Apoptotic cell clearance in Systemic Lupus Erythematosus (SLE)
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Reduced uptake of apoptotic cells by macrophages in Systemic Lupus Erythematosus (SLE): correlates with decreased serum levels of complement

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Departments of Rheumatology & Clinical Immunology and Pathology & Laboratory Medicine², University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ABSTRACT

Objectives: Defects in phagocytosis of apoptotic cells are supposed to play an essential role in the pathogenesis of autoimmune diseases. Decrease of phagocytosis of apoptotic cells has been described in systemic lupus erythematosus (SLE). Factors underlying decrease of phagocytosis are, presently, unknown. We, therefore, analyzed the expression of relevant membrane receptors of monocyte-derived macrophages (MDM) from SLE patients and assessed their capacity to phagocytose apoptotic cells in comparison to healthy controls. Additionally, we compared phagocytosis in the presence of SLE sera with that in normal serum.

Methods: Human peripheral blood monocytes were isolated from patients and controls, and cultured for 7 days to obtain MDM. Membrane expression of CD14, CD18, CD36 and CD51/61 was measured. MDM were incubated with apoptotic Jurkat cells in the presence of normal human serum (NHS) or serum derived from patients during active or inactive disease.

Results: No differences in phagocytosis capacity were found between MDM from patients and controls. Membrane expression of the respective receptors was comparable between patients and controls. However, when MDM from controls were incubated with apoptotic cells in patient serum, phagocytosis was significantly decreased in comparison to incubation in NHS. This effect was dependent on the state of disease activity of the patients and could be reversed by addition of normal human serum. Reduced uptake of apoptotic cells was associated with decreased levels of complement C1q, C4, and C3, but not with levels of complement factor B.

Conclusions: Reduced uptake of apoptotic cells by MDM from SLE patients is not an intrinsic defect but is serum dependent and associated with decreased levels of complement components C1q, C4 and C3.
INTRODUCTION

Disturbance in the clearance of apoptotic cells is considered one of the potential pathophysiological mechanisms underlying breakdown of tolerance and, subsequently, the induction of autoimmune disease (1). In animal models, the absence of the first components of the complement system triggers autoimmunity through delayed phagocytosis of apoptotic cells (2). C1q-deficient mice spontaneously develop antinuclear antibodies and glomerulonephritis (3,4). A feature of glomerulonephritis in these animals is the presence of increased numbers of apoptotic bodies in their glomeruli suggesting a role for C1q in the clearance of apoptotic cells (4). Additional in vitro studies supported the importance of C1q in the uptake of apoptotic cells as C1q binds to apoptotic cells (5,6). Phagocytosis of apoptotic thymocytes by inflammatory macrophages was shown to be significantly reduced in C1q knock-out mice, and also in 3 patients with inherited C1q deficiency (2). A kinetic defect in the uptake of apoptotic cells was found in these individuals (2). C1q, thus, seems essential for the adequate uptake of apoptotic cells by macrophages. However, inherited C1q deficiency in SLE patients is exceptional (7). Therefore, other mechanisms must explain the decreased phagocytic capacity of apoptotic cells as reported in SLE patients (8).

Phagocytosis of apoptotic cells is a complex process in which many membrane receptors and serum proteins are involved. These include the lipopolysaccharide (LPS) receptor (CD14), complement receptors (CD11a,b,c/CD18), the thrombospondin receptor (CD36), MER (membrane tyrosine kinase c-mer), the vitronectin receptor (CD51/CD61), CD44, and the phosphatidylserine receptor (9-12). Next to C1q, other complement components, phospholipase A2, and the pentraxins C-reactive protein (CRP), serum amyloid P (SAP), and pentraxin-3 (PTX3) bind to apoptotic cells and mediate their uptake by macrophages (13-17). Their relevance is demonstrated by a defect in the phagocytic uptake of apoptotic syngeneic thymocytes in C4-deficient mice (4).

