inflammatory infiltrate rich in T cells. It is not known whether recruited T cells induce fibrosis or react to developing fibrosis.

Objectives Chemokines (CC) are involved in cell recruitment into inflammatory sites. Our aim was to characterise the CC receptor (R) usage as well as the effector function of T cells infiltrating a developing fibrotic skin lesion in a SLE patient.

Methods T cells from a lesional skin biopsy were grown *in vitro* in the presence of IL-2. TcR, accessory molecules, and CCR expression were analysed by flow cytometry and RNase protection assay. The capacity to release interferon- γ (IFN- γ) and IL-4 was assessed upon CD3-crosslinking in phenotypically homogeneous T cell lines or clones. The chemotactic responses to synthetic CC was assessed. Immunohistochemistry was performed on the same tissue.

Results T cells infiltrating the affected skin were heterogeneous in terms of accessory molecules (CD4⁺, CD8⁺, and CD4CD8 double negative) as well as of TcR (a/b and g/d) expression. The majority preferentially produced IFN- γ , while a minority produced both IFN- γ and IL-4. Preferential expression of type-1-related CXCR3 (and response to relevant CC) was observed and confirmed by tissue immunohistochemistry. Interestingly, several T cells coexpressed type 1 and type 2-related CCR and migrated to an equivalent extent in response to specific ligands.

Conclusion The analysis performed on T cells infiltrating a novel fibrotic skin lesion from a SLE patient has revealed heterogeneous expression of CC receptors by T cells preferentially producing IFN- γ . Since IFN- γ exerts a potent inhibitory activity on ECM deposition by fibroblasts, these results hint to a reactive mechanisms aimed to control the extent of fibrosis.

REFERENCE

- 1 Chizzolini C, Rezzonico R, Ribbens C, Burger D, Wollheim FA, Dayer J-M. Inhibition of type I collagen production by dermal fibroblasts upon contact with activated T cells. Different sensitivity to inhibition of systemic sclerosis and control fibroblasts. *Arthritis Rheum.* 1998;**41**:2039–47

FRI0209 RATE OF CEREBRAL MICROEMBOLI IN APS CAN BE INFLUENCED VIA BLOCKADE OF GPIIb/IIIa PLATELET RECEPTOR WITH TIROFIBAN

¹C Specker, ¹A Perniok, ²T Menge, ²M Siebler, ¹M Schneider. ¹Department of Rheumatology; ²Department of Neurology, Heinrich-Heine-University, Düsseldorf, Germany

10.1136/annrheumdis-2001.290

Background Patients with antiphospholipid syndrome (APS) are prone to thromboembolism. So far, the only predictive parameters for further complications are their number in patient's history and perhaps the titre of aPL. Derived from clinical investigation of stroke and obvious analogies between cerebrovascular ischemia (CVI) in patients with carotid artery disease (CAD) and patients with APS, a novel non-invasive method using transcranial Doppler (TCD) is used for detection of so called 'microembolic signals' (MES) in the cerebral vasculature. In patients with CAD, these 'microemboli' proved to correlate with past and impending symptoms of CVI allowing for therapeutic stratification by their detectability. We found very similar signals in the cerebral bloodstream of APS patients with a history of CVI.

Detectability of MES in APS implies occlusive thromboembolism and not vasculitis as the underlying mechanism at least in arterial cerebrovascular manifestations of this state. However, Doppler signal properties (i.e. the reflected energy) do not allow

for direct estimation of the signal's substrate size or composition.

Objectives To further elucidate the pathophysiological substrate of MES in APS.

Methods Repeated TCD monitoring in a 38 year old female APS patient with ongoing transitory ischaemic attacks and high rate of MES before and after intravenous administration of Tirofiban, a potent, synthetic, highly selective and reversible inhibitor of platelet gpIIb/IIIa receptor which binds fibrinogen on the platelet's surface.

Results Within one hour the rate of MES decreased from ~50/hr to <5/hr on three successive occasions accompanied by cessation of clinical symptoms. Using flowcytometry, platelet-leucocyte aggregates found increased before administration of Tirofiban were also reduced considerably.

Conclusion The finding that MES are influenced by blockade of the platelet gpIIb/IIIa receptor and correlate with platelet-leucocyte aggregates argues for a central role of thrombocytes in APS with CVI. This might favour to look for thrombocyte targeted prophylactic therapy in APS. Whether detection of MES can also serve to monitor such therapies has still to be proved.

