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ORIGINAL ARTICLE

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Altered peptide ligands control type II collagen-reactive T cells from rheumatoid arthritis patients

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Abstract We previously reported that peripheral blood mononuclear cells from HLA-DRB1*0101 Japanese patients with rheumatoid arthritis (RA) were highly reactive to 256–271 peptide of type II collagen (CII). In this report, we tried to regulate the CII reactivity of T cells from RA patients with HLA-DRB1*0101 by altered peptide ligand (APL), which is a single amino acid substitution of the T-cell epitope on CII 256–271 peptide. Antagonistic activity of 21 APLs was assessed using three different T-cell lines. Results showed that 262 (G→A) APL of CII 256–271 exhibited antagonistic activity in all T-cell lines and it was suggested that the application of CII APL might be a new therapeutic strategy in the regulation of RA.

Key words Altered peptide ligand (APL) · Antagonist · Rheumatoid arthritis (RA) · T cells · Type II collagen

Introduction

T-cell activation depends on the ability of the T-cell receptor (TCR) to recognize 8–20 amino acid peptides that are bound to major histocompatibility complex (MHC) molecules. The way TCR recognizes peptide is flexible. If the amino acid residue of peptide ligands for TCR is substituted for a different amino acid and can still bind to MHC molecules (altered peptide ligands; APLs), these APLs could regulate the activation of T cells. Several studies have shown that APL had a potential to induce differential cytokine secretion, anergy, and antagonism of the response to the wild-type antigens.[1–3] Therefore, it is possible to use APL as a therapeutic agent against T-cell-mediated diseases such as autoimmune diseases and allergic disorders.

Rheumatoid arthritis (RA) is generally considered to be a T-cell-mediated autoimmune disease. Type II collagen (CII), a molecule abundant in articular cartilage, is an attractive candidate as a target antigen (autoantigen) responsible for pathogenicity of RA. We previously reported that peripheral blood mononuclear cells (PBMCs) from RA patients with HLA-DRB1*0101 haplotype, which is one of the major alleles in Japanese RA patients, reacted to CII 256–271 peptide and this CII fragment was suggested to be a major T-cell epitope in RA patients with this haplotype.[4] In the present study, we established three different CII256–271-specific T-cell lines (E01, H01, H07) from two HLA-DRB1*0101-positive RA patients and tried to regulate CII reactive T cells by inducing TCR antagonism using 21 different APLs.

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Patients and methods

Patients

Two RA patients, who met the 1987 classification criteria of the American College of Rheumatology,[5] were selected after confirmation that their PBMCs included CII 256–271 peptide-reactive T cells. Their HLA-DRB1 haplotypes were DRB1*0101/*0405 and DRB1*0101/*0901. They agreed to participate in the present study and written informed consent was obtained from these subjects before collection of blood samples. The study design was approved by the Ethical Committee of the University of Tsukuba.

