Two Distinct Epitopes on the Ovalbumin 323-339 Peptide Differentiating CD4⁺T Cells into the Th2 or Th1 Phenotype

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The epitopes for OVA323-339-specific CD4⁺T cells from OVA23-3 food allergy model and DO11.10 tolerant induction model mice were analyzed. We found that OVA23-3 CD4⁺T cells recognized the N-terminal region, showing strong proliferation and the Th2-phenotype, and that DO11.10 CD4⁺T cells recognized the C-terminal region, showing milder proliferation and a Th1-skewed response. These differences may regulate the responses of those mice to OVA-feeding, inflammation, and tolerance.

Key words: OVA323-339 amino acid residue; food allergy; tolerance; oral immunotherapy; epitope

T-cell receptor (TCR) transgenic (TCR-Tg) mice have been often used for examining the immune responses to orally administered antigens, because antigen-specific T-cell responses can be visualized. TCR-Tg mice play important roles for this characteristic in clarifying the mechanism or developing oral immunotherapy (OIT) in respect of the antigen-specific T-cell response with food allergy. There are two strains of OVA-specific TCR-Tg mice that are transgenic for OVA323-339-specific and I-A<sup>d</sup>-restricted TCR genes, DO11.10 (D10) mice⁵ and OVA23-3 mice.⁶ OVA23-3 mice have been reported as an effective model for food allergy inflammation by feeding an egg white diet (EW-diet) alone.³ It was suggested that IL-4 and the subsequent Th2 cytokine responses would play critical roles in inducing food allergy inflammation in EW-diet-fed OVA23-3 mice.³ On the other hand, D10 mice have been often used as an oral tolerance induction model.⁴ CD4⁺T cells, by recognizing the epitope peptides presented by MHC class II molecules, critically regulate the antigen-specific immune responses in food allergy or tolerance induction. We have therefore tried to determine CD4⁺T cell epitopes in mice or food allergic patients⁵ to develop effective induction of tolerance or a treatment for food allergy. We were also interested in the regions of OVA323-339 recognized by naïve CD4⁺T cells from the two strains which exhibited different characteristics by an oral administration of OVA. Since OVA323-339 was a dominant determinant of CD4⁺T cells from egg allergy patients,⁷ examining the epitopes of the two strains was thought to present important information for the development of OIT.

It has been reported that OVA323-339 contained at least two epitopes binding to the I-A<sup>d</sup> molecule by using distinct registers.⁸ The crystal structure of the I-A<sup>d</sup>-restricted OVA323-339 peptide complex was determined and the core epitope of OVA323-339 was clearly identified in the N-terminal region of the peptide.⁹ This suggested that the N-terminal region might contain the OVA-specific CD4⁺T cell epitope for OVA23-3 and D10 mice.¹⁰ However, some reports have indicated that an epitope for OVA-specific D10 CD4⁺T cells was in the C-terminal region of OVA323-339.¹¹ We therefore examined in this study whether each epitope would be different between CD4⁺T cells of OVA23-3 and that of D10 mice.

To effectively obtain OVA-specific CD4⁺T cells, we used RAG-2-deficient OVA23-3 and D10 (ROVA23-3 and RD10) mice which respectively were kindly given by Dr. S. Habu of Tokai University and by Dr. Y. Wakatuki of Kyoto University.¹² Both strains were maintained in a specific-pathogen-free room at the University of Tokyo. ROVA23-3 mice have also developed severe inflammation by feeding an EW-diet.³ The N-terminal 323-336 peptide and C-terminal 326-339 peptide derived from the original OVA323-339 peptide were designed according to previous reports (Fig. 1).¹,⁸,¹¹ These peptides were synthesized by Laboratory Center for Proteomics Research at The University of Tokyo. The CD4⁺T cells were purified by MACS CD4 beads with the Auto...
MACS cell separation system (Miltenyi Biotec, Bergish Gladbach, Germany). CD4+ T cells (1 x 10^5 cells/well), whose purity was more than 95%, were cultured with different concentrations of three synthetic peptides (0.00005 µM - 5 µM) and mitomycin-C-treated splenocytes from BALB/c mice (4 x 10^5 cells/well). Proliferation was analyzed by a tritiated thymidine (37 kBq/well) incorporation assay. The cytokine concentration in the 24-h culture supernatant was measured by ELISA (R&D Systems, MN, USA).

The proliferative response and cytokine production of the OVA-specific naïve CD4+ T cells are indicated in Fig. 2. In response to OVA323-339, ROVA23-3 CD4+ T cells showed significantly higher response than RD10 CD4+ T cells at concentrations of 0.05 µM and 0.5 µM. RD10 CD4+ T cells had relatively low potential to produce IL-4 at any concentration of OVA323-339, but showed a biased Th1 response producing IFN-γ. ROVA23-3 CD4+ T cells produced the highest level of IL-4 at 0.05 µM. However, the IL-4 production decreased with increasing peptide concentration, suggesting that the expression of Th2 cytokine transcription factors was inhibited by signaling via the IL-4-receptor and TCR signals in the naïve CD4+ T cells stimulated with the peptide at high concentrations. ROVA23-3 CD4+ T cells produced lower levels of IFN-γ at 0.5 and 5 µM, in contrast with the higher IFN-γ production of RD10 CD4+ T cells.

