Letters to the Editor

Apoptotic neutrophils and anticardiolipin antibodies

Sirs,

The autoantigens of human SLE are exposed on the surface of apoptotic cells and increased apoptotic cells may contribute to autoantigen generation (1). We have previously demonstrated that the presence of increased circulating apoptotic neutrophils in SLE is correlated with disease activity and antibodies to double-stranded DNA (2). Apoptotic cells have also been suggested as the antigenic stimulus for the production of antiphospholipid antibodies (3). We can now report that SLE patients with elevated anticardiolipin antibodies have increased circulating apoptotic neutrophils.

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies that bind to negatively charged phospholipids, including cardiolipin, phosphatidylserine, phosphatidylinositol and phosphatidic acid. Almost one-third of SLE patients have increased aPL (4), but a smaller percentage develop clinical complications.

Early during the process of apoptosis, cells lose their phospholipid membrane asymmetry which leads to externalization of phosphatidylserine (5). Annexin V is a calcium dependent phospholipid binding protein with high affinity for phosphatidylserine which can be used to quantify apoptotic cells by flow cytometry.

We studied neutrophil apoptosis in 49 SLE patients, of whom 16 had elevated anticardiolipin antibodies. Seven of these 16 patients also had clinical features of the antiphospholipid syndrome. Anticardiolipin antibodies were measured by ELISA (Sigma Chemicals, UK). A positive value for both IgG and IgM was 10 G/M PLU. The percentage of circulating apoptotic neutrophils was determined using Annexin V as previously described (2, 6). Annexin V positive necrotic cells were excluded by using Propidium Iodide.

The group of patients with elevated anticardiolipin antibodies had significantly increased apoptotic neutrophils (n=16, median 3.65%; interquartile range 2.5-10.3) compared to anticardiolipin negative patients (n=33) (median 3.0%; IQ range 1.9-4.2: p=0.034) (Mann Whitney U test) (Fig. 1). However, there was no significant difference in the percentage of apoptotic lymphocytes in the patients with elevated anticardiolipin antibodies (median 4.8%; interquartile range 3.0-6.9) compared to the SLE anticardiolipin negative patients (median 4.9%; interquartile range 1.9-6.2: p= 0.70) (Mann Whitney U test). There were no significant differences in disease activity (SLAM) between the two groups (p=0.15) (anticardiolipin positive; median 10.5, anticardiolipin negative; median 8.0).

This increase of apoptotic neutrophils in SLE patients with aPL has not been reported before. It has been reported that levels of aPL fluctuate with SLE disease activity (7). This may suggest that the presence of increased apoptotic neutrophils and elevated levels of aPL are markers of active disease which are not necessarily related. However, we did not find significantly increased disease activity in the patients with anticardiolipin antibodies. The alternative hypothesis is that apoptotic cells drive the production of aPL. The association between aPL and other conditions with increased apoptotic cells, such as HIV infection, would support this theory (8). However, it would be important to determine whether apoptotic cells expose the beta-2-glycoprotein-1-related epitopes recognised by the aPL detected.

In addition, anticardiolipin antibodies have been shown to have a role in the clearance of apoptotic cells by a process of opsonization (9). The relationship between the percentage of apoptotic cells and the presence of antiphospholipid antibodies may, therefore, be crucial to an understanding of leukocyte apoptosis in SLE.

We have suggested that apoptotic neutrophils may be an important source of autoantigen generation in SLE (10). The observation of increased circulating apoptotic neutrophils in SLE patients with elevated anticardiolipin antibodies would support this hypothesis.

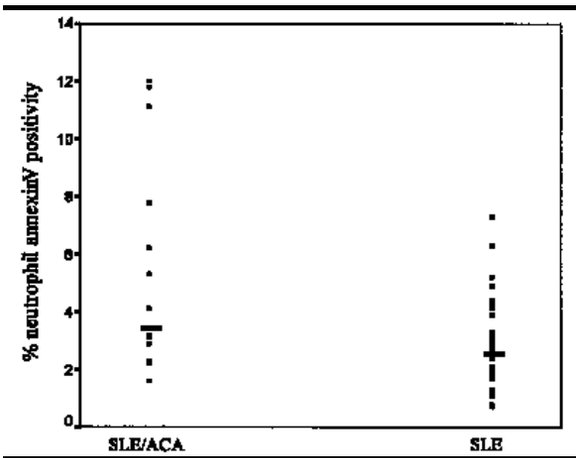

Fig. 1. Anticardiolipin antibodies (ACA) and apoptotic neutrophils. Percentage annexin V positive neutrophils in SLE patients stratified according to the presence of ACA. Each point represents an individual patient and medians are marked for each subgroup.

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