particles are targeted by autoantibodies from RA and other systemic rheumatic diseases.
Methods: Using a protein macroarray we identified JKTBP in humans and animal models of inflammatory rheumatic diseases. Bacterially expressed recombinant JKDBP proteins were used to confirm the obtained data. Epitope, TLR7/9 and MyD88 dependency was determined by ELISA. JKTBP expression in cultivated cells and synovial tissue was analysed by indirect immunofluorescence, immunoblot and immunohistochemistry.
Results: Anti-JKTBP autoantibodies were detected in 46% of the patients with systemic lupus erythematosus (n=103), in 20–30% of the patients with rheumatoid arthritis (n=286), in 10% of the patients with mixed connective tissue disease (n=20) or spondyloathropathy (n=20), and in <10% of patients with other autoimmune disorders (n=382). Sera positive to JKTBP as well as hnRNP-B1, revealed nearly two thirds of the RF IgM/ CCP2-seronegative patients as early RA patients. Combining sensitivities to all autoantigens tested (JKTBP, AUF1, hnRNP-B1), it was possible to identify 92% of the early RA patients (n=91). In the MRL/lpr mouse model of SLE, mice deficient of MyD88 or TLR7/9 lacked anti-JKTBP autoantibodies, whereas mice deficient of SIGIRR/TIR8 showed enhanced anti-JKTBP autoantibody production. These results show that autoantibody generation against JKTBP, AUF1, hnRNP-B1 is dependent on TLR 7 and TLR9 like rheumatoid factor different to TLR 7 dependent generation of snRNPs. For all tested autoantigens either their titter or generation are dependent on the activation of innate immunity genes MyD88 and SIGIRR/TIR8 gene.
In localization, experiments anti-JKTBP autoantibodies specifically stained stress granules (SG) in the cytoplasm. Immunohistochemical studies revealed JKTBP to be highly expressed in SG in the cytoplasm of RA synovial tissue different from OA and normal control tissue
Conclusions: These data identify SG as targeted particle in RA and JKTBP as a novel autoantigen in RA, SLE patients and mouse models of inflammatory rheumatic diseases. In combination with the hnRNPs AUF1 and hnRNP-B1, JKTBP autoantibodies close the sensitivity gap in RA left by rheumatoid factor and anti-CCP2 antibodies.
Disclosure of Interest: None declared
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SAT0005 DNA METHYLATION INHIBITORS PRODUCE SUSTAINED REMISSION OF ARTHRITIS IN MICE AND PROMOTE REGULATORY T CELL RESPONSES

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Background: Dysfunction of Tregs results in a breakdown of immunological tolerance and has been implicated in the pathogenesis of autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis (RA) and type 1 diabetes. Treg function is regulated by epigenetic factors and we have previously reported the presence of Tregs expressing an aberrant DNA methylation profile in RA.
Objectives: The aim of this study was to assess the potential utility of DNA methylation inhibitors for the treatment of RA, using collagen-induced arthritis (CIA) as an animal model.
Methods: DBA/1 mice were immunised with bovine type II collagen emulsified in complete Freund's adjuvant. The mice were treated with zebularine (400 mg/kg), decitabine (1 mg/kg) or psammaplin A (10 mg/kg) for 4 days, starting on the day of arthritis onset. Treatment was then stopped and the disease was monitored up to day 10 of arthritis. The expression of Treg genes was measured in lymph nodes on day 10 by qPCR. To assess the effect of DNA methylation inhibitors on generation of Tregs, naïve $CD4^+CD25^-$ T cells were cultured with mitomycin C treated APCs plus IL-2, TGFβ and anti-CD3 in the presence or absence of DNA demethylating agents and numbers of $CD4^+FoxP3^+$ Tregs were determined by FACS after 72h.
Results: Treatment with zebularine resulted in a sustained reduction of arthritis severity, accompanied by an increase in the expression of Treg associated genes, *Foxp3, Ctla4 and Tgfb1*, in draining lymph nodes. Treatment with decitabine produced a more profound reduction in disease severity whereas the therapeutic effect of psammaplin A was more transient. All three DNA methylation inhibitors could convert $CD4^+CD25^-$ T cells into $CD4^+FoxP3^+$ Tregs in a dose-dependent manner *in vitro*.
Conclusions: This study has shown that pulse treatment with DNA demethylating drugs produces a sustained reduction in the severity of arthritis and promotes the generation of Tregs. The findings raise the possibility that epigenetic drugs can be used on a short-term basis for re-setting tolerance and boosting Treg responses in human autoimmune disease.
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SAT0006 ANTI-TNF AGENTS INDUCE MACROPHAGES WITH PRO-RESOLVING PHENOTYPE