REFERENCES

- 1 Specker C, Rademacher J, Sohngen D, Sitzler M, Janda I, Siebler M, Steinmetz H, Schneider M. Cerebral microemboli in patients with antiphospholipid syndrome. *Lupus* 1997;**6**(8):638–44
- 2 Specker C, Perniok A, Brauckmann U, Siebler M, Schneider M. Detection of cerebral microemboli in APS – introducing a novel investigation method and implications of analogies with Carotid artery disease. *Lupus* 1998;**7**(Suppl 2):75–80

FRI0210 CARDIAC INVOLVEMENT IN SYSTEMIC SCLEROSIS (SSC) – DOPPLER ECHOCARDIOGRAPHY STUDIES IN 100 SSC PATIENTS

AV Volkov, GP Kotelnikova, ES Match. Department of Microcirculation and Inflammation, Institute of Rheumatology of RAMS, Moscow, Russia

10.1136/annrheumdis-2001.291

Background Cardiac involvement is a relatively common occurrence in systemic sclerosis. When this complication is clinically recognisable, the prognosis is usually poor. However, heart abnormalities are frequently asymptomatic and they can only be diagnosed by Doppler- echocardiography.

Objectives The aim of this study is to find out the frequency of different variants of cardiac involvements in SSC pts and connexion with other SSC manifestation.

Methods 100 SSC pts (18 male and 82 women, mean age $\pm$ SD 51,8 $\pm$ 14,1 years, ranging from 15 to 83 years, mean duration of disease $\pm$ SD 10,11 $\pm$ 9,22 years). All of the pts were clinically studied with Doppler Echocardiography.

Results According to the Doppler Echocardiography studies cardiac involvement was found in 82% occurrences, and was the most frequent symptoms of internal organ damage. Pericard, myocard and endocard were involved. Pericarditis was found in 30% pts, higher frequency (25%) in limited cutaneous SSC, and correlated with disease duration. Pericarditis pts have more frequently interstitial lung disease, pulmonary hypertension. The microcirculation changes such as tendency to decreasing of basal digital blood flow and the reduction of capillar loops density were noted in the pericarditis patients. The changes in the size of left ventricular were more frequent in pts with diffuse cutaneous SSC, shorter duration of the disease and arterial hypertension, the latter does not exclude the influence of age factors in evolution of these changes. Diastolic disfunction was found in 25%

and most frequently in the elderly pts with arterial hypertension. However, the fact of the correlation between diastolic dysfunction and high level of ESR ($r = 0,364$, $p < 0,05$) as well as fibrinogen concentration ($r = 0,325$, $p < 0,05$) and reduction of capillary loops do not exclude the influence of sclerodermic fibrosis on these abnormalities. Mitral (22%) and aortal (5%) regurgitations were the most frequent indication of endocard damage, however, there was no significant difference between these abnormalities and other clinical manifestations of SSc. Fifty one out of 100 pts (51%) were diagnosed as having pulmonary hypertension (PH), including 15 pts having isolated PH without other antecedent pulmonary or cardiac conditions. Correlations were found between PH and age of pts, high level of ESR and fibrinogen concentration. Twenty-six pts out of 100 did not complain on cardiac problem, but Doppler Echocardiography studies showed pericarditis in two cases, diastolic abnormality in three cases, mitral regurgitation in four cases and pulmonary hypertension also in four cases.

Conclusion Doppler Echocardiography is an important method in diagnosis of cardiac involvement in SSc pts including those without any symptoms of sclerodermic cardiac disease.

FRI0211 SERUM CARNITINE MEASUREMENT IN PRIMARY RAYNAUD'S PHENOMENON

¹L La Civita, ²G Pugliese, ¹P Fadda, ¹I Olivieri. ¹Rheumatology Department of Lucania; ²Lab Unit, San Carlo Hospital, Potenza, Italy

10.1136/annrheumdis-2001.292

Background Raynaud's phenomenon (RP) is a microcirculatory disorder characterised by deficient endothelial activity. Such endothelial dysfunction should be responsible for reduced release of NO, a potent vasodilatory agent. Since the cell function is dependent on energy production, it is though deficient endothelial activity could be due to the loss of energy production probably secondary to a low availability of Carnitine, an important cell metabolic carrier.

Objectives To conduct a preliminary evaluation of the serum Carnitine concentrations in primary RP patients (pRP pts.) compared to age- and sex-matched controls.

