts, and 30 8-week-old

female BALB/c mice were used for production of ascites. For the production of mAbs, female BALB/c mice were immunized with Der p extracts prepared as above. Blood was collected from the infra-orbital plexus every week to monitor the titer of Der p-specific antibodies by ELISA. Three days before fusion, the mouse with the highest antibody titer was injected i.v. with 100 µg of antigen in PBS. This animal was killed and its spleen cells were fused with FO-mouse myeloma cells as described [23]. Ten days after the fusion, culture supernatants from the fused cells were screened for anti-Der p-specific antibodies by ELISA with purified native Der p 1 in the solid phase. The positive hybrids were cloned and sub-cloned by limiting dilution. The mAb W108 was purified from hybridoma culture supernatants using standard protein G affinity chromatography (Sigma-Aldrich, USA). The isotype of the antibodies was determined by ELISA with anti-mouse subclass antisera (Nordic, Tilburg, Netherlands).

Inhibition assay

The inhibition of the binding of specific IgE to Der p extracts and/or native Der p 1 by mAb W108 was evaluated by competitive inhibition in a modified ELISA. Detection of a bound human-specific IgE antibody was performed using HRP-conjugated mouse anti-human IgE Ab (Zymed, USA).

2-DE immune-detecting and proteomic analysis

The Der p protein was suspended in 400 µl of sample solution (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer [immobilized pH gradient], pH 4–7, 0.003% bromophenol blue) and 2-DE was performed as described by Corti et al. [24]. Bound monoclonal antibodies were detected using peroxidase-labeled goat anti-mouse IgG antibodies (Zymed). Protein spots of interest were removed from the 2-D gel and transferred to 650-µl tubes (siliconized) for in-gel digestion and directly spotted onto matrix-assisted laser desorption (MALDI) plates for liquid chromatography tandem mass spectrometry (LS MS/MS) analysis. Mass analyses were undertaken using the PerSeptive Biosystems Voyager-DE STR MALDI-TOF mass spectrometer (Framingham, Danvers, MA, USA). Peptide mapping was performed by comparison with the deduced amino acid sequence of Der p 1 complementary DNA (cDNA). The amino acid sequence was translated from proteomic data using the MASCOT Tool from Matrix Science (<http://www.matrixscience.com>). Homology search of the predicted amino acid sequence was conducted using the BLAST network server at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

Recombinant Der p 1 peptide

The overlapping peptide fragments of Der p 1 were amplified by multiplex PCR methods. Gel-purified PCR products were ligated into pGEX-2T vector (Pharmacia Biotech, UK). The peptides were expressed as glutathione S-transferase (GST) fusion protein/peptides in *E. coli* by isopropyl-B-D-thiogalactopyranoside induction, as described previously [25]. The purification of recombinant proteins was performed by affinity chromatography using a glutathione-sepharose column (Sigma, USA) according to the protocol described in the GST purification module (Pharmacia Biotech).

RT-PCR amplification from mAb W108 clone

Approximately 1×10^6 W108 hybridoma cells were harvested by centrifugation and homogenized. Total RNA was prepared using the standard Trizol™ kit (Invitrogen, USA). PCR amplification was carried out using 1 µg of cDNA in each reaction, and the primers to amplify the complete kappa chain cDNA are listed in Table E1, and those to amplify the heavy chain Fd fragment cDNA are listed in Supplemental Table E2.

Molecular modeling

Using the 3D structure of the major house dust mite allergen Der p 1 (PDB accession number 1xkg), a model structure of Der p 1 was constructed using the SWISS-MODEL program (<http://swissmodel.expasy.org/SWISS-MODEL.html>). The solvent-accessible surface area of individual amino acids was calculated using the GETAREA program (www.scsb.utmb.edu/cgi-bin/get-a-form.tel). This program considers residues as buried if the ratio of the side-chain surface area to a random coli reference value per residue is <20% and the solvent as exposed if the ratio exceeds 50%. The solvent-accessible surface area on the mature protein covered by the peptide was calculated using the CCP4 program ArealMol [26]. All structural images were prepared with the program PyMOL (<http://www.pymol.org>).

Immunization of mice

Detailed procedures for mite allergen sensitization and the allergen challenge in the mouse model of asthma were previously reported [27]. The airway resistance of mice was measured in a single-chamber, whole-body plethysmograph (Buxco Electronics, Inc, Troy, NY, USA). Bronchoalveolar fluids (BALF) of treated and non-treated mice were centrifuged to count infiltrating cells, and the supernatants were collected for cytokine analysis. Inflammatory cell

infiltrates and lung architecture were assessed using light microscopy. Levels of the cytokines, IL-4, IL-5, IFN- γ , and eotaxin, in the BALF were assayed in ELISA kits (R&D Diagnostics, USA).

Results

mAb W108 inhibited Der p-specific IgE binding and protease activity of Der p 1

Our strategy was to screen for mAbs with the highest blocking activity by inhibition assays of the binding between serum Der p-specific IgE and Der p extracts. We found that mAb W108 (selected from over 700 positive antibodies) had the highest blocking activity (i.e., inhibited >70% of the binding of allergen-specific IgE in individual and pooled sera from nine Der p-sensitive

allergic asthmatic children, Fig. 1a, b). Western blot analysis showed that mAb W108 blocked the binding of serum Der p-specific IgE to 36-kDa bands in the Der p extract (Fig. 1c). Using ELISA plates coated with purified native Der p 1, mAb W108 was found to dose-dependently inhibit serum Der p-specific IgE binding to Der p 1 (data not shown). Using azocasein as a substrate, mAb W108 inhibited not only the binding of Der p 1, but also its cysteine protease activity in a dose-dependent manner (Fig. 1d).

