

Identification of T-cell epitope sequences on an important mite antigen

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Summary

Background T-cell epitopes on Der 1 and Der 2 groups, the major mite allergens, have been intensively analysed, while those on the other important allergens remain to be elucidated. We have cloned four cDNAs coding for important mite allergens on the basis of frequency and capacity of IgE binding. Stimulatory action of glutathione S-transferase-fused Mag1 on lymphocytes from mite-allergic patients was relatively high among them. **Objective** To identify T-cell epitopes on Mag1, we studied the stimulating activity of truncated Mag1 proteins and synthetic peptides on proliferative response of lymphocytes from mite antigen-immunized mice and mite-sensitive patients.

Methods Truncated Mag1 proteins were expressed as a fusion protein with β -galactosidase in *Escherichia coli* pop2136 carrying a variety of deleted Mag1 inserts. Murine T-cell epitope regions were estimated by the truncated antigen-induced lymphocyte proliferation assay. Overlapping peptides covering the whole sequence of the presumed T-cell epitope regions were synthesized to identify the epitope core sequences using murine and human Mag1-specific T-cell lines.

Results Amino acid range 56–70 on Mag1 molecule showed remarkable stimulatory action on murine T cells, while amino acid ranges 51–65 and 86–100 had potent stimulatory activity on human T cells.

Conclusions These results suggest that Mag1 is a valuable antigen suitable for studies on T-cell responses and T-cell epitopes in mice and humans.

Keywords: *Dermatophagoides farinae*, recombinant fusion antigen, T-cell proliferation, T-cell epitope

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Introduction

Allergens from house dust mites, *Dermatophagoides* (*D.*) *farinae* and *D. pteronyssinus*, are major agents causing allergic disorders such as bronchial asthma, atopic dermatitis, and perennial rhinitis [1,2]. The characterization of mite antigens has been made by biochemical, immunological, and genetic engineering methods [1–3]. Recently,

cDNAs coding for major or important mite antigens, Der 1 [4,5], Der 2 [6,7], Der 3 [8,9], Der p 5 [10,11], Der 7 [12,13], and Der p 8 [14] have been cloned and the corresponding recombinant antigens have become available. Human or murine T cell-reactive regions on Der p 1 [15–17] and Der p 2 [18–21] have been identified using recombinant truncated fusion proteins, synthetic peptide, and/or T-cell clones. However, because mite allergens are a complex mixture of diverse allergenic proteins, detailed analysis of T-cell epitopes of the other allergens will be important for establishing the feasibility of peptides for use in immunotherapy.

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We have cloned four cDNAs coding for additional important allergens from *D. farinae* library which were referred to as mite antigen gene (Mag) 1, 3, 29 and 44 [22–26]. Mag29 is nearly identical in sequence to heat shock protein 70, while Mag44 has been identified as mite tropomyosin. The recombinant antigens, Mag1, Mag3, and Mag44, bound with considerably high frequencies to IgE antibody in the sera from patients with bronchial asthma showing positive skin reactions to mite and house dust extracts, while Mag29 reacted to the IgE to less extent [25]. IgE-binding epitopes on Mag1 molecule were located in two regions (amino acid ranges 55–70 and 104–115) [23]. Recombinant Mag1 and Mag3 had the abilities to stimulate T cells from mite-sensitive patients to proliferate [27]. Moreover, much information on proliferative response of lymphocytes stimulated with these important antigens remains to be accumulated.

In this communication, we studied T-cell response to the recombinant mite antigen, Mag1, prepared in our laboratory using lymph node lymphocytes from immunized mice and demonstrated the importance of Mag1 as mite antigen in the aspect of T-cell stimulation. Furthermore, we identified the epitopes of murine and human T-cell lines using the truncated Mag1 proteins and the synthetic peptides.

Materials and methods

Preparation of mite antigens

Mite, *Dermatophagoides farinae*, body extract (Dfb) and faeces extract (Dff) were prepared as described previously [28].

Preparation of recombinant antigens

The truncated plasmids inserting deleted Mag1 cDNA into a plasmid pUEX2 were constructed and transformed into *Escherichia (E.) coli* pop2136 as described previously [23]. Heat-induced cells in 2 L of culture were harvested and sonicated in PBS. The β -galactosidase-fused protein, β -Gal-Mag1, and the truncated β -Gal-Mag1 proteins encompassing amino acid ranges of 1–49, 1–70, 1–85, 1–103, 1–115, and 1–194 on Mag1 molecule, which were referred to as Mag1(1–49), Mag1(1–70), Mag1(1–85), Mag1(1–103), Mag1(1–115), and Mag1(1–194), respectively, were purified by preparative disk gel electrophoresis (model NA-1800, Nippon Eido, Tokyo, Japan).

Glutathione S-transferase-fused protein, GST-Mag1, and GST were expressed in *E. coli* JM105 and purified by passing through a glutathione Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) column as described previously [25].