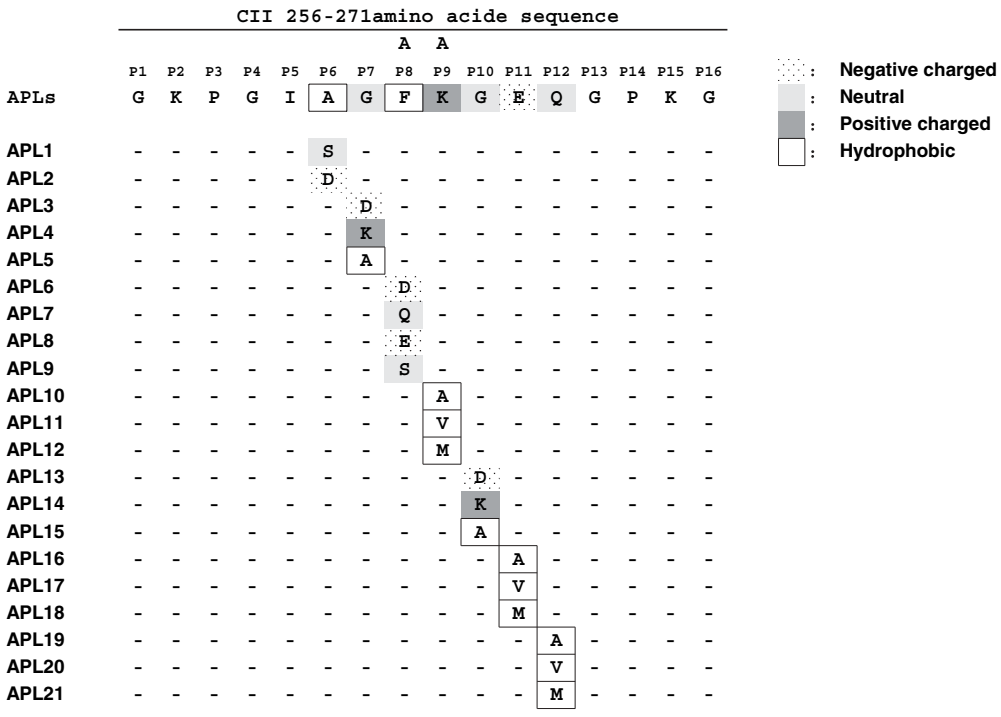

Fig. 1. Design for altered peptide ligands (*APL*). Two anchor positions on CII 256–271 peptide, CII 263 (F) and CII 264 (K), were reported and a single amino acid around these residues was substituted. Twenty-one analog peptides were designed. *A* indicates an anchor residue, which was a binding site to HLA molecule. The *dash* indicates the identical amino acid residue to that of CII 256–271 peptide

Peptides

It was reported that CII 263 (F) and CII 264 (K) were the dominant residues at the binding to DR1 molecule,[6] and therefore, the TCR contact site was considered to be around these residues. The amino acid sequence of APLs was designed so that a single amino acid of the TCR contact site, between CII 261 and CII 267, was changed to an amino acid that had a different charge and was of similar molecular size. Twenty-one APLs were set in this examination, and they were synthesized by Qiagen (Tokyo, Japan) including CII 256–271 wild-type peptide. Purity of each peptide was higher than 90%. The sequences of the peptides are shown in Fig. 1.

Generation of antigen-specific T-cell lines

Peripheral blood mononuclear cells from two RA patients were isolated using Ficoll-Paque (Pharmacia Biotechnology, Piscataway, NJ, USA). 2×10^6 PBMCs were suspended in RPMI-1640 containing 2 mM L-glutamine (Gibco BRL, Grand Island, NY, USA), 100 IU/ml penicillin/streptomycin (Gibco BRL), and 10% autologous serum, and cultured at 37°C in 5% CO_2 with 20 μg/ml of CII 256–271 peptide and interleukin (IL)-2 (30 U/ml, Immunace 35; Shionogi, Osaka, Japan). Cells were restimulated with the CII peptide and limiting dilution was carried out at 10, 50, or 100 cells/well in the presence of 2×10^4 feeder cells, which was the autologous B-cell line infected Epstein–Barr virus and irradiated (100 Gy), and pulsed with CII peptide. Cells were restimulated at 7-day intervals, and 6 lines (E01–E06) and 10 lines (H01–H10) were established from each patient. These T-cell lines were examined for antigen specificity by proliferative response to several concentration of CII 256–271 peptide. Restriction of the DRB1 molecule was confirmed using anti-DP, DR, and DQ antibody and L-cell transfectant expressing HLA-DR 1.[3] From the results, 1 line (E01) and 2 lines (H01 and H07) were established from each individual as DRB1-restricted T-cell lines.

Measurement of TCR antagonism by APLs

Whether APL could function as an antagonist for TCR was determined as previously reported.[1] Briefly, feeder cells were pulsed with a suboptimal concentration (5 μM) of CII 256–271 peptide for 2 h at 37°C under 5% CO_2, washed twice, and irradiated. Feeder cells prepulsed with the CII peptide were incubated with each APL (100 μM) for 12 h, and thereafter, antigen-specific T cells (1×10^5) were added. After incubation for 72 h, cell proliferative response was estimated. Antagonistic activity of APL was expressed as percentage of inhibition of the CII 256–271 peptide response. It was judged as positive when the percentage of inhibition was more than 80%.

Evaluation of cell proliferative response

Cell proliferative response was measured using a bromodeoxyuridine enzyme-linked immunosorbent assay (ELISA) system (Cell Proliferation ELISA kit; Roche Diagnostics, Mannheim, Germany).

