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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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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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.

Methods Using flowcytometric whole blood and platelet rich plasma double staining assay systems we analysed CD40 L expression. PE labelled CD40L (cloneTRAP1, Pharmingen) was employed, FITC-CD42b as detection system for platelets. 40 patients with diagnosis of SLE were included into this study, among them 10 with secondary APS and compared to 15 normal healthy donors (NHD). Results were analysed using Cellquest software (Becton Dickinson, San Jose, USA) differentiating single from aggregated platelets under nonstimulatory and stimulatory conditions (thrombin 30 μ M, ADP 10 μ M).

Results We found that CD 40 L expression was higher on aggregated compared to single platelets, where fluorescence means were close to baseline. We compared the whole blood method with platelet rich plasma and found lower background activity in whole blood test. Using established stimulators of platelets we could increase CD40L expression on platelets, especially on aggregated platelets. Analysing CD40L expression in SLE patients we found no significant difference between SLE and NHD, neither on single or on aggregated platelets nor upon stimulation with ADP or thrombin. High CD40 L expression was observed in APS with highly significant differences compared to NHD and SLE analysing single platelets ((NHD < APS ($p < 0,0001$) SLE < APS ($p < 0,0001$)) as well as aggregated platelets. Upon stimulation with thrombin or ADP results were comparable indicating significantly higher CD40 L expression on platelets.

Conclusion We first describe high CD40 L expression on single and aggregated platelets in APS establishing CD40L as a novel platelet activation marker in APS. Interplay between CD40L on platelets and endothelial cells may contribute to procoagulatory conditions in APS. On the other hand we could not show altered CD40L expression in patients with SLE without clinical APS.

OP0075 CHROMATIN CLEARANCE FROM NECROTIC CELLS BY A CONCERTED ACTION OF DNASE 1 AND SERINE PROTEASES RESEMBLES APOPTOTIC DNA-DEGRADATION

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Background Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by the presence of antinuclear antibodies (ANA) which tend to accumulate as immunodeposits in different tissues and cause inflammations. One main feature of SLE-pathogenesis seems to be a reduced elimination of cellular debris after cell death.^{1,2} The recently generated mice deficient for the endonuclease Dnase 1 has underscored the important influence of defects of chromatin clearance for the onset of SLE.³

Objectives

Methods Cell nuclei of MCF-7 cells were isolated and either incubated with serum from Dnase 1 wildtype and Dnase 1 deficient mice or with recombinant human DNASE 1 alone and in combination with different serine proteases. Subsequently the DNA was isolated and analysed by agarose gel-electrophoresis.

DNA-degradation of MCF-7 cells incubated with different amounts of native or heat inactivated bovine serum in DMEM was analysed under apoptotic (2 μ M staurosporin) and necrotic conditions (1 mM H₂O₂) or after complement lysis due to the

application of 6.7% (v/v) rabbit complement. Subsequently the DNA was isolated and analysed.

Results Recombinant human Dnase 1 could induce an apoptotic-like DNA-fragmentation pattern in isolated MCF-7 cell nuclei by a concerted action with different serine proteases like plasmin, trypsin, a-chymotrypsin, thrombin and kallikrein. Dnase 1 alone only induces an unspecific, time-retarded DNA-degradation. Serum from Dnase 1 wildtype mice also induced DNA-laddering in cell nuclei whereas serum from knockout mice did not due to the lack of Dnase 1. The laddering capacity of wildtype serum could be inhibited by serine protease inhibitors. In contrast to apoptotic cells native bovine serum and not heat-inactivated serum induces internucleosomal DNA-laddering in MCF-7 cells lysed by rabbit complement or by the cytotoxic action of H₂O₂.

Conclusion This study gives insight into a new function of Dnase 1 which could be linked with its protective role towards the induction of an anti-DNA immunoreaction and subsequently towards SLE-pathogenesis.^{2,3} Dnase 1 in connexion with serine proteases is able to effectively degrade the genomic DNA of nuclei and of cells lysed by complement or the cytotoxic action of H₂O₂. In contrast apoptotic cells were not influenced by serum Dnase 1 and serine proteases. Since apoptotic cells undergo a cell-autonomous intracellular DNA-degradation process one can conclude that Dnase 1 in connexion with serine proteases fulfills this process for necrotic cells.

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OP0076 KIDNEYS OF AUTOIMMUNE NZB/W MICE CONTAIN LONG-LIVED PC

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Background NZB/W mice develop a disease similar to human systemic lupus erythematosus (SLE), including autoantibody production, hypergammaglobulinaemia and inflammation of the kidneys. Large numbers of lymphocytes infiltrate the kidneys of these mice but the role of this organ for the production of antibodies is not clear.

Objectives Here, we compare the role of bone marrow, spleen and inflamed kidneys of NZB/W mice for the activation of B cells and for the persistence of antibody secreting cells (ASC).

Methods NZB/W mice were immunised i.p.i with Ovalbumin and then boosted 1 month later. The number of antigen-specific PC present in spleen, bone marrow and kidneys was determined by Elispot. The frequency of different cell types was determined by flow-cytometry. The distribution of PC in the different organs was investigated by microscopy.

Results ASC are present in the kidneys of mice with full blown disease, as many as in the spleen and bone marrow, and 50 times more than in the kidneys of normal mice. In the kidneys, ASC are located mainly in the outer medulla, close to B- and T cell infiltrates. The specificity of the ASC in the inflamed kidneys is not restricted to self-antigens. After immunisation of NZB/W