

News Release

Title

Transfusion of immunoregulatory M2 macrophages derived from bone marrow or iPS cells ameliorates crescentic glomerulonephritis

Key Points

- Administration of CD206⁺ M2 macrophages artificially differentiated from bone marrow or induced pluripotent stem cells (iPS cells) exerted a prominent therapeutic effect in a mouse model of crescentic glomerulonephritis (CGN) that leads to unfavorable outcomes in human patients.
- CD206⁺ M2 macrophages elicit a switch of M1 macrophages to the M2 phenotype and induce immunoregulatory T lymphocytes (Tregs) *in vitro*.
- Administration of CD206⁺ M2 macrophages promoted significant induction of Tregs in the spleen and kidney-draining lymph nodes in CGN mice.

Summary

Professor Shoichi Maruyama (Department of Nephrology), Associate Professor Naotake Tsuboi (Department of Nephrology), and graduate student Qiunna Du (Department of Nephrology), along with their collaborators at Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, MD, PhD), showed that the transfusion of CD206⁺ M2 macrophages, which were artificially differentiated from bone marrow or induced pluripotent stem (iPS) cells, successfully ameliorated mouse crescentic glomerulonephritis (CGN) characterized by rapid renal dysfunction.

Macrophages are scavenger cells that engulf foreign bodies; however, when they are excessively activated, they can induce tissue inflammation. In addition to the classically activated macrophages, which play effector roles in tissue injury (M1 macrophages), recent reports have identified alternatively activated M2 macrophages that are involved in the resolution of inflammation and tissue repair.

CGN is an unfavorable and intractable kidney disease that is characterized by rapid renal impairment. Corticosteroids and/or cyclophosphamides are commonly used to treat patients with CGN, but the adverse events, including infectious diseases, caused by immunosuppression often result in a negative outcome. Therefore, the research team investigated whether administration of immunoregulatory M2 macrophages might ameliorate the renal injury in a mouse model of CGN. They demonstrated for the first time that CD206⁺ M2 macrophages artificially differentiated from bone marrow or iPS cells exerted a prominent therapeutic effect against CGN. They also elucidated the therapeutic mechanism, in which CD206⁺ M2 macrophages elicit a switch of M1 macrophages to the M2 phenotype and induce immunoregulatory T lymphocytes (Tregs). Their results suggest that CD206⁺ M2 macrophage transfusion would be an effective therapeutic strategy for CGN patients. Moreover, besides bone marrow cells, iPS cells appear to be a promising source of M2 macrophages owing to their minimal invasiveness for collection.

Research Background

In addition to the classically activated macrophages that play effector roles in tissue injury (M1 macrophages), recent reports have identified alternatively activated M2 macrophages that are involved in the resolution of inflammation and tissue repair. Since macrophages predominantly accumulate in the kidneys of patients with glomerulonephritis and other renal diseases, they have been thought to be involved in the onset and progression of kidney injuries. In the present study, the renoprotective effect of transfused macrophages that were artificially differentiated to the M2 phenotype against rapidly progressive crescentic glomerulonephritis (CGN) was evaluated in a mouse model.

Research Results

The researchers attempted to differentiate M2 macrophages from bone marrow or induced pluripotent stem (iPS) cells cultured with interleukin (IL)-4 and IL-13. The cell types established from the different origins both expressed CD206 on the cell surfaces, as well as M2 macrophage-specific genes (bone marrow-derived CD206⁺ M2 macrophages, CD206⁺ M2BMM; iPS cell-derived M2 macrophages, CD206⁺ iPS-M2M). Then, they evaluated the therapeutic effect of the intravenous administration of CD206⁺ M2BMMs or iPS-M2Ms on day 4 after mouse CGN induction. When compared to treatment with parental bone marrow-derived macrophages, CD206⁺ M2BMM transfusion significantly ameliorated proteinuria and renal tissue injury, and reduced glomerular leukocyte accumulation and inflammatory cytokine production in the kidneys of the CGN mice (Figure 1A-D). By contrast, renal injury was significantly enhanced in the M1BMM-treated CGN animals. Interestingly, CD206⁺ iPS-M2M administration exerted a comparable renoprotective effect to that observed with CD206⁺ M2BMM administration (Figure 1E,F). Next, they co-cultured CD206⁺ M2BMMs with M1 macrophages and demonstrated that CD206⁺ M2BMMs promoted the phenotypic conversion of contacted M1 macrophages to the M2 phenotype (Figure 2). A co-culture experiment of CD206⁺ M2BMMs with spleen-derived T lymphocytes demonstrated the induction of immunoregulatory T lymphocytes (Tregs)(Figure 3A,B). Significant induction of Tregs was also observed in the lymph nodes draining the spleen and kidneys in CGN mice treated with CD206⁺ M2BMMs(Figure 3C,D). These results suggested that phenotypic conversion from M1 macrophages to M2 cells and induction of Tregs, which are both driven by the administered CD206 macrophages, were involved in the ameliorated renal injury in the treated group.

