# 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

CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY
Havana, Cuba

Lorenzo N[1], Torres AM[2], Henández MV[2], Gil R[2], Prada DM[2], Gómez JA[2], Garay H[1], Reyes O[1], Altruda F[3], Silengo L[3], Padrón GR[1] and Domínguez MC[1].

(1) Center for Genetic Engineering and Biotechnology, P.O. Box 6162, Havana 10600; (2) Institute of Rheumatology, Havana 10600, Cuba
(3) Molecular Biotechnology Center, Turin, Italy

email: mcarmen.dominguez@cigb.edu.cu

## 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 cytokine 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 inductor of RA in animal models, but immunization with conserved HSP60 epitopes increases resistance to arthritis induction in rats (4). In the spontaneous remitting form of oligoarticular JIA, T cells reactive to self HSP60 at the onset of disease are associated with disease remission having a regulatory phenotype (5). Thus, HSP60 self-reactive T cells may play a role in modulating RA, JIA and AA, underscoring their potential as candidate antigens to modulate the immune response in chronic arthritis.

Induction of peripheral tolerance by using epitopes derived from autoantigens involved in the autoimmune disease pathogenesis constitutes a novel therapeutic approach for treatment of these diseases. These epitopes can be modified in order to modulate their immunological properties. These modified peptides are named APL; which are similar to immunogenic peptides but with one or several substitutions in the essential contact positions with the TCR or the MHC, interfering the cascade of necessary events for the complete activation of T cells (6). Some authors have suggested that APLs can affect T cell differentiation and therefore the TH1/TH2 balance modifying disease outcome (7,8).

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 suppress 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.

## Amino acid sequence of the wild type peptide and the APLs

Wild type (E18-12): MGPKGRTVIIEQSWGSPKVTK

APL1: MGPKGRTVII**L**QSWGSPKVTK

APL2: MGPKGRTVIIEQS**L**GSPKVTK

APL3: MGPK**L**RTVIILQSWGSPKVTK

The amino acid modified in each APL has been represented in bold.

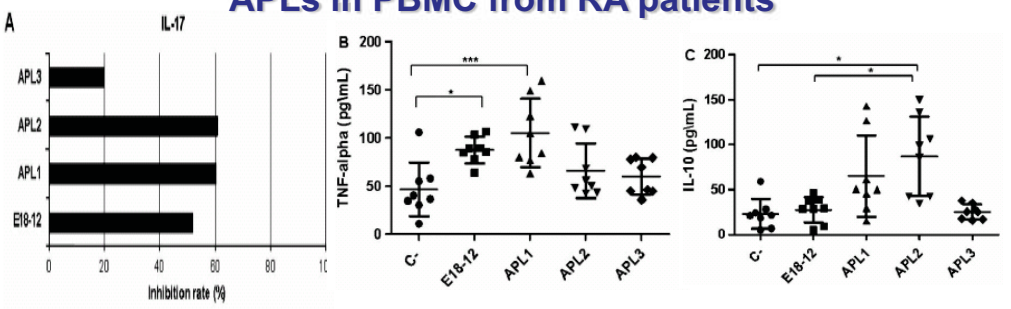
## Effect of E18-12 and APLs on cell cycle progression of CD4+ T cells from RA patients

| Stimulation | % Cells in G0/G1 | % Cells in S + /G2/M | % Cells in SubG1 |
|---|---|---|---|
| Unstimulated | 44.68 | 17.41 | 32.43 |
| E18-12 | 47.35 | 24.32 | 22.87 |
| APL-1 | 52.56 | 25.11 | 17.96 |
| APL-2 | 47.45 | 25.30 | 21.71 |
| APL-3 | 44.21 | 16.98 | 29.40 |
| CD3/CD28 | 61.27 | 29.36 | 4.54 |

Representative results obtained by PI staining of CD4 + T cells from a patient are shown.

**E18-12, APL1 and APL2 constitute a survival stimulus for CD4+ T cells inducing exit from G0/G1 and entry into S+G2/M phase of the cell cycle.**

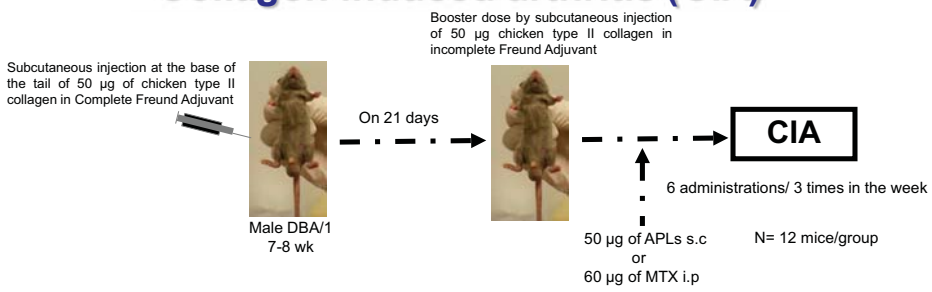
## Evaluation of IL-17, TNF-α and IL-10 levels induced by APLs in PBMC from RA patients




Inhibition rate of IL-17 is expressed as percentage inhibition against the PBMC without peptide stimulation (A). Concentrations of TNF-a (B) and IL-10 (C) are expressed as mean±deviation and were analyzed using the ANOVA and Tukey post-test (*, P<0.05, *** P<0.001).

