

Localization of T-cell determinants on bovine β -lactoglobulin

Noriko M. Tsuji^a, Jun-ichi Kurisaki^{a,*}, Koko Mizumachi^a and Shuichi Kaminogawa^b

^a*Department of Animal Products, National Institute of Animal Industry, Tsukuba Norindanchi P.O. Box 5, Ibaraki 305, Japan,*
and ^b*Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

(Received 12 November 1992, revision received 30 June 1993, accepted 25 July 1993)

1. Summary

T-cell determinants of bovine β -lactoglobulin (β -LG) in BALB/c(H-2^d), C57BL/6(H-2^b) and C3H/He(H-2^k) mice were identified using a set of overlapping synthetic peptides encompassing the entire primary structure of the protein. Lymph node cells from mice immunized with β -LG were subjected to cell proliferation assay in the presence of these peptides and uptake of ³H-labeled thymidine was measured. Determinant regions were indicated to lie in residues 42–56, 62–76 and 139–153 in BALB/c mice, residues 11–26, 72–86, 100–113 and 119–133 in C57BL/6 mice, residues 72–86, 91–104, 129–143 and 139–153 in C3H/He mice. Some of these fragments included the antigenic motifs predicted by hypotheses according to amphipathicity and sequential patterns of peptides. We reported elsewhere that residues 42–56 and 72–86 represent one of the B-cell antigenic determinants in BALB/c and C3H/He, respectively. These peptides serve as good models of co-linear T- and B-cell determinants as they contain both of T- and B-cell determinants

2. Introduction

Protein antigens are incorporated in antigen presenting cells (APC) and processed at the endo-

somal region to peptide fragments some of which encompass linear T-cell determinants [1]. These fragments are to associate with MHC class II molecules before MHC-antigen complexes on APC are recognized by relevant T cells.

Since the binding capacity of peptide fragments to MHC molecules and the capacity of being T-cell determinants have considerable relevance [2,3], stable association of peptide fragments with MHC molecules is most important to initiate T-cell responses. In previous studies, linear T-cell determinants are often represented by synthetic peptides and added as exogenous fragments. While resulted fragments by processing of protein antigens in APC are likely to associate with newly synthesized MHC class II molecules, most exogenous peptide fragments are to associate with external MHC class II molecules [4] which are already occupied with self-peptides or other protein fragments. It is considered that external association of antigenic peptides occurs as the result of competition with those peptides, which ensures the high binding capacity of the antigenic fragments to MHC molecules

The strategy using synthetic peptides in the investigation of T-cell determinants has become increasingly important as the fine chemistry and structure of MHC molecules are elucidated [5–7]. Consequently, a number of T-cell determinants on several soluble proteins as well as on viral proteins [8] were defined, enabling predictive hypotheses of determinants based on sequence pattern analysis [9–11]. Herein we identified T-cell determinants on bovine β -lactoglobulin (β -LG), using synthetic peptides and examined those identified antigenic peptides in respect to several predictive

Key words T-cell determinants, Synthetic peptide, β -Lactoglobulin

*Corresponding author Dr Jun-ichi Kurisaki, Department of Animal Products, National Institute of Animal Industry, Tsukuba Norindanchi P.O. Box 5, Ibaraki 305, Japan Tel 81-298-38-8687, Fax 81-298-38-8687

hypotheses.

β -LG is suspected to be one of the major allergens of cow's milk known for their conformational stability in the gastrointestinal tract [12]. It is important to clarify its antigenic structure both for those who are suffering with allergy as well as out of general interest in the antigenicity of proteins. Although there have been several studies on β -LG to solve this problem [13–18], complete localization of both B- and T-cell antigenic regions has not yet been achieved. We synthesized 16 overlapping peptides with 14–16 amino acids encompassing the entire primary structure of β -LG. These peptides were subjected to screen T-cell determinants in 3 strains of mice (BALB/c, C57BL/6, C3H/He) with different propensity of MHC molecules to the protein.

