A Microparticle Strategy to Induce Tolerogenic Dendritic Cells for Treatment of Rheumatoid Arthritis

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Statement of Purpose: Rheumatoid Arthritis (RA) is a debilitating autoimmune disease affecting ~1% of the population. It is characterized by inflammation of the joint synovium leading to bone and cartilage destruction. There is currently no cure and treatment options require lifelong management of immunosuppressive drugs. However, these drugs are accompanied by many adverse effects such as increased risk of infection and acute and chronic toxicities. The ongoing COVID-19 pandemic has underscored a critical need for new therapies that do not immunocompromise the patient. In this study, we have rationally designed a novel poly (lactic-co-glycolic) acid (PLGA)-based microparticle system, termed REGvac, that upon subcutaneous delivery facilitates generation of tolerogenic dendritic cells (tDC) to mediate antigen-specific tolerance towards rheumatic antigens. This is achieved through delivery of REGvac which is comprised of (1) a non-phagocytosable 30 um PLGA microparticle encapsulating transforming growth factor β1 (TGF-β1) and granulocyte-macrophage colony-stimulating factor (GM-CSF) to provide sustained release of a DC chemoattractant alongside an immunosuppressive cytokine at the site of injection, and (2) a phagocytosable 1 um PLGA microparticle encapsulating vitamin D3 (a potent tolerogenic factor) and the rheumatic antigens type II collagen (col II) and citrullinated fibrinogen to facilitate cytosolic delivery to the DC upon phagocytosis.

Methods: Fabrication of REGvac

Briefly, small PLGA microparticles encapsulating type II collagen (Sigma), citrullinated fibrinogen (Cayman Chemical), and vitamin D3 (EMD Millipore) were fabricated using a double emulsion solvent evaporation method, and large PLGA microparticles encapsulating recombinant human transforming growth factor-β1 (TGF-β1) (Peprotech) and recombinant mouse granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fisher Sci) were fabricated using an emulsion solvent evaporation method.

CIA/FIA induction in DBA/1J mice and REGvac treatment

Rheumatoid Arthritis was induced via the collagen-induced and citrullinated fibrinogen-induced arthritis model (CIA/FIA). DBA1/J mice (JAX) were immunized with type II collagen (Sigma) and citrullinated fibrinogen (Cayman Chemical) in Complete Freund's Adjuvant (Sigma). Onset of severe RA initiated therapy with REGvac (2.5 mg subcutaneous) or methotrexate (2.5 mg/kg intraperitoneally) in the abdominal region.

Clinical scoring of CIA/FIA mice

Each paw was assessed for erythema, redness, and swelling and assigned a score from 0, 1, 2, or 3, with 0 being none, 1 being mild, 2 being moderate, and 3 being severe. The scores in each paw were then summed for a maximum score of 12.

Results: REGvac induces RA remission in the CIA/FIA mouse comparable to methotrexate. To test whether or not REGvac is able to induce remission in arthritis that has already established and progressed to severe levels we initiated treatment with REGvac or methotrexate after a clinical score of 6 was achieved in the mice. We found that methotrexate treatment resulted in rapid alleviation of symptoms in the paw at d10 while REGvac treatment began to elicit therapeutic effects at week 5 and achieved methotrexate levels of remission by week 7 and 8 (**Fig 1**).

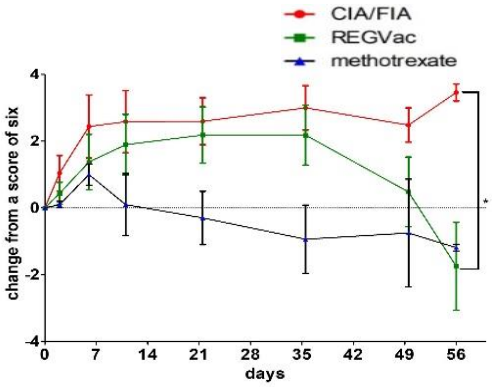

Figure 1. REGvac induces RA disease remission. Time-course of RA clinical scoring of REGvac versus methotrexate. Y-axis depicts the change of the clinical score from 6 once treatment has started, with remission being a negative change. X-axis is days since treatment has started. Day 0 denotes RA clinical score of 6 and initiation of treatment.

REGvac induces regulatory T cell expansion specific for type II collagen in the popliteal lymph node.

8 weeks post-treatment, inguinal LNs, popliteal LNs, and spleen were harvested for flow cytometric analysis of regulatory T (Treg) cells. Flow cytometric analysis revealed a 2-fold increase in %CD25+FOXP3+col II tetramer+ Treg cells of CD4+ cells, in the REGvac-treated mice, compared to the methotrexate-treated mice (**Fig. 2**).

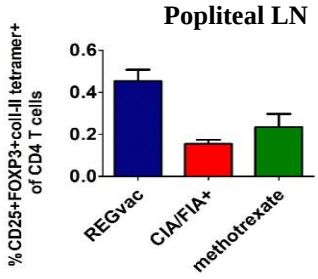

Figure 2. REGvac induces antigen-specific Treg expansion. Flow cytometry of %CD25+FOXP3+Col-II tetramer+ Treg cells of CD4 T cells in the popliteal lymph node.

Conclusion:

These results collectively demonstrate that REGvac treatment is able to induce antigen-specific Tregs that promote therapeutic effects and disease remission in RA comparable to the clinical standard methotrexate. This provides strong evidence for REGvac as a legitimate platform to induce antigen-specific tolerance, potentially allowing a tunable therapy for other antigen-driven autoimmune diseases.