Background IL10 is a pleiotrophic cytokine that inhibits the antigen specific activation of Th1 clones by reducing both the antigen presenting capacity and the production of pro-inflammatory cytokines by monocytes. It is also a potent activation and differentiation factor for B lymphocytes. SLE patients show increased IL10 serum levels associated with the pathogenesis of the disease. Peripheral blood mononuclear cells from SLE patients, and their relatives, constitutively produce high levels of the cytokine, suggesting an hereditary component in IL10 production. Anti-IL10 antibodies administration to SLE patients may be beneficial in the management of refractory cases. The IL10 gene promoter contains two dinucleotide repeats (microsatellites): IL10G and IL10R, close to transcription factor binding sites, probably playing an important role in the transcription regulation.

Objectives To evaluate the association of the IL10G microsatellite with SLE in Spanish patients.

Methods 80 unrelated SLE patients from Canary Islands (Spain) fulfilling at least four of the ACR criteria and 79 sex-matched healthy donors were included. A segment of the IL10 gene containing the microsatellite was amplified by PCR and the resulting products were electrophoresed in denaturing polyacrilamide gels. Chi-square test was used for statistical analysis.

Results No particular IL10G allele was found to be significantly associated with SLE. Nevertheless, we found an elevated risk of serositis in carriers of the allele 19 ($p = 0.007$), a significant association of the 19/23 genotype with seizures ($p = 0.018$), and a reduced risk of renal disorder ($p = 0.047$) and thrombocytopenia ($p = 0.049$) in carriers of the allele 23.

Conclusion Although IL10G alleles seem not to influence susceptibility to SLE in Spaniards, some of them may be related to the presence of several clinical manifestations of the disease.

FRI0090 THE PRESENCE OF MICROCHIMERISM IN SYSTEMIC LUPUS ERYTHEMATOSUS: PRELIMINARY DATA

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Background The development of microchimerism after solid organ transplantation, but also after blood transfusions and during pregnancy, is no longer considered controversial. Controversy still exists on the immunological significance of this phenomenon, such for example its role on the development of tolerance to the graft, or of some autoimmune conditions such as scleroderma (SSc). In this respect several studies have showed the presence of fetal cells not only in the peripheral blood but also in the skin of SSc patients. These observations, along with epidemiological (female prevalence, onset after childbearing years) and clinical (similarities with chronic graft versus host disease) data, have led to the hypothesis that microchimerism may play a role in the aetiopathogenesis of this disease.

Objectives The aim of our study was to evaluate the prevalence of microchimerism in a population of SLE patients in comparison with SSc patients followed at our Unit.

Methods Anticoagulated peripheral blood was obtained from 22 female patients (13 SLE and 9 SSc) who had a previous male pregnancy followed at our Unit. Genomic DNA was isolated from anticoagulated blood using a DNA extraction kit. A specific Y-chromosome sequence was detected by amplifying DNA in a nested-polymerase chain reaction (PCR) using published primers.

Results Microchimerism was present in 62% of SLE patients and 89% of patients with SSc, the mean time interval between the pregnancy and the analysis was of 27 years and no significative differences were observed between positive and negative patients. In all scleroderma patients the pregnancy occurred before the onset of the disease (onset of Raynaud's phenomenon). In SLE patients 38% of the pregnancies occurred before the onset of the disease, and 62% after the onset of the disease, however no differences in the development of microchimerism were observed.

Conclusion Since the literature data report a prevalence of 33% of microchimerism in healthy controls, these preliminary data may suggest that microchimerism develops more easily in patients with various autoimmune conditions. Further studies on extended populations of CTD patients and healthy controls appear necessary, in order to look for permissive factors for its development, such for example immunosuppressive therapy or immunological abnormalities.

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FRI0091 ANGIOGENESIS IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND PRIMARY ANTIPHOSPHOLIPID SYNDROME (PAPS)

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Background Angiogenesis is an essential process of the body's physiology and plays a role in several diseases such as rheumatoid arthritis, neoplasias or atherosclerosis. Vascular endothelial growth factor (VEGF) and angiogenin (Ang) are factors that promote angiogenesis *in vitro* and *in vivo*. However, new vessels formation seems limited to tissues with some degree of inflammation or with hypoxia. SLE and PAPS are characterised by endothelial damage and the mechanisms of angiogenesis have not been explored in these diseases.

Objectives In this study we determine the possible role of VEGF and Ang in the pathogenesis of SLE and PAPS.

Methods We included 28 SLE patients, 10 PAPS patients and 24 matched controls. VEGF and Ang plasma levels were measured by ELISA.

Results The VEGF plasma levels were significantly increased in SLE patients compared with PAPS patients and controls, $p < 0.01$. The Ang levels were similar in the three groups. No correlation between VEGF levels and clinical manifestations was found.

Abstract FRI0091 Table 1

	Gender (F/M)	Age (years)	VEGF (pg/ml) (median/range)
SLE Group	24/4	36.6 ± 16.1	70.25 (4.69-341.2)
PAPS Group	8/2	36.2 ± 8.0	34.1 (6.91-83.78)
Control	19/5	29.2 ± 8.5	23.48 (0-307.4)

Conclusion The raised levels of VEGF in SLE might be related to the process of endothelial repair present in different organs of lupus patients.