Proteomic analysis of Der p extracts with pooled sera from Der p-sensitized patients and mAb W108

A total of over 200 distinct spots were detected after silver staining and image analysis (BioRad PDQuest software) in 2D gel electrophoresis. Replicate gels gave spot reproducibility ranging between 86% and 91% ($n=5$ replicates each). Using a silver stain, approximately 275 distinct and

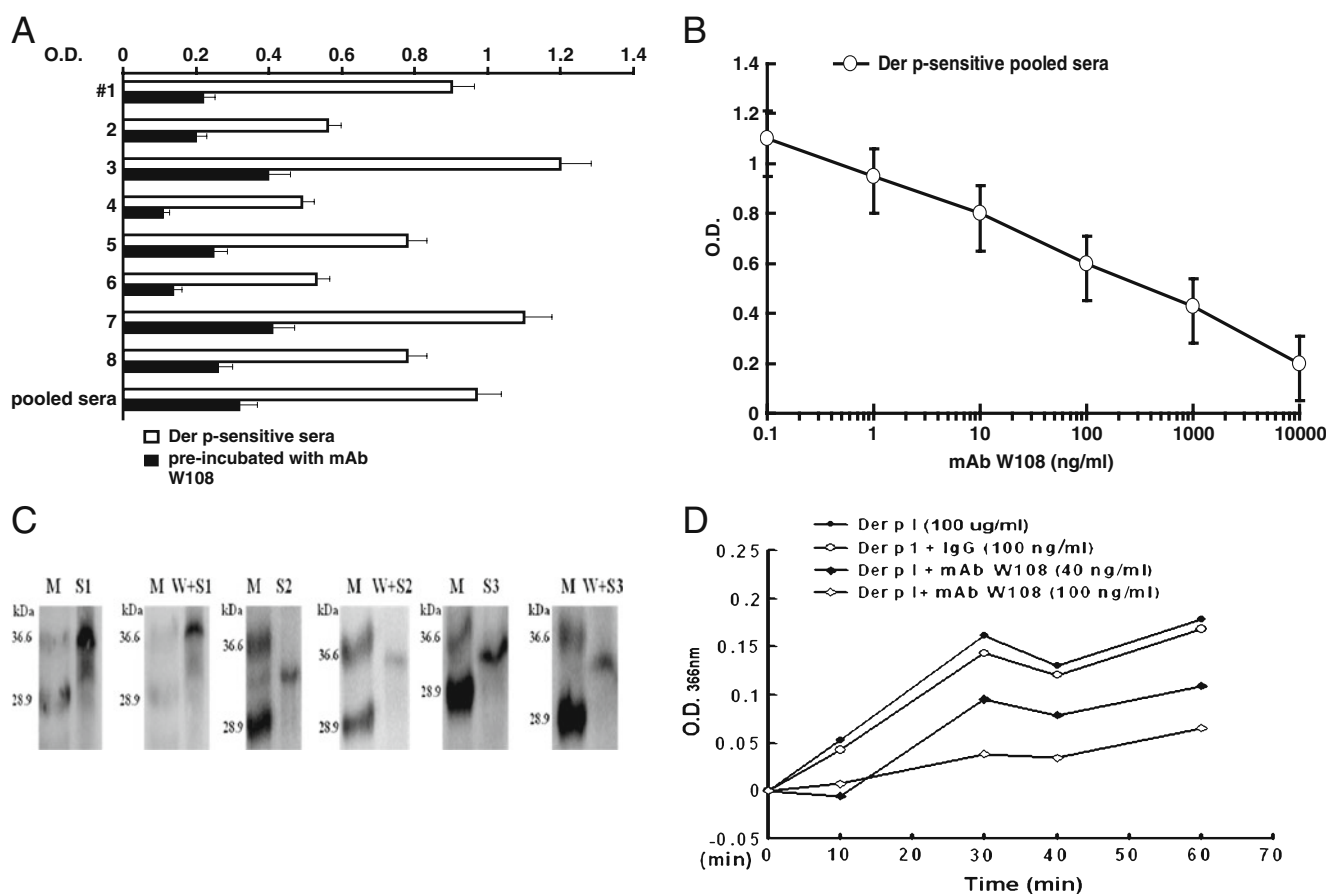


Fig. 1 The competitive binding analysis of monoclonal antibody W108 using patient IgE to Der p allergen. **a** Binding of Der p-sensitive sera (white bar) and pre-incubated with mAb W108 (black bar) detected using HRP-mouse anti-human IgE Ab, respectively. **b** The mAb W108 had the highest blocking activity (inhibited more than 70% of the allergen-specific IgE binding in the pooled sera [empty circle] of nine Der p-sensitive allergic asthmatic children). **c** Immunoblot analysis of mAb W108 and patient sera to Der p