3. Materials and Methods

3.1. Peptide synthesis

Peptides of 14–16 residues overlapping each other by 5 residues and corresponding to the entire sequences of the β -LG (genetic variant of A) were synthesized by the method of Merrifield's solid-phase synthesis and characterized as described elsewhere [19]. Cysteine residues at 66, 106, 119, 121 and 160 of β -LG were substituted by alanine to facilitate peptide synthesis and purification. The covalent structures of peptides used in this study are shown in Fig. 1.

3.2. Preparation of β -LG

Fresh milk from a Holstein cow of the genotype A for β -LG was obtained. β -LG was prepared by the method of Aschaffenburg and Drewry [20] and purified by ion-exchange chromatography on DEAE-Sephacel. Purity was confirmed by SDS-PAGE.

3.3. Animals and immunization procedure

BALB/c (H-2^d), C57BL/6 (H-2^b) and C3H/He (H-2^k) were purchased from Charles River Japan (Tokyo) at the age of 5 weeks. Eight- to 10-week-old mice were immunized by s.c. injections of β -LG (10–80 μ g/head) in saline emulsified with an

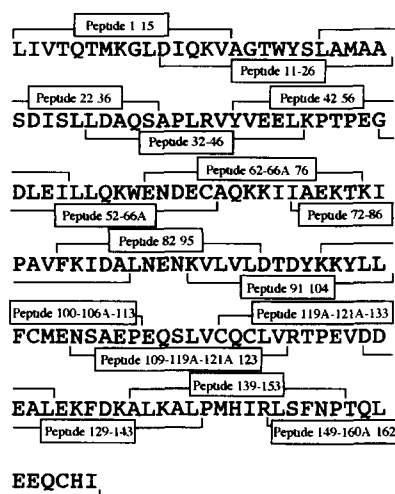


Fig. 1 Amino acid sequence of β -LG and peptides used in this study. Nomenclature of peptides according to the C- and N-terminal amino acid residue are shown in open boxes. Cysteine residues at 66, 106, 119, 121 and 160 are replaced by alanine in synthetic peptides.

equal volume of complete Freund adjuvant (CFA) containing *Mycobacterium tuberculosis*, strain H37Ra (Difco) at the base of tail and at foot pads.

3.4. Lymphocyte proliferation assays

Popliteal, inguinal and periaortic lymph node cells were prepared from mice immunized with β -LG 7 days before. Cells (5×10^5) were cultured in triplicate at 37°C under 5% CO₂ in 96-well flat-bottom plates containing one of the following antigens: 5 μ g/ml of ConA (Sigma), ovalbumin, β -LG and the synthetic peptides corresponding to the sequence of β -LG. Culture medium contained RPMI-1640 (Gibco) supplemented with 50 μ M 2-mercaptoethanol, 10 mM Hepes, 100 U/ml penicillin, 100 μ g/l streptomycin and 1% syngeneic mouse serum. Optimal doses of antigens were determined in preliminary experiments, using each protein at 10–400 μ g/ml and each synthetic peptide at 5–200 μ g/ml. Optimal length of culture was determined with cultivation for 3, 4 and 5 days. [³H]-labelled thymidine (1 μ Ci/well) was pulsed during the final 16-h incubation. After the harvest of cells by a cell harvester

(Skatron-AS), the incorporated radioactivity was counted by a liquid scintillation counter (Packard 3255).

3.5. Antibodies

Monoclonal antibodies produced by hybridomas 39-10-8 [21], ISCR3 [22] and 10-2-16 [23] specific to I-A^d, I-E^{k,d,t,p} and I-A^{k,r,s}, respectively, were used to block the Ia-restricted lymphocyte proliferation. Antibodies were precipitated from culture supernatants of the hybridomas by salting out with 50% ammonium sulfate and purified on protein A columns (AmpureTM PA Kit, Amersham).

