

## Macrophage-Targeted Alginate Nanoparticles as a Non-Condensing Murine IL-10 Gene Delivery System for the Treatment of Experimental Arthritis

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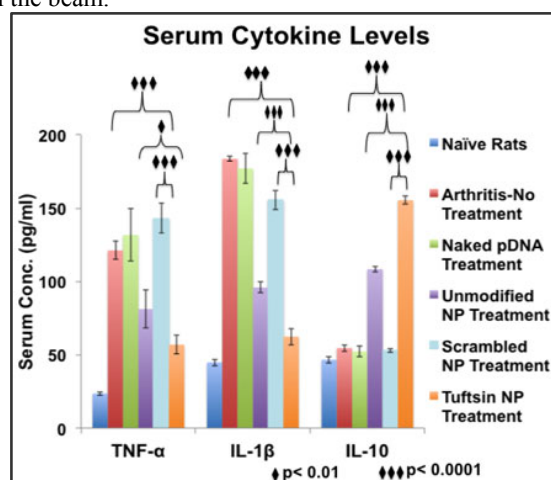
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**Statement of Purpose:** Rheumatoid arthritis (RA) is an auto-immune systemic disease that is associated with stiffness, pain, and swelling of several joints. The severity of RA has been directly linked to the number of macrophages present in the arthritic synovium<sup>1-2</sup>. Additionally, macrophages in the synovial lining are characterized by strong HLA-DR expression and found in an activated state. There is strong evidence indicating that synovial macrophages are capable of secreting pro-inflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ), growth factors (GM-CSF), chemokines and chemo-attractants (IL-8, macrophage inflammatory protein (MIP)-1, and monocyte chemo-attractant protein (MCP)-1) that contribute towards inflammation and joint destruction<sup>3</sup>. Thus, targeting macrophages from a therapeutic perspective is a rational approach given their presence in abundance and central role in cytokine mediated inflammation associated with RA. The therapeutic goal of this project is to develop a safe and effective non-viral gene delivery system for IL-10-expressing plasmid DNA and transfection for anti-inflammatory therapy for the treatment of experimental arthritis in male Lewis rats.

**Methods:** External gelation method was used to form calcium alginate nanoparticles encapsulating murine IL-10 plasmid DNA. Furthermore, surface of the particles was decorated with tuftsin peptide (TKPR) to target receptors on the surface of macrophages. Male Lewis rats (150-170g) were inoculated, intra-dermally at the base of tail, with 0.05ml of 10mg/ml of *heat-killed mycobacterium butyricum* suspended in incomplete Freund's adjuvant to induce arthritis. The control groups included naive rats (no arthritis), arthritic rats (no treatment), and animals treated with naked plasmid DNA alone. A total of five animals per treatment group were utilized for this study and were randomly distributed into different groups once the first signs of inflammation were observed. Plasmid DNA dose of 100 $\mu$ g was administered intra-peritoneally at day 12 post-adjuvant administration in each rat. All the animals were euthanized as per Northeastern IACUC guidelines at day 18 and paw tissues were excised for analysis. Increase in the paw width/depth was recorded using calipers to quantify edema before and after the treatment. Rats were subjected to beam walking test to analyze animal mobility post-treatment. Histology, Serum cytokine ELISA, and qPCR analysis were performed to evaluate the features of arthritis and panel of pro- and anti-inflammatory cytokines including TNF- $\alpha$ , IL-1 $\beta$ , and IL-10, respectively.

**Results:** Optimized peptide-modified nanoparticles formed were spherical in shape with an average size of 286.6 $\pm$ 1.36 nm and surface charge of 19  $\pm$ 0.4 mV. Peptide density on nanoparticle surface was calculated to

be 116.7  $\pm$  1.01  $\mu$ mol/cm<sup>2</sup> or 702.6  $\pm$  6.08 molecules of peptide/cm<sup>2</sup>. Cytokine ELISA studies (**Figure below**) were performed to quantify the serum levels of pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  also revealed significantly lower levels of these cytokines in tuftsin-peptide modified and unmodified calcium alginate nanoparticles as compared to arthritic rats with no treatment and scrambled-modified treatment groups ( $p < 0.0001$ ). Alternatively, the levels of anti-inflammatory IL-10 cytokine were reported to be ~150pg/ml (TNP) and 100pg/ml (UNP) and were approximately 3 fold and 2 fold higher than levels observed in other treatment groups, respectively. Lastly, beam-walking tests also showed that animals treated with tuftsin-modified and unmodified nanoparticles were able to traverse the entire length of beam (40 cm) in about 3-5 seconds throughout the course of the study and these readings were in comparison to the normal rats. However, the animals in other treatment groups struggled to maintain their balance on the beam.



**Conclusions:** In summary, these studies provide evidence on the ability of tuftsin-modified alginate based nanoparticles to suppress the inflammation in rats with adjuvant-induced arthritis. We are currently pursuing therapeutic studies extending up-to 28 days post adjuvant administration to determine the long-lasting transgene expression upon treatment with targeted nanoparticles.

### References:

1. (Imboden, J. B. Annual Rev Pathol.2009;4:417-434).
2. (Weyand, C. M., Goronzy, J. J. J Mol Med.1997;75: 772-785).
3. (Kinne, R. W., Brauer, R., Stuhlmuller, B., Palombo-Kinne, E., Burmester, G. R. Arthritis Res. 2000;2:189-202).