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Original article

The role of interleukin-6 trans-signalling on cardiovascular dysfunction in inflammatory arthritis

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Abstract

Objectives. Cardiovascular (CV) mortality in RA patients is 50% higher than in the general population. There is increasing recognition that systemic inflammation is a major driver of this. IL-6 is implicated in cardiovascular disease (CVD) in the general population but its role in CVD in RA is undefined. Of the two modes of IL-6 signalling, trans-signalling is pro-inflammatory whereas classical signalling is linked with inflammation resolution. This study examines the role of IL-6 trans-signalling in CVD in a mouse model and patients with RA.

Methods. Myography determined the effect of IL-6 trans-signalling blockade, using sgp130Fc, on aortic constriction in murine collagen-induced arthritis. Serum CCL2 and sVCAM-1 as soluble biomarkers of sIL-6R trans-signalling were investigated in a human cross-sectional study. An observational longitudinal study investigated the association between these biomarkers and progression of subclinical atherosclerosis in early RA by measuring carotid intima-media thickness (CIMT).

Results. sgp130Fc reduced arthritis severity, serum CCL2 and sVCAM-1 and restored vascular function in collagen-induced arthritis (CIA). In established RA, sVCAM-1 correlated with the 28-joint DAS (DAS28) and CV risk. In early RA, baseline DAS28 was associated with CIMT change at 6 months. CIMT 'rapid progressors' at 12 months had higher baseline sVCAM-1, haemoglobin A1c, cholesterol:high-density lipoprotein cholesterol ratio and LDL cholesterol.

Conclusions. IL-6 trans-signalling plays a pivotal role in vascular dysfunction in CIA. In early RA, sVCAM-1 was associated with progression of subclinical atherosclerosis. Inflammation from RA onset in CVD-susceptible individuals may accelerate atherosclerosis. IL-6 trans-signalling blockade may be beneficial to RA patients and perhaps for atherosclerosis in the general population.

Key words: RA, inflammation, cardiovascular diseases, experimental arthritis

Rheumatology key messages

- IL-6 trans-signalling blockade using spg130Fc improved arthritis severity and restored vascular function in murine CIA.
- In early RA, baseline sVCAM-1 was higher in patients who developed rapid progression of subclinical atherosclerosis.
- Blockade of IL-6 trans-signalling is promising for RA and its comorbidity of cardiovascular disease.

Introduction

Cardiovascular (CV) mortality in patients with RA is 50% higher than in the general population [1]. Although well

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established that the incidence of cardiovascular disease (CVD) is increased, the precise cause is unclear. There is increasing recognition that systemic inflammation is a major driver of increased CV risk [1–3]. CV risk is evident even before a clinical RA diagnosis is made [4]. IL-6 is implicated in CVD in the general population but its role in CVD in RA is not well defined. IL-6 can activate cells via two signalling pathways, IL-6 classical signalling and IL-6 trans-signalling. In classical signalling, IL-6 binds to membrane-bound IL-6 receptor (mIL-6R). The complex of IL-6/mIL-6R associates with the β-signalling receptor gp130, which dimerizes and instigates intracellular signalling [5]. IL-6R is only present on hepatocytes and some leucocyte subpopulations, including monocytes, neutrophils and some T cells and B cells [6]. Thus classical signalling only affects certain cells. In addition to signal transduction through mIL-6R, there is also 'trans-signalling'. Here, soluble IL-6R (sIL-6R), which is generated by either alternative splicing or ectodomain shedding [7], triggers gp130 signalling by binding to IL-6R [6]. As gp130 is ubiquitously expressed, trans-signalling enables IL-6/sIL-6R to activate cells that lack mIL-6R. Of the two modes of IL-6 signalling, evidence demonstrates that trans-signalling is pro-inflammatory whereas classical signalling has regenerative or anti-inflammatory effects [6, 8]. The aim of this study was to examine the role of IL-6 trans-signalling in CVD in RA by experimental and translational studies.

The well-characterized model of murine collagen-induced arthritis (mCIA) offers a method to study early systemic inflammatory changes in RA and associated vascular responses and is considered the gold standard *in vivo* model for RA studies [9]. Since increased CV risk precedes RA onset, mCIA offers an ideal opportunity to identify the earliest pathological changes within the vasculature that could subsequently contribute to CVD. To mechanistically investigate these tissues in human disease is difficult, if not impossible. Vascular dysfunction has been described in several animal models of arthritis, including adjuvant-induced arthritis [10] and mCIA [11]. We have previously characterized vascular responses in mCIA, observing decreased constriction responses in aortae [12, 13]. Soluble forms of gp130 (sgp130) are the natural inhibitors of IL-6 trans-signalling [14] and previous studies found that sgp130Fc improved arthritis severity in mCIA [15]. To test the hypothesis that targeting IL-6 trans-signalling can improve vascular dysfunction in mCIA, we used sgp130Fc and observed aortic constriction responses *ex vivo*.