4. Results

Sixteen overlapping peptides representing the primary structure of β -LG were subjected to cell proliferation assay. In pilot experiments (data not shown) the optimum dose of β -LG for immunization was determined as 40 μ g/head for 3 strains of

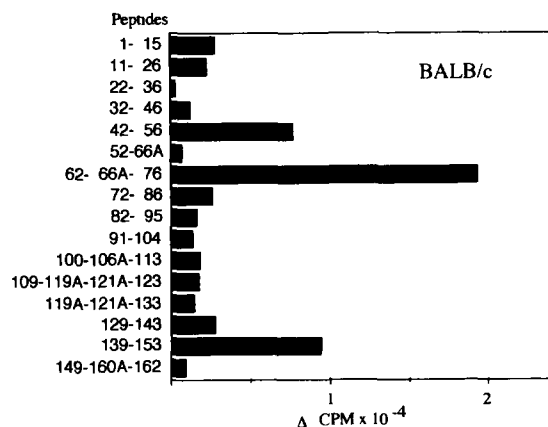


Fig 2 Proliferative response of β -LG-primed lymph node cells from BALB/c mice to the individual synthetic peptides forming the β -LG pool. The values are average of triplicate cultures. Stimulation dose of diagramed data: 5 μ g/ml for peptides 52-66A and 109-119A-121A-123, 10 μ g/ml for peptides 1-15, 72-86, 82-95, 119A-121A-133, 139-153 and 149-160A-162, 20 μ g/ml for peptides 91-104 and 129-143, 40 μ g/ml for peptides 11-26, 42-56 and 100-106A-113, 80 μ g/ml for peptides 22-36, 32-46 and 62-66A-76. SI of β -LG, ovalbumin and ConA were 8.20, 1.10 and 120.67, respectively. The basal rate of proliferation of unstimulated cells was 3359 ± 242 c.p.m.

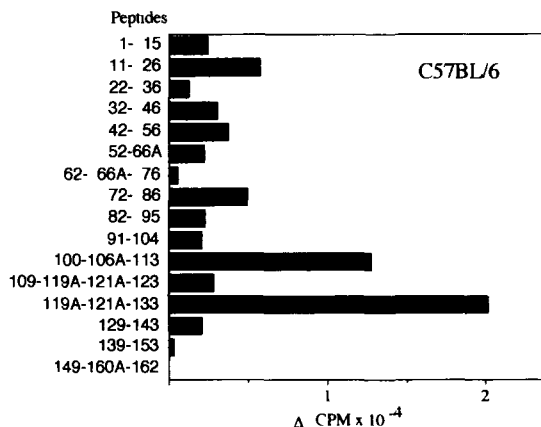


Fig 3 Proliferative response of β -LG-primed lymph node cells from C57BL/6 mice to the individual synthetic peptides forming the β -LG pool. The values are averages of triplicate cultures. Stimulation dose of diagramed data: 5 μ g/ml for peptide 149-160A-162, 25 μ g/ml for peptides 100-106A-113 and 139-153, 50 μ g/ml for peptide 119A-121A-133, 100 μ g/ml for peptides 22-36, 62-66A-76 and 82-95, 200 μ g/ml for peptides 1-15, 11-26, 32-46, 42-56, 52-66A, 72-86, 91-104, 109-119A-121A-123 and 129-143. SI of β -LG, ovalbumin and ConA were 6.41, 0.95 and 11.59, respectively. The basal rate of proliferation of unstimulated cells was 4695 ± 1108 c.p.m.

mice and the optimum culture length of lymph node cells was determined at 4 days.

Responses of cell proliferation to each synthetic peptide with an optimal dose are shown in Figs. 2-4. Each strain of different H-2 haplotype showed a different profile of T-cell determinants. Candidates of T-determinant peptides were selected in reference to stimulation indexes (SI) calculated as follows: $SI = E/C$, where E and C stand for the rate of proliferation of stimulated and unstimulated cells, respectively. In BALB/c (H-2^d) mice, the highest response was induced in the presence of peptide ⁶²ENDEAAQKKIIAEKT⁷⁶. Peptides ⁴²YVEELKPTPEGDL⁵⁶ and ¹³⁹ALKALPMHIRLSFNP¹⁵³ also stimulated T cells ($SI > 3$). All other peptides induced weak proliferative responses ($SI > 1.6$), indicating that every peptide is more or less antigenic. Among them, cell proliferation with SI greater than 2 was observed in the presence of peptides 1-15, 11-26, 72-86 and 129-143 (Fig. 2). Peptides 72-86 and 129-143 overlap with major determinant peptides 62-66A-76 and 139-153, respectively, indicating

that they may share the determinant regions.

