IXMYELOCEL-T THERAPY ALTERNATIVELY ACTIVATED MACROPHAGES POTENTIALLY EXERT ATHEROPROTECTIVE EFFECTS THROUGH IMMUNOMODULATION, EFFEROCYTOSIS, AND CHOLESTEROL EFFLUX

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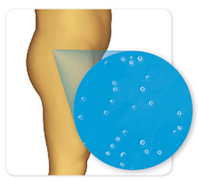
ABSTRACT

Ixmyelocel-T therapy, an expanded autologous multicellular therapy cultured from bone marrow mononuclear cells, includes a population of alternatively activated macrophages. Ixmyelocel-T therapy has shown clinical promise in the treatment of severe chronic ischemic and inflammatory diseases associated with atherosclerosis. Advanced atherosclerotic lesions are characterized by lipid accumulation, chronic inflammation, and defective efferocytosis, all characteristics associated with pro-inflammatory macrophages, therefore it might be beneficial to treat with alternatively activated macrophages where they might promote tissue repair. We herein investigated the atheroprotective potential of ixmyelocel-T's alternatively activated macrophages. Flow cytometry analysis and fluorescent microscopy imaging revealed that ixmyelocel-T macrophages express two well-known surface receptors of alternative activation: CD206 and CD163. ELISA analysis of cytokines revealed ixmyelocel-T macrophages secrete substantial levels of IL-10 (1060±278 vs.1978±313 pg/ml, $P < 0.05$) and IL-1ra (12673±1242 vs. 37576±3650 pg/ml, $P < 0.001$) before and after overnight LPS stimulation. Baseline secretion of the pro-inflammatory cytokines IL-1β (5.6±2.3 vs. 10.1±1.9 pg/ml), TNFα (133±32 vs. 234±50 pg/ml), and IL-12 (5.1±1.8 vs. 4.8±1.5 pg/ml) is minimal and remain low after LPS stimulation. Fluorescent microscopy and flow cytometry revealed that ixmyelocel-T macrophages readily ingest apoptotic cells. Additionally, ELISA analysis after exposure to oxidized LDL ixmyelocel-T macrophages remain anti-inflammatory secreting elevated levels of IL-10 (1087±154 vs. 1205±268 pg/mL) and IL-1ra (26284±2117 vs. 30245±2354.23 pg/mL). Real time PCR analysis revealed increase expression of the cholesterol transporters ABCA1 and ABCG1 after exposure to oxidized LDL suggesting a capacity to handle and efflux cellular cholesterol. This data suggests ixmyelocel-T therapy might exert beneficial effects on atherosclerotic lesions by providing alternatively activated macrophages which potentially limit atheroma development through secretion of anti-inflammatory cytokines, removal of apoptotic cells, and the ability to handle and efflux cholesterol.

INTRODUCTION AND BACKGROUND

Ixmyelocel-T is an expanded autologous multicellular therapy containing a mixture of cell types cultured from bone marrow mononuclear cells (BMMNC). The process used to generate the ixmyelocel-T expands the CD90+ mesenchymal stromal cells and CD14+ monocytes and macrophages while retaining many of the CD45+ cells found in the bone marrow. Recent clinical trials evaluating ixmyelocel-T therapy in the treatment of critical limb ischemia and dilated cardiomyopathy have shown clinical promise. The proprietary twelve day cell expansion process used to produce ixmyelocel-T therapy results in the generation and expansion of an alternatively activated macrophage population of cells. This population of cells could be beneficial in the treatment of chronic inflammatory diseases, including atherosclerosis, where tissue remodeling and immunomodulation are key components to successful clinical outcomes.

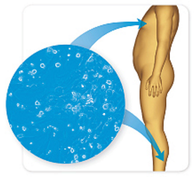


Day 1

A small quantity (approx. 50ml) of bone marrow is taken from the patient's hip in a 15-minute in-office (outpatient) procedure.

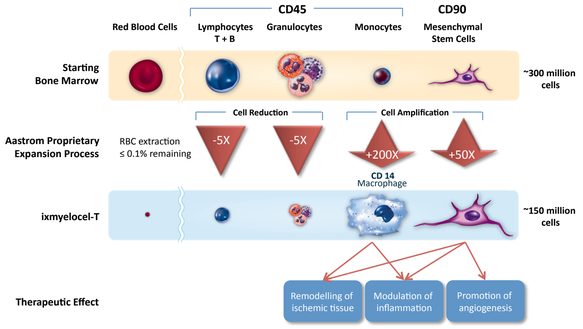
Days 2-13

Over a period of about 12 days, Aastrom's proprietary system expands the naturally occurring cell populations found in the extracted bone marrow. These cells are know to play key roles in tissue regeneration.



