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TITLE: Therapeutic Evaluation of Mesenchymal Stem Cells in Chronic Gut Inflammation

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The overall objective of this proposal is to evaluate the therapeutic efficacy of human, bone marrow-derived mesenchymal stem cells (hMSCs) in a mouse model of inflammatory bowel disease (IBD). As mentioned in my previous report, we encountered an unexpected problem with our mouse model of IBD following our relocation to TTUHSC. We have spent the last 2 years developing and characterizing a new model that has a much incidence of disease than what we observed following our relocation to TTUHSC. Unfortunately, this situation delayed the start of the studies outlined in Tasks 1 and 2. Nevertheless, we now have the studies outlined in Task 1 currently underway. We present new and exciting data demonstrating that the inflammation observed in our re-derived mouse model of IBD is characterized by the infiltration of much larger numbers of myeloid cells into the inflamed colon compared to our original model. In addition, we have made great progress in developing a more immunologically-relevant in vitro system to assess immuno-suppressive activity and mechanisms of suppression of hMSCs (Task 3). This antigen-specific, in vitro model more closely mimics the cellular and immunological interactions that occur in our in vivo mouse model of IBD. Surprisingly we find that hMSCs are much less effective at suppressing antigen-specific proliferation of mouse T cells compared to the immunosuppressive effects of mouse regulatory T cells or mouse MSCs. These data, together with our previous data demonstrating that hMSCs attenuate IBD in mice suggest that suppression of disease may not be due to suppression of T cell proliferation in vivo. We care currently exploring whether hMSCs suppress inflammatory cytokine generation by mouse T cells.
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1. **INTRODUCTION**

The inflammatory bowel diseases (IBD; Crohn’s disease; ulcerative colitis) are chronic inflammatory disorders of the small bowel and/or colon that affects approximately 1.5 million people in the US with a calculated annual cost for both medical expenses and work loss of almost $4 billion dollars. A recent study analyzing the Department of Veterans Affairs database from 1975-2006 reports that although rates of hospitalization for ulcerative colitis (UC) and Crohn’s disease (CD) have begun to stabilize over the past few years, there has been a disproportionate increase in rates of hospitalizations for nonwhite vs. white US military veterans for both UC and CD. Currently, there are only a handful of medical treatments available to treat these debilitating inflammatory disorders with only a few new therapies projected to be available in the near future. Thus, there is a clear need for the development of additional therapeutic agents to treat patients with IBD. A great deal of excitement has been generated from recent studies demonstrating that adoptive transfer of syngeneic, allogeneic or xenogeneic (human) MSCs suppress the inflammation and tissue injury observed in animal models of autoimmune encephalomyelitis, allograft rejection, arthritis and graft vs. host disease. Because MSCs can be grown and expanded in vitro and exert their immuno-regulatory activity across major histocompatibility complex barriers in vivo, we are in the unique position to evaluate the therapeutic efficacy of human MSCs in our mouse model IBD. The overall objective of this proposal is to evaluate the therapeutic efficacy of ex vivo-generated, bone marrow-derived human MSCs in a well-characterized mouse model of chronic colonic inflammation. **Hypothesis:** We propose that ex vivo-generated MSCs suppress chronic gut inflammation by homing to the mesenteric lymph nodes (MLNs) and/or colonic lamina propria where MSC-derived TGFβ suppresses/limits the generation of colitogenic T-cells and/or induces the formation of IL-10-producing Tregs. In order to test this hypothesis we propose the following three specific aims: a) Evaluate the ability of human MSCs to suppress the induction of chronic gut inflammation; b) Determine the therapeutic efficacy of human MSCs in reversing preexisting colitis; and c) Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis.

