BONE MARROW M2 MACROPHAGE CELL THERAPY DOES NOT INDUCE RENOPROTECTION IN UUO MICE MODEL

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INTRODUCTION

Alternatively activated macrophages (M2) have regenerative properties and shown promise as a potential cellular therapeutic strategy for acute and chronic kidney disease.

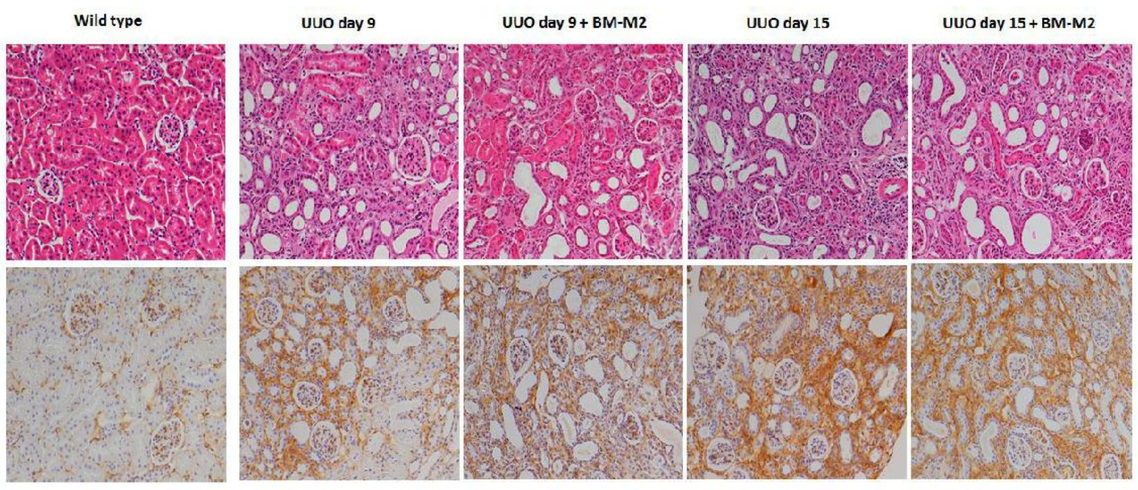
The aim of this study was to evaluate whether bone marrow-derived M2 macrophages (BM-M2) cell therapy could induce tissue repair in the Unilateral Ureteral Obstruction (UUO) mice model of chronic kidney damage.

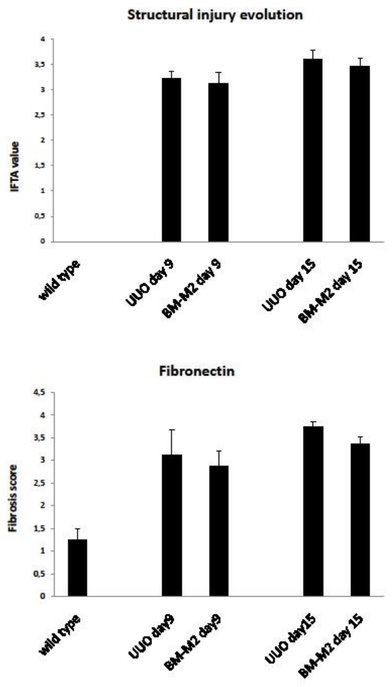
AIMS

1. To analyse the BM-M2 bio-distribution after infusion with especial emphasis on renal localization.
2. To analyse whether BM-M2 cell therapy modifies the pro-inflammatory environment resulting from UUO disease.
3. To analyse whether BM-M2 cell therapy improves renal functional parameters and induces renal injury regression.