Methods After verbal consent, 17 consecutive pRP pts (16 female, mean age 32.1 ± 11.3 yrs) and ten control subjects (9 female, mean age 34 ± 11 yrs) were enrolled for this study. Patients had to have a RP history no longer than 5 years. After acclimatisation, each subject underwent a venous blood drawing from same vessel at baseline and after a 5-minute cold-water test. All blood samples were stored $\geq 20^\circ\text{C}$. Then, Carnitine was measured in collected sera by enzymatic assay (SIGMA-TAU pharmaceuticals). T-test for paired data was used for statistical analysis.

Results Table 1 showed the mean values of serum Carnitine concentrations in two studied groups. After test, serum Carnitine concentration increased in 12/17 pRP patients and lowered in the remainders. Contrary, in the control group circulating Carnitine increased significantly after provocative test.

Conclusion Lower circulating Carnitine titres in our pRP pts could be associated with diminished availability of Carnitine in endothelial cells and consequently with their impaired activity. Although this study is preliminary, our results suggest Carnitine supplementation for promotion of endothelial activity in pRP pts may be worth investigating, particularly in RP subjects unresponsive to conventional treatments.

Abstract FRI0211 Table 1

	Baseline	After test	p-value
pRP (17, mg/L)	$34.4 \pm 16.7 \pm 1.9$	$35.9 \pm 6.9 \pm 1.7$	n.s.
Controls (10, mg/L)	$33.5 \pm 16.1 \pm 5.1$	$42.7 \pm 19.5 \pm 6.2$	0.0005

OP0036 MOLECULAR CLONING AND CHARACTERISATION OF AUTOANTIGEN RECOGNISED BY ANTI-WA ANTIBODIES IN PATIENTS WITH SYSTEMIC SCLEROSIS

¹Y Shirai, ¹T Nojima, ¹M Hirakata, ²H Yamagata, ³K Miyachi, ⁴T Mimori. ¹Internal Medicine, Keio University; ²Internal Medicine, Natl. Murayama Hospital, Tokyo; ³Internal Medicine, Keigo Clinic, Yokohama; ⁴Clinical Immunology, Kyoto University, Kyoto, Japan

10.1136/annrheumdis-2001.293

Background Anti-Wa antibodies were first reported in patients with systemic sclerosis (SSc) as autoantibodies reactive with a 48kDa transfer RNA-associated protein.¹ However, the nature of the Wa antigen has not been clarified so far.

Objectives To identify and characterise the Wa antigen by molecular cloning using anti-Wa serum as a probe.

Methods Molecular cloning of the target Wa antigen was performed using lambda gt11 cDNA library constructed from human liver mRNA and anti-Wa prototype serum as a probe. Bound antibodies were eluted from the fusion proteins of the isolated clones expressed in E.coli and were used for RNA-immunoprecipitation assay. The reactivity of fusion proteins prepared from lysogenic E.coli Y1089 carrying the recombinant phages were examined using five sera that were proved to have anti-Wa antibodies in double immunodiffusion assay. The cDNA inserts of the isolated clones were sequenced, and the detailed homologies of nucleotide and amino acid sequences were analysed through the BLAST server at NCBI DNA databank. Anti-Wa antibodies in sera from patients with various rheumatic diseases were detected by immunoblot analysis using the fusion protein from the positive clone.

Results 10 positive clones were recognised by the probe serum. In RNA-immunoprecipitation, the affinity-purified antibodies from only one clone (termed Wa-1) immunoprecipitated transfer RNA from HeLa cell extracts. In immunoblot study, the Wa-1 fusion protein was recognised by all five sera that were proved to have anti-Wa antibodies. These data confirmed that the Wa-1 clone encoded for the Wa antigen. The nucleotide sequence and deduced amino acid sequence of the 0.7-kb insert cDNA of Wa-1 were completely identical with the C-terminal part of NEFA (named after DNA-binding domain, two EF-hands, Acidic amino acid rich region)/nucleobindin-2.² In immunoblots using the Wa-1 fusion protein, autoantibodies to Wa (NEFA/nucleobindin-2) were detected in 3 of 21 SSc (21%), one of 10 RA (10%) and one of 16 SLE sera (6%). However, neither RA nor SLE sera immunoprecipitated transfer RNA from HeLa cells, while all 3 SSc sera immunoprecipitated transfer RNA, suggesting the diversity of autoepitopes among patient sera.

Conclusion These results demonstrated that the autoantigen recognised by anti-Wa antibodies were proved to be identical with NEFA/nucleobindin-2.

REFERENCES

- 1 Miyachi K, et al. *J Rheumatol.* 1991;**18**:373-8
- 2 Barnikol-Watanabe S, et al. *Biol Chem Hoppe Seyler* 1994;**375**:497-512