allergen. Pre-incubation with mAb W108 (S1/S2/S3+W) can block serum Der p-specific IgE (S1, S2, S3) bound to Der p 1 (36 kDa). **d** The cysteine protease activity of Der p 1 (100 μ g/ml) (using azocasein as a substrate) is inhibited by 100 ng/ml (empty diamond) and 40 ng/ml (filled diamond) of mAbW108. Der p 1 (100 μ g/ml) alone (empty circle); Der p 1 (100 μ g/ml) incubated with IgG (100 ng/ml)

reproducible spots in Der p extract were detected (Fig. 2a). Immunoblotting showed that mAb W108 reacted with four components of Der p extracts with a molecular mass of 36 kDa and *pI* values varying from 5 to 6 (Fig. 2b). These spots also reacted to pooled sera from Der p-sensitive subjects (Fig. 2c). In inhibition studies, specific binding to these protein spots by IgE in pooled sera from Der p-sensitive patients was blocked by pre-incubation with mAb W108 (Fig. 2d).

All four of these spots were then excised and subjected to in situ in-gel trypsin digestion for MALDI-TOF MS analysis. The MS profile showed multiple peaks in the range of 800–2,800 Da (Fig. 3a). One peak with a molecular mass 2,056.0 Da was selected for comparison with established databases, and the proteins with the highest correlation with the analyzed spot were the cysteine protease from Der p allergen (accession number gi1460058, score 162) and the major mite fecal allergen Der p 1 precursor (accession number gi730036, score 121), with sequence coverage of nearly 99%.

The 36-kDa spots (*pI* 5–6, labeled as spots 1, 3, and 4) had identical amino acid sequences, which showed their identity with the known partial sequence of the Der p 1 isoform 3 precursor, while spot 2 had a sequence identity with the known partial sequence of the Der p 1 isoform 5 precursor (Fig. 2b). When the internal sequences of the mAb were further characterized, W108 recognized Der p peptides, spots 1 and 2 were selected for LC MS/MS

analysis, and the generated fragment-ion spectrum identified these two peptides as having the same sequences—aa 209–224, aa 227–243, and aa 260–287 (Fig. 3b). All sequences of these fragments were identical with the Der p 1 precursor sequence (Table 1).

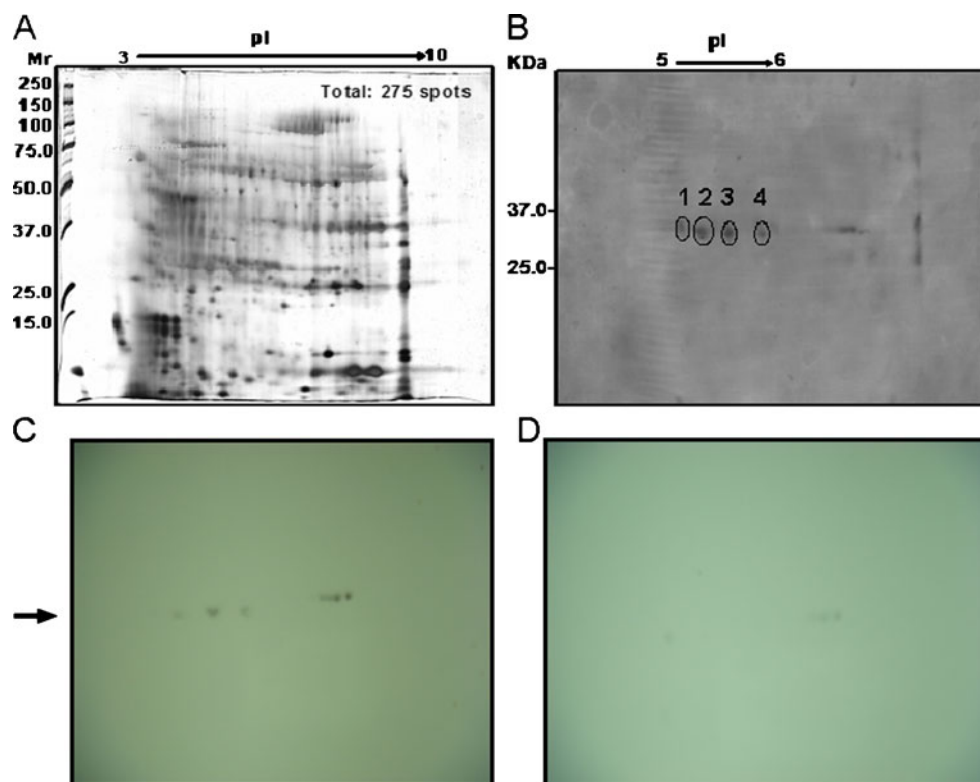
Identification of epitopes on Der p 1 recognized by mAb W108

To identify the linear mAb-binding epitopes on Der p 1 allergen recognized by mAb W108, peptides were constructed to cover the sequences of the IgE-binding epitopes of Der p 1 as previously reported [28] and predicted by the computer software (Fig. 4a). To test the binding of mAb W108 to these peptides, seven peptide fragments, covering almost the entire amino acid sequence of Der p 1, were screened by solid phase ELISA. Two peptide fragments (aa 151–197 and 286–320) showed the strongest binding to mAb W108 (Fig. 4b) and were highly bound to specific IgE antibodies from the pooled sera ($n=10$) of Der p-sensitized patients (Fig. 4b).