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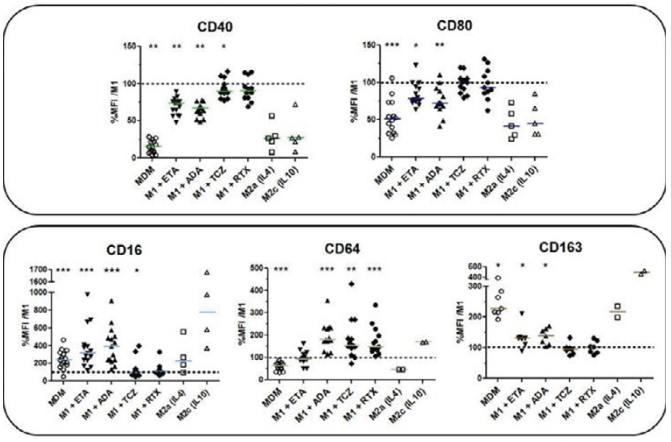
Background: Macrophages contribute to the rheumatoid arthritis (RA) pathogenesis. They can display various states of activation or " polarization ", characterized by distinct functions in inflammation, and reversibility depending on their environment [1]. M1 polarization corresponds to the "classical", pro-inflammatory activation as identified in RA. M2 "alternative" polarizations display pro-resolving or wound-healing properties.
Data concerning the effects of RA biological drugs (bDMARDs) on macrophage polarization are scarce.
Objectives: To assess in vitro modulation of macrophage polarization by RA bDMARDs.
Methods: Blood monocytes from 14 healthy donors were positively sorted by CD14+ magnetic selection. Macrophages were Derived from Monocytes (MDM) by 5 days of culture in the presence of MCSF, and activated or not for 24h as M1 pro-inflammatory MDM (by LPS + IFNγ) or as M2 alternative MDM (by IL10 or IL4). MDM were cultured with or without bDMARDs.
We evaluated 2 anti-TNF agents (etanercept (ETA), adalimumab (ADA)), 1 anti-IL6R agent (tocilizumab (TCZ)), and 1 anti-CD20 agent (rituximab (RTX)) used as control monoclonal antibody. bDMARDs effects were assessed separately on differentiation and activation phases by flow cytometric analysis of membrane markers. Functional aspects of polarization were assessed by analysis of cytokine production in supernatants (cytometric bead array) and phagocytosis (flow cytometry).
MDM cultured in the presence of bDMARDs were compared to untreated MDM by a Wilcoxon matched pairs test.
Results: We first validated *membranous polarization markers* in our culture model: CD40 and CD80 as M1 (LPS + IFNγ) markers; CD16, CD163 and CD64 as M2 (IL10) markers, CD206 and CD200R as M2 (IL4) markers.
Anti-TNF significantly modulated surface polarization markers of *MDM* obtained after differentiation. CD40 decreased with both anti-TNFs. CD16 increased with ADA, whereas opposite effects on CD64, decrease with ETA, increase with ADA, were observed. CD206 increased with ADA. CD200R increased with both anti-TNF. We observed a significant increase in CD64 with TCZ. RTX induced no modulation except an increase in CD64.
Concerning the effect of bDMARDs on *M1 activation* (M1 MDM) shown in Figure, anti-TNF agents induced a significant decrease in M1 markers and a significant modulation in M2 (IL10) markers. We observed a decrease in CD40 and CD80, an increase in CD16, CD163, a decrease in CD64 with ETA and an increase with ADA. TCZ induced a slight but significant decrease in CD40 and an increase in CD64. RTX only affected CD64 as observed for differentiation.
In *M2 (IL4) et M2 (IL10) activated MDM*, anti-TNF agents induced a similar trend toward M2 (IL10) phenotype.
Anti-TNF agents led to a significant decrease in *TNFα, IL6, IL12 and IL10 levels* measured in cell culture supernatant after M1 MDM. TCZ mainly affected IL6 and TNFα productions. No significant effect was observed with RTX.

Normalized polarization surface markers on M1 MDM



Data were normalized to M1 MDM (represented as a dotted line) for each donor. Statistical comparison of M1 MDM without bDMARD (dotted line) versus M1 MDM with bDMARD, using a Wilcoxon matched pairs test → * p < 0.05 ; ** p<0.005 ; *** p<0.0005