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Project Title	The study of immunological activation mechanism of umbilical cord-derived mesenchymal stromal cells
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Report

Umbilical cord-derived mesenchymal stromal cells (UC-MSCs) are activated to have the immunosuppressive potency, upon the adjacent of the activated T cells, and/or inflammatory cytokines. However, activation mechanisms of UC-MSCs in complicated environment remained unclear. The objectives of this study are to evaluate the influence of priming effect of with triptolide (TPL) isolated from Chinese herb, on UC-MSC in immunosuppression and anti-inflammation potency. Previously, we found that TPL-primed UC-MSCs exhibited stronger anti-proliferative effect for activated CD4+ and CD8+ T cells in the allogeneic mixed lymphocyte reaction assay than the non-primed UC-MSCs. TPL-primed UC-MSCs promoted the expression of IDO-1 and PD-L1 in the presence of IFN-γ, but TPL alone was not sufficient (Paper in revise).

Because of the Covid-19 pandemic, the collaborating researchers could not visit Japan in 2020. Therefore, we studied the experiments separately by e-mail communications.

In 2020, we studied Th1/Th2 cytokines secretion by ELISA in the supernatant of MLR co-cultured with 0.01uM TPL or 10ng/ml IFN-γ primed UC-MSCs. TPL-primed UC-MSCs significantly suppressed IL2,TNF-α and TNF-ß secretion in the MLR more than UC-MSC alone, while IFN-γ-primed UC-MSCs did not. Furthermore, TPL-primed UC-MSCs up-regulated IL4, IL5, IL6, and IL10 secretion compared with those by UC-MSCs alone and IFN-γ-primed UC-MSCs. These results suggested that TPL-primed UC-MSCs induced Th2 humoral immunity rather than UC-MSC alone. Also the mechanism to activate UC-MSC by TPL might be different from those by IFN-γ.

The osteogenic and adipocyte differentiation ability were evaluated. UC-MSCs, TPL primed UC-MSC, IFN-γ primed UC-MSCs, IFN-γ and TPL primed UC-MSCs groups were established. The osteogenic and adipocyte differentiation of TPL primed UC-MSCs did not show better differentiation ability compare with UC-MSCs, However, the osteogenic differentiation ability of UC-MSCs was obviously inhibited after IFN-γ treatment (IFN-γ primed UC-MSCs) and the inhibitory effect could reverse by TPL (IFN-γ and TPL primed UC-MSCs).

The immunoregulation ability of TPL primed UC-MSCs was studied in aGVHD mice. The mice were divided into NC group (negative control group), aGVHD group (positive

group), +UC-MSC group, +TPL primed UC-MSCs group, +IFN-γ primed UC-MSCs group, + IFN-γ and TPL primed UC-MSCs group, the peripheral blood of mice was drawn on the 0, 3rd, 6th, 9th, 12th, and 15th days to detect Th1 and Th2 cytokines. The mice were sacrificed on the 15th day, the lungs, liver, and small intestine were evaluated for HE staining. The results show that, compared with UC-MSCs, TPL primed UC-MSCs can better down-regulate Th1 cytokines (IL2, IFN-γ, TNF-α, TNFß) and up-regulate Th2 cytokines (IL4, IL5, IL6, IL10); TPL primed UC-MSCs could reduce the inflammation of lungs, liver, and small intestine by histopathological detection. IFN-γ primed UC-MSCs show a more severe inflammation, however, the severity of inflammation could reverse by IFN-γ and TPL primed UC-MSCs .

Our research confirms that TPL can promote the immunosuppressive effect of UC-MSCs, which provides a preliminary experimental basis for combining traditional Chin ese medicine monomers with MSC therapy to prevent and treat GVHD.