described, with some heterogeneity being observed across monocyte subsets. However, although previously linked to blood pressure control, the significance of ACE expression on monocytes remains obscure.

Objectives: to evaluate whether monocyte subsets and ACE expression were associated with surrogates of CV subclinical disease in very early RA patients.

Methods: patients were recruited upon early referral to the rheumatology department. All patients were untreated at the time of sampling. The frequency of classical (CD14+CD16-), intermediate (CD14+CD16+) and non-classical (CD14lowCD16+) monocyte subpopulations and ACE expression were assessed by flow cytometry in peripheral blood. Plaque occurrence, cIMT and stiffness parameters were analyzed by Doppler ultrasound in internal carotid, middle cerebral and basilar arteries.

Results: 53 patients were recruited, 47 fulfilling 2010 ACR/EULAR classification criteria for RA (36 women, 26 FR+ and 26 ACPA+) and 6 fulfilling criteria for Clinically Suspect Arthralgia (CSA) (6 women, 3 RF+ and 2 ACPA+). Non-classical and intermediate monocytes were positively associated with cIMT ($r=0.413$, $p=0.005$ and $r=0.353$, $p=0.018$, respectively), as well as with pulsatility ($r=0.332$, $p=0.026$ and $r=0.323$, $p=0.030$) and resistivity indices ($r=0.286$, $p=0.050$ and $r=0.277$, $p=0.045$) at the left internal carotid artery. Expansion of CD16+ monocytes was not associated with disease activity, measured as DAS28 or SDAI (all $p>0.050$), but frequency of intermediate monocytes paralleled duration of the symptoms ($r=0.324$, $p=0.020$).

ACE expression was higher in CD16+ monocyte subsets than in their classical counterparts. ACE expression was not associated with blood pressure, individual traditional CV risk factors, body mass index nor with mSCORE (all $p>0.050$). ACE expression on intermediate and classical monocytes was correlated with cIMT ($r=0.382$, $p=0.010$ and $r=0.349$, $p=0.019$, respectively). Moreover, ACE expression in all monocyte subsets was strongly associated with resistivity and pulsatility indices at the internal carotid artery (all $p<0.010$).

Conclusion: the expansion of CD16+ monocyte subsets and the ACE expression are associated with arterial wall thickening and vascular functionality in treatment-naïve RA patients, hence suggesting a very early role for monocyte traits in this scenario.

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SAT0065

WNT5A INCREASES RHEUMATOID SYNOVIOCYTE MIGRATION THROUGH RYK RECEPTOR AND RHO-ROCK PATHWAY

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Background: Fibroblast-like synoviocytes (FLS) are pivotal in inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive and invasive phenotype and secrete inflammatory mediators, metalloproteases and cathepsins that perpetuate inflammation and lead to cartilage and bone damage. Given the importance of FLS in the pathogenesis of RA, there is a growing interest in developing new therapies aimed to revert their aggressive phenotype. To this end, a better knowledge of molecular mechanisms involved in FLS behavior is needed. Recently, the role of non-canonical Wnt pathway has been highlighted in several processes as migration, invasion and inflammatory responses. The non-canonical Wnt signaling pathway included the planar cell polarity (PCP), with the activation of Rho and Rac GTPases, and the Wnt/Ca2+ pathways. We have previously demonstrated that Wnt5a contributes to the aggressive phenotype of FLS by increasing their migration and invasion ability, and by stimulating the inflammatory response.

Objectives: To analyse the molecular mechanisms involved in the enhancing effects of non-canonical Wnt5a pathway on the aggressive phenotype of rheumatoid synoviocytes.

Methods: The expression of receptors of non-canonical Wnt5a pathway was assessed in FLS from six RA patients by real-time PCR. Expression of these receptors was suppressed by siRNA transfection (Dharmacon) and the antibody (anti-RYK, Abgent). Pharmacological inhibitors were used to identify the signaling pathways. Thus, an inhibitor of ROCK pathway (Y-27632, Selleckchem) was used and a calcium chelator (BAPTA-AM, Enzo) for the inhibition of the PKC pathway. The expression of MAPKs was analyzed by western blot. Migration after stimulation with recombinant Wnt5a protein was analyzed by the wound healing assay

using Ibidi inserts. The occupation area was determined by Image J software.

