BIOMIMETIC DELIVERY OF IL-33 FOR TREATMENT OF CHRONIC HEART REJECTION

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INTRODUCCION

More than 5 million people experience congestive heart failure in which the heart fails to pump sufficient blood to the body. It often requires a transplant to restore much of the heart’s functionality. All transplants, or allografts, throughout their lifespan are susceptible to chronic heart rejection. Chronic rejection, also known as coronary allograft vasculopathy, has been thought to be the long-term consequence of repetitive acute rejection events that gradually deteriorate the heart over time. Chronic rejection is usually characterized by accelerated hardening and restricting of blood vessels in the heart, a condition referred to as atherosclerosis. Advanced atherosclerosis is difficult to reverse and leads to eventual graft failure. Despite improvements to immunosuppressive drugs, chronic heart rejection continues to be the major cause of patient morbidity and mortality after transplantation and remains the end result of all heart transplant patients.

Allograft rejection can be attributed to the upregulation of various proinflammatory cytokines that activate dendritic cells to direct effector T cells to the graft for recognition and destruction. Such processes are characteristic of the T_{H1} response, a pathway typically responsible for fighting against infections. An immediate goal for addressing chronic rejection is to influence the T_{H1} pathway by modulating levels of inflammatory proteins. In previous research it has been discovered that a cytokine known as IL-33 can indeed achieve this. IL-33 has been found to influence dendritic cells to induce the T_{H2} pathway normally activated during parasitic infections, asthma, and other allergic responses. Previous research has shown that induction of this T_{H2} pathway can reduce the production of competing T_{H1} proteins, diminishing graft inflammation and granting cardioprotective effects to the host. Furthermore, IL-33 has been found to be a powerful mediator of a class of helper T cells called regulatory T cells (Tregs) that suppress immune responses and limit damage caused by antibodies and effector cells. Thus, a possible method to improve allograft acceptance is to promote the production of these Tregs and reduce the recruitment of T_{H1} proteins through administration of IL-33 to the patient. While theoretically promising, prior literature has shown that IL-33 can have various side effects including end organ damage and pulmonary toxicity.

OBJECTIVE

Given the significant adverse effects of IL-33, there exists a need to develop an alternative delivery system that can suppress the immune system locally and controllably, protecting the integrity of the patient’s immune defenses. Microparticles composed of poly(lactic-co-glycolic) acid (PLGA) can be used to encapsulate IL-33 and release the therapeutic compound at predictive, nontoxic levels, thus offering a reliable method for delivery. The purpose of this study is to fabricate microparticles containing IL-33 (herein referred to as IL-33MP) and to measure release of IL-33 from the PLGA microparticles over 50 days to determine the feasibility of release of this cytokine from the drug delivery system. Furthermore, IL-33-MP was cultured with naive T-cells, and populations of the resulting T_{H2} cells produced were counted to determine the efficacy of the encapsulated IL-33 in inducing a protective T_{H2} pathway in-vitro. It was hypothesized that IL-33MP could stably release the therapeutic payload to heart allografts to induce the protective T_{H2} pathway, reduce coronary vasculopathy, and improve graft survival in the long term.

METHODS

An initial study of the release characteristics of an IL-33-MP system was necessary to establish a reliable delivery method for this cytokine. 20 μm diameter microparticles were fabricated using the double emulsion/evaporation technique. 200 mg of poly(lactic-co-glycolic) acid was dissolved in dichloromethane to form an organic oil layer. A stock solution of IL-33 was dissolved in a saline solution and stirred vigorously in the PLGA suspension using a sonicator. A homogenizer (Silverson L4RT-A) was used to homogenize the mixture at 3000 rpm for 1 minute. Excess solvent was allowed to dry using a lyophilizer (Virtis Benchtop K freeze dryer), resulting in a white powder containing the microparticles. Particle morphology and size were determined using scanning electron microscope and a Coulter Counter, respectively.

Release of IL-33 from the microparticles was determined by dissolving particles in phosphate buffered saline (PBS) and placing the solution on an end-over-end rotator overnight. The solution supernatant was collected everyday for 50 days, and IL-33 content within the supernatant was measured using an enzyme-linked immunosorbent assay (ELISA) to create a release profile for the system. Successful release was indicated by any increases in levels of IL-33 in the PBS over time.

