Objectives: To evaluate the quantitative statuses of peripheral CD4⁺T subpopulations in patients with gout and further investigate the effects of immunomodulatory combination therapies on those cells.

Methods: Total 247 patients who met the clinical criteria of gout from the American College of Rheumatology and 206 healthy controls (HCs) were enrolled in this retrospective cross-sectional study. Among those patients, 70 follow-up patients donated their peripheral blood after receiving immunomodulatory drugs (e.g., low-dose interleukin-2, rapamycin, metformin, retinoic acid, etc). The absolute numbers of Th1, Th2, Th17 and Tregs in peripheral CD4⁺T subsets were detected by flow cytometry combined with standard absolute counting beads.

Results: Compared with HCs, the absolute numbers of Th1 and Th17 were evidently increased in gout patients ($P < 0.001$), while the level of Tregs was significantly decreased ($P < 0.05$) (Figure 1). After immunomodulatory combination treatments, there were dramatical increases in a wide variety of CD4⁺T subsets such as Th1, Th17 and Tregs ($P < 0.05$). Interestingly, the increased amount of Tregs was much more than that of other T cells, leading to the decrease ratios of Tregs/Teffs such as Th2/Tregs, restoring immune homeostasis (Figure 2).

Conclusion: This cross-sectional study clarified the abnormal statuses of CD4⁺T subsets in gout patients, suggesting that CD4⁺T subsets, especially Tregs, might be relevant and play a crucial role in the pathogenesis of gout, thus providing a potential therapeutic target for gout patients. Immunomodulatory combination therapies effectively increase the number of Tregs and may help for gout patients' symptom remission.

Figure 1: Comparison of absolute counts of peripheral CD4⁺ lymphocyte subsets between patients with gout (n=247) and healthy controls (n=206). All the CD4⁺ lymphocyte subpopulations were analyzed by detected by flow cytometry combined with standard absolute counting beads. Data were presented as mean±SD and statistical analysis was determined by two-tailed unpaired t-test. Patients had lower levels of Tregs and Tregs/Teffs such as Th1 and Th17 compared with those of healthy controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2: Changes of peripheral CD4⁺ lymphocyte subpopulations after immunomodulatory combination treatments (n=70). Data were presented as mean±SD and statistical analysis was determined by two-tailed paired t-test. Absolute counts of Tregs and Tregs/Teffs such as Th1 and Th2 in gout patients were significantly increased after immunomodulatory combination treatments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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AB0033

CHARACTERIZATION OF THE PERIPHERAL B CELL COMPARTMENT IN PATIENTS WITH EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

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Background: Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare form of systemic vasculitis, which is characterized by bronchial asthma, hypereosinophilia, and systemic vasculitis. B-lymphocytes play a key role in EGPA as producers of IgE and anti-neutrophil cytoplasmic antibodies (ANCA). Indeed, the neutrophils that are targeted by these antibodies are widely described as the mechanism of endothelial damage in this disease. On the other side, the therapeutic response to rituximab in EGPA patients provides evidence for a role of B-cells in the pathogenesis of EGPA. Therefore characterizing B cell subpopulations may help in understanding the disease and the treatment.

Objectives: To characterize the peripheral B cell compartment in patients with EGPA and to analyze the in vivo potential of B lymphocytes to class-switch to IgE and to assess in vitro the differentiation potential of naïve B cells of EGPA patients into IgE-secreting plasmablasts.

Methods: Clinical characteristics of the patients, including organ involvement and treatment regimen were evaluated. Laboratory work-up included ANCA-status, eosinophils, IgE, IgG, IgA, IgM, and peripheral CD19⁺ B-cell count. For immunophenotyping isolated PBMCs were stained with monoclonal or polyclonal antibodies and B cells were classified into: naïve, marginal zone, class-switched memory B cells, unconventional memory B cells, transitional and plasmablasts. Furthermore, the expression of IgG+ and subclasses IgG1-4, IgA⁺, IgE⁺ B cells, BAFFR and TACI was quantified. For in vitro differentiation assays magnetically isolated B lymphocytes from EGPA patients and age-matched healthy controls were stimulated with CD40L, IL-21 and IL-4. Starting the culture with equal number of B cells, the absolute number of plasmablasts, and IgE class switched cells after 9 days was determined by counting the events in the CD27^{high}CD38^{high} gate or the IgG/A/D-IgE⁺ gate by flow cytometry. IgE secretion in the supernatant was measured by ELISA. JAK-STAT signalling pathway was analyzed in response to IL-4 and IL-21 stimulation and phosphorylation of STAT5 and 6 measured by flow cytometry.

