adiponectin. We also investigated whether these biomarkers correlated with various demographic, clinical and laboratory markers.

Methods We treated 37 RA patients with either etanercept (ETN) or certolizumab pegol (CZP) in a 12 month follow-up study. Assessments were performed at baseline, and 3, 6 and 12 months after treatment initiation. Serum chemerin and adiponectin concentrations were measured by commercially available ELISA kits (R and D System, MN and USA). PON1 and arylesterase activities were measured by spectrophotometry. In addition, age, disease duration, disease activity (DAS28), CRP, anti-CCP, IgM rheumatoid factor and plasma lipid levels were also assessed. Arterial flow-mediated vasodilatation (FMD), carotid intima-media thickness (cIMT) and arterial pulse-wave velocity (PWV) were assessed by ultrasound.

Results Anti-TNF treatment resulted in a significant decrease in the levels of chemerin (p<0.001) and adiponectin (p<0.007) after 12 months. There were no significant changes in the levels of other metabolic biomarkers. We found the following correlations between the baseline values: the PON1 levels correlated with the age of patients (R=0.466, p=0.004). The adiponectin correlated with the disease activity (R=0.385, p=0.030), HDL-C (R=0.417, p=0.012) and the triglyceride levels (R=0.481, p=0.003). The total cholesterol correlated with the PWV (R=0.449, p=0.021) and the levels of the LDL-C (R=0.911, p<0.001). The baseline triglyceride correlated with the IgM rheumatoid factor (R=0.343, p=0.021); and the levels of LDL-C correlated with the PWV values (R=0.444, p=0.023).

Conclusions Metabolic factors, such as certain adipokines, PON1 and arylesterase may play a role in oxidative stress and atherosclerosis associated with RA. Anti-TNF treatment may affect adipokine levels.

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Disclosure of interest None declared

A BIOASSAY TO MEASURE TGFβ ACTIVITY REVEALS DECREASED TGFβ ACTIVITY IN SYSTEMIC SCLEROSIS SERUM

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Methods

Introduction Systemic sclerosis (SSc) is a severe disease characterised by auto-immunity, vasculopathy and excessive fibrosis of connective tissues. The pathophysiology of SSc is still poorly understood, but its symptoms imply a role for dysregulated transforming growth factor β (TGFβ) signalling because this cytokine is known to regulate vascular and connective tissue biology. TGFβ circulates in blood in an inactive latent form bound to latency associated peptide and latent TGFβ binding proteins. This latent TGFβ has to be activated before it can become bioactive. With the use of a bioassay TGFβ’s bioactivity can be measured in complex mixtures like serum. This is not possible with an ELISA because this technique does not take (cellular) activation processes into account.

Objectives To determine the bioactivity of TGFβ in SSc serum compared to that of healthy control serum.

Methods Serum was collected of 10 SSc patients and 10 age and sex matched healthy controls. Primary human fibroblasts of 3 donors were transduced with CAGA12-luc which produces luciferase in response to TGFβ/Smad3 or BRE-luc which produces luciferase in response to BMP/Smad1/5. These cells were treated with 10% serum for 16 hour and luciferase activity was measured. To activate all TGFβ, sera were treated with 4M HCl for 1 hour at RT, after which pH was normalised with 4M NaOH. Controls were treated with HCl and NaOH simultaneously. To verify that TGFβ signalling was measured in this reporter assay, sera were treated with anti-TGFβ1/2/3 for 1 hour at RT before use.

Results Control sera significantly induced reporter activity by 4.5-fold. However, SSc sera only induced a 2.5-fold increase in luciferase activity, indicating significantly lower bioactivity of TGFβ (p<0.0001). This difference was not due to a difference in total TGFβ levels; after activation of all TGFβ both HC and SSc sera induced a similar 6-fold increase in signal strength. These data show that in HC sera approximately 75% of all TGFβ is bioactive compared to only 42% in SSc. Addition of anti-TGFβ1/2/3 inhibited BRE-luc reporter activity (p<0.0001) of both HC and SSc serum, and of both acidified and not acidified sera (p<0.0001), showing that our bioassay is indeed TGFβ-dependent. To investigate if reduced bioactivity is a more general phenomenon we measured BMP activity. BMP proteins are structurally closely related to TGFβ and also circulate in inactive form. Both HC and SSc sera induced a similar 8-fold increase in BRE-luc activity, and this activity was increased to a 16-fold induction after acidification for both groups. BMPs in SSc sera are thus not less bioactive. This illustrates the uniqueness of our observation on TGFβ bioactivity.