Identification of heavy and light chain paratopes in complementarity determining regions (CDR) of mAb W108

To characterize the amino acid sequences in the CDRs of mAb W108, a PCR amplification of mAb W108 cDNA was

Fig. 2 Two-dimensional immunoblot analysis of monoclonal antibody W108 binding to Der p 1 allergen. **a** Coomassie Brilliant Blue-stained blot from Der p extract. **b** Immunoblots were probed with mAb W108. The N-terminal sequences of spots 1, 2, 3, and 4 were determined and are presented in the text. **c** These spots reacted to pooled sera from Der p-sensitive atopic subjects. **d** The binding of IgE from the pooled sera of Der p-sensitive patients to these protein spots is specifically blocked by pre-incubation with mAb W108, as shown in two-dimensional PAGE and a semi-dry transfer to PVDF membranes



A MI205678 (MALDI)-MS



Table 1 The amino acid sequences of Der p 1 precursor that are recognized by mAb W108 (in bold characters), which were characterized by LC MS/MS (Sinica MS Center)

Match to: P08176-00-01-00; score: 195

(MMAL_DERPT) Splice isoform displayed; variant unnamed variant isoform 5; conflict displayed; from P08176 major mite fecal allergen Der p 1 precursor

Found in search of D:\data 10.2.2k3\MI206367.pkl

Matched peptides shown in bold

1 MKIVLAIASL LALSAVYARP SSIKTFEYK KAFNKSATF
EDEEAARKNF

51 LESVKYVQSN GGAINHLSL SLDEFKNRFL MSAEAFEHLK
TQFDLNAETN

101 ACSINGNAPA EIDLRQMRTV TPIRMQGGCG
SCWAFSGVAA TESAYLAYRN

151 QSLDLAEQEL VDCASQHGCH GDTIPRGIEY
IQHNGVVQES YYRYVAREQS

201 CRRPNAQRFG **ISNYCQIYPP** NVNKIREALA QTHSAIAVII
GIKDLDAFRH

251 YDGRTHIQRD **NGYQPNYHAV** NIVGYSSNAQG
VDYWIWRNSW DTNWGDNGYG

301 YFAANIDLMM IEQYPYVVIL

performed, using the heavy and light chain primers listed in Tables E1 and E2. Online repository Fig. E1 shows the results of representative cDNA clones for the heavy and κ -light chains, together with their corresponding PCR products. Each of the heavy chain primers, MHV7, MHV8, and MHV12, resulted in a single band of approximately 650 bp, which is the expected size for the Fd fragment of the heavy chain (Fig. E1A). Each of the κ -

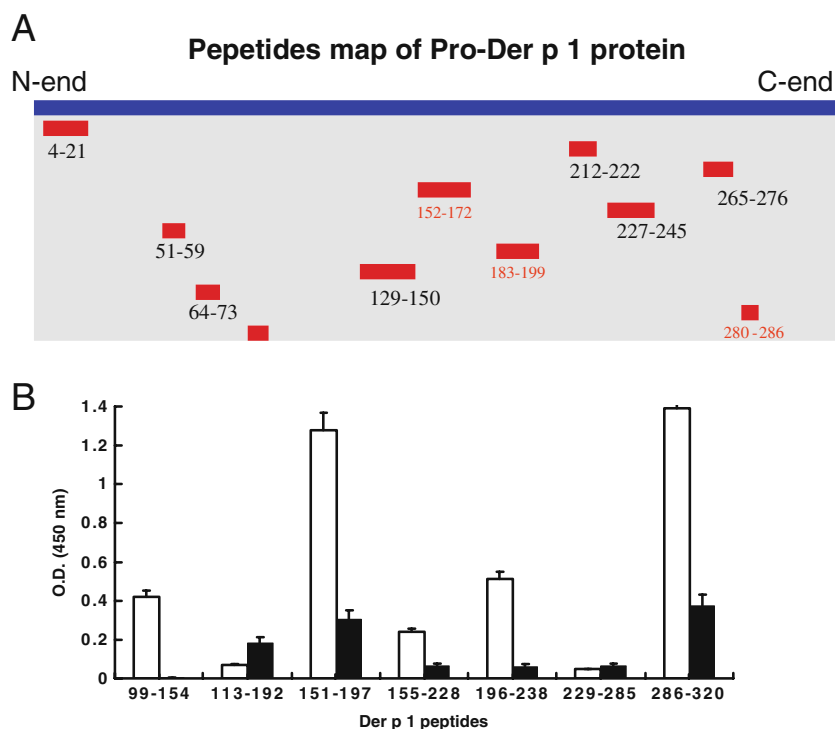
chain primers, MKV5, MKV9, MKV10, and MKV11, also resulted in a band of 450 bp when used in conjunction with the reverse κ constant primer (Fig. E1B).

These PCR products were cloned into the vector pCRTM 2.1, and 10 clones from each reaction were selected for an amino acid sequence analysis of the cloned plasmids. The first eight amino acids of the light chain and the first nine amino acids of the heavy chain were derived from the degenerate PCR primers. The amino acid sequences for the CDRs in the light and heavy chains of mAb W108 are shown in Fig. 5. The V-region protein sequences in the heavy and light chains of mAb W108 were compared with the homologous sequences in the V-BASE database, and a number of homologous V-genes were identified (Tables E3A and E3B). The heavy chain of mAb W108 had a >90% homology with a member of the VH3 family, D13203-IGHV3S4*01 (Table E3A). The amino acid sequence of CDR3 of the heavy chains was LSLHLLHSAPV (aa 105–115) as determined by automatic amino acid sequencing. Similarly, the light chain had a >80% homology with M15520 IGKV10-96*01, including the predicted CDR1 and CDR2, and the amino acid sequence of CDR3 was QQGNTLPVDVR (aa 105–115) in the light chain of mAb W108 (Table E3B).