Results: 34 patients with EGPA diagnosed according to ACR and CHC-criteria were included into the study. Ten of these patients were analysed separately because they received rituximab therapy. Peripheral B cell numbers in EGPA patients were markedly diminished. B cell subpopulation phenotyping showed in average 57.9% naïve B cells, 12.5 % marginal zone like B cells and 19.2% switched memory B cells. Plasmablasts constituted in average 1.15% of the peripheral B cell compartment, transitional B cells 2.0%. Interestingly, the expression of BAFF receptor and TACI in the memory B cell subset was significantly decreased in EGPA patients when compared with healthy donors. In vitro assays of isolated B cells from EGPA patients demonstrated an increased proportion of IgE-class-switched B cells after 9 days of culture under IL4 stimulation compared with controls. However, no differences were observed in the phosphorylation of STAT5 and STAT6 after stimulation with IL-4 or IL-21.

Conclusion: In the EGPA-patients we observed markedly diminished B-cells despite of normal lymphocyte counts. B cells showed a reduced expression of BAFF-R and TACI. Class switch to IgE is enhanced in EGPA patients.

Disclosure of Interests: None declared

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AB0034

EARLY IMMUNIZATION AGAINST TREATMENT IS ASSOCIATED TO POOR CLINICAL RESPONSE AT 6 MONTHS AND LOW NUMBER OF TRANSITIONAL B CELLS AT BASELINE IN RHEUMATOID ARTHRITIS PATIENTS TREATED BY ADALIMUMAB

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Background: Circulating anti-drug antibodies (ADAs) are detectable approximately in 33% of adalimumab treated rheumatoid arthritis (RA) patients, often within the first 6 months of therapy^{1,2}. Classically, circulating ADAs associate with their specific drugs to form immune complexes, increasing drug clearance, and by this mechanism reducing therapeutic effect³. B cell involvement leading to ADAs production is not yet well established.

Objectives: To study early ADAs formation according to clinical response to an adalimumab therapy in RA patients and the relationship between ADAs and circulating B cell subsets.

Methods: 28 RA patients and 13 healthy controls were included. Patients all presented inadequately controlled RA under conventional treatment, were naive of biotherapies, and started an adalimumab treatment at baseline (M0). Responder status was determined according to the DAS28CRP score (<3.2) at 3 (M3) and 6 months (M6). ADAs plasma concentration $>10\text{pg/mL}$ at M3 defined the immunized patient group. Circulating B cell subsets were quantified by flow cytometry at M0 and M3.

Results: 11 (42.3%) patients were immunized at M3. Among them, 4 (36.4%) were responders at M6 and 7 (63.6%) were non-responders. Presence and concentration of ADAs at M3 was associated to non-responder status at M6 ($p=0.043$; $p=0.042$). Immunized patients had lower transitional B cells count at M0 compared to non-immunized patients ($p=0.031$).

Conclusion: A high but classical proportion of RA patients developed ADAs after only 3 months of adalimumab treatment. This immunization was associated to non-responder status at M6 and to a low blood transitional B cells count at baseline. Our results suggest transitional B cells implication in

RA activity and biotherapy resistance due to immunization. Low concentrations of transitional B cells could be an early biomarker of immunization process against adalimumab.

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Table. Patients characteristics at baseline