Up till now, it is not clear whether the defect found in SLE patients is intrinsic to their macrophages, or acquired due to serum derived factors such as a decline of complement levels during active disease. We conducted this study to investigate whether an intrinsic defect in phagocytosis of apoptotic cells is present in SLE patients. We analyzed the expression of membrane receptors on macrophages involved in the uptake of apoptotic cells and studied the phagocytic capacity when incubated in normal serum. In addition, we analyzed the differential effects of SLE sera on this process in order to approach the in vivo situation.

METHODS

Patients and controls

Patients eligible for this study fulfilled at least 4 American College of Rheumatology (ACR) criteria for SLE (18). For monocyte isolation 16 consecutive patients with inactive disease were selected. Patient characteristics are shown in Table 1. Simultaneously, monocytes were collected from 16 healthy, age and sex matched, controls. To test the influence of serum in phagocytosis studies paired serum samples were used obtained from another 20 patients at the moment of active and inactive
disease, respectively. Patients with active disease had to fulfill criteria as previously described (19). Inactive disease was defined as the persistent absence of clinical disease activity for at least a 4 month period while patients were without or on a constant dose of immune modulating drugs. Characteristics of these patients are given in Table 2. For each participating patient SLEDAI-score was recorded (20).

**Complement levels**

Complement C3 and C4 levels were detected by radio-immuno diffusion according to the manufacturer’s instruction (Dade Behring, Marburg, Germany). Complement C1q and factor B were measured by nephelometry.

**Macrophage culture**

Peripheral blood mononuclear cells (PBMC) were isolated as described previously (21). PBMC from patients and controls were isolated and cultured pair wise to avoid differences in conditions.

**Expression of membrane receptors**

Monocyte surface marker analysis was performed by incubating 200 µl whole blood with 10 µl labeled MoAb (Table 3) for 15 minutes at room temperature (RT). Subsequently, 3 ml 1:10 FACS-lysing solution was added for 10 minutes at RT, samples were washed with PBS/heparin and taken up in 150 PBS/heparin. The same analysis was performed immediately after isolation of the mononuclear cell fraction and after 7 days of culture and measured on a Coulter Epics-Elite (Coulter Electronics, Mijdrecht, The Netherlands).

The level of expression of membrane markers was given as Mean Fluorescence Intensity (MFI), corrected for nonspecific binding using an irrelevant antibody. HUVEC were used as positive control for CD51/61 and monocyte derived dendritic cells (MoDC) for CD83 staining. MoDC were obtained by 7 days of culture in RPMI, supplemented with GM-CSF (800 IU/ml), IL-4 (100 IU/ml) and 10% FCS, and subsequent simulation with LPS (1µg/ml) for 2 days.

**Induction and detection of apoptosis**

Apoptosis in Jurkat cells was induced as described before (21). After 4 hours 54.0 ± 5.1% (mean ± SEM of 3 experiments) of Jurkat cells were positive for annexin V and negative for propidium iodide, while 5.3 ± 1.5% were positive for both markers.

**Phagocytosis assay**

Phagocytosis assay was performed as described before (21). In short, irradiated Jurkat cells (2 x 10⁶ cells/ well) and macrophages were incubated for 30 min at 37°C, in the presence of 30% pooled serum of (NHS) of normal healthy individuals (NHS). Subsequently, coverslips were washed gently to remove non-bound cells. Four sera from active SLE patient with resulting in low phagocytic capacity were reconstituted using a mix of 15% active sera and 15% NHS and compared to 30% active SLE sera alone, or 15% and 30% NHS.
Staining procedure and scoring of macrophage density and phagocytosis

Staining procedures were performed as described previously (21). Preparations were scored by two independent observers at 400x magnification using light microscopy. Density of macrophages was determined by averaging the number of macrophages in 3 microscopic fields. Phagocytosis was expressed as phagocytosis index (number of Jurkat cells internalized by 100 macrophages). All experiments were performed in duplicate.