Der p 1 epitopes localized by mAb W108

To localize the mAb W108 epitopes on Der p 1, a 3D structural model of Der p 1 was constructed using the major house dust mite allergen Der p 1 (PDB accession number

Fig. 4 The epitope mapping of antibodies which binds Der p 1 precursor amino acid sequences was via an ELISA analysis, and the three-dimensional structure of Der p 1 shows the active site and the epitope site. **a** The peptide map of the Der p 1 precursor was via computer prediction. **b** The two peptide fragments (aa 151–197 and 286–320) with the strongest binding to mAb W108 are shown. The mAb W108 (white bar) and pooled patient sera (black bar)



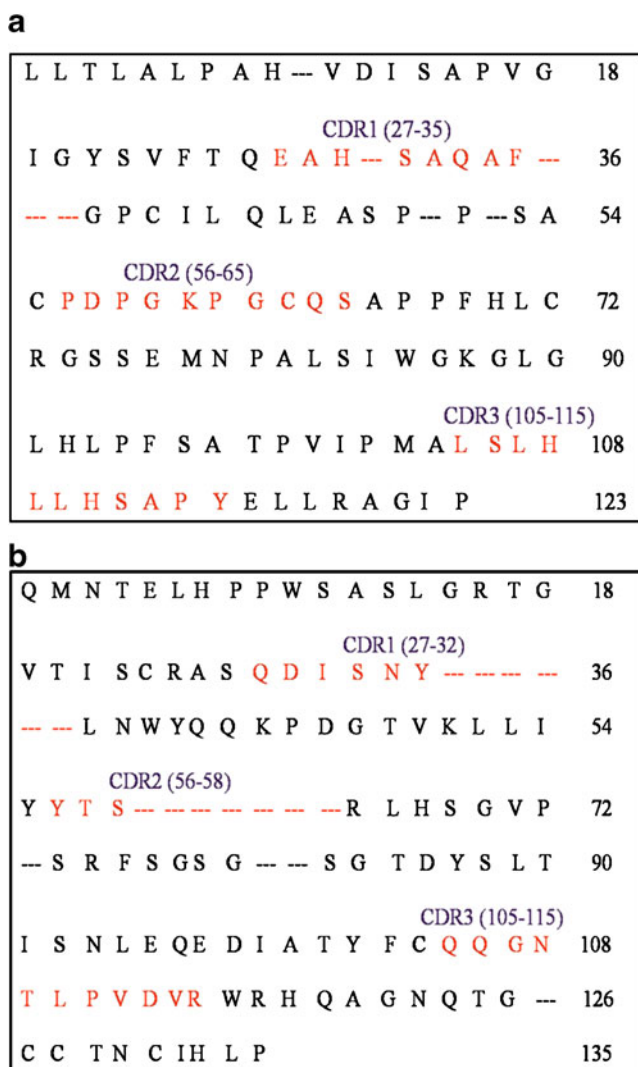


Fig. 5 The amino acid sequences of the CDR regions are in heavy (a) and light (b) chains of mAb W108, and dashes indicate missing amino acids

1xkg). The mature region, or pro-Der p 1, was folded to form a globular protein with two interacting domains which delimit a cleft on the surface (Fig. 6). An examination of the structural environment for solvent accessibility showed that the core of the Der p 1 was α -helices and β -sheets, while the outer surface was mainly inter-connecting loops, of which Ser164–Ser180 and Val280–Tyr286 were the longest. Like other papain-like members of family C1 with a known structure, the predicted active sites of cysteine protease activity on Der p 1 were located at C132, H268, and N288 (Fig. 6a), and the two peptide segments of Der p 1 identified by mAb W108 (aa 151–197 and 286–320) were parts of the inter-connecting loops located in the substrate-binding cleft and on the surface of the domain comprising mainly β -sheets. From the predicted interaction between the amino acid sequence in the CDR3 of mAb W108 and

Der p 1-binding epitopes (Figs. 4b and 5), the possible binding sites for mAb W108 to Der p 1 may sterically hinder the IgE epitope, as well as the active site of cysteine protease activity (N280), as shown in red by the steric arrangement of the epitope regions aa 151–197 (left) and the aa 286–320 (right) after the binding of mAb W108 (Fig. 6b).

mAb W108 attenuates Der p-induced airway inflammation in a mouse model of asthma

The in vivo effect of mAb W108 on the Der p-induced airway inflammation was studied in Der p-sensitized mice. Weekly doses of mAb W108 (1 μ g/kg) injected i.p. during allergen sensitization for 2 weeks and 6 h before challenge attenuated airway inflammation (Fig. 7a); lowered airway hyperreactivity (Fig. 7b) and titer of Der p-specific IgE in serum (Fig. 7c); infiltrated eosinophil (Fig. 7d), IL-4, and IL-5; and produced eotaxin in BALF (Fig. 7e). In contrast, Der p-specific IgG2a in serum and INF- γ in BALF were increased in mAb W108-treated mice as compared with non-treated mice or mice sensitized with non-specific IgG (Fig. 7). There were similar inhibitory effects of mAb W108 on Der p-induced airway inflammation (Fig. 8a), airway hyperreactivity (Fig. 8b), Der p-specific IgE production (Fig. 8c), inflammatory cell infiltration (Fig. 8d), and Th2 and inflammatory cytokine production in BALF when administered after allergen challenge in Der p-sensitized mice.