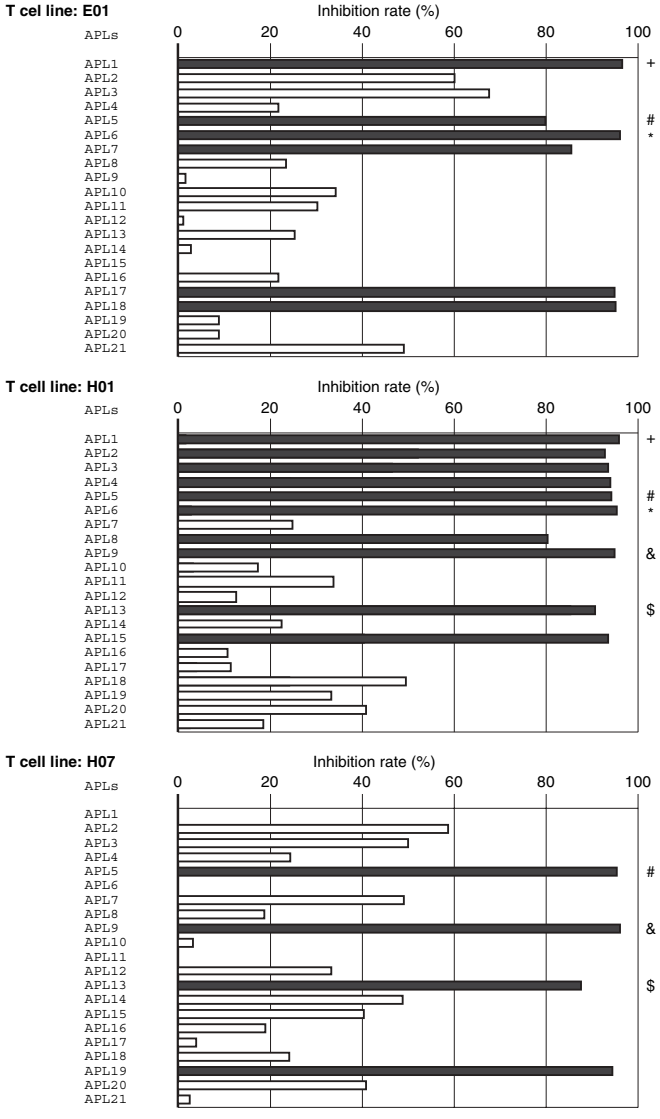

Fig. 2. Antagonistic activity of altered peptide ligands (*APL*) to T-cell lines. Three T-cell lines were established from two rheumatoid arthritis patients with DRB1*0101 and antagonistic activity of each APL was evaluated. The antagonistic activity was expressed as percentage of inhibition of the CII 256–271 peptide response. It was judged as positive when the percentage of inhibition was more than 80%. The *symbols* *, +, &, and $ designate APL inducing antagonistic activity in two T-cell lines; # designates APL inducing antagonistic activity in three T-cell lines

Results and discussion

The results demonstrated that seven APLs suppressed CII-specific T-cell response in E01 T-cell line, 10 APLs in H01, and four APLs in H07. Especially, APL5 (CII 262; G→A) inhibited the CII response in all three T-cell lines. Four other analog peptides, APL1 (CII 261; A→S), APL6 (CII 263; F→D), APL9 (CII263; F→S), and APL13 (CII 265; G→D), decreased T-cell proliferation in two T-cell lines. Although CII 263 (F) was considered an anchor residue, which is a binding site to DR1 molecule, some APLs induced T-cell suppression by antagonism when the residue was substituted. This might imply that there was another anchor residue on the peptide. Actually, CII 260 (I) was predicted as an anchor residue using the systems of prediction of MHC binding peptide (MHC Pred: http://www.jenner.ac.uk/MHCPred, RANKPEP: http://www.mifoundation.org/Tools/rankpep). CII 263 (F) might compose a TCR contact site when the peptide is bound to HLA molecule at CII 260 (I) or CII 264 (K).

To date, there have been some trials to change the T-cell response to CII. Fridkis-Hareli et al. reported that analog peptides based on CII 261–273 could block the binding of CII 261–273 peptide to DR1 molecule and suppress T-cell response to CII 261–273 peptide.[7] Myers et al. showed that CII 256–276 peptide substituted CII 263 (F→N) and CII 266 (E→D) induced lower IFNγ production and higher IL-4 and IL-10 production from splenocytes of DR1 transgenic mice, and collagen-induced arthritis was suppressed using this peptide.[8] On the other hand, we used antagonistic activity to change the T-cell response. This strategy is characterized by controlling the activation of antigen-specific T cells directly. Since RA patients do not necessarily have CII-reactive T cells, the antigen-specific therapy targeted to several autoantigens is expected to be an efficient method to control RA. This examination was probably the first trial using T cells of RA patients.

In conclusion, we observed evidence that at least four APLs of CII (256–271) were able to suppress the CII-specific T-cell proliferation. Further trials using more T-cell lines established from RA patients are required; however, these findings should shed light on a new therapy for RA in the antigen-specific fashion.

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