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.

Disclosure of Interests: Daniele Mauro: None declared, Giuliana Guggino Grant/research support from: Laborest, Pfizer, Consultant for: Novartis, Abbvie, Speakers bureau: Sandoz, Angelo Ferrante: None declared, Cristiano Alessandri: None declared, SERENA FASANO: None declared, Daniela Iacono Speakers bureau: PFIZER, Marianna Lo Pizzo: None declared, Riccardo Alessandro: None declared, Aroldo Rizzo: None declared, Francesco Ciccio Grant/research support from: CELGENE, PFIZER, Consultant for: UCB, NOVARTIS, CELGENE, PFIZER, LILLY, Paid instructor for: UCB, NOVARTIS, CELGENE, PFIZER, LILLY, JANSSEN, Speakers bureau: UCB, NOVARTIS, CELGENE, PFIZER, LILLY, JANSSEN, ROCHE, AMGEN

DOI: 10.1136/annrheumdis-2019-eular.4072

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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Disclosure of Interests: Sei Muraoka Grant/research support from: Novartis, Speakers bureau: Pfizer, Chugai, Kaichi Kaneko: None declared, Natsuko Kusunoki: None declared, Shinichi Kawai Grant/research support from: Nippon Zoki, Chugai, Ono, Ayumi, Hisamitsu, Eisai, Japan Tobacco, Nippon Kayaku, Daiichi Sankyo, Astellas, Yutoku, and Mitsubishi Tanabe, Consultant for: Santen, Japan Tobacco, Speakers bureau: Ayumi, Chugai, Ono, Astellas, Pfizer, Celltrion, Toshihiro Nanki Grant/research support from: Chugai, Eisai, Takeda, Teijin, Eli Lilly, Bristol-Myers, AbbVie, Ono, Novartis, Asahikasei, Mitsubishi-Tanabe, Astellas, Ayumi, Pfizer, Daiichi Sankyo, Shionogi, Sanofi, Nippon Kayaku, Yutoku, Actelion, UCB, Bayer, Nihon Pharmaceutical, Consultant for: UCB, Eisai, Chugai, Ono, Gilead, Speakers bureau: Mitsubishi-Tanabe, Chugai, Eisai, Takeda, Astellas, Janssen, Eli Lilly, Ayumi, Pfizer, Asahikasei, Sanofi, Daiichi Sankyo, Otsuka, AbbVie, Ono, Teijin, Nippon Kayaku, UCB.

DOI: 10.1136/annrheumdis-2019-eular.3046

THU0047 IA-14069, A NOVEL SMALL-MOLECULE INHIBITOR DIRECT-TARGETING TUMOR NECROSIS FACTOR- α , ATTENUATES COLLAGEN INDUCED ARTHRITIS

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Background: Rheumatoid arthritis (RA) is systemic autoimmune disease that is characterized by autoreactive immune cells and various cytokines-mediated inflammation in multiple joints, leading to cartilage degradation, bone erosion and finally irreversible joint destruction¹. The inflammatory cytokine tumor necrosis factor- α (TNF- α) is known to play a central role in several chronic immune-mediated inflammatory disorders².

Objectives: Despite the great success of anti-TNF- α biological drugs in the treatment of RA, no chemical drug targeting TNF- α is available. Here we report that IA-14069, a novel small molecule inhibitor, binds directly to TNF- α and inhibits TNF- α activities both *in vitro* and *in vivo*.

Methods: IA-14069 was screened and identified by competitive binding assay using TNF- α and TNF receptor. *In vitro* neutralization activity of IA-14069 against TNF- α was determined using MTT assay. The direct binding of IA-14069 to TNF- α was demonstrated by surface plasmon resonance and bead pull-down assays. The inhibition of TNF- α -TNFR interactions by direct binding of IA-14069 to TNF- α was analyzed by flow cytometry. Levels of phosphorylated I κ B α (p-I κ B α) and NF- κ B p65 were analyzed by western blot. IA-14069 was orally administered to TNF- α -transgenic (TNF- α -TG) RA mice at 3.3 or 33 mg/kg twice per week or at 25, 50 or 100 mg/kg 3 times per week for preventive or therapeutic effect, respectively. *In vivo* therapeutic efficacy of IA-14069 or methotrexate was evaluated in collagen-induced arthritis (CIA) mice immunized with bovine type II collagen (CII) emulsified in complete Freund's adjuvant, and boosted with CII emulsified in incomplete Freund's adjuvant.

