Methods CD14-positive monocytes from healthy donors were cultured in presence of M-CSF, IFN- γ , IL-4 or IL-10. The expression of the *PADI2* and 4 genes was measured by RT-qPCR and the expression of PAD2 and 4, evaluated by immunoblotting of total cell extracts.

Results *PADI2* and 4 mRNAs are expressed in monocytes while PAD2 and 4 proteins are also immunodetected in these cells. In the different M Φ subsets, *PADI2* mRNAs are less detected than in the related monocytes except for the IFN- γ M Φ subset where it is detected at a higher relative rate. By contrast, *PADI4* gene expression is suppressed in all M Φ subsets except the IFN- γ M Φ where *PADI4* mRNAs are weakly detected. Consistently with mRNA analysis, PAD2 is detected in all polarised M Φ and more strongly expressed in IFN- γ M Φ whereas PAD4 is no longer detectable except faintly for the IFN- γ M Φ .

Conclusions This study shows that PAD2 is expressed at various degrees in the four analysed M Φ phenotypes but PAD4, only in the IFN- γ M Φ , both enzymes being expressed in monocytes. This reinforces the hypothesis of a role for monocytes and M Φ in genesis of ACPA epitopes in the ST.

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P006

ANTI-RA33 (HNRNP-A2/B1) AUTOANTIBODIES ARE ASSOCIATED WITH THE THERAPEUTIC RESPONSE TO METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction Besides the determination of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), anti-RA33 antibodies (which are directed to the nuclear antigen hnRNP-A2) could be of additional diagnostic and/or prognostic value in patients with rheumatoid arthritis (RA) because they are also found in RF/ACPA negative patients.¹

Objectives So far, published data on anti-RA33 antibodies refer only to the IgG isotype.² It was therefore the aim of this study to measure the prevalence of anti-RA33 IgG, IgM and IgA antibodies in patients with RA and to determine their potential prognostic value regarding prediction of response to treatment.

Methods To determine the diagnostic sensitivity and specificity of anti-RA33 subtypes sera from 255 RA patients, 258 disease controls and 100 healthy subjects were tested by a prototype anti-RA33 EliA (Thermo Fisher Scientific) for the presence of anti-RA33 IgG, IgA and IgM antibodies. ACPA and RF were routinely measured by EliA and nephelometry, respectively. All RA patients had initially been treated with conventional synthetic drugs (mostly methotrexate, MTX) and were subsequently treated with at least one TNF inhibitor (TNFi). Therapeutic responses to MTX and TNF blocking biologicals

were analysed in an inception cohort (n=104) who had started their DMARD therapy at our clinic. To define therapeutic responses the simplified disease activity index (SDAI) and American College of Rheumatology (ACR) responses were calculated.

Results Diagnostic specificity was >96% for all 3 anti-RA33 subtypes. Among the 255 RA patients, 11% tested positive for anti-RA33 IgG antibodies, 15% for IgM antibodies and 6% for IgA antibodies. Altogether, 62 patients (24%) had at least one type of anti-RA33 antibody: among these, 24 patients were RF-negative, 26 were ACPA-negative and 18 were RF/ACPA double negative. Thus, in 32 patients (13%), anti-RA33 was the only antibody specificity. Regarding responses to MTX therapy, the percentage of SDAI50 or ACR20 responders, respectively, was significantly higher (p=0.034 for SDAI50 and p=0.005 for ACR20) among anti-RA33 positive patients (with or without RF/ACPA) compared to anti-RA33 negative (but RF/ACPA positive) patients and RF/ACPA negative patients. Thus, 60% of the anti-RA33 positive patients as compared to 37% anti-RA33 negative patients showed a SDAI50 response; similar values were seen for ACR20 responses.

Conclusions Apart from their added diagnostic value anti-RA33 antibodies may have also prognostic value for prediction of therapeutic responses to MTX treatment. Therefore anti-RA33 antibodies may be taken into consideration as additional markers that might become helpful tools in therapeutic decision making.

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P007

IN RA, BECOMING SERONEGATIVE OVER THE 1ST YEAR OF DMARD TREATMENT DOES NOT TRANSLATE TO BETTER CHANCES OF DRUG-FREE REMISSION IN THE LONG-TERM

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Introduction Patients with RA harbour autoantibodies of various isotypes and directed against several post-translational modifications. Baseline seropositivity is a poor prognostic factor for sustained drug-free remission (SDFR). However, autoantibody levels may change and patients may become seronegative under treatment. It is unknown how often this happens, and whether decreasing levels or becoming seronegative early in disease, indicating disappearance of serological autoimmunity, improves chances of SDFR.

Objectives To longitudinally characterise the levels and presence of autoantibodies in RA patients and to investigate

whether changes in these levels and/or presence associates with SDFR.