Discussion

A molecular analysis of the IgE-binding epitopes of Der p 1 and Der f 1 using correctly folded and fully active recombinant proteins has recently been reported [20, 21, 29]. Previously, others have reported mouse monoclonal antibodies with properties similar to a major component of the human IgE response to Der p 1 [21, 22]. Our study found a blocking monoclonal antibody (mAb W108) which inhibited the binding between serum-specific IgE and Der p 1 (Figs. 1 and 2). Moreover, mAb W108 administration inhibited the cysteine protease activity of Der p 1 (Fig. 1d), Der p allergen-induced airway inflammation, and other immunological changes in the mouse model of asthma (Figs. 7 and 8). In this respect, it performs better than other reported mAbs which are claimed to share epitope specificity with human anti-Der p 1 IgE [22]. It is difficult to ascertain whether the inhibitory effects of these monoclonal antibodies are due to competition for the same epitopes, or to steric hindrance. As previously shown, unlike natural allergens, synthetic peptides and recombinant fragments expressed in *E. coli* have little or no IgE-binding activity [16, 28], suggesting that binding could depend on the presence of conformational IgE epitopes.

Fig. 6 **a** Schematic representation of the mature Der p 1, including the visible part of the α -helices (blue coils), β -sheets (blue arrows), the side chain of the active site residues C132, H268, and N288 (green sticks), and the side chain of the epitope site residues aa 151–197 and aa 286–320 (red sticks). **b** A molecular model of the epitope-binding regions of mAb W108, including the epitope-binding region for aa 151–197 (left) and aa 286–320 (right). The epitope-binding regions are shown in red

