Signal Transduction Events in Health and Disease

Abstract

Overproduction of nitric oxide causes oxidative stress in joint tissues. Under this stress, synovial cells are more prone to pro-inflammatory cytokine stimulation, which could result in the development of Rheumatoid Arthritis. This laboratory has discovered that linear shared epitope peptides can induce the production of nitric oxide in cells. They have begun testing different concentrations of a cyclic peptides (derived from the shared epitope sequence) in order to check if cyclic peptides maintain the same ability to trigger nitric oxide signaling as the linear counterpart; cyclic peptides have longer half-lives than linear peptides. For detecting nitric oxide response in cells, DAF-2 DA dye is loaded into M1 cells at 37^0C for 1 hour; then cells are treated with different concentrations of cyclic peptides. Linear peptides are used as positive control, and no peptide treatment as the negative control. Nitric oxide levels are continually monitored by a fluorescence micro-plate reader during an eight hour time period. The results obtained from this experiment will be used to design a cyclic peptide to stimulate the signaling cascade that leads to the increase of nitric oxide in cells. Eventually, these data will help us to further understand the genetic causes of Rheumatoid Arthritis.

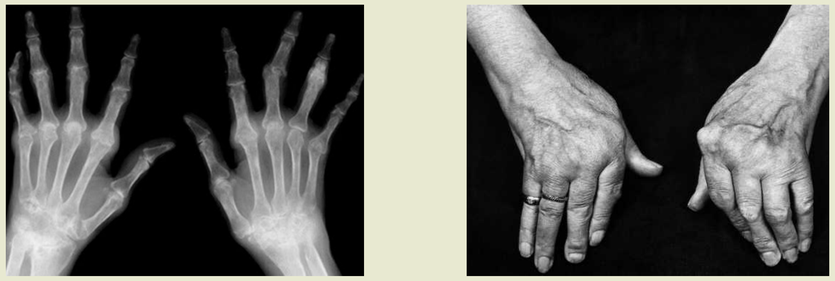
Introduction

Shared epitope positive individuals are more prone to rheumatoid arthritis, and other autoimmune diseases. The linear peptide 65-79*0401 has been shown to increase nitric oxide production in cells. High levels of nitric oxide in cells cause cell death, and in rheumatoid arthritis patients can cause extreme discomfort due to the swelling of joints. The aim of this project is to compare the relative NO production rate between the linear, and cyclic peptide. The results of this experiment will be used to further understand the signaling cascade that occurs in shared epitope positive individuals that leads to the development of rheumatoid arthritis.

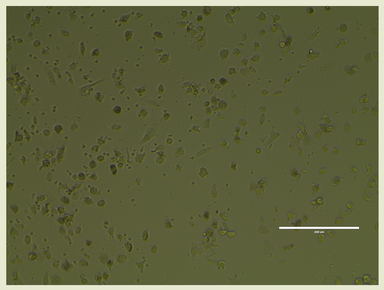
Methods

NO Assay:
3×10^4 human fibroblasts were loaded in each well of 96-well plates one day before experiment in order to allow the cells to attach to the surface. The supernant was removed, and cells were washed with Phenol-red free DMEM buffer. Cells were first loaded with 20 µM of the fluorescent NO probe 4,5-diaminofluorescein diacetate (DAF-2DA), and then incubated in the dark at 37°C for 1 hour. After dye loading the supernant was removed, and the cells were once again washed. Each well in the 96- well plate received 50 µl of Phenol-red free medium, and 50 µl of either more medium to act as control wells, the linear peptide, or the cyclic peptide in varying concentrations (.1,1, 10,100,1000 µM). NO signal was recorded by using a Fusion αHT system at an excitation wavelength of 488 nm and emission wavelength of 515 nm.

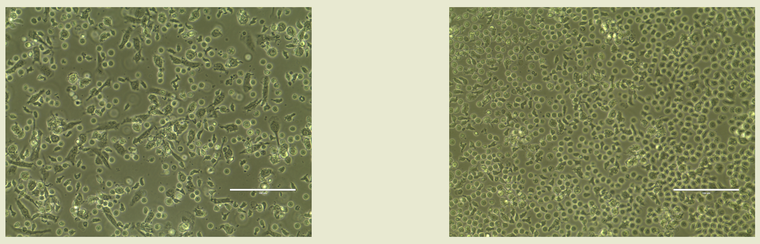
ROS Assay:
ROS production was quantified similarly, with the exception that cells were loaded with 10µM CM-H2DCFDA for 30min.



Individuals suffering from rheumatoid arthritis experience pain, inflammation, and deformities of the joints due to degradation of the bone.



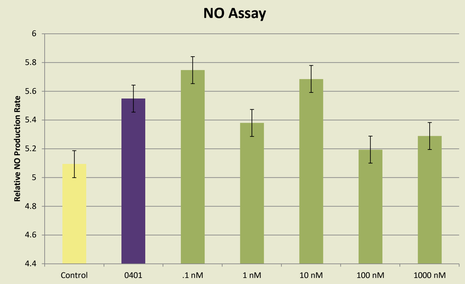
Immature Human Dendritic Cells

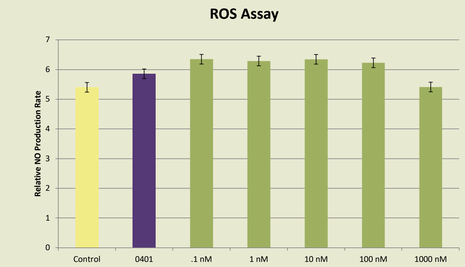


Human PBMC depleted of CD4+ and highly adherent cells after 3 days of culture. The picture on the left is of cells treated with 50µg/ml DRB1-0401 on day 0. The picture on the right shows cells treated with 50µg/ml DRB1-0402 on day 0. Current research is continuing with Luz P. Blanco, PhD. in order to fully asses the actions of DRB1-0401, and DRB1-0402 in the signaling cascade that occurs in shared epitope positive individuals.

Results

Analysis of the resulting NO, and ROS Assay showed that cells treated with 57mM 65-79*0401, and the cyclic peptide had higher relative NO production rates compared to the control cells. The highest NO production rates were found at concentrations of .1 nM and 10nM. The cyclic peptide in concentrations of .1 and 10 nM induced greater NO production in the M1 cells than the linear57 mM 65-79*0401 peptide. While cells treated with concentrations of 1, 100, and 1000 nM had relative NO production rates lower than those of cells treated with 57 mM 65-79*0401.





Conclusion

The data collected from NO and ROS assays supports our hypothesis that peptide 65-79*0401 induces nitric oxide production in human fibroblasts cells. The data also shows that the cyclic peptide is fully capable of producing relative NO production in rates exceeding those of the linear peptide 65-79*0401. 10 nM of the cyclic peptide has a more potent ability to stimulate NO signaling than the 57 mM linear peptide, which indicates that the cyclic peptide is 5000 folds more potent than the cyclic peptide. It should be added that the cyclic peptide has a more stale structure, and longer half-life. The knowledge gained from these experiments is currently being used in studies with human dendritic cells to fully understand the signaling cascade that occurs in shared epitope positive individuals.