Results: IA-14069 potentially inhibits both TNF- α -induced cytotoxicity (IC₅₀ < 0.7 μ M) which directly binds to TNF- α and TNF- α -triggered signaling (p-I κ B α and NF- κ B p65) activities. The therapeutic as well as preventive anti-RA effects of IA-14069 were demonstrated in TNF- α -TG and CIA models. IA-14069 and MTX had synergistic effects in the CIA therapeutic model. According to pharmacokinetic analysis, IA-14069 showed significant bioavailability. In addition, no *in vivo* toxicity was observed even under treatment of excessive amount of IA-14069.

Abstract THU0047 – Figure 1

Conclusion: The data indicate that IA-14069 can be a novel and potential TNF- α inhibitor for the treatment of RA and other inflammatory diseases.

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Disclosure of Interests: Sung-Dong Park Employee of: MOGAM Institute for Biomedical Research, Hyung Gun Maeng: None declared, Yeon-Hwa Park: None declared, Kye-Jung Shin: None declared, Tae-Hwe Heo: None declared

DOI: 10.1136/annrheumdis-2019-eular.5478

THU0048 2ND LINE TREATMENT WITH LESINURAD AND ALLOPURINOL VERSUS FEBUXOSTAT FOR MANAGEMENT OF HYPERURICEMIA: A COST-EFFECTIVENESS ANALYSIS FOR SPANISH PATIENTS

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Background: Lesinurad, a medication that inhibits uric acid reabsorption, has been recently approved in combination for the treatment of patients with gout which do not reach therapeutic serum urate target with xanthine oxidase inhibitors monotherapy

Objectives: To assess the incremental cost-effectiveness ratio of adding lesinurad to allopurinol as 2nd line therapy, compared to febuxostat for the management of patients with gout in Spain.

Methods: A Markov model comprising 6 health-states representing the disease evolution was used to estimate in 6-months cycles, the lifetime accumulated cost and benefits in terms of quality-adjusted-life-year (QALY) in an hypothetical cohort of patients stratified according to serum uric acid levels observed on pooled CLEAR trials: 18.8% (<6 mg/dL); 63.7% (6-8 mg/dL); 15.2% (8-10 mg/dL) and 2.3% ($\geq$ 10 mg/dL). During the simulation, patients could either continue with 2nd line treatment with lesinurad (200mg/daily) plus allopurinol (400mg/daily) or febuxostat (80mg/daily), switch to allopurinol monotherapy (271mg/daily) in case of intolerance or discontinue treatment. Proportion of tophaceous gout (18.9%) was considered at each health-state. The efficacy of treatments was captured in the transition probabilities between health states which were derived from findings on CLEAR 1, CLEAR 2 and EXCEL clinical trials. Quality of life related to gout severity (tophaceous or non-tophaceous) and flare frequency was considered by means of utilities estimated from SF-36 scores of the pooled CLEAR trials which were mapped to EQ-5D values. The total cost estimation (€, 2018) included drug acquisition cost (retail prices with mandatory deduction applied), disease monitoring (€377.03 for first 6-month period, €329.95/subsequent year) and flare management cost (€301.69). Unitary costs derived from local cost databases and literature. A 3% annual discount rate was applied for cost and outcomes. Sensitivity analyses (SA) were carried out.

Results: Lesinurad added to allopurinol provided higher QALYs (14.79) than febuxostat (14.69). Total accrued cost per patient were lower (€-6,819.13) with lesinurad and allopurinol (€9,469.27) compared to febuxostat (€56,478.40). Lesinurad plus allopurinol resulted a dominant option (more effective and less costly) compared to febuxostat. SA results confirmed the model robustness.

Conclusion: These results suggest that treatment with lesinurad 200 mg/day plus allopurinol 400 mg/day compared to febuxostat 80 mg/day is an effective second option for the management of hyperuricemia in patients with gout who did not reach therapeutic serum urate target to previous allopurinol monotherapy, associated to cost-savings for the Spanish National Health System.

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Disclosure of Interests: María Presa Consultant for: I am employee of PORIB a consultant company which has received financial support from Grünenthal for development of this project, Fernando Perez-Ruiz Grant/research support from: Cruces Rheumatology Association, Consultant for: Grünenthal, Menarini, Horizon, Speakers bureau: Menarini, Grünenthal; Spanish foundation for rheumatology, Itziar Oyagüez Consultant for: I am employee of PORIB a consultant company which has received financial support from Grünenthal for development of this project

DOI: 10.1136/annrheumdis-2019-eular.3231