Day 14

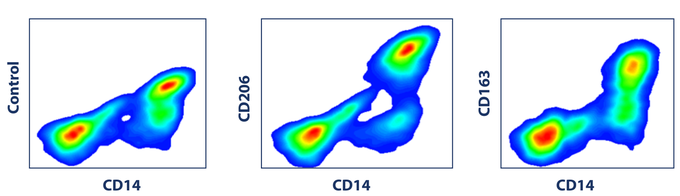
In a single inpatient procedure, the expanded mixed-cell therapy produced from this process is then administered to the same patient to promote healing of the affected tissues.

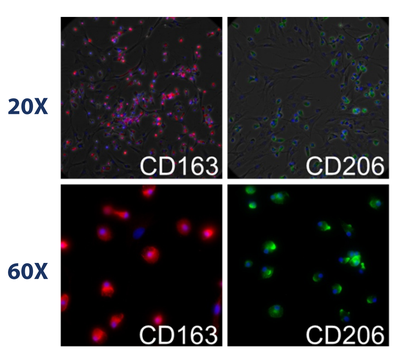


MATERIALS AND RESULTS

IXMYELOCEL-T MACROPHAGES ARE CHARACTERIZED BY SURFACE EXPRESSION OF TWO WELL CHARACTERIZED MARKERS OF ALTERNATIVELY ACTIVATED MACROPHAGES: CD206 AND CD163.

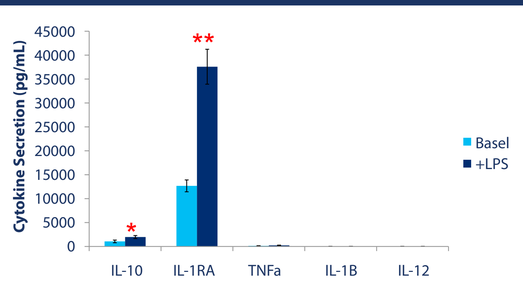
Flow cytometry analysis and fluorescent microscopy imaging revealed that ixmyelocel-T macrophages express two well-known surface receptors of alternative activation: CD206, the macrophage mannose receptor, and CD163, the haptoglobin-hemoglobin scavenger receptor.





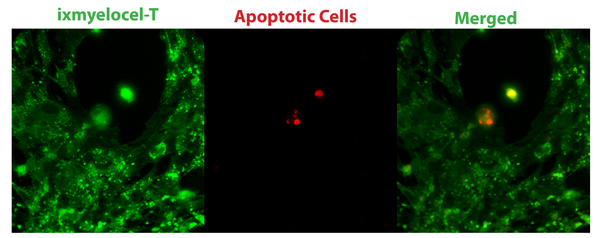
IXMYELOCEL-T MACROPHAGES SECRETE ELEVATED AMOUNTS OF ANTI-INFLAMMATORY CYTOKINES, AND MINIMAL AMOUNTS OF PRO-INFLAMMATORY CYTOKINES

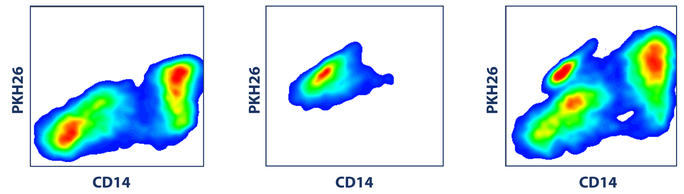
IL-10, IL-r1a, TNFα, IL-1β, and IL-12 were quantified in MACS sorted CD14+ sorted ixmyelocel-T supernatants treated with and without LPS ($n > 3$). Ixmyelocel-T macrophages secrete elevated levels of anti-inflammatory cytokines, before and after LPS stimulation, while pro-inflammatory cytokine secretion remains minimal. * $P < 0.05$ vs. basal,** $P < 0.001$ vs. basal. Values are presented as mean ± SEM relative to control.