It is difficult to quantify the level of IL-6 trans-signalling in mouse models and RA patients; levels of IL-6, sIL-6R, sgp130, IL-6/sIL-6R complex and IL-6/sIL-6R/sgp130 complex can be measured but the affinity of IL-6 to IL-6R is low, in the range of 1 nM [16]. Therefore serum proteins linked to IL-6 trans-signalling (VCAM-1 and CCL-2) were measured as potential biomarkers of both trans-signalling and disease activity and vascular function in CIA and validated in patients with established and early RA. VCAM-1 and CCL2 are associated with CVD in the general population. Previous work using a BioMAP system found that sIL-6R regulates the release of VCAM-1 and CCL2 [17].

Rapid progression of CVD occurs soon after RA onset [18]. Importantly, the current modified systemic coronary risk evaluation (mSCORE) index recommended by the EULAR is predicted to underestimate CVD risk in RA patients [19]. Several validated non-invasive imaging techniques are now available as surrogate markers for determining subclinical atherosclerosis in RA [20]. Of these, ultrasonographic assessment of carotid intima-media thickness (CIMT) and the presence of plaques is capable of stratifying RA patients with high CV risk. In this study, CIMT and CIMT progression over time were used as surrogate markers of CVD and CVD progression.

In short, to examine the role of IL-6 trans-signalling in CVD in RA we investigated whether blocking IL-6 trans-signalling using sgp130Fc, without inhibiting IL-6 classical signalling, restores vascular function in CIA and whether proteins regulated by IL-6 trans-signalling (VCAM-1 and CCL-2) are associated with vascular dysfunction in CIA and markers of CVD in RA patients.

Methods

Animals

Experiments were undertaken in 8-week-old male DBA-1 mice (Charles River, Margate, UK). Procedures were performed in accordance with Home Office–approved project license 30/2928 and personal licence I5/6CC73C0.

mCIA

DBA-1 mice were induced with mCIA as previously described [9, 21]. Mice were immunized on two occasions, 21 days apart, with 100 μL intradermal injections of emulsion containing 1 mg/ml type II chicken sternal collagen and 2.5 mg/ml Freund's complete adjuvant (both from Sigma-Aldrich, Gillingham, UK). Temgesic (0.4 mg/ml) was administered *ad libitum* via drinking water on day 20 and continued until the end of each experiment.

Assessment of arthritis

Joint swelling was assessed daily following immunization on day 21 as previously described [9]. Hind paw swelling was recorded using an analogue micrometre. Arthritis severity in each paw (paw score) was assessed using an established scoring system from 0 to 5 [9] (Supplementary Data S1, available at *Rheumatology* online). The sum of scores for all four paws provided the clinical score for each mouse.

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sgp130Fc therapy in CIA

Mice were randomly assigned to one of three treatment groups on day 21. Animals received either i.v. sgp130Fc (2.5 mg/kg), etanercept (2.5 mg/kg; Amgen, Cambridge, UK) or PBS on day 21 and day 28 ($n = 10$ in each group). Mice were killed on day 30. Ten mice served as age- and sex-matched non-immunized controls.

Collection of experimental samples for mCIA

At the experimental endpoint, mice were killed by inhalation of CO_2. Subsequently the aorta was exposed and vented in the abdomen. The left ventricle was perfused with 500 μL physiological Krebs solution (mmol/l: NaCl 10.92, KCl 2.68, KH_2PO_4 1.78, $MgSO_4 \bullet 7H_2O$ 2.49, $NaHCO_3$ 25.10, glucose 10.99, $CaCl_2 \bullet 2H_2O$ 1.98). The thoracic aorta was dissected and immediately placed in Krebs solution for myography.

ELISA

Approximately 1 ml of whole blood was obtained from mice after sacrifice. After centrifuging at 4°C at 10 000 rpm for 10 min, 200 μl of serum was aliquoted. Serum CCL2, sVCAM-1 and sgp130 were measured using ELISA kits (R&D Systems, Abingdon, UK) in accordance with manufacturer's instructions.

Assessment of aortic constriction in murine CIA

Myography was used to investigate vascular constriction to serotonin (5-HT) in isolated sections of thoracic aorta. Aortic rings without perivascular adipose tissue (2 mm thick) were bathed in physiological Krebs solution at 37°C and gassed with 95% O_2/5% CO_2. Rings were set to baseline tension of 5 mN. Following equilibration, rings were exposed to 60 mmol/l potassium Krebs (mmol/l: NaCl 39.36, KCl 59.83, KH_2PO_4 1.18, $MgSO_4 \bullet 7H_2O$ 2.49, $NaHCO_3$ 25.10, glucose 10.99, $CaCl_2 \bullet 2H_2O$ 1.98; Sigma-Aldrich). Once constriction had reached a plateau, tissues were washed and allowed to return to baseline tension. Cumulative concentration responses to 5-HT (1 nmol/l–10 mmol/l) were then performed. Contractile response was measured using MyoDak software, analysed using MyoData and calculated as developed tension (in mN).