RESULTS

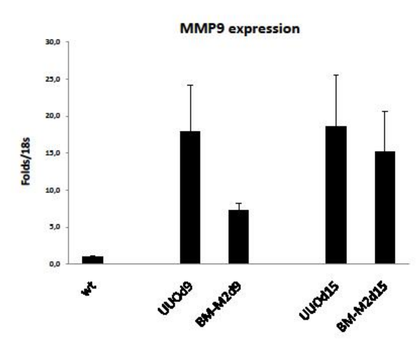
1. immunohistochemistry

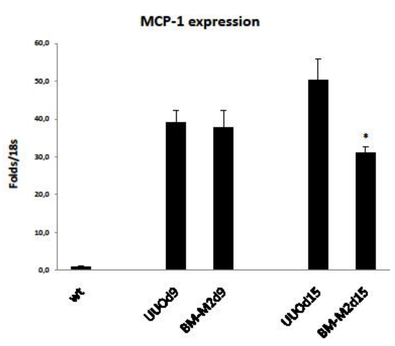


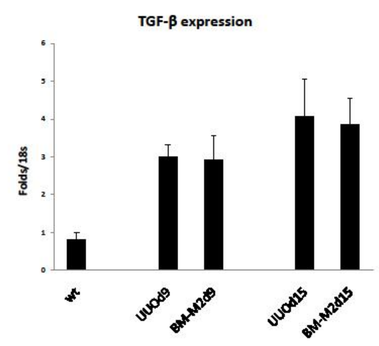


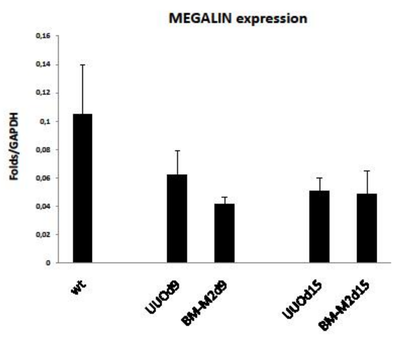
Representative Hematoxylin&Eosin and Fibronectin staining in treated BM-M2 macrophages group and non-treated group at day 9 and day 15. Both groups show similar renal lesions. Fibrosis and IFTA values were assessed and quantified in a blinded and semi quantitative manner graded on a 0-4 scale: lowest to highest degree.