Research Summary and Future Perspective

The current study demonstrated the effectiveness of transfused CD206⁺ M2 macrophages against CGN and elucidated at least part of the underlying therapeutic mechanism (Figure 4). Previous studies have described the improvement of renal injury following transfusion of spleen-derived M2 macrophages; however, the spleen is not a realistic cell source for clinical practice. The therapeutic efficacies of bone marrow- or iPS cell-derived M2 macrophages demonstrated in the current study have significance for the clinical application of M2 cell therapy. The research team is currently evaluating whether M2 macrophage transfusion shows comparable therapeutic potential for other

autoimmune glomerulonephritis and inflammatory disorders as observed for CGN, along with further exploration of the detailed mechanistic aspects. Their research goal is to establish a novel therapy of autologous transplantation with bone marrow- or the less invasive iPS cell-derived M2 macrophages from patients.

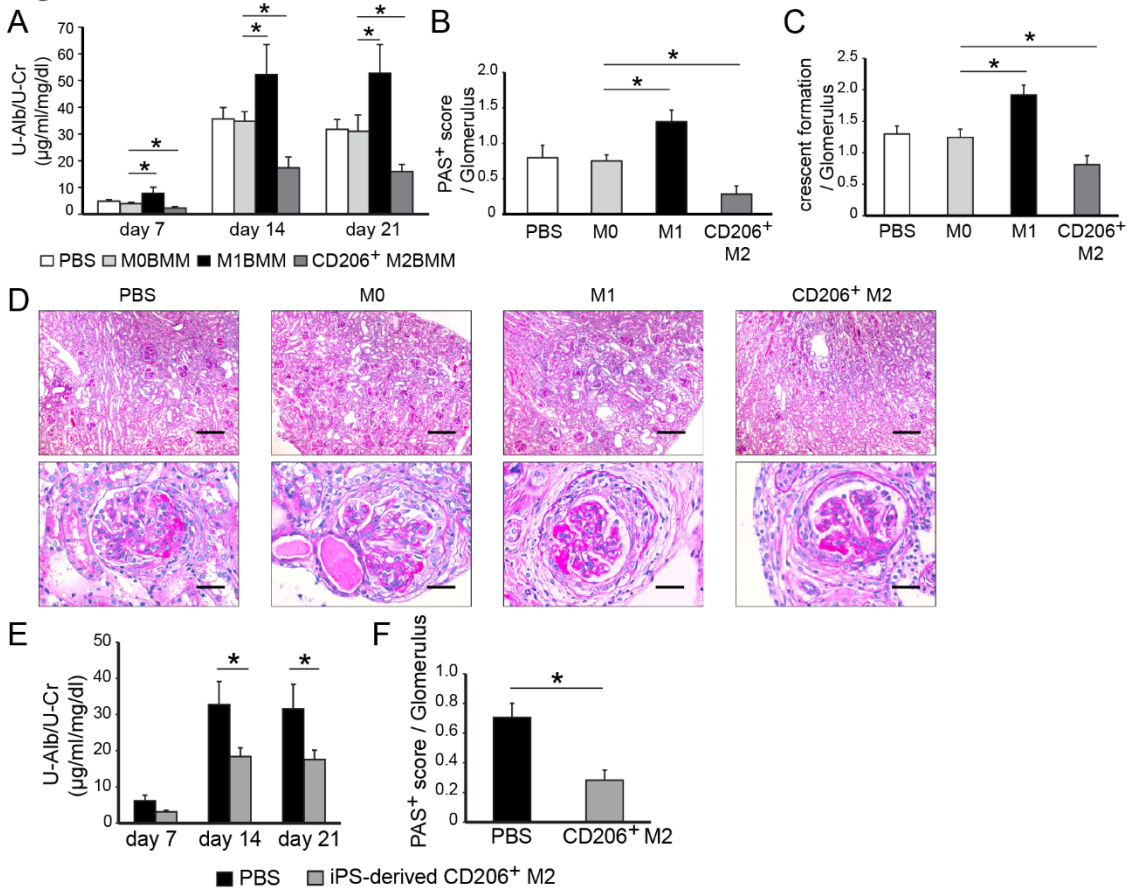
Publication

Du Q, Tsuboi N, Shi Y, Ito Y, Sugiyama Y, Furuhashi K, Endo N, Kim H, Katsuno T, Akiyama S, Matsuo S, Isobe K, Maruyama S. Transfusion of CD206⁺ M2 macrophages ameliorates antibody-mediated glomerulonephritis in mice. *The American Journal of Pathology*, November 14,2016.

Japanese ver.

http://www.med.nagoya-u.ac.jp/medical/dbps_data/material/nu_medical/res/topix/2016/macrophages_20161114jp.pdf

Figure 1



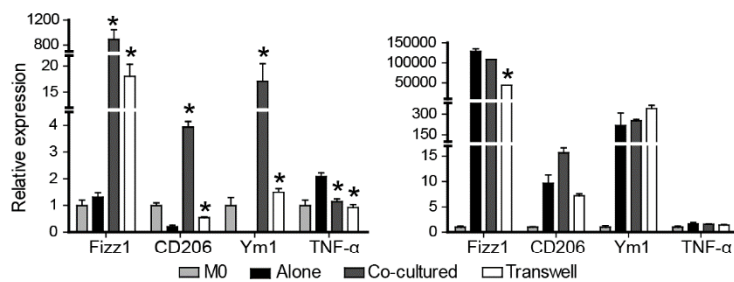
Amelioration of mouse crescentic glomerulonephritis (CGN)

by transfusion of bone marrow-derived CD206⁺ macrophages (A-D).