2. **KEYWORDS**

inflammatory bowel disease; mesenchymal stem cells; Tregs; IL-10, TGFβ; colitis; intestinal inflammation; immuno-suppression;

3. **ACCOMPLISHMENTS**

**Major Goals of the Project**

**Task 1.** Evaluate the ability of human MSCs to suppress the induction of chronic gut inflammation (months 1-12).

**Task 2.** Determine the therapeutic efficacy of human MSCs in attenuating preexisting colitis (months 12-24).

**Task 3.** Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis (months 24-36).
Accomplishments for the Current Reporting Period

Tasks 1 and 2. Evaluate the ability of human MSCs to suppress the induction of chronic gut inflammation

In order to assess the therapeutic efficacy of human MSCs in a mouse model of chronic gut inflammation we will use our well-characterized T cell transfer model of chronic colitis. We induce chronic disease by adoptive transfer of naïve (CD4⁺CD45RB<sup>high</sup>) T-cells obtained from healthy wild type donors into recombinase-activating gene-1 deficient (RAG-1<sup>−/−</sup>) mice. We have more than 15 years of experience with this mouse model in which we have routinely demonstrated that ~85% of the reconstituted mice develop moderate-to-severe colonic inflammation by 6-8 weeks post T cell transfer. Shortly after my relocation from LSU Health Sciences Center (LSUHSC) to my current position at Texas Tech Health Sciences Center (TTUHSC), we were surprised to find that only 30-40% of the RAG-1<sup>−/−</sup> mice housed in the TTUHSC animal facility developed chronic colitis at 8 weeks following T cell transfer compared to our historical incidence (at LSUHSC) of ~85%. We have spent the past 2 years attempting to determine the reason(s) for this significant reduction in disease incidence. After ruling out animal vendor, housing conditions (ventilator, micro-isolator cages) and T cell preparations/administration, we determined, as reported in the previous Progress report, that the reduced incidence appeared to be due to large and significant differences in the microbial composition of mice housed in the animal facilities at the 2 different institutions. Thus, we have spent most of last year attempting to enhance the incidence of disease for our in vivo studies outlined in Tasks 1 and 2. We found that if we colonize (via gavage) RAG-1<sup>−/−</sup> mice housed at TTUHSC with feces obtained from colitic mice generated at LSUHSC for 1 wk prior to T cell transfer, we can induce severe colitis in >90% of these mice following T cell transfer (referred to as LSUHSCc→TTUHSC mice)(please see previous Progress report). Studies performed during the current funding cycle revealed that serial transfer of feces obtained from colitic LSUHSCc→TTUHSC mice into RAG-1<sup>−/−</sup> mice housed at TTUHSC (called TTUHSCc→TTUHSC mice) produced moderate but not severe disease following T cell transfer that was localized more to the proximal rather than distal colon. We did observe significantly more disease in RAG-1<sup>−/−</sup> mice colonized with feces obtained from colitic TTUHSCc→TTUHSC mice than in mice colonized with feces obtained from healthy RAG-1<sup>−/−</sup> recipients housed at TTUHSC (Figure 1). Another major finding we made during the current funding cycle was that the colonic inflammation in TTUHSCc→TTUHSC mice contained significantly more myeloid cells when compared to mice that received healthy feces from animals housed at TTUHSC (Figure 2). We are currently exploring the reasons for
this interesting observation. While the initiation of studies outlined in Tasks 1 and 2 was delayed much longer than we anticipated due to the unexpected loss of disease phenotype, we felt that it was critical to invest a significant amount of time to either re-establish our mouse model of IBD or modify the original protocol to produce a more consistent model of chronic colitis before proceeding with our in vivo studies. In fact, the studies proposed in Tasks 1 and 2 are currently ongoing with the first results expected in mid-September 2015. We will forward these new data when they become available. Preliminary data in our original application demonstrated proof of concept that weekly injections (i.p.) of 5 million hMSCs for six weeks following T cell transfer significantly suppressed the development of chronic colitis in our mouse model of IBD. However, new clinical information has become available over the past year suggesting that the “dose” of MSCs we used in our preliminary studies (166 million/kg for a 30 gram mouse) is excessive and will never be used in the clinics to treat human IBD. Current clinical studies are using either “low dose” (2 million/kg) or “high dose” (8 million/kg) hMSCs to treat autoimmune and chronic inflammatory diseases (http://clinicaltrials.gov/show/NCT00294112). Furthermore, intraperitoneal administration of human MSCs will never be used in patients with IBD. In view of these clinical realities, our current, ongoing studies utilizes intravenous administration of clinically-relevant numbers of hMSCs to assess their therapeutic efficacy in our mouse model of IBD.

