Therapeutic effect of an altered peptide ligand derived from heat-shock protein 60 by suppressing of inflammatory cytokines secretion in two animal models of rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by persistent inflammatory synovitis leading to various degrees of cartilage destruction, bone erosion, and ultimately joint deformity and loss of joint function. Although the etiology of RA is not totally understood, many studies have shown that T lymphocytes, macrophages, and proliferating synovial cells play a major role in the pathogenesis of this disease (1, 2). In particular, inflammatory cytokines produced by these cells appear to be critically involved in the initiation and perpetuation of RA. Therefore, regulation of inflammatory cytokines levels in patients with RA may also be a useful treatment for this disease (3).

The heat-shock protein 60 (HSP60), a protein belonging to the HSP family, is an autoantigen in RA. This protein can be used as an inducer of RA in animal models, but immunization with conserved HSP60 epitopes increases resistance to arthritis induction in rats (4). In the spontaneously occurring form of ankylosing spondylitis, JIA T cells reactive to self-HSP60 at the onset of disease are associated with disease remission having a regulatory phenotype (5).

In the present study, a novel T cell epitope was predicted by bioinformatics tools located in the N terminal region (amino acids 55–75) of human HSP60. This epitope, named E18-12, was used to design three APLs: E18-12, APL1 and APL2 induce the activation of CD4+ T cells, but only APL2 increases the IL-10 levels and suppresses the IL-17 secretion in PBMCs from RA patients. Moreover, APL2 inhibits efficiently the course of arthritis in two animal models for RA. Therapeutic effect of this peptide was similar to MTX, the standard treatment for RA. These results suggest that APL2 is a potential therapeutic treatment for controlling RA.

Evaluation of IL-17, TNF-α and IL-10 levels induced by APLs in PBMC from RA patients

Amino acid sequence of the wild type peptide and the APLs

Wild type (E18-12): MGPKGRTVIIEQSWGSPKVTK

APL1: MGPKGRTVIIISMGPSVT

APL2: MGPKGRTVIIEQS

APL3: MGPK

Effect of E18-12 and APLs on cell cycle progression of CD4+ T cells from RA patients

Collagen induced arthritis (CIA)

Mice were clinically examined 3 times/ week. Arthritis severity was graded as follows:

- 0, no disease
- 1, one finger inflamed and swollen
- 2, more than one finger, but not entire paw, inflamed and swollen or mild swelling of entire paw
- 3, entire paw inflamed and swollen

The severity of arthritis in each paw was determined according to an established scoring system as follows:

- 0, no disease
- 1, slight swelling of the ankle or digit without redness and inflammation of at least one finger, independently of the number of affected fingers
- 2, moderate redness and swelling of the ankle and digit
- 3, severe redness and swelling of the entire paw
- 4, maximum swelling and disability of the paw involving multiple joints

Histopathologic analysis of AA rats and CIA mice

Therapeutic evaluation of APL2 in two animal model

Treatment with APL2 caused significant reduction of arthritis in CIA and AA

Concluding remarks

- APL2 increases the IL-10 levels and suppresses IL-17 secretion in PBMC from RA patients
- APL2 efficiently inhibits the progression of AA and CIA with a significant reduction of the clinical sign and histopathological damages
- APL2 reduces inflammatory response (TNF-α/IL-17) in AA rats and CIA mice, supporting the potential use of APL2 as a therapeutic agent in RA patients

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