SUPPLEMENTAL MATERIAL

Urbonavicuite et al., http://www.jem.org/cgi/content/full/jem.20081165/DC1

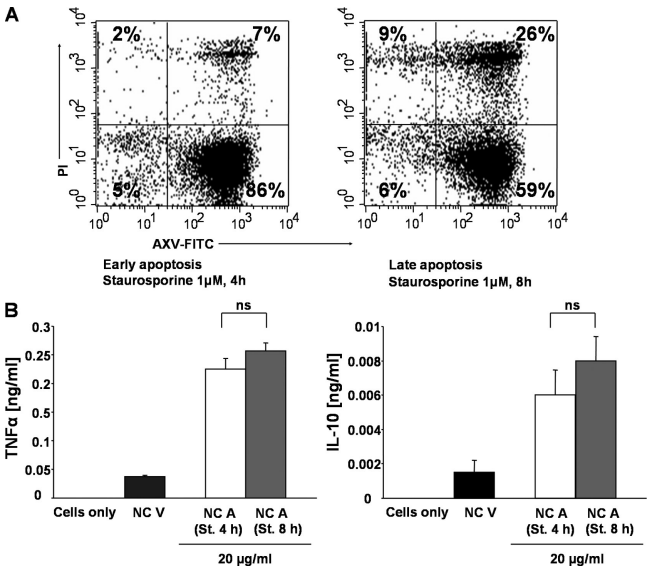

Figure S1. Nucleosomes purified from early and late apoptotic cells have similar proinflammatory potential. (A) Evaluation of apoptotic cell death. Jurkat cells were treated with 1 μM staurosporine either for 4 or 8 h. 10^5 cells were then stained with Annexin V–FITC/propidium iodide and analyzed by flow cytometry. Percentages of Annexin V–FITC/propidium iodide double-negative, Annexin V–FITC-positive/propidium iodide–negative, Annexin V–FITC-negative/propidium iodide–positive, and Annexin V–FITC/propidium iodide double-positive cells are depicted on dot plots. A representative dot plot is shown. (B) Nucleosomes purified from early and late apoptotic cells (see A) induce TNF-α and IL-10 release from human macrophages. Human macrophages were cultured in the absence or in the presence of 20 μg/ml of nucleosomes, purified from viable cells (black) as well as early (white) and late (gray) apoptotic cells. Untreated human macrophages served as a negative control. Cell culture supernatants were harvested after 24 h and concentrations of TNF-α and IL-10 were measured by ELISA. Mean values and SD were calculated from triplicates. Student's *t* test was used for statistical analysis. One representative experiment of three independent experiments is shown.

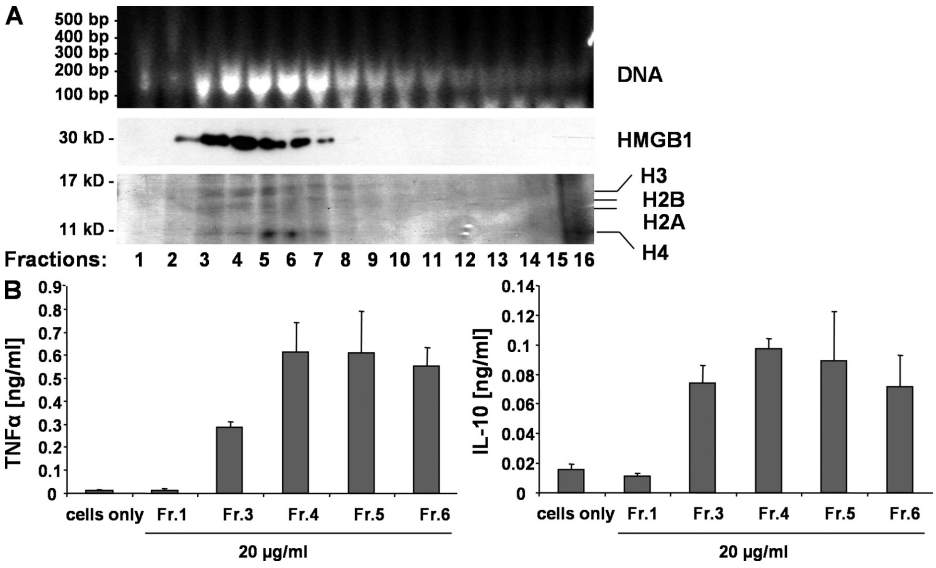

Figure S2. HMGB1–nucleosome complexes spontaneously released during secondary necrosis display proinflammatory properties. (A) Isolation of nucleosomes spontaneously released into cell culture supernatant. Apoptosis in Jurkat cells was induced by UVB irradiation. After 48 h, when virtually all cells had undergone secondary necrosis, the supernatants were collected, filtered through a 0.2-μm sterile filter, and concentrated 100× using ultrafiltration columns (Millipore) with a molecular mass cutoff of 100 kD followed by sucrose gradient ultracentrifugation. 16 fractions were collected from the top (1) to the bottom (16) of the gradient and were analyzed by 1.5% agarose gel electrophoresis in the presence of 1% SDS for detection of DNA. HMGB1 was visualized by Western blotting. The protein composition of chromatin was revealed by SDS-PAGE and Coomassie blue staining. (B) Spontaneously released HMGB1–nucleosome complexes potently stimulate TNF-α and IL-10 secretion by primary human macrophages. Human macrophages were cultured in the absence or in the presence of HMGB1–nucleosome complexes containing fractions 3–6 (20 μg/ml of protein). Fraction 1, containing neither DNA nor HMGB1, served as a negative control. Cell culture supernatants were harvested after 24 h and concentrations of TNF-α and IL-10 were measured by ELISA. One representative experiment out of three is shown. Mean values and SD were calculated from triplicates.