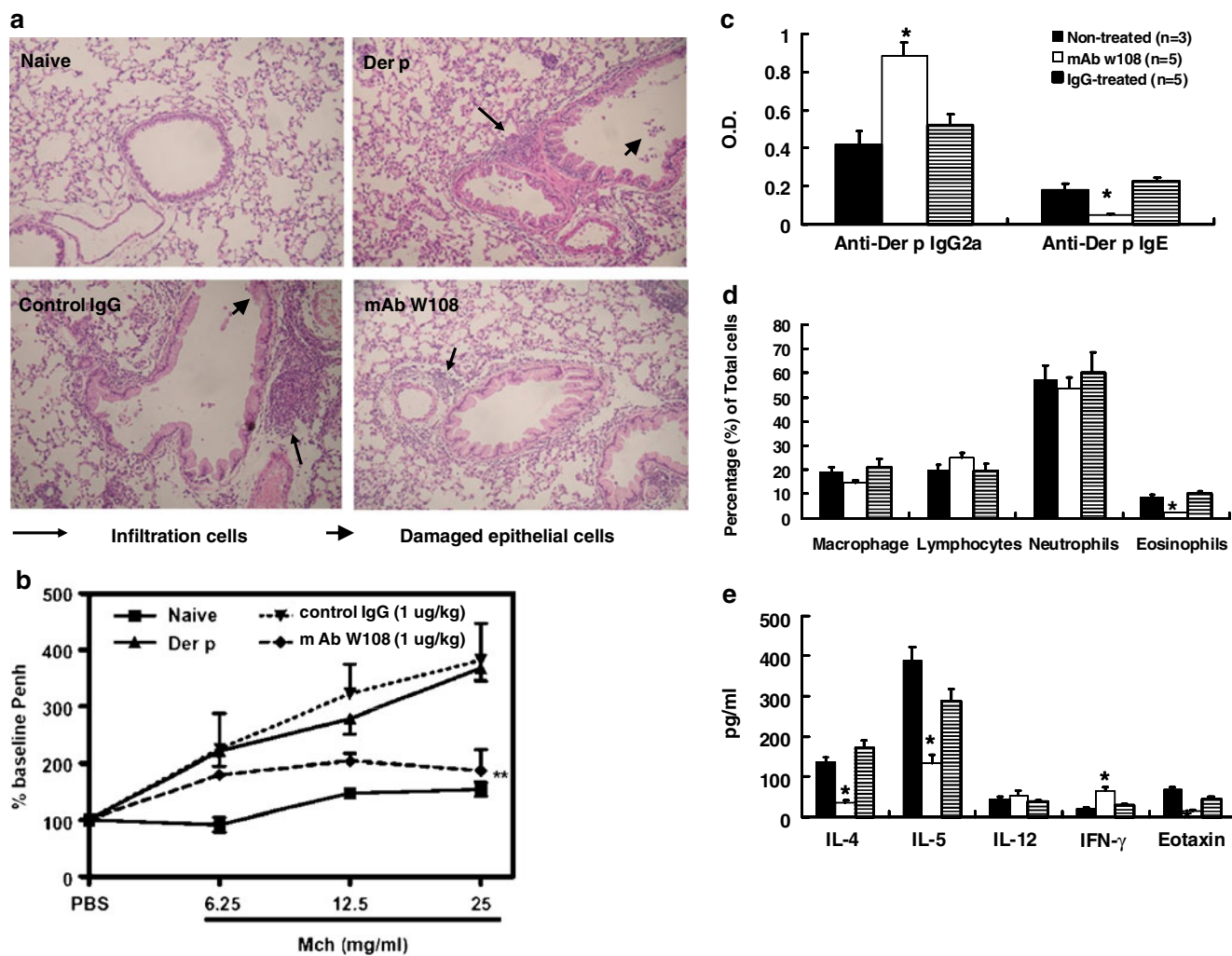
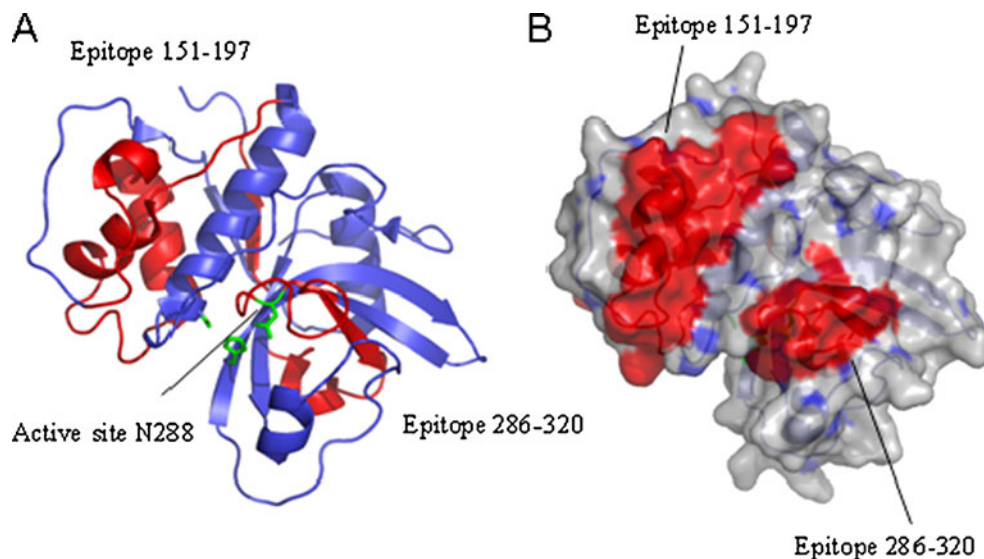
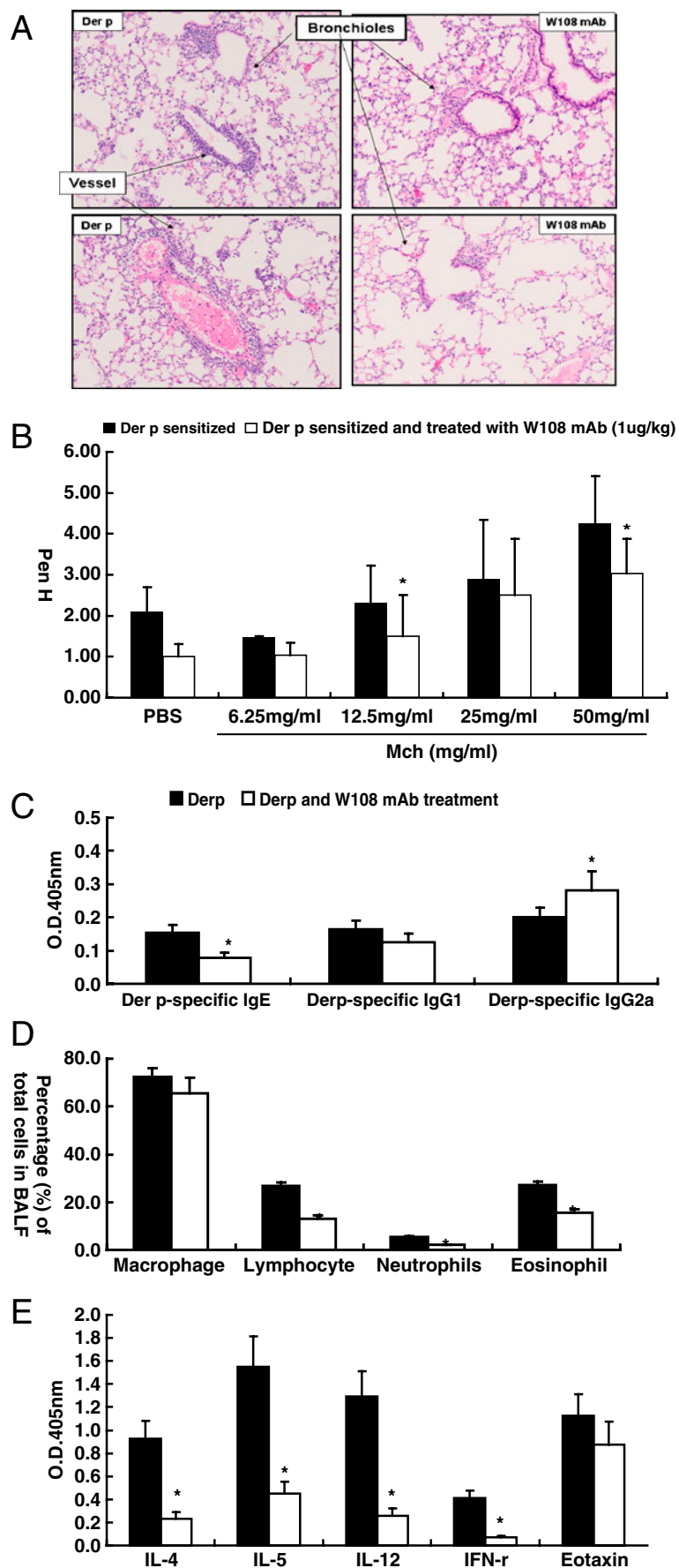