RA patients

A total of 182 patients with established RA, defined by ACR/EULAR 2010 criteria [22] and diagnosis >1 year, were recruited to a cross-sectional study (Research Ethics Committee for Wales reference no. 12/WA/0045). Demographic details are shown in Table 1. Forty-five patients with early RA (symptoms <6 months) were recruited. Demographic details are shown in Table 2. Patients were assessed every 6 months for 12 months. A total of 35 patients were followed up at 6 months and 18 patients were followed up at 12 months. Ethnicity was 91% Caucasian, 4% Indian, 2% Pakistani and 2% Czech (Research Ethics Committee for Wales reference no. 11/WA/0326).

Table 1 Demographic and clinical data for 182 patients with established RA

Variables	Values
Age, years, mean (S.E.M.)	60 (1.2)
Gender, %	
Female	67
Male	33
Disease duration, years, mean (S.E.M.)	13.1 (1.0)
Rheumatoid factor positive, %	63.9
Anti-CCP antibody positive, %	66.3
CRP, mg/l, mean (S.E.M.)	11.9 (2.1)
ESR, mm/h, mean (S.E.M.)	22 (1.7)
DAS28, mean (S.E.M.)	3.6 (0.2)
Systolic BP, mmHg	129 (2.2)
Cholesterol:HDL ratio, mean (S.E.M.)	3.8 (0.1)
QRISK2, %, mean (S.E.M.)	16 (1.5)
Framingham, %, mean (S.E.M.)	13 (0.9)
Taking any DMARD, %	70
Taking methotrexate, %	49
Taking any biologic, %	21
Taking tocilizumab, %	10
Taking corticosteroids, %	22

Table 2 Demographic and clinical data for 45 patients with early RA at baseline

Variables	Values
Age, years, mean (S.E.M.)	56.1 (2.2)
Female, %	75
Disease duration, months, mean (S.E.M.)	4.0 (0.2)
RF positive, %	59
Anti-CCP antibody positive, %	75
CRP, mg/l, mean (S.E.M.)	11 (2)
ESR, mm/h, mean (S.E.M.)	24 (3)
DAS28, mean (S.E.M.)	3.87 (0.20)
Extra articular features, %	15
Systolic BP, mmHg, mean (S.E.M.)	136 (3.2)
QRISK2, %,mean (S.E.M.)	16.9 (2.5)
QRISK2 >10%, %	54
Framingham score, %,mean (S.E.M.)	14.5 (2.2)
SCORE, %, mean (S.E.M.)	1.1 (0.4)
Smoking, %	
Never smoked	39.5
Former	34.9
Current	25.6
BMI, mean (S.E.M.)	27.3 (0.9)
HbA1c, mmol/mol, mean (S.E.M.)	40.2 (1)
Family history of CVD <60 years, %	18
Taking any DMARD, %	63
Taking methotrexate, %	58
Taking biologics, %	0
Taking two DMARDs, %	30
Taking NSAIDs, %	22
Taking corticosteroids, %	24

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Patient assessments

The 28-joint DAS (DAS28) [23] was calculated. CV risk was calculated for each patient using the QRISK2, Framingham and SCORE algorithms.

Carotid US

A total of 45 patients with early RA underwent carotid US. Examinations were performed by the same investigator in a quiet, temperature-controlled room. Patients were positioned supine after resting for 30 min. The left and right common carotid arteries were imaged longitudinally 1 cm proximal to the carotid bifurcation. Images were focused on the posterior wall of the artery and magnified. Several 10 sec cine loops were recorded in DICOM format and downloaded for offline analysis, performed by a single observer. An automated carotid analyser (Carotid Analyzer, Medical Imaging Applications, Iowa City, IA, USA) was used to measure CIMT. Three end-diastolic frames were selected and CIMT measured, defined as the interface between lumen-intima and media- adventitia, for the right and left carotid arteries. The mean of three end-diastolic frames was calculated, then the mean of the left- and right-sided readings was calculated. The internal and external carotid arteries and bifurcation were scanned and the presence/absence of plaque noted. CIMT >0.90 mm and/or carotid plaques were used as the gold standard test for subclinical atherosclerosis and high CV risk as per Corrales *et al*. [24]. These patients were classed as 'US positive'. Those without plaque and CIMT <0.9 mm were 'US negative'. A previous study by Södergren *et al*. [25] in 27 patients with early RA found that the mean increase in CIMT at 18 months was 0.05 mm (S.D. 0.15). For this study, 'rapid progressors' were defined as those who had an increase in CIMT >0.05 mm. The carotid distensibility coefficient in 10^{-3}/kPa was calculated using the equation from Dijk *et al.* [26]:

$$(2 \times \Delta D/D_d)/PP) \times 1000.$$

Measurement of IL-6, sIL-6R, IL-6/sIL-6R complex, CCL2 and sVCAM-1

Blood was taken into Vacutainer serum separation tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 1300–1800 *g* for 5 min at 4°C. Serum was aliquoted and frozen at −80°C. After thawing, IL-6, sIL-6R, IL-6/sIL-6R complex, CCL2 and sVCAM-1 were measured using ELISA kits (R&D Systems) in accordance with the manufacturer's instructions.