Table 1: Characteristics of SLE patients (n=16) of whom monocytes were isolated during inactive disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean, range)</td>
<td>45.7 (26-77)</td>
</tr>
<tr>
<td>Male/female</td>
<td>2/14</td>
</tr>
<tr>
<td>Disease duration (years, mean, range)</td>
<td>14.2 (4-44)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.2 ± 1.1</td>
</tr>
<tr>
<td>Anti-dsDNA (Farr, IU/ml)</td>
<td>51.9 ± 21.6</td>
</tr>
<tr>
<td>Complement C3 (g/l)</td>
<td>0.87 ± 0.05</td>
</tr>
<tr>
<td>Complement C4 (g/l)</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Cumulative ACR criteria</td>
<td>n (%):</td>
</tr>
<tr>
<td>Malar rash</td>
<td>5 (31)</td>
</tr>
<tr>
<td>Discoid rash</td>
<td>3 (19)</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>9 (56)</td>
</tr>
<tr>
<td>Serositis</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Nephritis</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Hematological</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>14 (88)</td>
</tr>
<tr>
<td>Anti-Smith antibodies</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Anti-nuclear antibodies</td>
<td>16 (100)</td>
</tr>
</tbody>
</table>

LE-cell phenomenon and/or false positive syphilis test are not included in the table because neither was routinely analyzed in our center. Results are given as mean ± SEM, unless depicted otherwise.

Statistics

Levels of statistical significance were calculated using GraphPad Prism™ (version 3.0; GraphPad software). Comparison between groups was performed using Mann-Whitney U test, Wilcoxon signed rank test or one-way analysis of variance for selected pairs of columns (ANOVA) with Bonferroni’s correction for multiple comparisons as appropriate. Correlations were calculated using Spearman rank correlation test.
Table 2: Characteristics of SLE patients (n=20) of whom serum was obtained during inactive and active disease.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Inactive</th>
<th>Active</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI</td>
<td>2.2 ± 0.4</td>
<td>11.8 ± 1.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>6.5 ± 2.3</td>
<td>38.3 ± 13.4</td>
<td>0.0194</td>
</tr>
<tr>
<td>Anti-dsDNA (IU/ml)</td>
<td>28.8 ± 8.4</td>
<td>201.4 ± 67.8</td>
<td>0.0007</td>
</tr>
<tr>
<td>Complement C3 (g/l)</td>
<td>1.20 ± 0.08</td>
<td>0.79 ± 0.06</td>
<td>0.0003</td>
</tr>
<tr>
<td>Complement C4 (g/l)</td>
<td>0.19 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.0005</td>
</tr>
<tr>
<td>Complement C1q (g/l)</td>
<td>0.14 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.0007</td>
</tr>
<tr>
<td>Complement factor B (g/l)</td>
<td>0.33 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>0.1418</td>
</tr>
</tbody>
</table>

Results are given as mean ± SEM. P values were calculated using Wilcoxon signed rank test. Normal C3 and C4 values are 0.77-1.84 and 0.16-0.40 g/l, respectively. Normal values of C1q and factor B are 0.10-0.25 and 0.19-0.40 g/l, respectively.

Table 3: Membrane markers used.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Label</th>
<th>Cellular distribution</th>
<th>Monoclonal Antibody</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>PE</td>
<td>Monocytes/macrophages/granulocytes (weak)</td>
<td>IgG2a</td>
<td>IQP</td>
</tr>
<tr>
<td>CD18</td>
<td>FITC</td>
<td>All types of leukocytes</td>
<td>IgG1</td>
<td>Imm. tech</td>
</tr>
<tr>
<td>CD36</td>
<td>FITC</td>
<td>Monocytes/macrophages/platelets/erythrocyte precursors</td>
<td>IgG1</td>
<td>IQP</td>
</tr>
<tr>
<td>CD51/61</td>
<td>FITC</td>
<td>Endothelium/platelets/macrophages</td>
<td>IgG1</td>
<td>BD</td>
</tr>
<tr>
<td>CD83</td>
<td>FITC</td>
<td>Dendritic cells</td>
<td>IgG1</td>
<td>BD</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>PE</td>
<td>Antigen presenting cells</td>
<td>IgG2a</td>
<td>BD</td>
</tr>
</tbody>
</table>

FITC: fluorescein isothiocyanate; PE: phycoerythrin; IQP: Groningen, The Netherlands; Imm. tech: Immunotech, Marseilles, France; BD: Becton Dickinson, Mountain View, USA.