**Task 3. Define the immuno-regulatory mechanisms utilized by MSCs to attenuate chronic colitis**

To investigate the cellular and immunological interactions that occur between human MSCs and mouse immune cells in our mouse model of IBD, we have developed a novel xenogeneic in vitro system to quantify these interactions in a more controlled and immunologically-relevant environment. This is a very important, yet under-appreciated aspect of MSC therapeutic research in which human MSCs are administered to mice with acute or chronic inflammation. The vast majority of published studies that utilize hMSCs to treat mice or rats with chronic inflammation have used syngeneic in vitro assays to model the in vivo studies. That is, investigators have assessed the ability of human MSCs to suppress activation of human lymphocytes (e.g. T and B cells) in vitro. We believe this may be a critical shortcoming in interpreting results obtained from these studies because the more immunologically-relevant in vitro system would assess the immuno-modulatory effects of hMSCs on mouse immune cell function. As reported in our previous Progress Report, we demonstrated that hMSCs are capable of suppressing proliferation of mouse T cells activated with CD3 monoclonal antibody (mAb). While CD3 mAb-mediated proliferation of T cells in vitro has been used for many years to mimic lymphocyte activation in vivo, investigators agree that the mechanisms responsible for CD3 mAb-induced T cell activation/proliferation may be very different than those that occur...
during antigen-specific activation in vitro and in vivo. Over the past year, we have assessed a number of different in vitro systems to more closely recapitulate the cellular and immunological interactions that would occur in vivo between mouse immune cells and hMSCs in our mouse model of IBD. These studies have led to the development of a novel, antigen-specific and immunologically-relevant assay system that quantifies immuno-modulatory activity of human MSCs towards mouse immune cells. For this new assay, we activate mouse splenocytes obtained from OT2 transgenic (tg) mice with ovalbumin peptide (OVA) and quantify T cell proliferation in vitro. The T cell receptors (TCR) on CD4\(^+\) T cells in OT2 tg mice recognize only OVA presented by the major histocompatibility complex II (MHC II) expressed on mouse antigen presenting cells (APCs; dendritic cells, macrophages). Thus T cell activation/proliferation in this novel xenogeneic assay is antigen-specific. The utility of this new system can be seen in Figure 3 in which we observe a potent, dose-dependent suppression of T cell proliferation by regulatory T cells (Tregs) flow purified from the spleens of OT2 mice. This antigen-specific assay system will have great utility and be very important for us as we will be able to quantify the immunosuppressive activity of virtually any mouse or human MSC clone. Surprisingly, when we activated mouse OT2 splenocytes with OVA in the presence of increasing numbers of un-manipulated or irradiated hMSCs, we observe little or no suppression of T cell proliferation (Figures 4 and 5).
We confirmed that the lack of suppressive activity was not due an artifact with using \(^3\)H-thymidine incorporation to quantify cell proliferation as flow cytometric analyses revealed no significant reductions in CD4\(^+\) T cell proliferation (Figure 6).

The lack of suppression of T cell proliferation appeared to be specific for hMSCs as we demonstrated that mouse MSCs (also derived from bone marrow) were very effective in suppressing OVA-induced T cell proliferation (Figure 7). These data contrast with those presented in our previous Progress Report where we show dose-dependent suppression of CD3 mAb-mediated T cell proliferation by hMSCs. Taken together with our previous data demonstrating suppression of CD3 mAb-induced proliferation mouse T cells by hMSCs suggest that the signaling pathways involved in T cell proliferation induced by nonspecific crosslinking of the TCR (via CD3 mAb) may be very different than those induced in an antigen-specific manner. Indeed, these differences may be critical for defining the mechanisms by which hMSCs suppress IBD \textit{in vivo}.
Opportunities for Training and Professional Development
Although the primary purpose of this application was not to provide training and professional development, my graduate student and postdoctoral fellow have received extensive training in immunology, use of mouse models of IBD and flow cytometry.

Dissemination of Results
Because of the time required to develop and characterize our new mouse model of IBD at our new institution, we have not, until recently, been able to fully implement the in vivo studies outlined in Tasks 1 and 2. However, we have presented our new model data in a departmental seminar and at the national MHSRS meeting that was held August 17-20, 2015 in Ft. Lauderdale, FL.