Synthetic peptides

Overlapping peptides were synthesized by using a Multipin Peptide Synthesis kit (Chiron Mimotopes Pty Ltd, Clayton Victoria, Australia). These peptides were composed of 15-mer amino acids with an interval of five residues which were supposed as T-cell epitopes in the proliferative response.

Mice and immunization

C3H/HeN mice were obtained from Seiwa Experimental Animal Co. (Yoshitomi, Ohita, Japan) and maintained on purina rodent chow and water ad libitum. Both male and female mice were used at 6–8 weeks of age. Mice were immunized twice with 100 μ g of mite antigen (Dfb, Dff, or GST-Mag1) in Freund's complete adjuvant (Difco laboratories, Detroit, MI, USA) into foot pads and subcutaneous at 2 week intervals [29].

Subjects

Blood samples were obtained from 10 asthmatic patients with positive skin reaction or RAST > 1, who came to the Third Department of Internal Medicine, Hiroshima Prefectural Hospital (Hiroshima, Japan) as regular out-patients and a healthy volunteer insensitive to house dust mites.

Enzyme-linked immunosorbent assay (ELISA) and ELISA inhibition

Mite antigen-specific antibodies in immune mice and the content of Mag1 in mite antigen preparations were detected by ELISA and ELISA inhibition, respectively [30].

In brief, a flat-bottomed microtitre plate (Sumitomo Bakelite, Tokyo, Japan) was coated with Dfb, Dff, or GST-Mag1 (5 μ g) and blocked with 1% bovine serum albumin in PBS. The washed wells were overlaid with mouse anti-Dfb or anti-Dff serum (1000 $\times$), followed by incubation with peroxidase-conjugated sheep anti-mouse IgG antibody (1000 $\times$; The Binding Site, Birmingham, UK). Absorbance at 490 nm was measured after 30 min of the enzyme reaction in 10 mM citrate buffer (pH 4.2) containing 5.5×10^{-1} mM *o*-phenylenediamine dihydrochloride (Wako Jyunyaku, Osaka, Japan) and 0.01% H_2O_2 by Immuno Reader NJ-2000 (Japan Intermed, Tokyo, Japan).

A flat-bottomed microtitre plate was coated with GST-Mag1 (5 μ g) and blocked with the blocking solution. Mouse anti-Dfb or anti-Dff serum (1000 $\times$) was preincubated in the presence or absence of a series of properly diluted GST-Mag1, Dfb, Dff, or bovine serum albumin and added to the antigen-coated wells. The washed wells were overlaid with peroxidase-conjugated sheep anti-mouse IgG antibody. Absorbance at 415 nm was measured in 10 mM citrate

Table 1. The existence of Mag1-reactive antibodies in murine anti-Dfb and anti-Dff sera

Antisera	Antibody titre —	*(Absorbance at 405 nm) against		
		Dfb	Dff	GST-Mag1
Control	6.4	7.2	5.6	4.6
Anti-Dfb	10.3	105.6†	15.7	83.9†
Anti-Dff	7.8	32.4†	33.6†	10.3

* Antibodies in murine sera to Dfb, Dff, and GST-Mag1 were detected by ELISA at 1000× dilution. The results are expressed as absorbance × 10³.

† Significantly enhanced ($P < 0.05$).

buffer, pH 4.2, containing 0.1% 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS; Boehringer Mannheim, Mannheim, Germany) and 0.03% H₂O₂.

Fractionation of murine lymph node lymphocytes

Lymph node lymphocytes were treated with anti-mouse Thy 1.2 monoclonal antibody (100×; Olac 1976, Blackthorn Bicester, UK) or rabbit anti-mouse Ig sera (20×; Cedarlane Laboratories, Hornby, Canada) and rabbit complement (6×; Cedarlane Laboratories) at 37°C for 1 h to obtain B cell- or T cell-enriched fraction, respectively. After the treatment, Thy 1⁺ cells and Ig⁺ cells decreased to less than 1% and 5%, respectively, as detected by flow cytometry using fluoresceinated antibody, while the untreated spleen cells contained about 40% Thy 1⁺ cells and 40% Ig⁺ cells.

Furthermore, T cells were purified by passing lymphocytes through a nylon wool column as described previously [31]. After the nylon wool column-passing, Ig⁺ cells decreased to less than 1%.

Mag1-specific T-cell lines

To detect clearly proliferative response to Mag1, we constructed Mag1-specific T-cell lines. Murine lymph node cells sensitized with GST-Mag1 or human peripheral blood lymphocytes (PBL) from mite-allergic patients (2×10^5 cells/well) were cultured with GST-Mag1 (2.5 µg) for a week. The short-term cultures were continued in the presence of GST-Mag1 (2.5 µg) and mitomycin C (50 µg/ml; Kyowahakko Kogyo, Yokohama, Japan)-treated spleen cells or PBL as antigen presenting cells (1×10^6) for additional 3 weeks and every week, antigen presenting cells and GST-Mag1 were added. In this way, we established Mag1-specific T-cell lines.