A: Proteinuria B: PAS⁺ glomerular deposition C: glomerular crescentic formation

D: kidney histology (PAS stain)

Figure 2

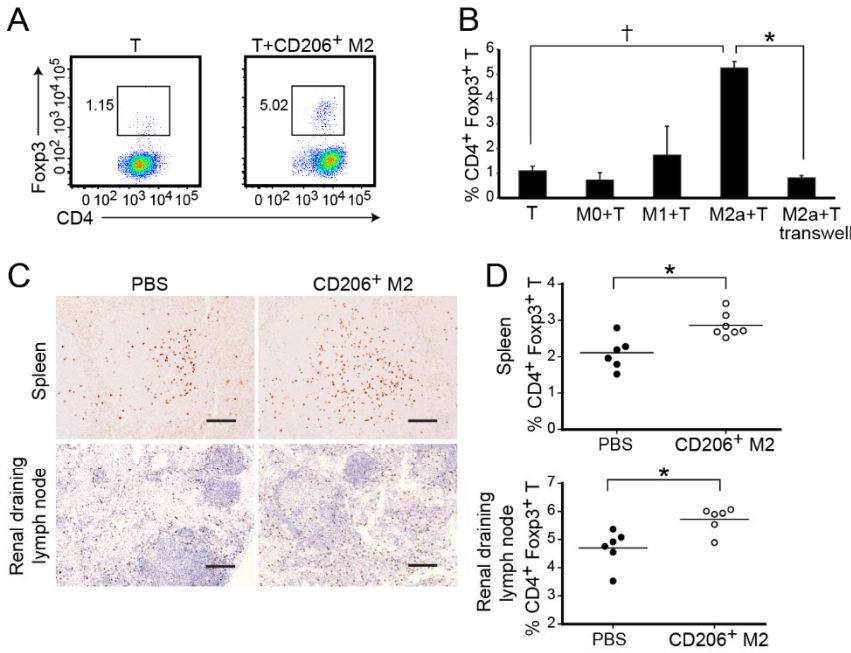


Bone marrow-derived CD206⁺ M2 macrophages convert contacted M1 macrophages to M2 phenotype cells (Co-culture experiment).

left: Induction of M2-specific genes (*Fizz1*, *CD206*, *Ym1*) and reduction of M1 gene (*TNF- α*) in M1 macrophages co-cultured with bone marrow-derived CD206⁺ macrophages.

right: Maintenance of M2 specific genes in bone marrow-derived CD206⁺ macrophages contacted with M1 macrophages.

Figure 3



Bone marrow-derived CD206⁺ M2 macrophages promote immunoregulatory T lymphocytes (Tregs) in a co-culture experiment.

A: Cell surface expression of Fxp3

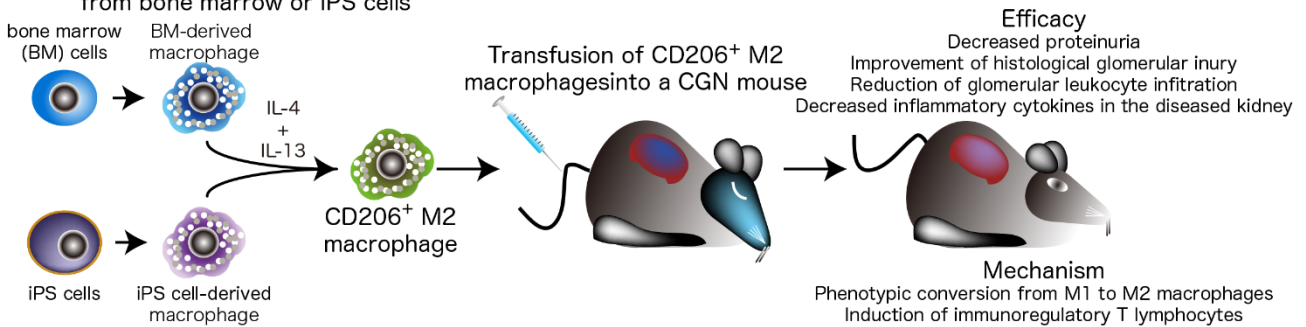
B: Induction of Fxp3⁺ Tregs

C: Accumulation of Fxp3⁺ Tregs in the spleen (upper) and kidney-draining lymph nodes (lower) from CGN mice treated with CD206⁺ M2 macrophages

D: Quantitative analysis for Fxp3⁺ Tregs as in C.

Figure 4

Establishment of CD206⁺ M2 macrophages from bone marrow or iPS cells



Therapeutic strategy for CGN by transfusion of CD206⁺ M2 macrophages derived from bone marrow or iPS cells.