Lymph node cells from C57BL/6 (H-2^b) mice proliferated significantly in response to peptides ¹¹DIQKVAGTWYSLAMAA²⁶, ⁷²IAEKTIP-
AVFKIDA⁸⁶, ¹⁰⁰KKYLLFAMENSAEP¹¹³ and ¹¹⁹AQALVRTPEVDDEAL¹³³ (SI > 2). There were also weak signs of proliferation (SI > 1.6) in the presence of peptides 32–46 and 42–56, indicating the minor determinant region 32–56 (Fig. 3).

Peptide ⁹¹KVLVLDTDYKKYLL¹⁰⁴ elicited a strong response of C3H/He (H-2^k) cells. Cell proliferation was also significant in the presence of peptides ⁷²IAEKTIPAVFKIDA⁸⁶, ¹²⁹DDEA-
LEKFDKALKAL¹⁴³ and ¹³⁹ALKALPMHIRL-
SFNP¹⁵³. Since peptides 129–143 and 139–153 are overlapping by 5 residues, the overlapped sequence may be recognized as T-cell determinant. Peptides 82–95 and 119A–121A–133 induced weak responses (SI > 1.6). Both of them are overlapping with major determinant peptides, indicating that these regions share determinants with peptides 72–86 and 91–104 or peptide 129–143, respectively (Fig. 4).

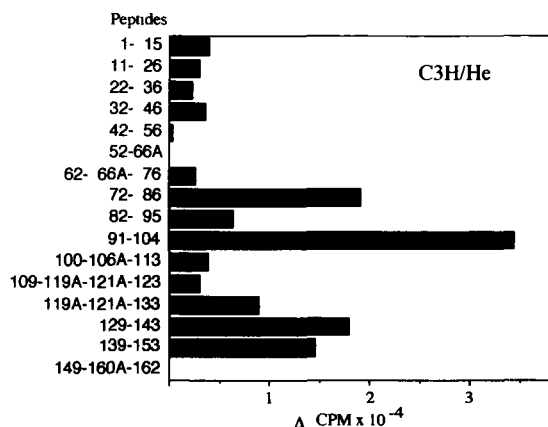


Fig. 4 Proliferative response of β -LG-primed lymph node cells from C3H/He mice to the individual synthetic peptides forming the β -LG pool. The values are averages of triplicate cultures. Stimulation dose of diagrammed data: 5 μ g/ml for peptide 22–36, 10 μ g/ml for peptide 42–56, 25 μ g/ml for peptide 52–66A, 50 μ g/ml for peptides 1–15 and 100–106A–113, 100 μ g/ml for peptides 82–95, 129–143 and 139–153, 200 μ g/ml for peptides 11–26, 32–46, 62–66A–76, 72–86, 91–104, 109–119A–121A–123, 119A–121A–133 and 149–160A–162. SI of β -LG, ovalbumin and ConA were 6.39, 1.13 and 14.32, respectively. The basal rate of proliferation of unstimulated cells was 12126 ± 1062 c.p.m.