Results: We analyzed the expression of Wnt5a receptors, FZD1, FZD2, FZD4, FZD5, FZD7, ROR1, ROR2 and RYK of RA FLS. Although all receptors were expressed in these cells, whereas ROR2 was weakly expressed, RYK showed the highest expression. Migration assays after receptor suppression by siRNA transfection or blocking antibodies showed the involvement of RYK on Wnt5a signaling in RA FLS. In effect, Wnt5a-induced migration was reduced by more than 25% in FLS treated with the anti-RYK antibody. In addition, Wnt5a-induced migration in FLS treated with ROCK inhibitor (Y-27632) was 32% lower than in FLS not treated. No change in spontaneous migration was observed in FLS treated with Y-27632. However, the treatment with the calcium chelator, BAPTA-AM, did not show any differential effect between the spontaneous or the Wnt5a-induced migration. In addition, we found the activation of MAP kinases, ERK and P38, as well as, AKT and GSK3 β in FLS treated with rWnt5a. Nevertheless rWnt5a did not activate JNK. These effects were reversed after treatment with the ROCK inhibitor or after blocking the RYK receptor.

Conclusion: These results indicate that Wnt5a contributes to the aggressive phenotype of RA FLS by binding to RYK receptor, through Rho-ROCK pathway and the activation of MAPKs, ERK and P38, as well as the activation of AKT and GSK3 β .

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SAT0066

DEFICIENCY OF TRANSCRIPTIONAL FACTOR T-BET ACCELERATES THE DEVELOPMENT OF COLLAGEN-INDUCED ARTHRITIS BY INDUCING T HELPER TYPE 17 DIFFERENTIATION

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Background: The transcriptional factor T-bet is required for Th-1 differentiation and important for regulating Th-17 differentiation, which plays a critical role in the development of collagen-induced arthritis (CIA). Previous studies reported that overexpression of T-bet suppresses Th-17 differentiation via inhibition of retinoic acid-related orphan receptor (ROR) γ t and aryl hydrocarbon receptor (AhR) (1, 2). However, the function of T-bet in the development of CIA remains largely unknown.

Objectives: To clarify the role of T-bet on the pathogenesis of CIA.

Methods: 1) T-bet knockout (T-bet^{-/-}) mice (C57BL/6, B6 background) were generated as previously described (3). T-bet^{-/-} mice were immunized with CII with CFA, and the incidence and severity of CIA was assessed. 2) Anti-CII antibodies in the sera from T-bet^{-/-} mice on day 60 after the first immunization were measured. 3) Lymph node cells from B6 or T-bet^{-/-} mice immunized with CII were analyzed by flow cytometry using antibodies specific for CD3, CD4, CD8, CD11b, CD11c, CD19, Gr1 and NK1.1. 4) The lymph node cells described above were cultured in the CII containing medium, then the cytokine production was assayed by ELISA.

Results: 1) CIA developed in the T-bet^{-/-} mice was more severe than that of B6 wild-type mice and the incidence of arthritis was around 1.5-fold higher. 2) The levels of total IgG, IgG1, IgG2b, IgG3 antibodies against native chicken CII were significantly higher in T-bet^{-/-} mice. However, IgG2a antibody production was significantly lower in T-bet^{-/-} mice compared to B6 mice. 3) The number of CD4+ T (CD3+CD4+), CD8+ T (CD3+CD8+), B (CD19+), dendritic cells (CD11c+), macrophage (CD11c-CD11b+Gr1-), neutrophil (CD11c-CD11b+Gr1+), NK cell (CD3-NK1.1+) in the lymph node was not different but the number of NKT (CD3+NK1.1+) cells was significantly decreased in T-bet^{-/-} mice. 4) The level of IL-17 and IL-22 in the culture supernatant was significantly higher in T-bet^{-/-} mice, whereas that of IFN γ was comparable in the two groups.