Once a feasible delivery system was in place, in-vitro functional experiments were performed to determine if the encapsulated IL-33 retained its biological effect compared to soluble, unencapsulated IL-33. In-vitro work was carried out using 20 μm particles and 5 μm diameter microparticles, fabricated to effect greater intracellular delivery into dendritic cell cytoplasm compared to the larger particles. Dendritic cells were isolated from bone marrow of mice limbs and treated with IL-33-MP for 6 days. On day 7, cells were cocultured with naïve T cells obtained from the spleens of mice and allowed to incubate overnight. An ELISA was performed on the culture media to determine levels of the T_{H2} cytokine IL-5 produced. Treatment controls included an untreated control, a negative control (LPS), and a positive control (soluble IL-33). Flow cytometry was carried out on culture plates to measure populations of T_{H2} cells generated from the naïve T cells.

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increase in IL-5 levels and TH2 cell populations after IL-33MP treatment were considered indicative of a TH2 response.

RESULTS
All release samples were performed in triplicates (n = 3), and IL-33 concentrations were averaged to calculate a mean release for each day. IL-33MP was found to have an acceptable release profile, releasing a total of 1.747±0.0022 ng IL-33 / mg microparticle over 50 days, with 1.680±0.001 ng IL-33 / mg microparticle releasing within the first 6 days. ELISA results from the DC/T-cell coculture indicated large levels of IL-5 cytokines produced in the culture by the large (~20 μm) microparticles. This was not statistically significant (p < 0.005) to the positive control, soluble IL-33. The small microparticles (~5 μm) caused production of IL-5 to a lesser extent compared to the positive control. The increases in IL-5 produced by the IL-33MP over the negative control LPS indicated successful induction of TH2 response.

Flow cytometry showed contradictory results. The percentage of TH2 cells in the large IL-33MP treated group was 2.88%. Similarly, the percentage of TH2 cells in the small IL-33MP treated group was 2.04%. These values were significantly lower than that of the untreated control, 29.0% TH2 cells, possibly indicating little to no elicited TH2 response from the IL-33MP or procedural errors that resulted in inconsistent data.

DISCUSSION
The experimental design of this project was generally successful, and the results supported the hypothesis to a limited degree. Gradual release of IL-33 from PLGA microparticles was evidence that the drug delivery system was capable of cytokine release as was predicted. Microparticle systems formulated prior to this study showed similar release behavior, and so levels of IL-33 released here were deemed sufficient for in-vitro application. The released IL-33 was found to be stable and functional as indicated through the DC/T cell cultures. The greater concentrations of IL-5 produced in groups treated with IL-33MP compared to the negative controls suggested that the payload continued to function as a potent TH2 inducer. No deviations in behavior were observed between the IL-33MP and the positive control, and so encapsulation in PLGA microparticles had no unfavorable effects on the overall efficacy of the system.

Given these findings, it was expected that production of IL-5 would induce differentiation of naïve T cells into a TH2 phenotype. However, the flow cytometry data was unconvincing in this regard. There were very small populations of TH2 cells in groups treated with IL-33MP. The untreated controls had much larger populations of TH2 cells, which were unexpected, as these cells received no signaling molecules to allow for differentiation into any phenotype. Thus, it was concluded that the inconsistent data was attributed to poor cell staining and unreliable reagents. Additional trials will be required in order to observe larger populations of TH2 cells in the IL-33MP groups analogous to the hypothesized behavior.

The delivery model tested here was ultimately limiting in its clinical relevance. The microparticles released much of the IL-33 over a short time duration. Many treatment regimens used clinically require extended administration over much longer time periods, and so prolonged release of IL-33 will be required. This may be achieved by fabricating reduced-porosity microparticles that offer slower release of the payload, and so this model will be adjusted in the future to obtain more long-term release profiles.

CONCLUSION
Alternative treatment modalities such as controlled release immunoregulatory systems have the potential to make a substantial impact in the field of transplantation by promoting long term graft survival and minimizing the dosing and duration of systemic immunosuppression. Presented here are initial in-vitro findings for a novel drug delivery system IL-33MP. Given the observable cytokine release and induction of a TH2 response, IL-33MP has the potential for translation into an in-vivo model and eventually to patient’s bedside as a new treatment strategy for chronic rejection.

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