Plans for the Next Funding Period
We plan to devote our full attention to Tasks 1 and 2 for the next funding period. We plan to determine the therapeutic efficacy of clinically-relevant numbers of hMSCs in suppressing the development of chronic gut inflammation. In addition, we plan to assess the ability of hMSCs to reverse or attenuate preexisting disease in mouse model of IBD. Finally, we will initiate studies to assess the role of MLNs in hMSC-mediated suppression of disease.

4. Impact

Impact on discipline
We believe that data generated from our proposed studies will impact greatly those investigators using mouse models of IBD to evaluate therapeutic efficacy of different biologics and cell-based therapies. Our studies will represent the first studies to assess the ability of hMSCs to suppress chronic gut inflammation in a mouse model of IBD. All previous and current studies have used acute, self-limiting models of chemically induced IBD and hMSCs or have used the T cell transfer model of chronic colitis with mouse MSCs. The immunoregulatory effects reported in studies using MSCs have varied greatly with studies demonstrating protective effects while others have shown no effect or even exacerbation of colitis in different mouse models of IBD. In addition, data generated from studies that will be performed during current funding cycle will greatly impact how investigators perform and interpret their in vitro data describing the immunosuppressive properties of hMSCs in mouse models of autoimmune and chronic inflammatory diseases. The vast majority of published studies using human MSCs in mouse models of disease have not examined directly the immuno-regulatory properties of human MSCs towards mouse immune cells in vitro. We believe that it is critical to model the interactions between MSCs and immune cells in the more immunologically-relevant system using human MSCs and mouse immune cells. Data generated in the current funding cycle together with those present in our previous Progress Report describing the how activation of hMSCs by human or mouse inflammatory cytokines induce the dramatic up-regulation of certain immunosuppressive mediators that have not been interrogated in animal models of chronic inflammation will also have a major impact in the field of MSC therapy. Our studies may reveal new therapeutic targets for drug development.
Impact on other disciplines
Nothing to report

Impact on technology transfer
Nothing to report

Impact on society
Nothing to report

5. Changes/Problems

Changes in Approach
No major changes. Based upon new data from clinical studies using human MSCs in the treatment of different inflammatory diseases, we will concentrate our efforts on evaluating the immunosuppressive properties of clinically-relevant doses of human MSCs.

Problems and Delays
Because of the time required to develop and characterized our new mouse model of IBD, we were not able to fully implement the in vivo studies outlined in Tasks 1 and 2. We have however recently initiated the first of these studies with our new and improved model of IBD.

Changes that had a significant impact on expenditures
No major impact on expenditures

Changes in use of vertebrate animals
We have reestablished our mouse model of IBD.

6. Products

Publications

Fang K, Grisham MB, Kevil CG. Application of Comparative Transcriptional Genomics to Identify Molecular Targets for Pediatric IBD; Front Immunol. 2015 Apr 8;6:165.

Presentations
Data generated from these studies have been presented as seminars at Texas Tech University Health Sciences Center, Georgia Reagents University, Vanderbilt University and East Carolina University. I have also presented some of this work as part of a symposium at the American Gastroenterological Association at Digestive Diseases Week in Washington, DC (May 2015).

7. Participants and Other Collaborators
Individuals working on project

Name: Matthew Grisham, PhD  
Project Role: PI  
Nearest person months: 4 months  
Contribution: Dr. Grisham is involved in designing, implementing and interpreting the experiments  
Funding support:

Name: Iurii Koboziev, PhD  
Project Role: Postdoctoral fellow  
Nearest person months: 6 months  
Contribution: Dr. Koboziev performs all cell preparations, molecular and in vitro assays and assists with the flow cytometry studies and animal model  
Funding Support:

Name: Kathryn Furr, MS  
Project Role: Laboratory Scientist  
Nearest person months: 5 months  
Contribution: Ms. Furr is my Lab Manager and Flow Cytometry Core Manager. She performs all flow cytometry experiments; She is generates the mouse model of IBD and assists with some of the MSC cell culture experiments.  
Funding Support: TTUHSC/State Account

8. Special Reporting Requirements  
Nothing to report

9. Appendices