Conclusion: CIA was significantly severe in T-bet^{-/-} mice. The mechanisms of severe arthritis might be mediated through the up-regulation of IL-17 and IL-22 by Th-17 differentiation in the T-bet deficient and IFN γ independent manner or the decrease of NKT cells.

REFERENCES

- [1] Kondo Y, et al. Overexpression of T-bet gene regulates murine autoimmune arthritis. *Arthritis Rheum* 2012;64:162-72.

- [2] Yokosawa M, et al. T-bet over-expression regulates aryl hydrocarbon receptor-mediated T helper type 17 differentiation through an interferon γ -independent pathway. *Clin Exp Immunol* 2016;188:22–35.
- [3] Szabo, et al. Distinct effects of T-bet in TH1 lineage commitment and IFN γ production in CD4 and CD8 T cells. *Science*. 2002;295:338–42.

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SAT0067

SECRETORY ANTIBODIES TO CITRULLINATED PEPTIDES IN PLASMA AND SALIVA FROM RHEUMATOID ARTHRITIS PATIENTS AND THEIR UNAFFECTED FAMILY MEMBERS

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Background: Mucosal involvement in early phases of rheumatoid arthritis (RA) pathophysiology has emerged as an attractive hypothesis, supported by several findings. Elevated levels of antibodies against citrullinated peptides/proteins (ACPA) can be found during a long pre-symptomatic period, preceding manifest arthritis. Secretory antibodies are produced at mucosal surfaces, but can also be detected in the circulation. Secretory ACPA in plasma has been demonstrated in patients with RA (1). First-degree relatives (FDRs) of patients with RA can be regarded as potential pre-RA patients or at-risk individuals. In a previous study, a higher prevalence of ACPA was found in FDRs than in healthy controls (2), with IgA-ACPA being more common than IgG-ACPA.

We hypothesized that formation of secretory ACPA is an early step in RA development, preceding the occurrence of regular non-secretory ACPA, and consequently secretory ACPA would be prevalent in a large proportion of FDRs.

Objectives: To evaluate secretory ACPA in plasma and saliva from patients with RA and FDRs.

Methods: We analyzed secretory antibodies to 2nd generation cyclic citrullinated peptides (anti-CCP) in plasma from 194 patients with RA and 191 FDRs unaffected by RA and salivary samples from 25 RA patients, 21 first-degree relatives and 11 controls.

In plasma, cutoff for secretory ACPA was set at the 99th percentile of healthy blood donors. In saliva, a positive test was defined as a difference between optical density values for IgA anti-CCP and IgA anti-cyclic arginine peptide (delta OD value) >2 SD above the mean delta OD value of the controls.

Mann-Whitney U test was used for continuous variables and Pearson Chi-square for categorical variables.

Results: Secretory ACPA occurred in 37 (19.1%) of RA patients but only in 2 (1%) of FDRs (table 1). Salivary IgA ACPA was found in 3/25 (12%) of patients with RA, but not in any of the 21 FDRs. 27% of FDRs were positive for regular non-secretory IgA ACPA in plasma, and out of them, only 2 individuals (5%) were positive for secretory ACPA in plasma. Among FDRs negative for regular ACPAs, no one was positive for secretory ACPA. Secretory ACPA had the highest PPV for identifying patients, while IgG ACPA had the highest negative predictive value (table 2).