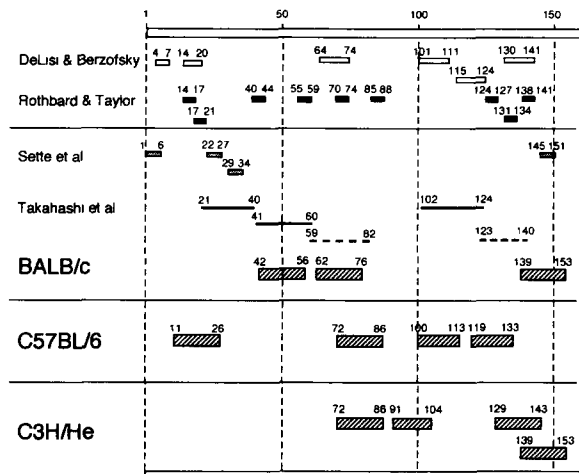


Fig. 5 Summary of data on the specificity of the response of β -LG-primed lymph node cells from BALB/c, C57BL/6 and C3H/He mice. T-cell determinant regions are shown for each mouse strain as hatched bars. The segments with highest propensity to form amphipathic α -helices [10] are in open bars, those containing a T-cell epitope motif of Rothbard and Taylor [9] are in filled bars. The H-2^d restricted antigenic regions predicted by the methods of Sette et al. [11] are shown as shaded bars. Solid lines and hatched lines represent antigenic peptides from tryptic and chymotryptic digestion, respectively, previously found to be immunogenic in BALB/c mice by Takahashi et al. [16].

We concluded that H-2^d-restricted T-cell determinants were contained in peptides 42–56, 62–66A–76 and 139–153 while H-2^b-restricted T determinants were encompassed in peptides 11–26, 72–86, 100–106A–113 and 119A–121A–133 and H-2^k-restricted T determinants were in peptides 72–86, 91–104, 129–143 and 139–153 (Fig. 5). All of the responses induced by these peptides were statistically significant as $P < 0.01$ by Student's t test.

The effect of anti-Ia monoclonal antibodies to the proliferation of lymph node cells against H-2^d and H-2^k restricted T-cell determinants were also analyzed as shown in Table 1. The proliferation against peptide 139–153 in BALB/c mice was blocked by anti-I-A^d antibody. Anti-I-A^d antibody also reduced the proliferation against peptide 62–66A–76 where anti-I-E antibody did not. The proliferation against peptide 42–56 was inhibited by both anti-I-A^d and anti-I-E antibodies. Anti-I-A^k antibody blocked the proliferation

TABLE 1

Analysis of Ia restriction of T-cell determinant peptides

Lymph node cells from mice immunized with β -lactoglobulin were cultured with T-cell determinant peptides in the presence or absence of anti-Ia antibodies. Stimulation dose of diagrammed data was 5 μ g/ml for peptide 42–56, 50 μ g/ml for peptides 91–104 and 139–153, 200 μ g/ml for peptides 62–66A–76, 72–86, 129–143 and 139–153. The basal rates of proliferation of unstimulated cells were 2970 ± 165 and 1814 ± 113 c.p.m. for BALB/c and C3H/He mice, respectively.

BALB/c (H-2 ^d) antibody	Stimulator		
	42–56	62–66A–76	139–153
	Δ c.p.m. $\pm$ SE		
–	5303 $\pm$ 1566	1348 $\pm$ 596	7464 $\pm$ 1146
39–10–8 ^a	1932 $\pm$ 748	838 $\pm$ 358	3137 $\pm$ 460
ISCR3 ^b	1018 $\pm$ 34	2195 $\pm$ 323	8670 $\pm$ 152

C3H/He (H-2 ^k) antibody	Stimulator			
	72–86	91–104	129–143	139–153
	Δ c.p.m. $\pm$ SE			
–	5156 $\pm$ 72	2599 $\pm$ 331	4591 $\pm$ 717	2915 $\pm$ 892
10–2–16 ^c	–346 $\pm$ 247	–776 $\pm$ 239	–739 $\pm$ 142	–679 $\pm$ 509
ISCR3	757 $\pm$ 539	1450 $\pm$ 125	–163 $\pm$ 160	1096 $\pm$ 651

^a39–10–8, specific to I-A^d

^bISCR3, specific to I-E^{k d,t,p}

^c10–2–16, specific to I-A^{k r,s}

against all 4 H-2^k restricted T-cell determinants in C3H/He mice while anti-I-E antibody inhibited the proliferation induced by peptides 72–86 and 129–143 stronger than that induced by other 2 peptides.