We further evaluated the OVA-specific naïve CD4+ T-cell responses by using C-terminal peptide OVA323-339 and N-terminal peptide OVA323-336. The proliferative response at 0.05 µM of OVA323-336 was significantly higher in the ROVA23-3 CD4+ T cells than in the RD10 CD4+ T cells. The response to OVA323-339 at 0.05 µM was significantly higher in the RD10 CD4+ T cells than in the ROVA23-3 CD4+ T cells. These results indicate OVA323-336 to be a more strictly defined epitope than OVA323-339 for ROVA23-3 CD4+ T cells, while OVA323-339 was the case for RD10 CD4+ T-cells. The proliferation level of ROVA23-3 CD4+ T-cells in response to OVA323-336 at a concentration of 0.05 µM was significantly higher than that of RD10 CD4+ T cells in response to OVA323-339. These results demonstrate that, even in response to their respective strictly defined epitope peptides, the response of ROVA CD4+ T-cells was stronger than that of RD10 CD4+ T-cells. In respect of the cytokine profile, ROVA23-3 CD4+ T-cells showed marked IL-4 production when stimulated with N-terminal OVA323-336. The amount of IL-4 decreased at concentrations of 0.5 and 5 µM, reflecting the inhibited expression of Th2 cytokine transcription factors as already described. Stimulated by OVA323-339, IL-4 production by ROVA23-3 CD4+ T cells was detected at 5 µM, but IFN-γ production was suppressed. RD10 CD4+ T-cells, however, maintained marked IFN-γ production in response to OVA323-336 as well as to OVA323-339 at 0.5 µM. These results clearly indicate that, in response to epitopes as well as original peptide OVA323-339, ROVA23-3 CD4+ T cells showed the Th2 phenotype, while RD10 CD4+ T cells showed the Th1 phenotype. OVA323-339 therefore contained two epitopes for OVA-specific CD4+ T-cells, each epitope-specific CD4+ T-cell respectively exhibiting the Th2 or Th1 response. The present results indicate that OVA23-3 mice, which have naive CD4+ T cells preferentially differentiating to Th2-type CD4+ T cells, had intestinal inflammation induced by the oral administration of OVA, while D10 mice with naive CD4+ T cells

![Fig. 1. Amino Acid Sequences of the OVA Peptides.](image)

OVA323-339 peptide, N-terminal OVA323-336 peptide and C-terminal OVA326-339 peptide were synthesized by the f-moc system and purified by HPLC.

![Fig. 2. Proliferation and Functions of Naïve OVA-Specific CD4+ T Cells from ROVA23-3 Mice and Those from RD10 Mice in the Immediate Response to OVA323-339, OVA326-339 and OVA326-339 Peptides.](image)

CD4+ T cells were cultured in the presence of OVA peptides and mitomycin-C-treated splenocytes from BALB/c mice to examine the proliferation and cytokine production. Proliferation was analyzed by a tritiated thymidine incorporation assay. Culture supernatants were collected and the cytokine production was determined by ELISA. The dose-response curves were confirmed by three repeated experiments. Each point is presented as the mean ± SD. The Mann-Whitney U test was used for comparisons between 2 groups, and differences are considered statistically significant when p values were <0.05. *p < 0.01, **p < 0.05.
preferentially differentiating to the Th1 type showed immediate tolerance induction. The continuous oral administration of OVA to D10 mice has been reported to induce a Th2 response.\(^1\) The tolerance to orally administered OVA being immediately acquired in D10 mice without facing a risk of severe inflammation might have been due to excess Th2-skewing being inhibited by the immediate naïve Th1 CD4\(^+\) cell responses. It has been suggested that an excess production of Th2 cytokines might affect Foxp3 molecule expression in the intestinal tissues,\(^2\) so that activating C-terminal epitope-specific naïve Th1 CD4\(^+\) cells may provide an appropriate environment to induce regulatory T cells as well as to inhibit inflammatory responses.

Table 1 shows a summary of this study. CD4\(^+\) T cells recognizing different epitopes exhibited contrasting functions. The affinity between each epitope and each TCR on CD4\(^+\) T cells may be higher in ROVA23-3 mice than in RD10 mice. Some molecules associated with TCR signaling influence the function of CD4\(^+\) T cells. This strong affinity between TCR and the epitope bound to the I-A\(^b\) molecule may therefore affect TCR signaling, subsequently inducing significant Th2 responses, in CD4\(^+\) T cells from ROVA23-3 mice. OIT has recently been recognized as one of the effective treatments for food allergy inflammation,\(^3\) although many people fear the side effects of this treatment.\(^4\) One idea for developing effective OIT may be using immunodominant T-cell epitope peptides to avoid this risk.\(^5\) However, it has been reported that an intranasal treatment did not succeed when using OVA323-339 in a food allergy model.\(^6\) Our results may suggest the necessity of using suitable and strictly determined epitopes to effectively induce tolerance and avoid inflammation. We should examine the effect of each N-terminal and C-terminal epitope by using the food allergy model of BALB/c mice to confirm this hypothesis.

### Table 1. Comparison of OVA23-3 and DO11.10 Mice Immune Responses

<table>
<thead>
<tr>
<th>Mouse</th>
<th>TCR</th>
<th>Epitope</th>
<th>CD4(^+) T-cell responses</th>
<th>Characteristics of the mice with oral administered OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA23-3</td>
<td>Vα3, Vβ15</td>
<td>OVA 323-336</td>
<td>Th2</td>
<td>Intestinal inflammation</td>
</tr>
<tr>
<td>DO11.10</td>
<td>Vα13, Vβ8.2</td>
<td>OVA 326-339</td>
<td>Th1</td>
<td>Tolerance induction</td>
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</table>

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### References