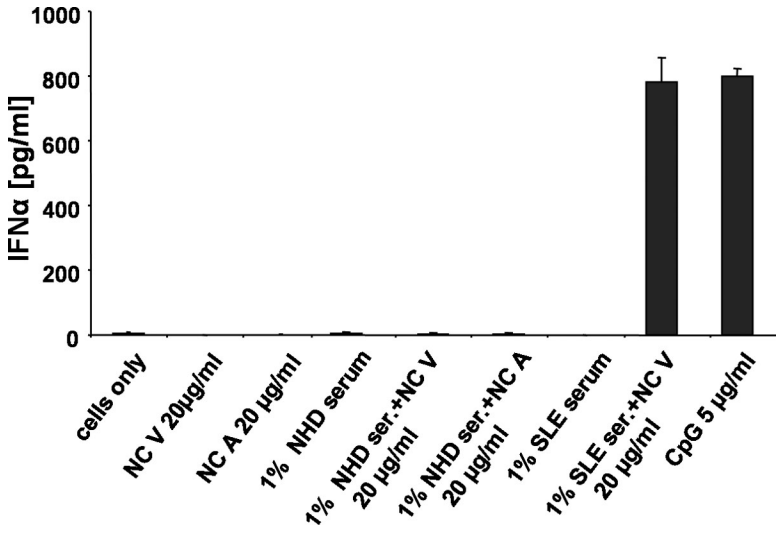

Figure S3. HMGB1–nucleosome complexes alone do not induce IFN-α secretion from pDC; however, IFN-α release is caused by a combination of nucleosomes and anti-dsDNA–containing SLE serum. pDC were isolated using the BDCA-4 kit (Miltenyi Biotec), plated at a density of 2×10^4 cells per well in 96-well plates, and incubated with 20 µg/ml of nucleosomes purified from viable (NC V) or apoptotic (NC A) Jurkat cells, or with 1% of NHD or SLE serum alone or 1% of NHD or SLE serum together with 20 µg/ml of nucleosomes. Untreated pDC or pDC stimulated with 5 µg/ml CpG oligodeoxynucleotides (ODN 2216; InvivoGen) served as negative and positive controls, respectively. After incubation for 48 h, concentrations of IFN-α within the supernatant was measured by ELISA (Bender MedSystems). One representative experiment of three is shown. Mean values and SD were calculated from triplicates.

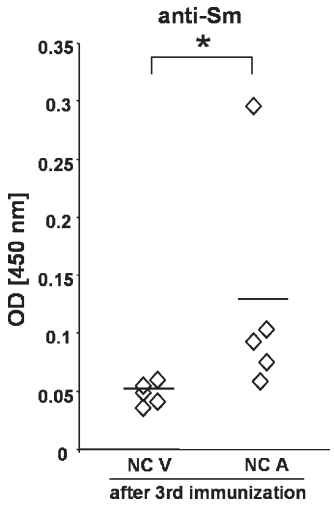

Figure S4. Increased anti-Sm response after immunization with apoptotic nucleosomes. Groups of five BALB/c mice each were i.v. injected with 50 µg of purified nucleosomes from viable (NC V) and apoptotic cells (NC A) in intervals of 3 wk. 8 wk after the third immunization, anti-Sm autoantibodies were quantified in serum samples (dilution 1:25) by ELISA (GE Healthcare). All sera were tested in the same assay. Bars indicate the mean values. Statistical analysis was performed using the nonparametric Mann-Whitney *U* test for unpaired samples. *, $P \leq 0.05$.

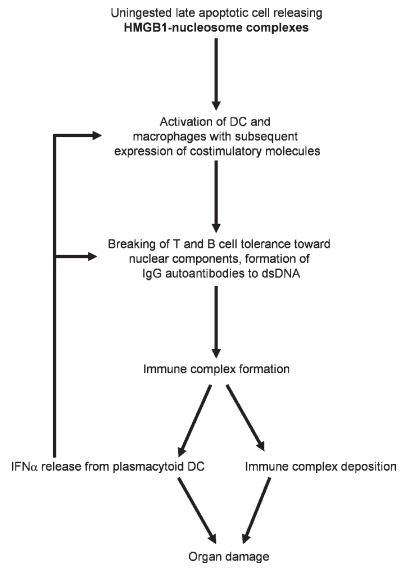

Figure S5. Model of the role of HMGB1–nucleosome complexes in immunopathogenesis of SLE. HMB1–nucleosome complexes released from uningested late apoptotic cells lead to activation of DC and macrophages presenting otherwise poorly immunogenic nucleosomes and other nuclear components. After breaking T and B cell tolerance, high-affinity IgG antibodies to dsDNA/nucleosomes are produced, which form immune complexes with dsDNA/nucleosomes. On one side, these immune complexes can induce IFN-α release from plasmacytoid DC augmenting the autoimmune reaction; on the other side, the immune complexes form proinflammatory deposits within glomeruli of kidneys and within blood vessels, leading to local inflammation and organ damage.