THU0045

IL-25/IL-17RB AXIS IS ACTIVATED AND ASSOCIATED WITH ILC2 EXPANSION IN GRANULOMATOSIS WITH POLYANGIITIS (GPA)

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Background: Pathogenesis of Granulomatosis with polyangiitis (GPA) is still unknown. However, it has been observed a skewing of circulating CD4+ T cells toward the Th17 and Th2 phenotype. The pro-inflammatory cytokine interleukin 25 (IL-25) is a member of IL-17 cytokine family associated to the Th2 immune phenotype. Through the receptor IL17RB, IL-25 further sustains the Th2-type immune response and elicits the expansion of the type 2 innate lymphoid cells (ILC2) and M2 macrophages. A pathogenic role of the innate lymphoid cells in GPA has been recently demonstrated; however, the relevance of IL-25 in this condition remains unexplored.

Objectives: Aim of the study was to evaluate the expression of IL-25/IL-25R axis and its functional relevance in patients with granulomatosis with polyangiitis (GPA).

Methods: Thirty patients with a diagnosis of GPA fulfilling the Chapel Hill Consensus Conference and ACR classification criteria, and 20 age-matched healthy controls (HC) were included in this study: age range 30-66 years, 40% female in GPA group and 32-63 years, 20% female in HC. IL-25 and IL-33 serum levels were measured by ELISA at the diagnosis and after Rituximab (RTX) therapy (n=15). Immunohistochemical staining for IL-25, IL-33, IL-17B was performed on sections of renal biopsies obtained from GPA patients (n=10). Kidney ILC2 infiltration was assessed by confocal microscopy. Frequencies ILC1, ILC2 and ILC3 were evaluated by flow cytometry analysis by CD45, Lyn, CCR2, GATA3, Tbet, CD56, Nkp44, IL-22, IL-23R, RORc. Expression levels of IL-5 and IL-13 in kidney were quantified by quantitative real-time PCR (qRT-PCR).

Results: Increased pre-RTX serum levels of IL-25 and IL-33 at baseline were observed in GPA compared to HC (p<0.0001). Both serum IL-25 and IL-33 correlated with the Birmingham Vasculitis Activity Score (BVAS) (r²0.51 p<0.0001 and r²0.14 p<0.05 respectively). IL-25, IL-17RB and IL-33 expressing cells were increased in GPA renal biopsies vs HC (p<0.0001). Frequencies of circulating ILC1 and ILC3 were not significantly increased in GPA. Conversely, the frequency of ILC2 was expanded in GPA vs HC (p<0.0001) and correlated with BVAS (r²0.60 p<0.0001). The number of ILC2 and the levels of expression of IL-5 and IL-13 in GPA kidney were increased (p<0.0001). RTX therapy led to a reduction in IL-25 and IL-33 serum levels and in the frequency of circulating ILC2.

Conclusion: Our data confirm the increases in IL-33 levels previously reported by other authors. The increased circulating and tissue levels of IL-25 and IL-25 receptor-expressing cells, including ILC2, suggests a role for the IL-25/IL-17RB axis in GPA pathogenesis. Treatment with anti-CD20 Rituximab may lead to modulation of IL-25/IL-17RB axis and reduction in ILC2.

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THU0046

FRACTALKINE PROMOTES DIFFERENTIATION INTO OSTEOCLASTS FROM HUMAN PERIPHERAL BLOOD MONOCYTES

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Background: Fractalkine (FKN) also known as chemokine (C-X3-C motif) ligand 1 plays important roles in the pathogenesis of rheumatoid arthritis (RA). Clinical trials of monoclonal antibody against FKN for RA are currently in progress. Although FKN has been reported to be involved in the migration of osteoclast precursor cells, the direct effect of FKN on osteoclast differentiation is not clear.

Objectives: In this study, we aimed to clarify the effect of FKN on differentiation into osteoclasts from human peripheral blood monocytes.

Methods: CD14-positive monocytes were purified from peripheral blood obtained from healthy donors by magnetic beads. CD16-positive and CD16-negative peripheral blood monocytes were isolated from the CD14-positive monocytes. The purified peripheral blood monocytes were incubated with M-CSF + RANKL with or without FKN for 1 week. The cells were stained for tartrate-resistant acid phosphatase (TRAP). TRAP-positive multinucleated (three or more nuclei) cells were counted as osteoclasts. The cells were seeded onto plates coated with calcium phosphate thin films, and the resorption lacunae were examined. Next, CD14-positive monocytes were incubated with GM-CSF + IL-4 with or without FKN for 1 week to differentiate into dendritic cells. The surface markers of these cells were analyzed by flow cytometry. In addition, the monocyte-derived dendritic cells were further incubated with M-CSF + RANKL with or without FKN. Then the cells were stained for TRAP. The research procedure has been approved by the ethics committee of Toho University School of Medicine (Approval number: 27060).

Results: CD16-negative peripheral blood monocytes differentiated into osteoclasts by incubation with M-CSF + RANKL. The osteoclast differentiation was enhanced by additional stimulation with FKN in a concentration-dependent manner. In addition, calcium resorption was also enhanced by addition of FKN. On the other hand, CD16-positive monocytes treated with M-CSF + RANKL did not differentiate into osteoclasts with or without FKN. CD14-positive monocytes differentiated into dendritic cells by stimulation with GM-CSF + IL-4. Expression of CD14 was decreased and CD1a expression was increased on the monocyte-derived dendritic cells. CD16-negative monocyte-derived dendritic cells differentiated into osteoclasts by further stimulation with M-CSF + RANKL. By co-stimulation with FKN, the osteoclast differentiation from CD16-negative monocyte-derived dendritic cells was also enhanced.

Conclusion: FKN promotes osteoclast differentiation from human peripheral blood CD16-negative monocytes and also from CD16-negative monocyte-derived dendritic cells. FKN might contribute bone destruction in the affected joints of RA as well as inflammatory cell accumulation into the synovial tissue.

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