Conclusions TGFβ in SSc serum is less bioactive than in control serum whereas BMPs are not less bioactive.

Disclosure of interest None declared

EFFECTS OF IL-17 AND THE HEPATOCYTE–MONONUCLEAR CELL INTERACTIONS IN THE HEPATIC INFLAMMATORY RESPONSE

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Methods Human HepaRG cell line and primary human hepatocytes (PHH) were cultured with or without IL-6, anti-IL-6 receptor (anti-IL-6R) antibody, IL-17 and/or TNFα. For co-cultures, PBMC from healthy donors activated or not with phytohemagglutinin (PHA) were added on hepatocytes at a 10% ratio. Transwell system was used to study the contribution of the direct cell-cell contact. Cytokine expression and production were quantified by qRT-PCR and ELISA respectively and
the secretion of C-reactive protein (CRP) by an automated analyzer.

Results IL-17 and TNFα induced in synergy the hepatic IL-6 production (≥8 fold, p<0.01) and CCL-20 expression (≥100 fold, p<0.01). CRP secretion was induced by IL-17/TNFα and the anti-IL-6R inhibited this induction. CCL-20 expression was not increased by IL-6 stimulation or reduced by the addition of the anti-IL-6R to the IL-17/TNFα treatment. In HepaRG cell-PBMC cultures, IL-6 and IL-17 were produced only in PHA-activated conditions and the IL-6 and IL-17 levels were higher in co-cultures vs PBMC mononucleotides (≥14 and ≥2 fold respectively, p<0.01). Transwell system that avoids direct cell-cell contact decreased the IL-6 and IL-17 secretion by 4- and 2-fold respectively in PHA-activated HepaRG cell-PBMC cultures. CRP production increased when PHH were cultured in presence of PHA-activated PBMC compared to PHH alone with PHA.

Conclusions The IL-17/TNFα synergistic effect on hepatocytes mediates systemic inflammation by inducing CRP secretion through the IL-6 pathway and mononuclear cell recruitment by acting on CCL-20 expression in an IL-6 independent manner. Direct and indirect hepatocyte-PBMC interactions contribute to the hepatic inflammatory response by increasing the IL-6 and IL-17 secretion. The induction of Th17 cell differentiation by IL-6 and the increase of CCL-20 mediated Th17 cell recruitment by IL-17 may lead to a vicious and chronic inflammatory cycle.

Disclosure of interest None declared

A GENETIC VARIANT OF IL-32 IS ASSOCIATED WITH THE EX VIVO CYTOKINE PRODUCTION OF ANTI-TNF TREATED PBMCs ISOLATED FROM RHEUMATOID ARTHRITIS PATIENTS

Introduction Since the introduction of biologics in the treatment of rheumatoid arthritis (RA) disease outcome improved. Still, about 40% of RA patients do not respond to therapy with TNFα blockers. Previously, a strong link between TNFα and interleukin (IL)–32 has been reported in RA.

Objectives We hypothesise that a promoter single nucleotide polymorphism (SNP) in IL-32 can affect clinical responsiveness to anti-TNFα treatment in RA patients, functioning as a new biomarker in treatment of RA.

Methods Peripheral mononuclear cells (PBMCs) from RA patients and healthy individuals were stimulated with RPMI or recombiant human (rh)TNFα to study the mRNA and protein expression of IL-32 and other pro-inflammatory cytokines. Moreover, disease activity scores (DAS28), ‘in vitro response’ and clinical response to anti-TNFα therapy (etanercept, adalimumab), of RA patients were measured and all were stratified for the IL-32 SNP (C/T).

Results Stimulation of PBMCs from RA patients was followed by higher IL-32 protein production and a tendency towards higher IL-32β and IL-32γ mRNA expression compared to healthy individuals. When data was stratified for the IL-32 promoter SNP, patients bearing the CC genotype showed higher IL-32 protein expression. Of interest, these patients also produced more cytokines. Even though the DAS28 did not depend on the presence of the promoter SNP, the ‘ex vivo’ cytokine response did have a different pattern in clinical responders depending on the genotype.

Conclusions IL-32 mRNA and protein production was higher in RA patients compared to healthy individuals, with a trend towards higher concentrations in patients bearing the CC genotype. Regardless of the fact that the promoter SNP was not associated with disease activity, IL-1 beta production in the CC-genotype might predict clinical response to either etanercept or adalimumab.

References

Disclosure of interest None declared