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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10.1136/annrheumdis-2001.1234

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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10.1136/annrheumdis-2001.1235

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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10.1136/annrheumdis-2001.1236

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

interfere with complement-mediated immune processes. Patients with systemic lupus erythematosus (SLE) have increased serum levels of calreticulin.⁴ C-reactive protein (CRP) facilitates the clearance of apoptotic cells in the presence of C1q,⁵ but CRP levels are inappropriately low in SLE.

Objectives To measure the expression of calreticulin on the surface of peripheral blood monocytes and neutrophils in SLE patients compared to normal and rheumatoid arthritis (RA) subjects, and correlate these with disease activity.

Methods Peripheral blood was sampled from 28 SLE, 17 normal, and 19 RA subjects. Calreticulin expression on monocytes and neutrophils was measured by immunofluorescence labelling and flow cytometry, and the density of surface expression of calreticulin expressed as mean channel fluorescence (MCF) values.

Results Calreticulin was expressed on the surface of over 90% of monocytes and neutrophils in all three groups. No differences in the percentage of cells expressing calreticulin were seen when the three groups were compared. The density of surface expression of calreticulin on monocytes was significantly higher in SLE (median MCF = 4.45) compared with normal subjects (median MCF = 3.25, $p = 0.014$). Density of surface expression of calreticulin on neutrophils was also higher in SLE (median MCF = 19.6) compared with normal subjects (median MCF = 14.50, $p = 0.002$). Calreticulin expression did not correlate with SLAM or BILAG scores. There were trends towards higher surface calreticulin expression on monocytes and neutrophils in SLE compared with RA, but these did not reach statistical significance.

Conclusion Calreticulin expression is increased on monocyte and neutrophil cell surface membranes in SLE. The combination of increased monocyte and neutrophil surface calreticulin expression (which can interfere with C1q-mediated clearance), and inappropriately low levels of serum CRP and/or C1q (which facilitate this clearance), may contribute to the increased circulating apoptotic leukocytes seen in SLE.

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OP0073 HISTONE RELEASE INTO THE CYTOPLASM OF HUMAN PBMCs AND PREAPOPTOTIC LYMPHOBLASTS

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10.1136/annrheumdis-2001.1237

Background Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by a wide range of antibodies reacting with nuclear antigens. Abnormalities in the apoptotic cell death process and apoptotic cell clearance may play an important role in generating these antibodies. Especially antibodies to histones in serum are frequently associated with SLE.

Objectives The aims of this study were to evaluate the presence of histones in the cytoplasm of fresh peripheral blood mononuclear cells (PBMCs) and in activated lymphoblasts. The activated lymphoblasts cultured without IL-2 served as a model for apoptosis induction.

Methods Human PBMCs were isolated from heparinized peripheral blood of normal donors ($n = 6$) and activated with 1 µg/ml phytohemagglutinin (PHA) and 10 U/ml IL-2 for the first 5 days. The following two days, the cells were expanded in IL-2 containing medium (0 h). Lymphoblasts were then incubated in IL-2 free medium for 8 or 24 h as well as for 24 h with IL-2 (10 U/ml). Furthermore, lymphoblasts were treated with IL-2 after 8 or 24 h IL-2 deprivation. PBMCs and lymphoblasts were lysed in a modified RIPA-buffer, 20 µg of protein were subjected to denaturing SDS-PAGE. Western blot detection was performed with antibodies against all 5 histones H1, H2A, H2B, H3 and H4 as well as against phosphorylated H3 and acetylated H4.

Results Only very weak signals of the histones H2A, H2B, H3 and H4 were detected in the cytoplasm of freshly isolated PBMCs, but H1 was undetectable. The activated lymphoblasts showed elevated amounts of all five histones compared to PBMCs. Furthermore, we observed an increased amount of the histones H2A, H2B, H3 and H4 in the cytoplasm in cell culture after another 8 or 24 h without IL-2. Interestingly, the signal intensity of these histones in the cytoplasm decreased significantly after addition of IL-2 to the lymphoblasts after 8 or 24 h IL-2 deprivation.

However, no difference of the detectable amount of H1 was noted in activated lymphoblasts compared to preapoptotic lymphoblasts incubated for another 8 or 24 h without IL-2. In addition, for H1 no effect of addition of IL-2 was observed.

Lymphoblasts activated with IL-2 for 24 h showed the same signal intensities for the histones H2A, H2B, H3 and H4 as observed for the cells at 0 h.

No bands for acetylated H4 or phosphorylated H3 were detected in cytoplasm samples in contrast to strong signals in nuclear preparations.

Conclusion These results suggest that histones were released from the nucleus into the cytoplasm of preapoptotic human lymphoblasts. The mechanism of the transport of the histones into the cytoplasm is until now unknown. Acetylated H4 and phosphorylated H3 were only detected in nuclear extracts which excludes nuclear membrane fragility and nuclear contamination as cause for our findings. In addition, our data indicate that IL-2 can reduce the release of histones from the nucleus into the cytoplasm.

OP0074 PLATELET CD40 L EXPRESSION IN SLE AND ANTIPHOSPHOLIPID SYNDROME

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10.1136/annrheumdis-2001.1238

Background CD40-CD40L interaction is a crucial costimulatory factor in T-cell mediated B-cell activation. Intervention into this system allows efficient modulation of immune response in transplantation and autoimmunity e.g. systemic lupus erythematosus (SLE). Recently CD40L (CD154) has been described as a novel antigen on activated platelets with impact towards thrombus formation and interplay with endothelial cells. Altered platelet CD40 L expression was already described in procoagulatory situations like instable angina pectoris.

Objectives As platelet activation by phospholipid antibodies is found in SLE and especially in Antiphospholipid syndrome (APS) we analysed CD40L expression in SLE and secondary APS.