Table 1. Demographic and laboratory characteristics of the study participants

	FDRs (n=191)	RA patients (n=194)	P value
Age (years), median (IQR)	60 (26)	66 (18.3)	<0.001
Female n (%)	111 (58.1)	136 (70.1)	0.014
Shared epitope, n (%)	83 (53.9)	116 (71.2)	0.001
Smoker ever, n (%)	81 (47.9)	109 (58.0)	0.057
IgG ACPA+, n (%)	34 (21.7)	140 (85.9)	<0.001
IgA ACPA+, n (%)	42 (26.8)	118 (72.4)	<0.001
IgM ACPA+, n (%)	35 (22.3)	74 (45.4)	<0.001
Secretory ACPA+, n (%)	2 (1)	37 (19.1)	<0.001
IgM RF+, n (%)	22 (14)	121 (74.2)	<0.001
IgA RF+, n (%)	35 (22.3)	123 (75.5)	<0.001

Table 2. Performance of circulating antibodies to identify patients with RA vs first-degree relatives

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Secretory ACPA+, n (%)	19.1	99.0	94.9	54.6
IgG ACPA+, n (%)	72.2	82.2	80.5	74.4
IgA ACPA+, n (%)	60.8	78.0	73.8	66.2
IgM ACPA+, n (%)	38.1	81.7	67.9	56.5
IgM RF+, n (%)	62.4	88.5	84.6	69.8
IgA RF+, n (%)	63.4	81.7	77.8	68.7

Conclusion: Secretory ACPA in plasma and saliva was rare in FDRs. Circulating secretory ACPA had the highest PPV of the RA-related antibodies tested to identify RA in these families. This implies that mucosal production of ACPA is not common in an at-risk population, but may be important in the transition from increased risk to actual RA disease.

REFERENCES

- [1] Roos K, et al. *Arthritis Res Ther*. 2016;18(1):119.
- [2] Young KA, et al. *Arthritis Rheum*. 2013;65(8):1995–2004.

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SAT0068

CIRCADIAN RHYTHMS OF IMMUNE SYSTEM IN HEALTHY INDIVIDUALS AND PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Chronobiological aspects play an important role in rheumatoid arthritis (RA), with major symptoms such as joint pain and stiffness follow a day-night-cycle that peak in the early morning. The circadian behavior of symptoms has been attributed to the rhythmic expression of cytokines and hormones^{1,2}. Animal models of autoimmune inflammation demonstrated that immune cells, which belong to the vast producers of such cytokines, circulate in a circadian manner and that the circadian rhythm of circulating immune cell is different in health and disease³. Moreover, coordinating timing of glucocorticoid therapy (chronotherapy) results in a greater reduction of morning stiffness and pain compared with the same glucocorticoid dose taken in the morning⁴.

Objectives: Here, we aim to analyse the circadian rhythms of circulating immune cell populations in the periphery of healthy individuals and RA patients in order to optimize treatment strategies.

Methods: We performed a 24-hours study involving 14 eligible RA patients and 12 healthy individuals to monitor the dynamic occurrence of diverse immune cells in the periphery. A week prior to study day, the biological rhythms were synchronized by scheduled activities and food intakes. On the study day, blood was drawn every 2 hours throughout 24 hours using peripheral venous catheter. The participants were provided with regular meal, allowed to eat snacks ad libitum and carry passive activities. Blood samples were subsequently analyzed by flow cytometry using TruCount Beads to count absolute cell numbers. RNA from CD14⁺ monocytes was analyzed by real-time PCR to monitor the circadian clock genes expression.

Results: We investigated major immune populations and found chronobiological differences in NK T cells, NK cells, CD8 T cells, CD4 T cells and regulatory T cells (Tregs) of healthy individuals and patients with RA. In RA Tregs showed a lag phase of 5 hours, while CD8 and CD4 T cells showed a mild reduction of the amplitude. NK T cells and NK cells did not circulate in a circadian manner in healthy individuals, but they exhibited circadian pattern in RA. In addition, the qPCR data also indicated a disrupted circadian rhythm on RNA level. Among ten clock genes that were examined, *REVERB α* , *PER1*, and *PER3* showed altered expression in RA patients. The expression of *REVERB α* was suppressed by 50% and the rhythm of *PER1* was diminished in RA patient.