Statistical analysis

Statistics used were dependant on the experiments performed. Variables were checked for normality; normally distributed data are presented as the mean (S.D.) and non-normally distributed data are expressed median (interquartile range). Differences between groups were analysed using paired (for comparison between the same patients) or unpaired (for comparison between distinct test subjects) *t*-tests for normally distributed data. Differences between groups with non-normally distributed data were analysed using the Mann–Whitney *U* test. Where multiple groups were compared, a one-way analysis of variance and *post hoc* Bonferroni test were performed. Binary logistic regression was used to examine the effect of multiple variables on particular outcomes. Differences with p-values <0.05 were considered significant. Statistics were calculated using SPSS version 26 (IBM, Armonk, NY, USA).

Patient and public involvement

Patients were involved in the design and conduct of this research. A local ambassador for the National Rheumatoid Arthritis Society was consulted on study design and updated on study progress and findings.

Results

I.v. sgp130Fc reduced arthritis incidence and severity in immunized DBA-1 mice and restored vascular function in CIA

To determine whether blockade of IL-6 trans-signalling improved arthritis severity and restored vascular function, i.v. sgp130Fc was administered to mice immunized with CIA. Arthritis incidence on day 29 was 90% for PBS-administered mice, 50% for sgp130fc-administered mice and 50% for etanercept-administered mice (Fig. 1A). The mean total paw score for PBS-administered mice [4.3 (S.D. 3.4)] was significantly higher than mice administered sgp130Fc [1.5 (S.D. 1.8)] and etanercept-administered mice [1.2 (S.D. 1.7), $P < 0.05$] (Fig. 1B). There was a significant reduction in maximal developed tension in PBS-administered mice [5.8 mN (S.D. 2.4)] compared with non-immunized control mice [8.5 mN (S.D. 1.8), $P < 0.05$]. There was no significant difference in mean maximal developed tension between sgp130Fc- [8.1 mN (S.D. 2.0)] and etanercept-treated [7.7 mN (S.D. 1.9)] mice and non-immunized controls (Fig. 1C).

sgp130Fc reduced serum CCL2 and sVCAM-1 levels in immunized mice compared with those administered PBS

Given that sgp130Fc improved indices of arthritis severity and vascular function during CIA, we measured serum sVCAM-1 and CCL2, both known to be associated with atherosclerosis and regulated by IL-6 trans-signalling [17]. Serum CCL2 was significantly higher in PBS- [117.3 pg/ml (S.D. 14.5)] and etanercept-administered [116.7 pg/ml (S.D. 36.7)] mice compared with sgp130Fc [41.87 pg/ml (S.D. 12.1)] and non-immunized controls [16.9 pg/ml (S.D. 4.8); $P < 0.001$] (Fig. 2A). Serum sVCAM-1 was significantly higher in PBS-administered mice [3144 ng/ml (S.D. 366)] compared with controls [1861 ng/ml (S.D. 160)], mice administered etanercept [2077 ng/ml (S.D. 173) and mice administered i.v. sgp130Fc [1979 ng/ml (S.D. 136); $P < 0.01$] (Fig. 2B).