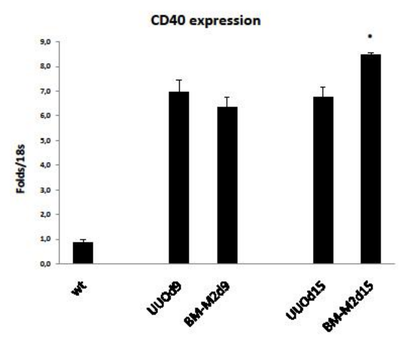
2. Renal mRNA expression

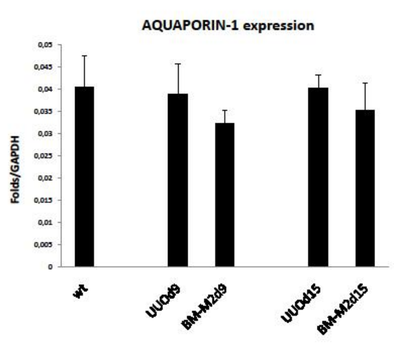


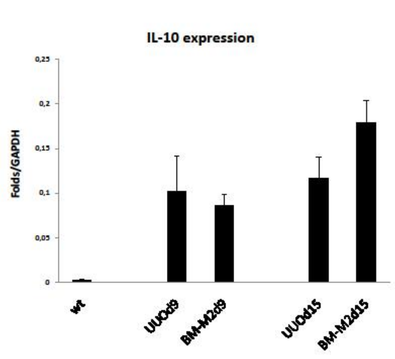






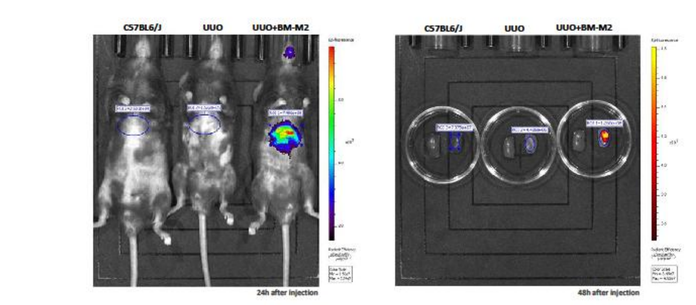






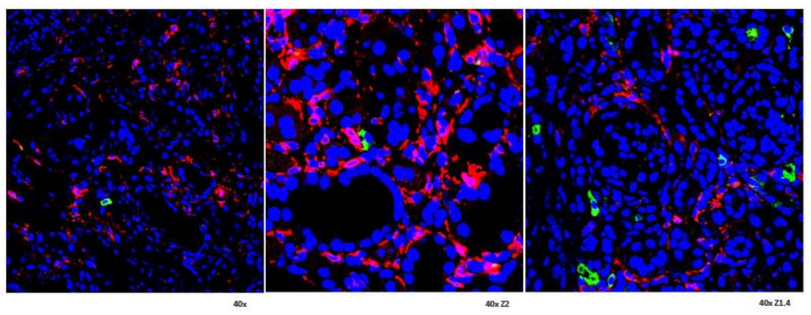
Effect of BM-M2 macrophage infusion on UUO mice. The real time PCR shows that BM-M2 treated group does not reduce the pro-fibrotic and pro-inflammatory cytokines levels and also shows a reduction of the epithelial preservation markers.

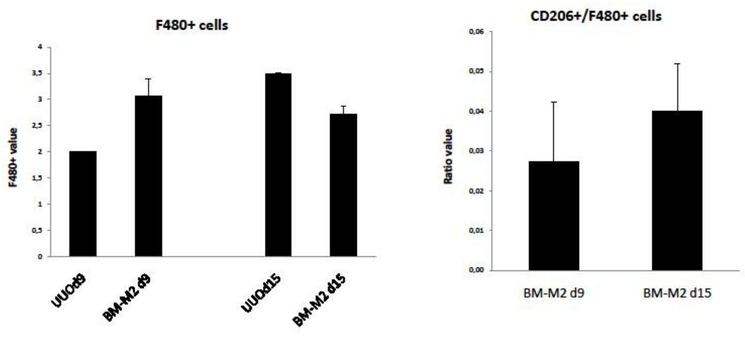
3. *In vivo* tracking



Tracking transfused-BM-M2. Fluorescent labeled macrophages were mainly recruited in liver 72h after infusion. BM-M2 macrophages coming from cell therapy eventually reached the obstructed kidney.

4. Immunofluorescence





Immunofluorescence of F480+ (red) and CD206+ (green) cells. Overall, there is a high amount of F480+ cells (macrophage) although there are few co-expressing CD206 + (anti-inflammatory phenotype).

METHODS

UUO surgery was performed on 8 week-old C57BL6J male mice. At day 7 after surgery, 1x10^{6}cells/animal were injected via the tail vein. Transfused BM-M2 macrophages were examined by immunofluorescence staining and in *vivo* tracking. Mice were killed and evaluated on day 9 and day 15 after UUO surgery, and divided into five groups: **wild type** (n=4); **UUO-d9,** killed at day 9 (n=9); **UUO-d15,** killed at day 15 (n=9), **UUO+BM-M2-d9,** animals with BM-M2 macrophage infusion and killed at day 9 (n=9); **UUO+BM-M2-d15,** animals with BM-M2 macrophage infusion and killed at day 15 (n=9). Tubular injury, interstitial fibrosis and pro-inflammatory cytokines were evaluated. A group of n=1 mice were used for *in vivo* BM-M2 macrophage cell therapy tracking.

CONCLUSIONS

1. BM-M2 macrophage cell therapy does not improve structural renal damage and fibrosis.
2. BM-M2 macrophage cell therapy is not able to reduce the pro-inflammatory milieu in the obstructed kidney.
3. Seldom BM-M2 macrophages coming from the cell therapy reached the obstructed kidney, however the majority of these macrophages are retained in liver.
4. The vast majority of macrophages in the obstructed kidney do not show the anti-inflammatory phenotype.
5. Our results suggest that BM-M2 macrophages from cell therapy switched their phenotype due to the renal pro-inflammatory milieu.

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