Regulation of CD4⁺FOXP3⁺ T cells by CCL20/CCR6 axis in early unexplained recurrent miscarriage patients

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ABSTRACT. Expression and function of CCR6/CCL20 in CD4⁺FOXP3⁺ regulatory T cells (Tregs) was investigated in unexplained recurrent miscarriage (URM) patients. Flow cytometry, reverse transcription-polymerase chain reaction, enzyme-linked immunosorbent assay, western blots, and Transwell migration assays were used to analyze the expression and function of regulatory T cells in peripheral blood (PB) and decidual samples of women with URM and of healthy controls. Proportions of CD4⁺FOXP3⁺ T cells and CCR6⁺CD4⁺FOXP3⁺ T cells were lower in URM patients than in healthy controls for both PB lymphocytes and decidual samples (P < 0.05). Expression levels of FOXP3 and CCR6 mRNA were lower in URM patients than in control subjects for PB and decidual samples (P < 0.05). CCL20 protein levels were lower in URM patients than in controls (P < 0.05). An effect of Treg migration was significantly blocked (by 89.13%) using a neutralizing anti-CCL20 antibody in vitro. Furthermore, CCL20-stimulated Tregs exhibited a 3.21-fold increase in migration and this was blocked using a neutralizing anti-CCL20 antibody. IL-10 concentration in culture supernatants of
CD4⁺CD25⁺CD127⁻ Tregs of URM patients was significantly lower than that in controls. Anti-CCL20 antibody inhibited IL-10 and IL-4 expression but increased IFN-γ and IL-17 levels when there was cell-cell contact between PB CD4⁺CD25⁺ T cells and CD4⁺CD25⁻ T cells. No difference was detected when cell-cell contact was prevented by a semi-permeable Transwell membrane. CCL20-CCR6 could drive immune activity of CD4⁺FOXP3⁺ Tregs, followed by their migration to the feto-maternal microenvironment. These results elucidated the mechanism by which Tregs exert this suppressive effect.

Key words: CD4⁺FOXP3⁺ regulatory T cell; Chemokine receptor; Chemokine; Unexplained recurrent miscarriage