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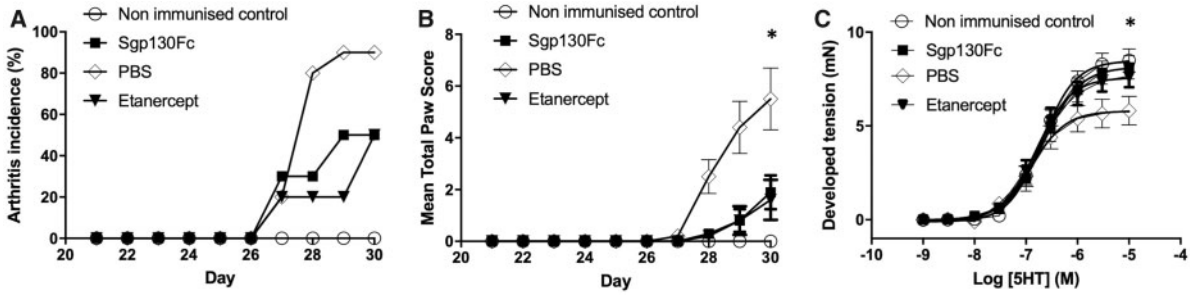
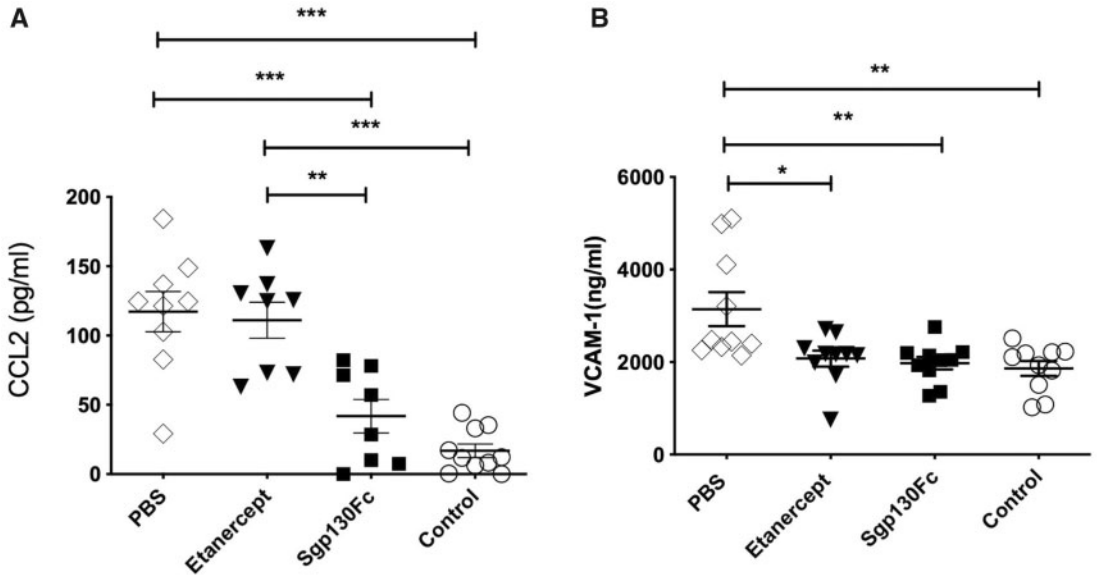
FIG. 1 Intravenous sgp130Fc reduced arthritis incidence and severity in immunized DBA-1 mice and restored vascular function in CIA



(**A**) Arthritis incidence and (**B**) paw score over time for mice immunized with CIA and administered i.v. sgp130Fc, etanercept or PBS. There was a significant reduction in mean total paw score at day 30 in mice administered sgp130Fc or etanercept compared with those administered PBS. (**C**) Vasoconstriction concentration–response curves to 5-HT in aortic rings. There was a significant reduction in maximal developed tension in mice with CIA administered PBS compared with non-immunized control mice. No significant difference in mean maximal developed tension was seen between sgp130Fc- and etanercept-treated mice and non-immunized controls. $n = 10$ in each group. $^*P < 0.05$.

FIG. 2 sgp130Fc reduced serum CCL2 and sVCAM-1 levels in immunized mice compared with those administered PBS



(**A**) Significantly higher serum CCL2 was seen in mice with CIA administered PBS or etanercept compared with sgp130Fc and non-immunized controls. $n = 9$ in each group. (**B**) Significantly higher serum sVCAM-1 was seen in PBS-treated mice compared with controls, mice administered etanercept and mice administered sgp130Fc. $n = 10$ in each group. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

In immunized DBA-1 mice there was a significant positive correlation between paw score and both serum sVCAM-1 and serum CCL2 level.

Serum sVCAM-1 is associated with disease activity and CV risk in established RA

To evaluate the relevance of sVCAM-1 in clinical disease, we measured serum sVCAM-1 in 182 patients with established RA and assessed the relationship between this and disease activity and CV risk. There was a significant positive correlation between the DAS28 and sVCAM-1 ($r = 0.27$, $P = 0.017$) and between the QRISK2 score and serum sVCAM-1 ($r = 0.32$, $P = 0.0025$). Patients defined as low CV risk according to the QRISK2 had significantly lower serum sVCAM-1 [977 ng/ml (S.D. 600) *vs* 1247 (588)] than those classified as high risk.