Methods In sera of 399 seropositive RA patients in the IMPROVED study,¹ we measured, at 4 month intervals over the first year of treatment: IgG, IgM, and IgA isotypes for anti-cyclic citrullinated peptide-2 (anti-CCP2) and anti-carbamylated protein antibodies (anti-CarP), IgM and IgA for rheumatoid factor (RF), and IgG autoantibodies against 4 citrullinated and 2 acetylated peptides. We investigated whether changes in antibody levels and seroconversion from positive to negative for each individual antibody was favourable for SDFR (drug-free DAS44 <1.6 lasting ≥1 year until last follow-up).

Results For all 14 antibodies, median levels decreased significantly between baseline and 4 months and then stabilised. Most seroconversion to negative happened within the first 4 months of treatment (with prednisone and methotrexate), after which some patients converted back to seropositive. The prevalence of seroconversion varied substantially depending on the autoantibody. The percentage of baseline autoantibody positive patients that became seronegative by 12 months was: anti-CCP2 IgG (2%), IgM (29%), IgA (29%); RF IgM (21%), IgA (31%); anti-CarP IgG (51%), IgM (49%), IgA (66%); anti-acetylated-lysine-vimentin IgG (24%), anti-acetylated-ornithine-vimentin IgG (10%); anti-cit-vimentin 59–74 IgG (21%); anti-cit-fibrinogen α27–43 IgG (45%), anti-cit-fibrinogen β36–52 IgG (20%), and anti-cit-enolase 5–20 IgG (22%). We hypothesised that greater level decreases and seroconversion to negative, reflecting disappearance of the underlying immunopathology, might be favourable for the long-term outcome SDFR, but surprisingly, greater median decreases in levels were not associated with higher chance of SDFR for any antibody. Furthermore, we found no evidence that rates of SDFR were higher in patients who seroconverted to negative compared those who stayed seropositive, for any of the 14 antibodies analysed.

Conclusions Autoantibody levels decrease and seroconversion from positive to negative occurs under treatment, but these changes do not translate to apparent clinical long-term benefits with regard to SDFR. This suggests that the disappearance of measurable serological autoimmunity does not lead to eradication of disease.

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P008

METHOTREXATE AND BAFF INTERACTION PREVENTS IMMUNISATION AGAINST TNF-A INHIBITORS BY INCREASING ADENOSINE AND REGULATORY B-CELLS

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Introduction TNFα inhibitors (TNFi), frequently used in patients with autoimmune diseases, can induce anti-drug antibodies (ADA) in one third of cases, leading to secondary resistance. Methotrexate (MTX) is known to decrease

immunisation against TNFi. In BAFFtg mice, a model of autoimmune disease in which immunisation against biologic drugs is high, we investigated a new way of tolerization using a single course of MTX and we identified the mechanisms involved in this tolerization.

Objectives To assess the potential interaction between BAFF and tolerization induced by MTX.

Methods BAFFtg mice treated with TNFi with or without MTX were monitored for drug concentration and ADA. WT and BAFFtg mice were compared for B-Cell surface markers involved in MTX-related purinergic metabolism, adenosine production or B-regulatory cells (Bregs). Then, BAFF levels, MTX treatment and ADA were assessed in the human ABIR-ISK cohort of patients with chronic inflammatory diseases.

Results In BAFFtg but not in WT mice, a single course of MTX prevented immunisation against TNFi and maintained drug concentration for over 52 weeks. BAFFtg mice B-cells expressed more CD73 and CD39 leading to more adenosine production and to increase in Breg. MTX-induced tolerization was reversed *in vivo* using anti-CD73 antibodies. In patients treated with TNFi for chronic inflammatory diseases, high BAFF serum level was protective against ADA formation to TNFi only in patients co-treated with MTX but not in patients on TNFi monotherapy.

Conclusions This data supports an interaction between MTX and BAFF via increase in CD73 to prevent ADA formation in mice and humans. Treatment increasing CD73 could potentialize the role of MTX to prevent immunisation.

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P009

TRANSCRIPTOMIC ANALYSIS OF PLASMACYTOID DENDRITIC CELLS FROM RHEUMATOID ARTHRITIS PATIENTS REVEALS NOVEL TARGETS FOR THERAPY

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Introduction Reestablishing immune tolerance and long term remission represent major therapeutic goals in rheumatoid arthritis (RA). Our laboratory previously demonstrated that plasmacytoid dendritic cells (pDCs) from RA patients in remission have the ability to induce IL-10 producing regulatory T cells *in vitro*. However, the molecular pathway of RA pDC-mediated Treg induction remains elusive.

Objectives Herein, we sought to identify the molecular mechanism through which pDCs contribute to restoration of tolerance in RA.

Methods pDCs were isolated from peripheral blood of RA patients responding to anti-TNF therapy (DAS28 <5.1) and