5. Discussion

T-cell determinants of β -LG in inbred mice strains were identified with the use of synthetic overlapping peptides corresponding to the entire primary sequence of the protein. Although β -LG has been considered to be one of the most causative antigens in milk allergy, the profile of T-cell determinants has not been investigated with the use of peptides short enough to allow the localization of determinant regions. The length of peptide 14–16 residues we used was considered reasonable, because naturally occurring peptides associated with MHC class II molecules are reported to be 13–17 residues or longer [24,25]. Although there may still be undiscovered T-cell determinants that attribute to deficiencies of adequate

peptide conformation or specific amino acid residue, the 16 peptides which overlap each other by 5 residues should, however, yield considerable information by reason that 5 residues is the minimal length of T-cell determinant peptides capable of displacing fragments on MHC molecules [26].

Studies on different strains of mice with different H-2 haplotypes resulted in considerably different mapping of peptides containing T-cell determinants on β -LG. No common region was found among T-cell determinant peptides restricted to 3 different haplotypes, although BALB/c (H-2^d) and C3H/He (H-2^k) mice commonly recognized peptide 139–153. Considering that most of MHC binding peptides have the capacity to bind to more than 1 MHC molecule [2,27] in spite of polymorphism of the putative antigen binding groove of different MHC molecules [5], more general antigenic regions are expected. The considerable difference in profiles among strains may be ascribed to differences in the T-cell repertoire in addition to the different propensity of MHC mo-

lecules to β -LG.

The profile of H-2^d restricted antigenic regions on β -LG determined with tryptic and chymotryptic fragments of reduced carboxymethylated β -LG reported by Takahashi et al. [16] are shown in Fig. 5. T-cell determinant regions 42–56 and 62–76 are located in a tryptic and a chymotryptic fragment, respectively. Region 139–153 was newly identified in the present study. Synthetic peptides corresponding to tryptic fragments 21–40 and 102–124 and chymotryptic fragment 123–140 were not identified as highly antigenic in this study. This might be due to the nature of the synthetic peptides used here in which cysteine at residues 106, 119 and 121 is replaced by alanine. Although short peptides would bind MHC class II molecules without further processing, we should also bear in mind that cysteine-rich segments are apt to contain T-cell determinants because they are resistant to proteolytic cleavage by cathepsins [28]. This study presented a fine mapping of determinant regions, although there is still a possibility that unidentified T-cell determinants could be found by adapting other strategies such as the multi-pin peptide synthesis system [29,30].

On many protein antigens, T-cell determinants have been investigated using inbred mice strains of differing MHC haplotype and a number of sequence patterns are accumulated [8]. On the basis of those sequences, classification and prediction of T-cell determinants have been suggested by several groups [9–11]. Rothbard and Taylor [9] proposed general T-cell determinant patterns as 4 (charged or glycine, hydrophobic, hydrophobic, polar or glycine) and 5 amino acids (charged or glycine, hydrophobic, hydrophobic, hydrophobic or proline, polar or glycine) with different flanking residues contiguous to the motif according to haplotypes. The motifs contained in the β -LG sequence are indicated in Fig. 5. There are 9 antigenic motifs on the protein and 6 of those are located on T-cell determinant peptides identified in this study. Specific residues at flanking positions proposed to be characteristic of each haplotype were not observed in these sequences.

As a general T-cell determinant structure, Delisi and Berzofsky [10] pointed out a high correlation between T-cell antigenic sites and amphipathicity.

The hypothesis suggests that T-cell determinants are likely to fall in amphipathic regions which are apt to form α -helix-like structures. Predicted T-cell antigenic sites in β -LG according to the method are shown in Fig. 5. Residue 62–76 for BALB/c mice, residues 100–113 and 119–133 for C57BL/6 mice, and residue 129–143 for C3H/He mice are comprised of amphipathic regions.