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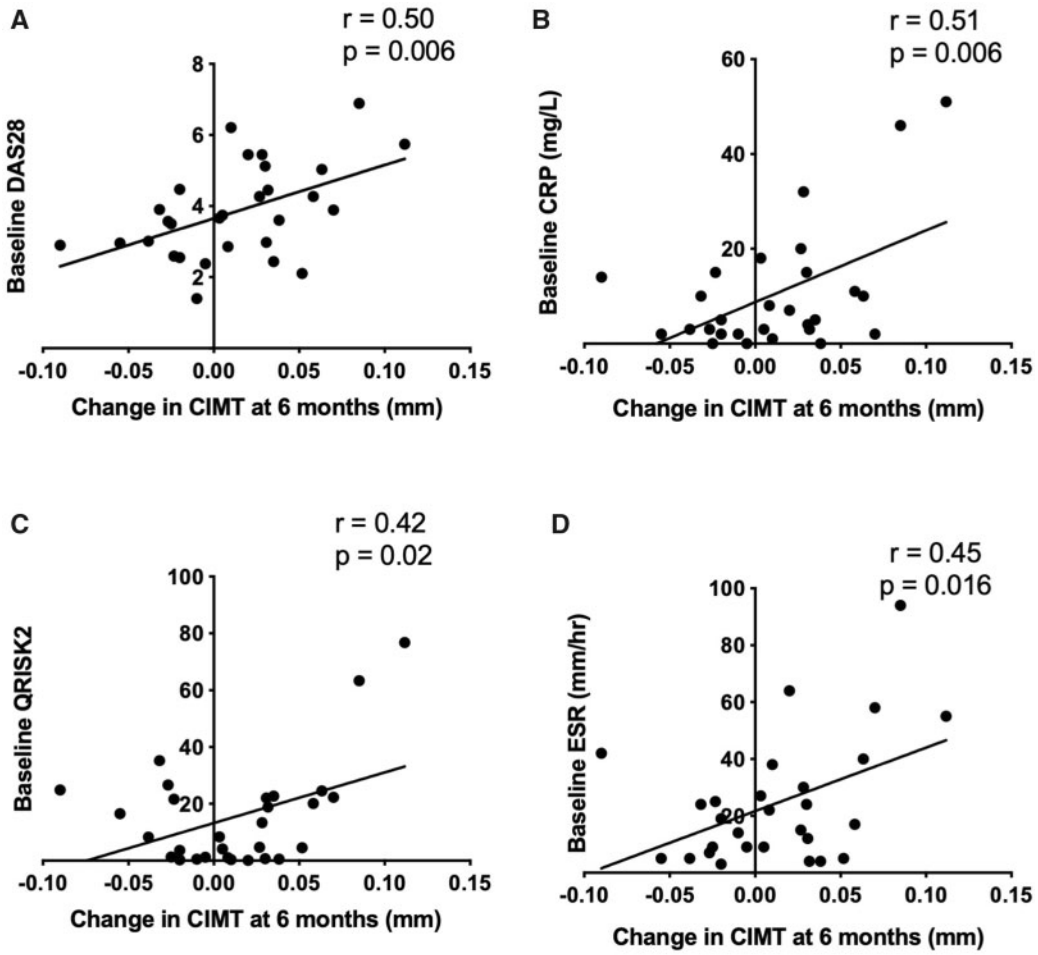
FIG. 3 Significant positive correlation between the change in CIMT at 6 months and (**A**) baseline DAS28 ($r = 0.50$, $P = 0.006$), (**B**) baseline CRP ($r = 0.51$, $P = 0.006$), (**C**) baseline QRISK2 ($r = 0.42$, $P = 0.02$) and (**D**) baseline ESR ($r = 0.45$, $P = 0.016$).



Traditional risk factors are higher in carotid US–positive patients with early RA

A total of 41% of patients were carotid US positive. These patients had significantly higher age, BMI, systolic blood pressure (BP), total cholesterol, cholesterol:HDL ratio and LDL cholesterol as well as higher QRISK2, Framingham, SCORE and American College of Cardiology/American Heart Association scores than US-negative patients (see Supplementary Data S2, available at *Rheumatology* online). Multivariate logistic regression analysis including age, BMI, systolic BP, total cholesterol, cholesterol:HDL ratio, LDL cholesterol and ESR as covariates found that total cholesterol, LDL cholesterol, age, BMI and systolic BP were significant independent variables.

There was no significant difference in CIMT at 12 months [0.706 mm (S.D. 0.048)] compared with 6 months [0.729 mm (S.D. 0.041)] or baseline [0.708 mm (S.D. 0.033)]. There were significant positive correlations between baseline DAS28, baseline CRP, baseline ESR and baseline QRISK2 and change in CIMT at 6 months (Fig. 3).

Baseline sVCAM-1 higher in rapid progressors and those with plaque

Rapid progressors were defined as those who had an increase >0.05 mm in CIMT; 3 of 18 patients (17%) were classified as rapid progressors at 12 months. Baseline total cholesterol, cholesterol:HDL ratio, LDL cholesterol, haemoglobin A1c (HbA1c) and sVCAM-1 were significantly higher in rapid progressors compared with non-rapid progressors (Fig. 4). Baseline QRISK2, Framingham and SCORE scores were significantly higher in rapid progressors and the arterial distension coefficient was significantly lower in rapid progressors (see Supplementary Data S3, available at *Rheumatology* online).