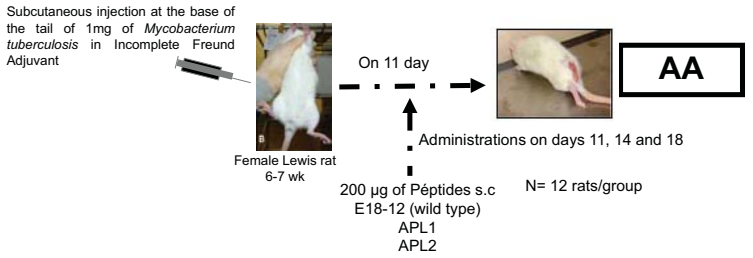
**APL-2 increases IL-10 levels and suppresses IL-17 secretion in PBMC from RA patients**

## Collagen induced arthritis (CIA)



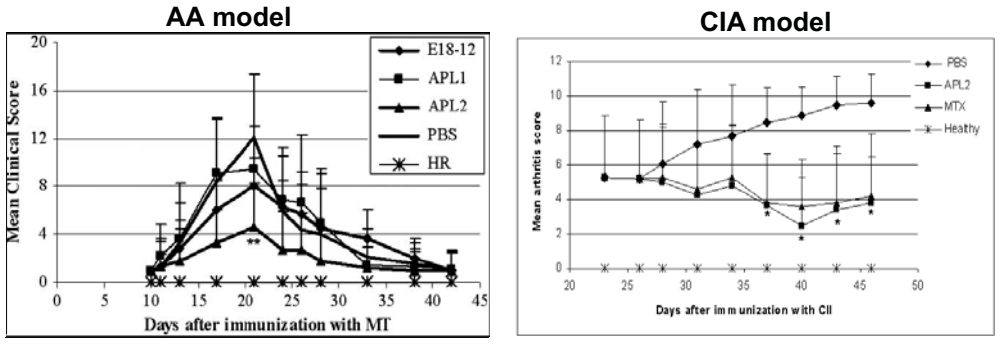

Mice were clinically examined 3 times/ week. Arthritis severity was graded as follows:
0, normal paw
1, one finger inflamed and swollen
2, more than one finger, but not entire paw, inflamed and swollen or mild swelling of entire paw
3, entire paw inflamed and swollen
4, very inflamed and swollen paw or ankylosed paw. If the paw is ankylosed, the mouse cannot grip the wire top of the cage
The score of each animal was the sum of scores for all four paws.

## Adjuvant induced arthritis (AA)




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 doll, or visible redness and inflammation of at least one finger, independently of the number of affected fingers
2, moderate redness and swelling of the ankle and the doll
3, severe redness and swelling of the whole paw including the fingers
4, maximum swelling and deformity of the paw involving multiple joints
Therefore, each rat can receive a maximum score of 16 points.

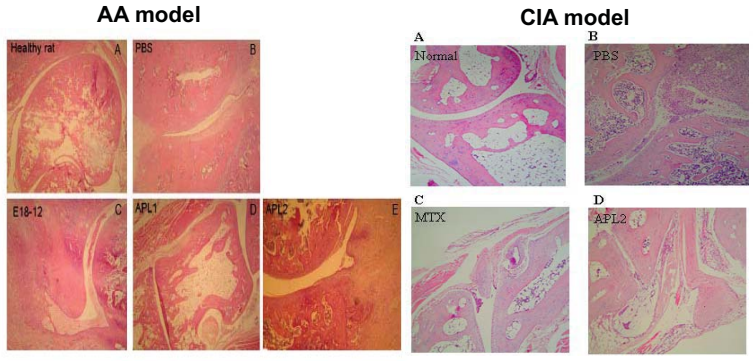
## Therapeutic evaluation of APL2 in two animal model




Data were analyzed using the ANOVA and Tukey post-test (*P<0.05; **P < 0.01). HR: healthy rat.

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

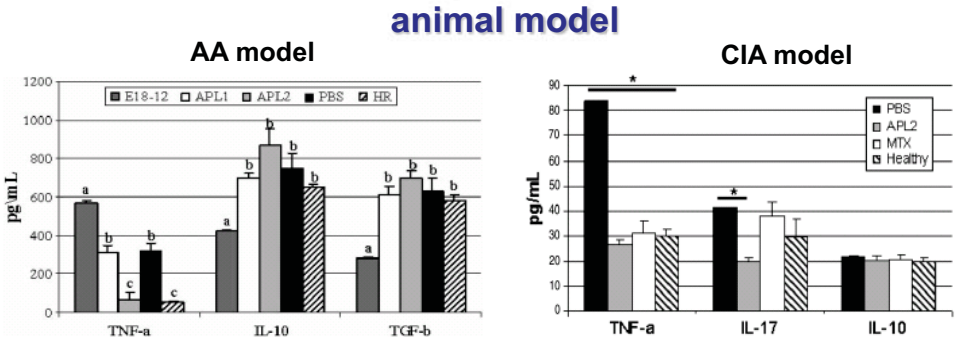
## Histopathologic analysis of AA rats and CIA mice




**Representative Hematoxilin and Eosin stained joint tissue sections from AA rats and CIA mice**
**Normal**: joint with a conserved synovial space
**PBS**: control mice with pannus infiltration, diminished synovial space and cartilage damage
**E18-12 and APL1**: massive cellular infiltration and moderate synovitis;
**MTX**: mild synovitis with hyperplastic membrane, no inflammatory reaction
**APL2**: joint with a conserved synovial space.

**Treatment with APL2 significantly prevented of histological damage in ankle joints from AA rats and CIA mice.**

## Effect of APL2 on cytokine production in two animal model




The results were analyzed using Kruskal-Wallis and Dunn post-test. Different letters indicate statistically significant differences and *P<0.05 .

**Treatment with APL2 led to significant reduction of TNF-α and IL-17 levels**

## Concluding remarks

- APL2 increases the IL-10 levels and suppress 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 damges

- 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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