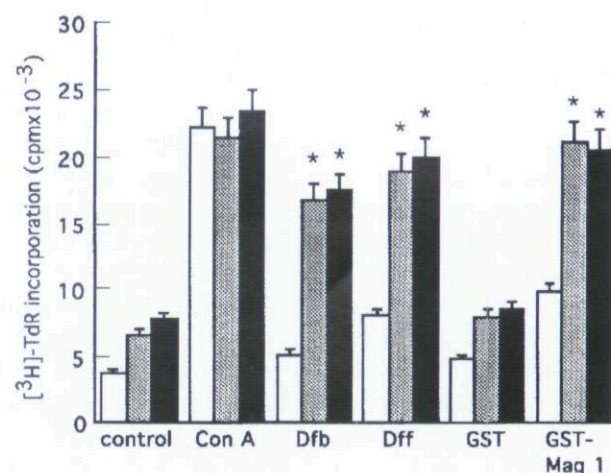


Fig. 1. Stimulatory action of GST-Mag1 on murine lymphocytes. 2×10^5 lymph node lymphocytes from normal mice (□) and from mice immunized with Dfb (▨) or Dff (■) were cultured *in vitro* with 10 µg/mL of Con A, mite antigens, GST, or recombinant mite antigen for 6 days and were pulsed with 18.5 kBq of [³H]-TdR for the last 18 h. [³H]-TdR incorporated was counted by a liquid scintillation counter. *Significantly enhanced as compared with non-immunized mice ($P < 0.05$).

Assay of proliferative responses

Lymph node lymphocytes (2×10^5 cells/well) from immunized mice or PBL (2×10^5 cells/well) from mite-allergic patients were cultured with 10 µg/mL of mite antigens, recombinant mite antigen, concanavalin A (Con A; EY Laboratories, San Mateo, CA, USA), lipopolysaccharide from *E. coli* (LPS; Sigma, St Louis, MO, USA), or 0.1 µg/mL of synthetic peptides in 0.2 mL of RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) or Eagle-Hanks amino acid medium described by Corradin *et al.* [32] containing 10% fetal calf serum (Grand Island Biological, Grand Island, NY, USA) in wells of flat-bottomed microtitre culture plates (Falcon 3072; Becton Dickinson and Co., Oxnard, CA, USA) at 37°C for 6 days in 5% CO₂ and 95% air. The cells were labelled during the last 18 h with 18.5 kBq of tritiated thymidine ([³H]-TdR, specific activity, 222 GBq/mmol; Amersham International plc, Buckinghamshire, UK) and were harvested with the aid of a semiautomated cell harvester (Abe Kagaku, Chiba, Japan). The amount of radioactivity incorporated into DNA in lymphocytes was measured with a liquid scintillation counter [33]. The results were expressed as the mean counts per minute (cpm) of triplicate cultures with standard error. The optimal concentrations of antigens were used for proliferative response.