OP0009 SLE-RELATED T CELL AUTOIMMUNITY TO HISTONES AND NUCLEOSOMES IS AN INHERENT PROPERTY OF THE NORMAL IMMUNE SYSTEM

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Background Systemic lupus erythematosus (SLE) is characterised by the generation of pathogenic B cell and T cell autoimmunity to DNA and nucleosomes. Human polyomavirus T-antigen may play a central role in initiating B cell and T cell autoimmunity to nucleosomes. Recent data demonstrate that nucleosome-T-antigen complexes stimulate nucleosome-specific B cells and T-antigen specific T cells through a hapten-carrier-like process, also indirectly resulting in termination of non-responsiveness of histone-specific T cells. This process is relevant to both SLE and normal individuals.

Objectives This work focuses on whether immune (T-antigen specific) and autoimmune (histone-specific) T cells from normal individuals share functional, structural and genetic characteristics with those deriving from SLE.

Methods Histone-specific T cell clones were generated by stimulation of peripheral blood mononuclear cells with nucleosome-T-antigen complexes and subsequently maintained by pure histones. T-antigen specific T cell clones were initiated and maintained by polyomavirus T-antigen. The frequencies of circulating T cells specific for these antigens were determined in healthy individuals and SLE patients by limiting dilution of blood mononuclear cells. T cell receptor gene usage and structure of their variable regions were determined by cDNA sequencing. The sequences were compared between the T cell populations, and between normal and SLE subjects for both T-antigen and histone specificity.

Results The frequencies of circulating T-antigen- or histone-specific T cells in normal individuals were similar to those of SLE patients. Some of the histone-specific T cells, derived from normal individuals, were clonally expanded, indicating that these cells are functionally activated *in vivo* in healthy individuals. Although heterogeneous for variable region structure and gene usage, histone specific T cells from healthy individuals and SLE patients selected glutamic and/or aspartic acids at position 99 and 100 of the Vb-CDR3.

Conclusion Autoimmune T cells induced among peripheral blood mononuclear cells from normal individuals were highly similar to those from SLE, demonstrating that T cell autoimmunity to nucleosomes is an inherent property of the normal immune system.

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OP0071 A PATHOGENIC ANTI-DNA ANTIBODY BINDS TO ALPHA-ACTININ IN RENAL MESANGIAL CELLS

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Background Anti-DNA antibodies have been demonstrated to play an important role in the pathogenesis of nephritis in systemic lupus erythematosus. One mechanism by which anti-DNA antibodies may contribute to nephritis is by cross-reactivity with renal antigens. However, the identity of this renal antigen remains elusive.

Objectives To identify the target antigen bound by a pathogenic anti-DNA antibody in renal tissue.

Methods Western blotting and immunoprecipitations of total cell lysate from mesangial cell lines derived from the autoimmune MRL-lpr/lpr mouse (lpr-mc) and a non-autoimmune BALB/c mouse (mc), with R4A, a pathogenic murine IgG2b anti-DNA antibody, and with 95, a non-pathogenic antibody derived from R4A by site-directed mutagenesis. The immunoprecipitated protein was identified by matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS) of tryptic digests.

Results The pathogenic anti-DNA antibody R4A, but not control isotype-matched antibodies, bound to and immunoprecipitated a 100 kD protein from mesangial cell lysates. Binding was more pronounced using lpr-mc rather than non-autoimmune mc lysate. Little effect on binding was seen after treatment of the cell lysate or R4A with DNase, while proteinase K treatment abolished binding. Preliminary MALDI-MS analysis revealed that alpha-actinin, an actin bundling protein that plays a role in experimental nephritis models, is present in the protein band immunoprecipitated by R4A. R4A, but not 95, binds to immobilised alpha-actinin by ELISA; Furthermore, double stranded DNA significantly inhibited the binding of R4A to the 100 kD mesangial cell protein, as well as to purified alpha-actinin. Serum of MRL-lpr/lpr lupus mice with active disease contain high-titer antibodies to alpha-actinin.

Conclusion The nephritogenicity of some anti-DNA antibodies may be mediated via cross-reactivity with a glomerular antigen. Variations in the display of the cross-reactive antigen in the target organ between individuals may underlie differential susceptibility to anti-DNA antibody induced renal disease.

OP0072 INCREASED CALRETICULIN EXPRESSION ON SURFACE MEMBRANES OF MONOCYTES AND NEUTROPHILS IN SYSTEMIC LUPUS ERYTHEMATOSUS: RELEVANCE TO APOPTOTIC CELL CLEARANCE

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Background Increased numbers of apoptotic leukocytes¹ and impaired clearance of apoptotic cells² have been demonstrated in SLE. Patients with C1q deficiency almost invariably develop SLE, probably because C1q is important in the clearance of apoptotic cells by monocyte/macrophages.³ Calreticulin has been shown to bind to the early complement component C1q, and