X-ray crystallographic analysis revealed that the conformation of β -LG contains only one 3-turn helix (130–140) [31]. Peptide 129–143 contains the α -helical region as well as 2 T-cell determinant motifs (131–134 and 138–141) deduced from the method of Rothbard and Taylor [9] and the amphipathic region (131–141). The region was recognized by C3H/He. There are 3 other peptides which contain both antigenic motifs and amphipathic regions. It is interesting that residues 62–76 (peptide 62–66A–76) and 119–133 (peptide 119A–121A–133), which were the most potent T determinants in BALB/c and C57BL/6, respectively, contain both 1 antigenic motif and 1 amphipathic region.

There are some reports that H-2^d prefers peptides as α -helical [32–34]. Although α -helical residues on β -LG conformation (130–140) were recognized by BALB/c mouse with only a weak T-cell proliferative response, two-thirds of H-2^d restricted determinants contained amphipathic sequences. The frequency was 2/4 for H-2^b restricted determinants and 1/4 for H-2^b restricted determinants.

As for preferences of H-2^d MHC molecules, Sette et al. [11] proposed a simple method to search for antigenic regions by sequence pattern analysis. According to the method, residues 1–6, 22–27, 29–34 and 145–151 were suspected to have high affinity to I-A^d as shown in Fig. 5. Peptide 139–153, one of the determinant peptides for BALB/c mice, contained residues 145–151 and the lymphocyte proliferation against the peptide was shown to be I-A^d restricted using anti-Ia monoclonal antibodies (Table 1). Residues 22–27 and 29–34 were found in enzymatic fragments which have been reported to be antigenic [16]. Typical I-E^d sequence pattern proposed by Sette et al. as 'basic-basic-noncharged-basic' motif was not detected in β -LG although the lymphocyte

proliferation against peptide 42–56 was inhibited by anti-I-E antibody as much as by anti-I-A^d antibody

In the study of milk allergy, β -LG is important because of its intensive antigenicity. To uncover the complete immunogenic profile of this protein, we have been investigating B-cell antigenic determinants as well as T-cell determinants. We have reported elsewhere [19] the B-cell determinant peptides to which high titers of antibodies were recognized in serum from 3 strains of β -LG immunized mice and 3 species of animals (outbred mouse, rabbit and goat). Among T-cell determinants identified in this study, major T-cell determinant peptides 42–56 and 72–86 were also recognized as B-cell determinants in relevant strains of mice. Those peptides may become potent immunogenic peptide fragments when β -LG is taken in a degraded form. They may also serve as good models of co-linear T- and B-cell determinants derived from one of the edible proteins which can cause food allergy. Although the antigenic structure was elucidated in animals in our study, we expect that the findings are also useful to analyze food allergy in human.

Acknowledgements

We are indebted to ex-President of NIAI, Prof. Dr. T. Morichi, Nihon University, and Department Director M. Yoshitake for their advice and encouragement throughout this work. This study was supported in part by a Grant-in-Aid (Bio Media Program) from the Ministry of Agriculture, Forestry and Fisheries (BMP 92-VI-1-3).