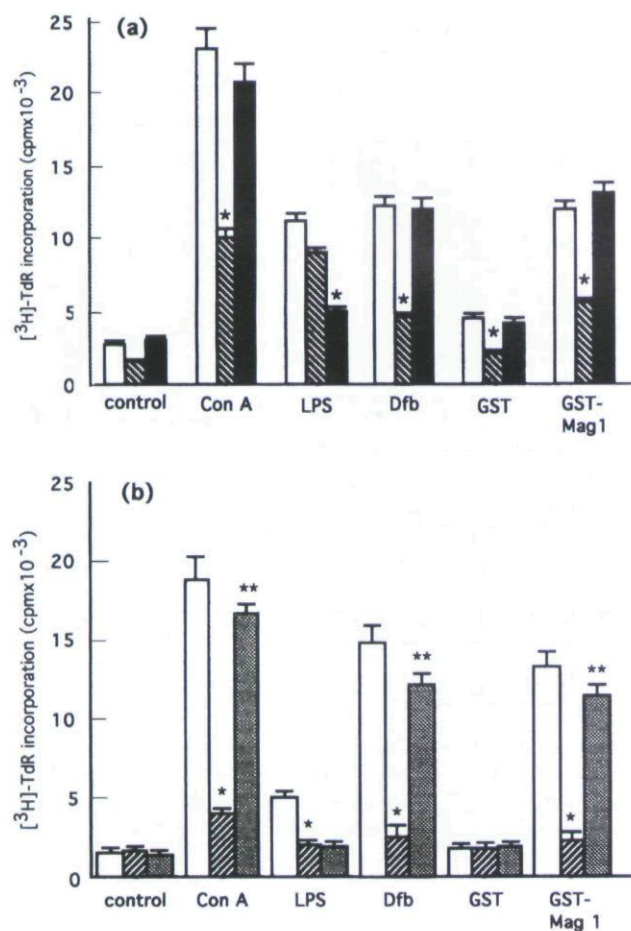


Fig. 2. Responder lymphocytes are T cells for the stimulation with mite antigen and recombinant antigen. (a) 2×10^5 lymph node lymphocytes treated with C alone (□), anti-Thy1 antibody and C (▨), or anti-Ig antibody and C (■) were cultured with $10 \mu\text{g/mL}$ of Con A, LPS, Dfb, GST, or GST-Mag1 for 6 days and $[^3\text{H}]\text{-TdR}$ incorporation was determined. *Significantly suppressed from the group treated with C alone ($P < 0.05$). (b) 2×10^5 lymph node lymphocytes (□), 10^5 nylon column-purified T cells alone (▨), or 10^5 nylon column-purified T cells plus 5×10^5 mitomycin C-treated spleen cells as accessory cells (▩) were cultured with $10 \mu\text{g/mL}$ of Con A, LPS, Dfb, GST, or GST-Mag1 for 6 days and $[^3\text{H}]\text{-TdR}$ incorporation was determined. *Significantly decreased from the corresponding control group by nylon column-passing ($P < 0.05$). **Significantly increased by the addition of mitomycin C-treated accessory cells ($P < 0.05$).

Statistics

All experiments were repeated at least three times and one representative result was shown as a Table and a Figure. Statistical analysis between control values and stimulated ones was performed by Student's *t*-test. A confidence level of < 0.05 was considered significant [34].

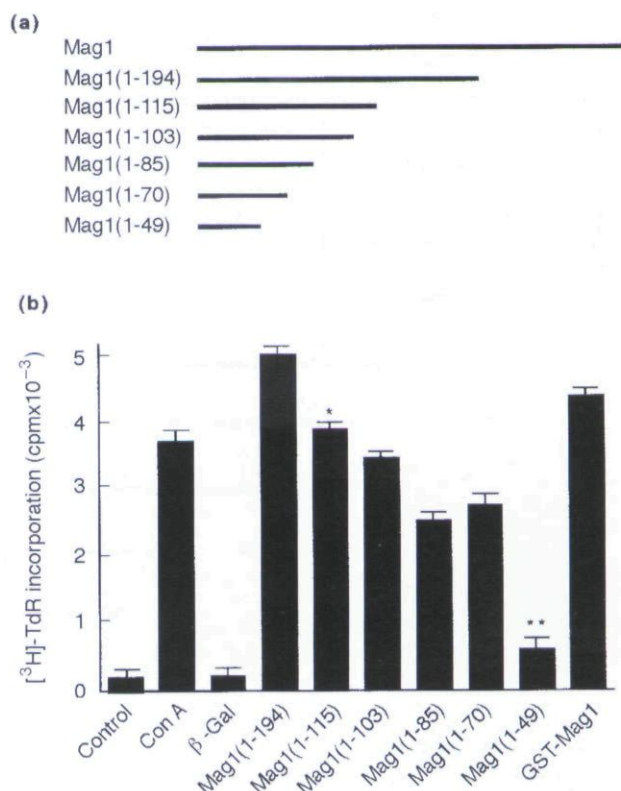


Fig. 3. Identification of murine T-cell epitope regions by changes in the ability of the truncated β -Gal-Mag1 proteins to induce the proliferation of lymphocytes. (a) The horizontal lines below Mag1 (amino acid residues 1–315) illustrate the extents of the truncated Mag1 fragments. (b) Proliferative response of GST-Mag1-specific T cells was induced by a series of truncated β -Gal-Mag1 proteins. 2×10^5 lymph node lymphocytes from GST-Mag1-immunized mice were cultured *in vitro* with $10 \mu\text{g}$ of Con A, β -Gal, truncated β -Gal-Mag1 proteins, or GST-Mag1 for 6 days and $[^3\text{H}]\text{-TdR}$ incorporation was determined. *Significantly decreased as compared with the protein of Mag1(1–194) ($P < 0.05$). **Significantly decreased as compared with the protein of Mag1(1–70) ($P < 0.01$).

Results

Induction of anti-recombinant mite antigen antibody by the immunization with mite antigens

Murine sera raised against Dfb reacted markedly with GST-Mag1 and murine anti-Dfb sera also bound weakly to the recombinant mite antigen (Table 1). However, the amounts of Mag1 were estimated to be about 4% in Dfb and Dff preparations in the inhibition test using GST-Mag1, Dfb, or Dff as competitor in an ELISA (GST-Mag1/mouse anti-Dfb or anti-Dff serum) (data not shown). These results suggest that Mag1 should be present as potent immunogens in both mite antigen preparations.