Characteristics	All RA patients (n=28)	M6 responders (n=16)	M6 non-responders (n=10)
Age (years)	60.5 [47-78]	65.5 [47-76]	54 [47-78]
Sex ratio M/F (% of F)	0.4 (71.4%)	0.5 (68.8%)	0.3 (80.0%)
Disease duration, (years)	5.6 [0.7-43.0]	6.8 [1.0-43.0]	2.9 [0.7-31.0]
Oral steroid use, (%)	18 (64.3%)	9 (56.2%)	8 (80.0%)
Oral steroids, dose (mg/day)	5.0 [2.0-15]	5.0 [4.0-12.5]	8.5 [2.5-15.0]
Methotrexate use, (%)	24 (50.9%)	14 (87.5%)	8 (80.0%)
Methotrexate, dose (mg/week)	20 [10-25]	20 [10-25]	15 [10-20]*
Leflunomide use, (%)	3 (10.7)	1 (6.3%)	2 (20.0%)
Leflunomide, dose (mg/day)	20 [20-20]	20 [20-20]	20 [20-20]
CRP, (mg/dL)	5.5 [1.0-57.0]	6.6 [1.0-46.8]	3.6 [1.0-57.0]
DAS28CRP score	4.3 [3.3-5.7]	4.1 [3.3-5.2]	4.5 [3.4-5.7]
RF positive, (%)	20 (71.4%)	12 (75.0%)	6 (60.0%)
RF, (U/mL)	116 [19-640]	88 [21-640]	181 [19-336]
ACPA positive, (%)	25 (89.3%)	15 (93.8%)	8 (80.0%)
ACPA, (U/mL)	340 [11-340]	340 [14-340]	340 [11-340]

Values are medians with ranges and frequencies with percentages. * $p=0.050$.

Figure 1. Graph 1 Immunization against treatment at 3 months and clinical response at 6 months in RA patients (n=26). Presence of ADAs at 3 months is associated to non-responder status at 6 months. Fisher exact test. R, responders at 6 months; NR, non-responders at 6 months; ADA+, immunized patients at 3 months; ADA-, non-immunized patients at 3 months.

Figure 2. Graph 2 Absolute number of transitional B cells at baseline in RA patients (n=28) according to immunized status at 3 months. Immunized patients at 3 months had lower transitional B cells at baseline than non-immunized patients. ADA+, immunized patients at 3 months; ADA-, non-immunized patients at 3 months. Data represent the mean; * $p<0.05$ by Mann-Whitney U test.

Disclosure of Interests: None declared

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AB0035 TWO DIFFERENT ABNORMAL BEHAVIORS IN CD4+T LYMPHOCYTES IN FIBROMYALGIA PATIENTS AND FIBROMYALGIA ASSOCIATED TO SJÖGREN'S SYNDROME

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Background: Primary fibromyalgia syndrome is a prevalent rheumatic condition characterized by widespread pain and whose etiopathogenesis is not well understood. Fibromyalgia can also be secondary to other rheumatic diseases like Sjogren's syndrome; however, its relation to this disease is unknown. It has been suggested that the immune system is involved in their pathogenesis. The role of activation stages and cytokines profiles of CD4+T lymphocytes in fibromyalgia or fibromyalgia secondary to Sjogren's syndrome are completely unclear and could play a key role in the pathophysiology of these diseases.

Objectives: The objective of this study is to investigate the counts and distribution of the CD4+T lymphocyte activation subsets and their pattern of cytokine production in women with primary fibromyalgia, fibromyalgia secondary to Sjogren's, Sjogren's syndrome and healthy controls (HC). The counts and distribution of naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}) and effector (T_E) CD4+T lymphocyte subsets were analyzed in these diseases. Furthermore, we investigated their pattern of IL-4, IL-10, IL-17A, IFN γ , and TNF α production.

Methods: Counts and distribution of CD4+T subsets (T_N , T_{CM} , T_{EM} , T_E) and their cytokine producing capacity were measured using multiparametric flow cytometry in peripheral blood mononuclear cells (PBMC) from 20 primary fibromyalgia, 15 fibromyalgia associated to Sjogren and 15 primary Sjogren patients and 15 female controls. Fibromyalgia and/or Sjogren's syndrome were diagnosed based on ACR criteria. CD4+T cell activation stages were analyzed by the expression of the CD3, CD4, CD45RA, CD27 and CCR7 antigens. Cytokine CD4+T producing cells subsets were assayed stimulating PBMC during 6 hours, fixed, permeabilized and simultaneously stained with IL-4, IL-10, IL-17A, IFN γ , and TNF α intracellular cytokines.

Results: Fibromyalgia patients showed a significant increase in the CD4+T, T_N and T_{CM} cells counts with compared to fibromyalgia secondary to Sjogren, Sjogren's syndrome and HC. The counts of IL-17A, IL-4 and IFN γ producing