References

- [1] Schwartz, R H (1985) *Annu Rev Immunol* 3, 237
- [2] Buus, S, Sette, A, Colon, S M, Miles, C and Grey, H M (1987) *Science* 235, 1353
- [3] Schaeffer, E B, Sette, A, Johnson, D L, Bekoff, M C, Smith, J A, Grey, H M and Buus, S (1989) *Proc Natl Acad Sci USA* 86, 4649
- [4] Davidson, H W, Reid, P A, Lanzavecchia, A L and Watts, C (1991) *Cell* 67, 105
- [5] Bjorkman, P J, Saper, M A, Samraoui, B, Bennett, W S, Strominger, J L and Wiley, D C (1987) *Nature* 329, 506
- [6] Bjorkman, P J, Saper, M A, Samraoui, B, Bennett, W S, Strominger, J L and Wiley, D C (1987) *Nature* 329, 512
- [7] Brown, J H, Jardetzky, T, Saper, M A, Samraoui, B, Bjorkman, P J and Wiley, D C (1988) *Nature* 332, 845
- [8] Milich, D R (1989) *Adv Immunol* 45, 195
- [9] Rothbard, J B and Taylor, W R (1988) *EMBO J* 7, 93
- [10] DeLisi, C and Berzofsky, J A (1985) *Proc Natl Acad Sci USA* 82, 7048
- [11] Sette, A, Buus, S, Appella, E, Smith, J A, Chestnut, R, Miles, C, Colon, S M and Grey, H (1989) *Proc Natl Acad Sci USA* 86, 3296
- [12] Korits, T N, Suzuki, S and Coombs, R R A (1987) *Int Archs Allergy Appl Immun* 82, 72
- [13] Haddad, Z H, Kalra, V and Verma, S (1979) *Ann Allergy* 42, 368
- [14] Kurisaki, J, Nakamura, S, Kaminogawa, S and Yamauchi, K (1982) *Agric Biol Chem* 46, 2069
- [15] Kurisaki, J, Nakamura, S, Kaminogawa, S, Yamauchi, K, Watanabe, S, Hotta, K and Hattori, M (1985) *Agric Biol Chem* 49, 1733
- [16] Takahashi, T, Kaminogawa, S, Kuwata, T, Ando, O and Yamauchi, K (1988) *Agric Biol Chem* 52, 2485
- [17] Takahashi, T, Yamauchi, K and Kaminogawa, S (1990) *Agric Biol Chem* 54, 691
- [18] Adams, S L, Barnett, D, Walsh, B J, Pearce, R J, Hill, D J and Howden, M E H (1991) *Immunol Cell Biol* 69, 191
- [19] Kurisaki, J, Mizumachi, K, Tsuji, N M and Kaminogawa, S, submitted
- [20] Aschaffenburg, R and Drewry, J (1957) *Biochem J* 65, 273
- [21] Sachs, D H, Mayer, N and Ozato, K (1981) in *Monoclonal Antibodies and T-Cell Hybridomas* (Hammerling, G J, Hammerling, U and Kearney, J F, Eds) pp 95–101, Elsevier, Amsterdam
- [22] Watanabe, M, Suzuki, T, Taniguchi, M and Shinohara, N (1983) *Transplantation* 36, 712
- [23] Oi, V T, Jones, P P, Goding, J W and Herzenberg, L A (1978) *Curr Top Microbiol Immunol* 81, 115
- [24] Rudensky, A Y, Preston-Hurlburt, P, Hong, S-C, Barlow, A and Janeway Jr, C A (1991) *Nature* 353, 622
- [25] Hunt, D F, Michel, H, Dickinson, T A, Shabanowitz, J, Cox, A L, Sakaguchi, K, Appella, E, Grey, H M and Sette, A (1992) *Science* 256, 1817
- [26] Reddehase, M J, Rothbard, J B and Koszinowski, U H (1989) *Nature* 337, 651
- [27] Buus, S, Sette, A and Grey, H M (1987) *Immunol Rev* 98, 115
- [28] Mouritsen, S, Meldal, M, Hansen-Ruud, J and Werdelin, O (1991) *Scand J Immunol* 34, 421
- [29] Maeji, N J, Bray, A M and Geysen, H M (1990) *J Immunol Methods* 134, 23
- [30] Gammon, G, Geysen, H M, Apple, R J, Pickett, E, Palmer, M, Ametani, A and Sercarz, E E (1991) *J Exp Med* 173, 609
- [31] Papiz, M Z, Sawyer, L, Eliopoulos, E E, North, A C T, Findlay, J B C, Sivaprasadarao, R, Jones, T A, Newcomer, M E and Kraulis, P J (1986) *Nature* 324, 383
- [32] Roy, S, Scherer, M T, Briner, T K, Smith, J A and Gefter, M L (1989) *Science* 244, 572
- [33] Gao, X-M, Liew, F Y and Tite, J P (1989) *J Immunol* 143, 3007
- [34] Brett, S A, Cease, K B, Ouyang, C S and Berzofsky, J A (1989) *J Immunol* 143, 771