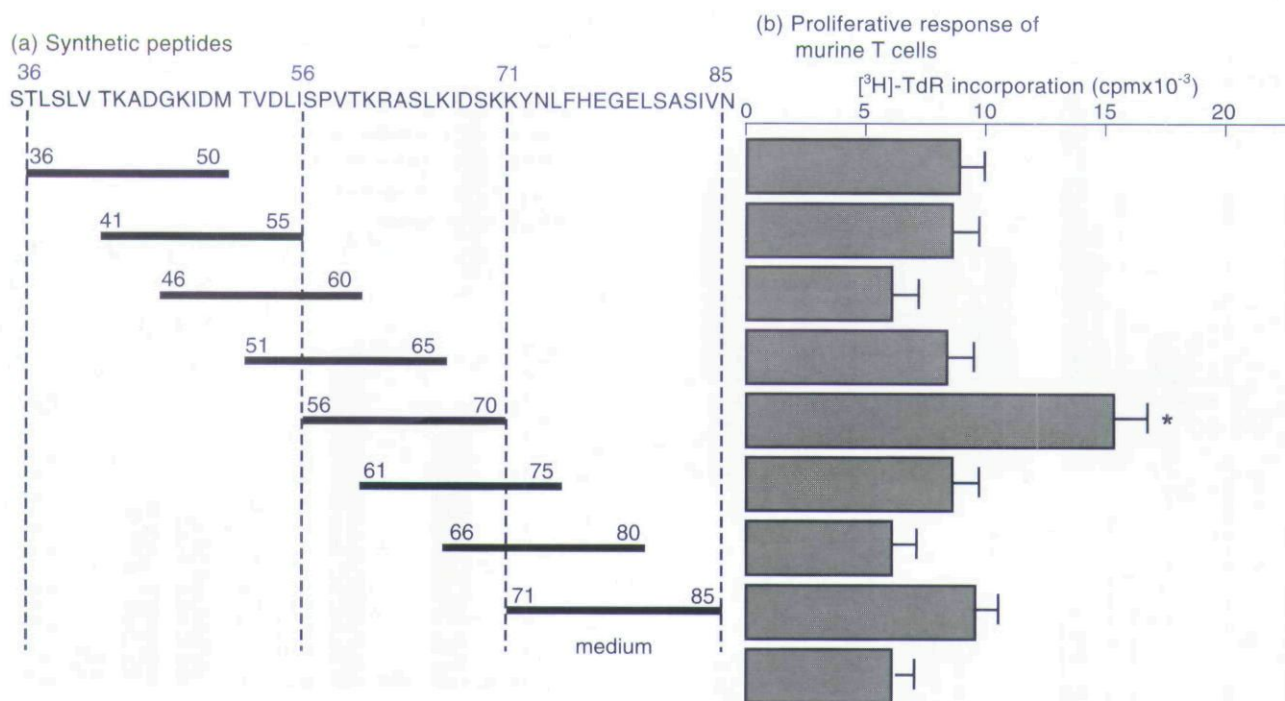


Fig. 4. Analysis of murine T-cell epitope. (a) Synthetic peptides were shown as bold lines and the number of amino acid residues was shown above the lines. (b) Stimulatory activity of synthetic overlapping peptides on short-term cultured Mag1-specific murine T-cell lines. 2×10^4 T cells were cultured *in vitro* with $0.1 \mu\text{g/mL}$ of peptides and 10^5 mitomycin C-treated spleen cells as antigen presenting cells for 6 days and $[^3\text{H}]\text{-TdR}$ incorporation was determined. *Significantly enhanced as compared with the other peptides ($P < 0.05$).

Stimulatory activity of recombinant mite antigen on lymphocytes from mice immunized with Dfb and Dff

From the time course of proliferative responses of lymph node lymphocytes from Dfb- and Dff-immunized mice, the peak response was observed on day 6 after the stimulation with Dfb or Dff and then the response gradually decreased (data not shown). Therefore, in the following experiments we assayed on day 6. As shown in Fig. 1, lymphocytes from Dfb- and Dff-immunized mice clearly proliferated in response to the stimulation of GST-Mag1 when compared with those from normal mice ($P < 0.05$). GST expressed similarly in *E. coli* did not significantly stimulate proliferative response of murine lymphocytes. These results suggest that Mag1 as well as mite antigens exerted a potent ability to activate the lymphocytes sensitized with Dfb or Dff.

Responder lymphocytes to antigens are T cells

To identify which lymphocytes commenced proliferating in response to the stimulation of antigens, murine lymphocytes sensitized with Dfb were treated with anti-Thy 1.2 antibody

or anti-Ig sera and rabbit complement to enrich them with B cells or T cells, respectively (Fig. 2a). The treatment of lymphocytes with anti-Thy1 antibody and complement reduced the Con A, Dfb, and GST-Mag1 responses, but not the LPS response. On the other hand, the treatment of lymphocytes with anti-Ig antibody and complement decreased the LPS response, but not the Con A, Dfb, and GST-Mag1 responses. These results implied that T cells would contribute to the proliferative response to these antigens. More obvious result was that nylon-purified lymphocytes responded to Con A, mite antigen, and recombinant mite antigen but not to LPS in the presence of mitomycin C-treated spleen cells as accessory cells (Fig. 2b). The result suggests that responder lymphocytes to these antigens are T cells.