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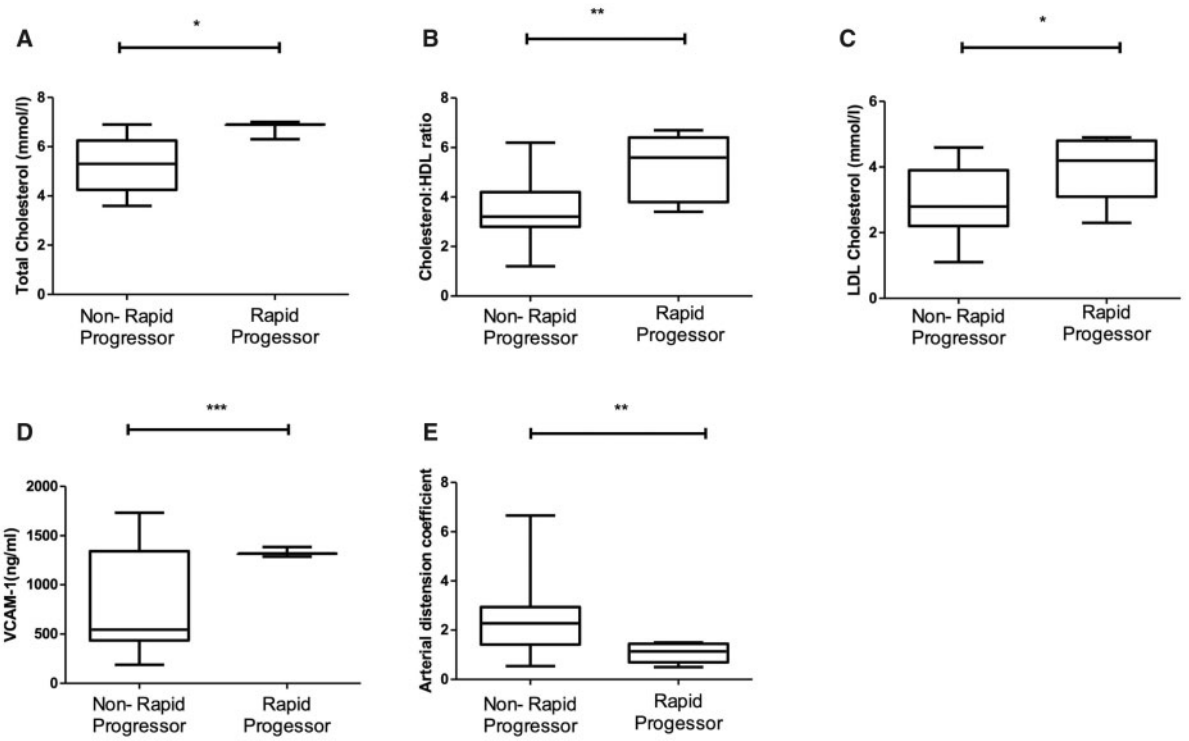
FIG. 4 Significantly higher baseline. (**A**) total cholesterol [6.7 mmol/L (S.D. 0.2) *vs* 5.2 (0.3)], (**B**) cholesterol:HDL ratio [3.7 (S.D. 0.3) *vs* 5.5 (0.7)], (**C**) LDL cholesterol [3.1 mmol/L (S.D. 0.3) *vs* 4.4 (0.3)], (**D**) sVCAM-1 [734 ng/ml (S.D. 126) *vs* 1328 (28)] in rapid progressors compared with non-rapid progressors. (**E**) Significantly lower arterial distension coefficient was seen in rapid progressors [18.8 (S.D. 1.8)] compared with non-rapid progressors [11.2 (S.D. 2.2)].



*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Baseline sVCAM-1 was significantly higher in those with plaque at baseline [1049 ng/ml (S.D. 315) *vs* those without plaque at baseline [653 ng/ml (S.D. 436)]. Baseline sVCAM-1 was also significantly higher in those with plaque at 6 months [1082 ng/ml (S.D. 341)] than those without plaque at 6 months [547 ng/ml (S.D. 380)]. At both 6 and 12 months, age and LDL cholesterol were higher in those with plaque, but there was no significant difference in other variables including IL-6, sIL-6R, CRP, ESR, IL-6/sIL-6R complex and CCL2.

Discussion

Prior studies have shown that mCIA is associated with aortic contractile dysfunction [9, 13]. The work here validates this finding but, for the first time, we show that selective blockade of IL-6 trans-signalling using sgp130Fc reduces arthritis severity and prevents vascular dysfunction associated with mCIA. This improvement in vascular function may be due to the effect of IL-6 trans-signalling on macrophage recruitment into the vessel wall and surrounding adipose tissue. Previous work by Williams *et al*. [22] found that mice with mCIA have increased macrophages in the aorta and perivascular adipose tissue. Kraakman *et al*. [27] found IL-6 trans-signalling recruited macrophages to adipose tissue in high fat diet–induced obesity in mice and that sgp130Fc prevented macrophage accumulation in adipose tissue. The improvement in vascular function in mice treated with sgp130Fc in our study was associated with a reduction in serum CCL2 and sVCAM-1. The effect of sgp130Fc on vascular function was similar to TNF-α blockade. TNF-α can stimulate release of IL-6, but this study did not directly compare TNF-α with IL-6 trans-signalling inhibition on vascular function. However, our results suggest inhibition of IL-6 trans-signalling alone can restore vascular dysfunction in CIA.