Fig. 7 The effect of mAb W108 on Der p-sensitized and allergen-challenged mice. The data show that treatment with mAb W108 (1 μ g/kg) i.p. during Der p sensitization and 6 h before allergen challenge. Non-treated (black bar), mAb W108 treated (white bar), and IgG treated

(striped bar). **a** Lung histology (H & E stain $\times 100$); **b** airway resistance induced by various dosages of methacholine and measured by PenH; **c** anti-Der p IgG subclass and IgE antibodies in sera; **d** differential cell counts in BALF; **e** cytokine production in BALF. $*p < 0.05$

Fig. 8 The effect of mAb W108 on Der p-sensitized and allergen-challenged mice. The data show that treatment with mAb W108 (1 $\mu\text{g/kg}$) i.p. 6 h after allergen challenge in Der p-sensitized mice. Non-treated (black bar); mAb W108 treated (white bar); **a** lung histology (H & E stain $\times 100$); **b** airway resistance induced by various dosage of methacholine and measured by PenH; **c** anti-Der p IgG subclass and IgE antibodies in sera; **d** differential cell counts in BALF; **e** cytokine production in BALF. * $p < 0.05$



Chua et al. [14] reported a sequence analysis of the Der p 1 cDNA clone and the similarity between its inferred amino acid sequence and the group of cysteine protease (aa 98–308) (Table E4). Moreover, studies also suggest that the epitopes on Der p 1 which bind IgE may not be continuous in nature [30, 31]. In this study, the two peptide segments of Der p 1 which bind to mAb W108 (aa 151–197 and 286–320) are parts of inter-connecting loops located in the substrate-binding cleft and on the surface of the domain, consisting mainly of β -sheets. Simulating the interaction of the amino acid sequence in the CDR3 of mAb W108 with Der p 1-binding epitopes suggested that the binding sites for mAb W108 on Der p 1 may be epitopes 151–197 and 286–320 (containing the active site of cysteine protease activity, N288), as shown in Fig. 6. However, these two separate non-linear regions do not correspond with the IgE-binding sequence of Der p 1, Leu245–Gln258 (a β -sheet region within the Der p structure), which was previously identified by screening a phage peptide library with an inhibitory anti-Der p 1 mouse mAb [22]. These results do not conflict with ours because the sequence 245–258 in Der p 1 is located at the pro-segment binding loop of our Der p 1 model (Fig. 4a). We believe that mAb W108-binding epitopes are protruding loops; therefore, it is capable of inhibiting IgE binding to Der p 1 (Figs. 1 and 2) and cysteine protease activity through steric hindrance. Nevertheless, Wan 108 may have competition as well as steric hindrance inhibitory effect on the IgE binding to Der p 1. To clarify this point, we need more information and studies regarding how polyclonal allergen-specific IgE interact with specific epitope(s) on their respective allergens.

An amino acid sequence analysis of CDRs in the heavy and light chains of mAb W108 revealed a >90% homology with two members of the VH3 family, D13203-IGHV3S4*01 (Supplemental Table 3A) and M15520 IGKV10-96*01 (Supplemental Table 3B). The V-BASE directory contains gene segments commonly found in humans [32, 33]. Presumably, antibodies similar to mAb W108 could be generated as part of the human repertoire for clinical use, particularly for combinatorial effects, and also somatic mutations of the CDRs within the rearranged genes. However, this hypothesis needs further investigation.

The clinical application of mAb W108 against Der p 1 to block its IgE-binding epitope and cysteine protease activity is a straightforward approach. Our model studied the molecular interactions involved in IgE-mediated responses. Although the IgE-binding epitope(s) of an allergen are not necessarily the allergen-specific motifs recognized by immune cells involved in the allergic (Th2) immune response [34], the anti-Der p 1 mAb W108 attenuated Der p allergen-induced airway inflammation and the Th2

cytokine response in our mouse model of allergic asthma. We therefore suspect that the binding of mAb W108 to Der p 1 also interferes with the interaction between allergen-specific motifs of Der p and the corresponding receptors of these in immune cells. However, further study is needed for confirmation.

In summary, the availability of three-dimensional models of the mAb and Der p 1, combined with data about the epitope specificity of mAb W108, will facilitate studies of IgE antibody responses to this important allergen. Such efforts may lead to new ways of suppressing Der p 1-specific IgE synthesis and effector functions by, for instance, anti-idiotypic antibodies and/or small molecules which block Der p-induced airway inflammation.

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Conflict of interests The authors declare no conflict of interests related to this study.

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