Analysis of T-cell epitope regions on Mag1 molecule

We purified several truncated $\beta\text{-Gal-Mag1}$ proteins and studied their stimulatory action toward T cells to determine the region of T-cell epitope. As revealed in Fig. 3, murine lymphocytes sensitized with GST-Mag1 were stimulated with GST-Mag1 to proliferate, but not with $\beta\text{-galactosidase}$.

Mag1(1–194) stimulated lymphocytes as well as GST-Mag1, suggesting that all T-cell epitopes on Mag1 molecule are localized in the area at amino terminus between amino acid residues 1 and 194. Significant decreases in proliferative response of lymphocytes were observed in the ranges of both amino acid residues 116–194 (between Mag1(1–194) and Mag1(1–115)) and 50–70 (between Mag1(1–70) and Mag1(1–49)) ($P < 0.05$).

Murine T-cell response to overlapping synthetic peptides

Since the decrease in proliferative response between Mag1(1–70) and Mag1(1–49) was more marked than that between Mag1(1–194) and mag1(1–115), we focused on amino acid range 50–70 as a preferentially recognized region for T cell. Overlapping peptides with a span of 15-mer amino acids were synthesized to encompass the entire sequence of the amino acid range 36–85 and their stimulatory action on T cells was studied (Fig. 4). The synthetic peptides 56–70 stimulated clearly T cells to proliferate, suggesting that murine T-cell epitope on Mag1 molecule resides predominantly in the amino acid range 56–70.

Human T-cell epitope regions on Mag1 molecule

Finally we performed the same approach using lymphocytes from 10 mite-allergic patients and one healthy volunteer. As shown in Fig. 5a, the stimulatory action of truncated Mag1 proteins on lymphocytes from a patient sensitive to mites significantly decreased both Mag1(1–103) to Mag1(1–85) and Mag1(1–70) to Mag1(1–49), suggesting that the epitope regions of human T cell correspond to the amino acid ranges 86–103 and 50–70, respectively. Similarly, significant decreases in proliferation of lymphocytes from the allergic patients in response to the stimulation of the truncated Mag1 proteins occurred at two stages in eight cases (Fig. 5b). In eight, nine and one out of 10 patients (A–J) studied, proliferative responses were elicited to the ranges 50–70, 86–103, and 116–194 in whole amino acid sequence of Mag1 molecule, respectively, while any stimulatory action of all truncated Mag1 proteins on lymphocytes from a healthy donor was not observed. These results indicate that human T-cell epitopes are immunodominantly located in both amino acid residues 50–70 and 86–103.

A series of overlapping synthetic pentadecapeptides encompassing the presumed T-cell-reactive sites was used to examine their abilities to induce T-cell activation (Fig. 6). Two T-cell epitope sequences were clearly identified which map to the regions spanning amino acid residues 51–65 and 86–100 using the synthetic peptide-mediated lymphocyte proliferation assay.

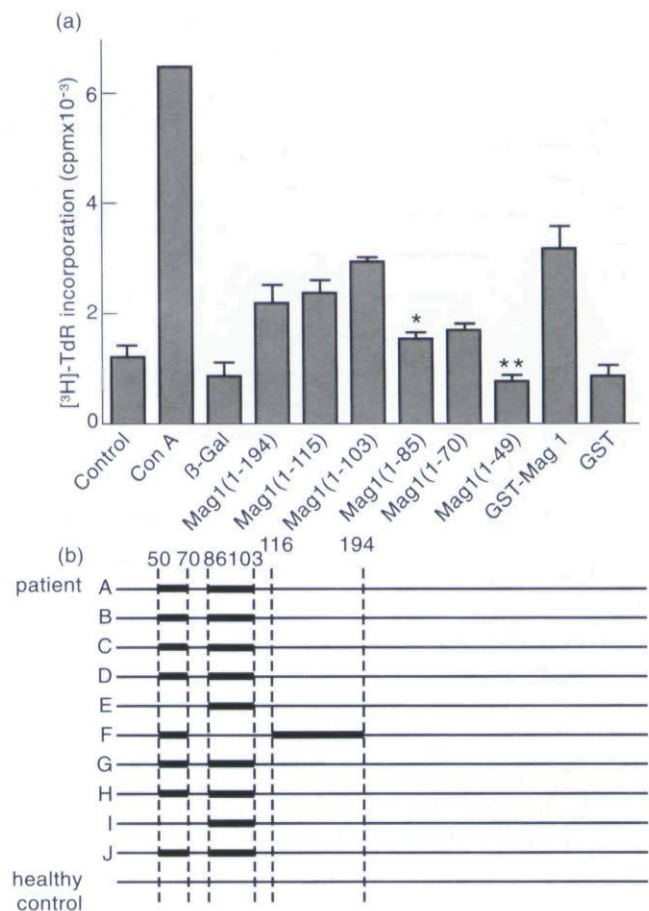


Fig. 5. Detection of human T-cell reactive regions using truncated Mag1 proteins. (a) 2×10^5 PBL from mite-allergic patient (patient A) were cultured *in vitro* with $10 \mu\text{g/mL}$ of Con A, β -Gal, truncated β -Gal-Mag1 proteins, GST-Mag1, and GST for 6 days and $[^3\text{H}]$ -TdR incorporation was determined. *Significantly decreased as compared with the protein of Mag1(1–194), Mag1(1–115), or Mag1(1–103) ($P < 0.05$). **Significantly decreased as compared with the protein of Mag1(1–85) or Mag1(1–70) ($P < 0.05$). (b) The same truncated antigen-induced lymphocyte proliferation assays as (a) were carried out for mite-allergic patients A–J. T-cell epitope regions were shown as bold horizontal lines.