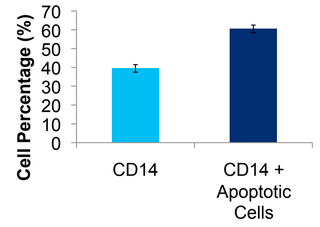


IXMYELOCEL-T ALTERNATIVELY ACTIVATED MACROPHAGES READILY PHAGOCYTIZE APOPTOTIC CELLS

Adherent ixmyelocel-T stained with PKH67 was incubated with PKH26 labeled apoptotic cells. Apoptotic cells were washed away, and healthy ixmyelocel macrophages were analyzed using fluorescence microscopy. PKH26 labeled apoptotic mononuclear cells were added to healthy ixmyelocel-T, efferocytosis was measured by flow cytometry. Healthy ixmyelocel-T macrophages were stained with anti-CD14 antibody. 60% of ixmyelocel-T CD14+ cells phagocytosed apoptotic cells ($n = 5$). Values are presented as mean ± SEM relative to control, *$P < 0.001$ vs. CD14. Magnification: 60X.

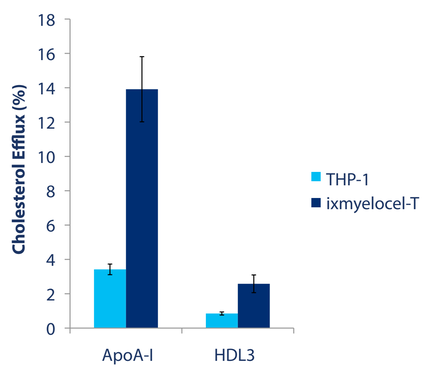


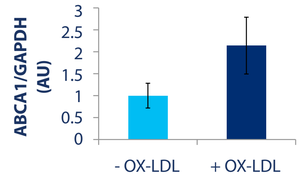


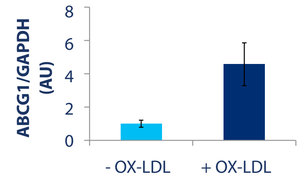


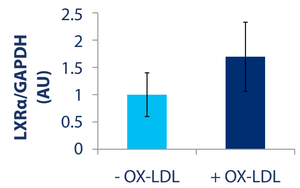
IXMYELOCEL-T TAKES UP AND EFFLUXES MODIFIED CHOLESTEROL

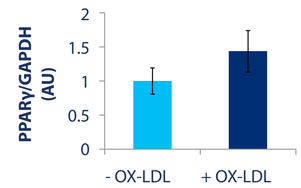
The ability of ixmyelocel-T to efflux cholesterol was measured with an in vitro cholesterol efflux assay. Ixmyelocel-T and THP-1 cells ($n=4$) were loaded with free cholesterol using acetylated LDL (acLDL). Ixmyelocel-T demonstrated a robust increase in ABCA1-mediated cholesterol efflux, as seen by the increase in efflux to apoA-I. Real time PCR analysis revealed increase expression of the cholesterol transporters ABCA1 and ABCG1 in ixmyelocel-T macrophages after exposure to oxidized LDL, as well as increase in the transcription factors LXRα and PPARγ. Additionally, ELISA analysis after cholesterol loading ixmyelocel-T macrophages revealed that the cells remain anti-inflammatory secreting elevated levels of IL-10 and IL-1ra.

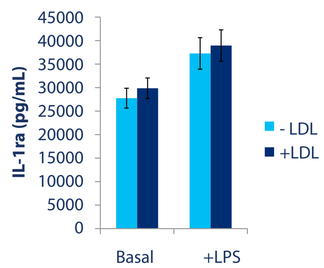


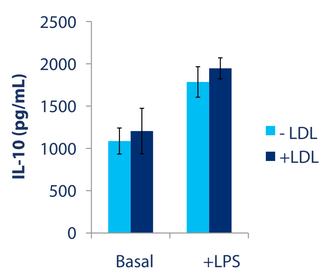


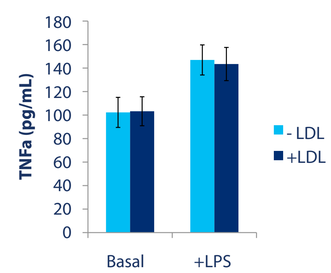












CONCLUSIONS

Theses biological properties of ixmyelocel-T macrophages suggest that this cellular therapy may exert beneficial effects on atherosclerotic disease by providing macrophages which potentially limit atheroma development through secretion of anti-inflammatory cytokines, promotion of cholesterol efflux, and removal of apoptotic cells.

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