It is known that in early RA, CV risk is increased. We confirm the high prevalence (41%) of subclinical atherosclerosis in patients with early RA. Carotid US–positive patients had several traditional risk factors that were significantly higher than in carotid US–negative patients: age, BMI, systolic BP, total cholesterol, cholesterol:HDL ratio and LDL cholesterol. We confirm previous findings that some traditional risk factors (HbA1c, total cholesterol, cholesterol:HDL ratio and LDL cholesterol) predict CIMT progression in early RA. However, we also show

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the novel finding that baseline serum sVCAM-1 is elevated in RA patients who become rapid progressors in terms of subclinical atherosclerosis. This adds weight to the finding that patients with established RA classified as high CV risk had higher serum sVCAM-1 than those classified as low CV risk. It should be noted that the number of rapid progressors in this study was low, and further work to validate this finding in larger cohorts is needed. We also show that patients with plaque at baseline and at 6 months have higher sVCAM-1, and baseline disease activity correlates with the change in CIMT at 6 months. Taken together, these findings suggest that in RA, inflammation from disease onset may accelerate atherosclerosis in susceptible individuals.

In this study, sVCAM-1 (a product of a shedding reaction by ADAM17 [28], which also sheds TNF-α and IL-6R [29]), was associated with disease states. Restoration of vascular function in mice immunized with CIA and treated with sgp130Fc was associated with reduced serum sVCAM-1. Serum sVCAM-1 correlated with disease activity and CV risk in established RA, and in early RA, baseline sVCAM-1 was higher in patients who developed rapid progression of subclinical atherosclerosis. What remains to be elucidated is whether serum sVCAM-1 can predict CV events in RA patients. In addition, these consistent findings of association of sVCAM-1 in disease states prompt consideration about the potential role of blockade of sVCAM-1 in the treatment of RA and atherosclerosis.

Studies have consistently shown that tocilizumab (monoclonal antibody against the IL-6R) is associated with increased lipid levels in the context of decreased inflammatory markers in RA patients [30, 31]. The ratio of LDL:HDL remains stable after treatment with tocilizumab due to the parallel increase of LDL and HDL. The ENTRACTE study compared the rates of major CV outcomes in RA patients treated with tocilizumab or etanercept [32]. Total cholesterol, LDL, HDL and triglycerides increased significantly in the tocilizumab arm compared with the etanercept arm, but there was no difference in major CV events over the follow-up of 3.5 years. Tocilizumab has also been shown to improve endothelial function, leading to a greater increase of effective myocardial work than conventional DMARDs plus glucocorticoids through a reduction of inflammatory burden and oxidative stress [33]. In a mouse model, serum cholesterol levels and atherosclerotic lesion formation were significantly increased in ApoE$^{-/-}$IL-6$^{-/-}$ mice compared with ApoE$^{-/-}$ and wild-type mice [34]. Conversely, mice with hyperactive gp130/IL-6/STAT3 signalling (gp130$^{F/F}$:ApoE$^{-/-}$ mice) have reduced aortic plaque development and triglyceride levels [35]. In another mouse study, sgp130Fc treatment led to significant regression of advanced atherosclerosis in Ldlr$^{-/-}$ mice [36]. In a Swedish cohort of 60-year-olds, the ratio between functional moieties of IL-6 trans-signalling, IL-6/sIL-6R and IL-6/sIL-6R/sgp130 was associated with CV event risk [37]. These observations outline a specific role for IL-6 in regulating lipid metabolism and CV risk.

The advantage of blocking IL-6 trans-signalling over IL-6R inhibition (e.g. tocilizumab) is that although the latter is a very effective treatment for RA, several side effects have been reported. These include abnormalities in liver function tests, an increase in infection rates and a risk of gastrointestinal perforation if the patient has pre-existing diverticulitis [38]. IL-6R inhibition blocks both IL-6 classical and trans-signalling, whereas sgp130Fc uniquely blocks trans-signalling. gp130 is a shared receptor utilized by several related cytokines, including IL-6, IL-11, IL-27, leukaemia inhibitory factor, oncostatin M and cardiotrophin 1. In mammals, gp130 plays a critical role during development and gp130-deficient mice are embryonically lethal [39]. sgp130Fc blocks IL-6 trans-signalling by binding to the complex of IL-6/sIL-6R so that this cannot bind to membrane-bound gp130. Thus other cytokines can still signal via gp130, including IL-6. Therefore classical signalling and its associated regenerative and protective effects can continue [6].

IL-6 trans-signalling appears to play a pivotal role in vascular dysfunction in mCIA. Results in mCIA were more consistent than in human studies in terms of association of disease states with VCAM-1 and CCL-2, likely due to the fact that RA is more heterogeneous than mCIA. In humans, VCAM-1, which is regulated by IL-6 trans-signalling, was associated with CV risk scores in established RA and progression of subclinical atherosclerosis in early RA. These findings suggest that blockade of IL-6 trans-signalling may be beneficial to RA patients, and perhaps for atherosclerosis in the general population. Sgp130Fc successfully underwent phase 1 clinical trials in 2014 and is currently under the name Olamkicept in phase 2 clinical trials for use in the treatment of inflammatory bowel disease [40]. This could also have therapeutic applicability in the management of RA and CVD.

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Data availability statement

Data are available upon reasonable request by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). All data relevant to the study are included in the article.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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