Discussion

The structure and function of the major or important allergens has been studied using proliferation assays and molecular cloning techniques with a view to identifying and manipulating the important IgE-binding determinants (B-cell epitopes) and T-cell-reactive regions (T-cell epitopes) on the proteins responsible for provoking allergic disease. Especially, detailed analysis of T-cell epitopes of these allergens will be necessary to pursue the possibility of peptides for use in desensitization. O'Hehir *et al.* [15]

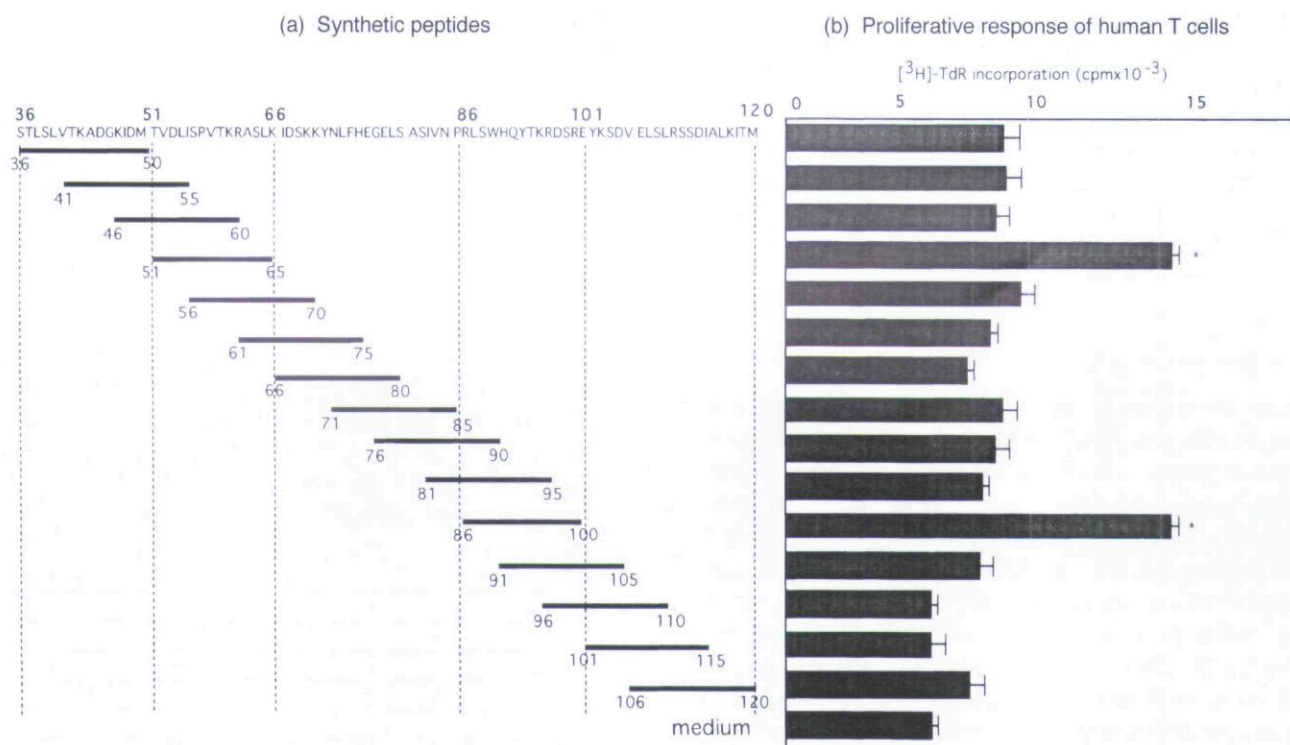


Fig. 6. Identification of human T-cell epitope sequences. (a) Synthetic peptides were shown as bold line and number of amino acid residues was shown above the lines. (b) Stimulatory activity of synthetic overlapping peptides on short-term cultured human T-cell lines. 2×10^4 T cell were cultured *in vitro* with $0.1 \mu\text{g/mL}$ of peptides and 10^5 mitomycin C-treated peripheral blood lymphocytes as antigen presenting cells for 6 days and $[^3\text{H}]\text{-TdR}$ incorporation was determined. *Significantly enhanced as compared with other peptides ($P < 0.05$).

demonstrated epitope sites on Der p 1 molecules between positions 45–73, 89–117, 85–109, and 111–139. Yssel *et al.* [16] localized epitopes to regions 45–67, 94–104, and 117–143. O'Brien *et al.* [17] reported that major immunogenic sites were contained within the 56 amino acids of the N-terminus although T-cell-reactive regions were located throughout the molecule. On the other hand, Der p 2 contains multiple T-cell epitopes that are present in all regions of the protein and that may differ from individual to individual [18–20]. By clonal analyses, distinct immunodominant T-cell epitopes have been identified, located within residues 116–129 and 29–42 [18], 16–31, 22–40, 82–100, and 111–129 [19], and 61–86, 78–104, 11–50, 81–96, and 91–101 [20]. Moreover, O'Brien *et al.* [17] emphasized that the studies with truncated recombinant allergens complemented with shorter synthetic peptide is essential for more precise localization of epitopes.

A mite antigen gene, Mag1, is a representative gene that we could detect with the highest frequency on screening from mite cDNA libraries with anti-Dfb sera. Its recombinant expression products, $\beta\text{-Gal-Mag1}$ and GST-Mag1 , exhibited on an average as high IgE-binding activity as the major allergens, although an objective valuation toward

Mag1 varied with the methods to assess IgE-reactivity in comparison with the major allergens as reference [22]. Furthermore, Mag1 had relatively potent ability to activate human T cell among our four recombinant fusion proteins [27]. In this study, we identified murine and human T-cell epitopes on the important allergen, Mag1, using truncated fusion proteins and synthetic peptides.

Murine sera sensitized with mite antigens reacted with GST-Mag1 , rabbit antisera to Mag1 bound to a high-molecular-size antigen (data not shown), and the magnitude of proliferation of the lymphocytes in response to the stimulation of GST-Mag1 were almost the same as mite antigens, suggesting that the natural antigen and its decomposed substances sharing IgG-binding determinants with Mag1 should be contained as potent immunogens in mite antigen preparations. These antigens were contained at relatively high concentration (about 4%) in a Dfb preparation, although these antigens have been estimated to be present at a concentration of less than 1/400th by weight in a different Dfb preparation in histamine release test and ELISA inhibition (Dfb/rabbit anti-Dfb serum) test as previously reported [22]. The discrepancy in the contents of Mag1 in these different Dfb preparations would reflect on the difference in assays (Dfb/rabbit anti-Dfb serum and

GST-Mag1/mouse anti-Dfb serum) and/or lots of whole mite cultures.

Mag1 stimulated antigen-specifically (gray and black columns in Fig. 1) and non-specifically (white columns) lymphocytes to proliferate. The magnitude of the antigen-specifically proliferative response of Mag1 was comparable to that of mite antigens. The lymphocytes proliferated in response to the stimulation of Mag1 were found to be T cells, because antigen-specific proliferative response was depleted by the treatment with anti-Thy1 antibody and complement and nylon-purified T cells could respond to mite antigens in the presence of accessory cells.

Next we analysed murine T-cell epitope regions using truncated Mag1 fusion proteins. The proliferative response of T cells was preferentially elicited to two regions, amino acid residues 50–70 and 116–194. Since the magnitude of stimulatory action of amino acid residues 50–70 was stronger than that of amino acid residues 116–194, we focused on the region of amino acid residues 50–70 and synthesized overlapping pentadecapeptides encompassing the presumed T-cell epitope region and studied their T cell stimulatory activity. In these synthetic peptides, only one kind of peptides, amino acid residues 56–70 had T cell stimulatory activity which correspond to amino acid sequence SPVTKRASKIDSKK.

Using truncated Mag1 proteins and overlapping synthetic peptides, we also analysed immunodominant regions for human T-cell epitope to identify two regions of amino acid residues 51–65 (TVDLISPVTKRSLK) and 86–100 (PRLSWHQYTKRDSRE) on the basis of the magnitude and frequency of T-cell reactivity. It is of interest that T-cell epitope of amino acid residues 56–65 is shared in mice and humans. Aki *et al.* reported that IgE-binding epitopes of Mag1 resided in amino acid residues 56–70 and 104–115 [23]. The former region is very close to T-cell epitopes demonstrated in this study. The primary sequences of the epitopes on Mag1 molecule found in this study are completely different from those of the epitopes on Der p 1 and Der p 2 [15–21].

It is generally accepted that for T-cell activation, antigens should be presented by antigen-presenting cells in the context of the products of the major histocompatibility complex (MHC) [35,36]. After the processing in antigen-presenting cells, the antigenic peptides bind to the groove of the MHC products and then presented to T cells. Thus, antigenic peptides have two functional sites; combining site for T-cell receptor and the MHC groove. Which amino acid residues in the mite antigen are combining sites for T-cell receptor and the MHC groove are under the investigation using the substitution and deletion of each amino acid residue. The identification and structural analysis of epitopes on an important antigen, Mag1, may be a useful strategy for the rational

design of peptide-based immunotherapeutic agents for mite allergy.

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