RESULTS

Phagocytosis of apoptotic cells

For phagocytosis experiments monocytes were isolated from 16 SLE patients, with inactive disease, and 16 healthy age and sex matched controls, and differentiated into macrophages (MDM). To detect intrinsic defects in the capacity to internalize apoptotic cells, MDM from patients with inactive disease and controls were incubated with apoptotic Jurkat cells in the presence of normal human serum (NHS). No differences in phagocytic capacity could be demonstrated between patients and controls (Fig 1A). As it has been reported that the density of MDM can influence their capacity to internalize apoptotic cells, density of MDM after culture was determined. No differences between patients and controls could be detected in the density of macrophages (Fig 1B).
Apoptotic cell clearance: role of macrophage and complement

Figure 1: Phagocytosis of apoptotic Jurkat cells by monocyte derived macrophages (MDM) and density of MDM on coverslips from 16 healthy controls (open circles) and 16 SLE patients (closed circles) with inactive disease. A) Phagocytosis occurred in the presence of 30% pooled serum of normal healthy individuals. Phagocytosis index was calculated as the number of apoptotic Jurkat cells internalized by 100 MDM. B) Density of MDM was scored after phagocytosis and depicted as number of MDM per microscopic field. Horizontal bars denote the median.

Expression of membrane receptors

To analyze whether the expression of membrane receptors, relevant for binding and subsequent internalization of apoptotic cells, differed between patients and controls membrane expression of CD14, CD18, CD36 and the vitronectin-receptor (CD51/61) was measured on mononuclear cells by flowcytometry. For this purpose, part of the MDM from 8 patients and 8 controls, as prepared for the phagocytosis experiments, were used. Membrane expression was measured in whole blood and after

Table 4: Membrane expression of several receptors measured on monocytes before and after isolation and on monocyte derived macrophages after 7 days of culture

<table>
<thead>
<tr>
<th>Marker</th>
<th>Whole blood</th>
<th>After Isolation</th>
<th>After 7 days culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>1045.0 ± 97.9</td>
<td>816.0 ± 106.2</td>
<td>709.2 ± 115.5</td>
</tr>
<tr>
<td>CD18</td>
<td>147.4 ± 16.6</td>
<td>134.4 ± 13.4</td>
<td>253.0 ± 62.6</td>
</tr>
<tr>
<td>CD36</td>
<td>395.6 ± 52.8</td>
<td>384.0 ± 41.0</td>
<td>419.9 ± 71.9</td>
</tr>
<tr>
<td>CD51/61</td>
<td>1.80 ± 0.56</td>
<td>2.33 ± 0.78</td>
<td>8.3 ± 6.6</td>
</tr>
<tr>
<td>CD83</td>
<td>2.22 ± 0.49</td>
<td>1.78 ± 0.46</td>
<td>3.9 ± 2.3</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>328.1 ± 39.4</td>
<td>547.2 ± 220.8</td>
<td>255.8 ± 68.9</td>
</tr>
</tbody>
</table>

Results are given as mean ± SEM and represent the mean fluorescence intensity of the respective markers, corrected for nonspecific binding of an irrelevant antibody and the conjugate. No differences in membrane expression could be demonstrated between patients and controls (using Mann-Whitney U test). Differences between results on whole blood and after isolation are depicted as * (P<0.05) and ** (P<0.01); differences between results directly after isolation and after 7 days of culture are depicted as # (P<0.05) and ## (P<0.01) as analyzed by Wilcoxon signed rank pairs test.
isolation of mononuclear cells in order to analyze the influence of isolation. No differences were found in the percentage of CD14, CD18, CD36 or CD51/61 positive cells between patients and controls (data not shown). Also, the level of membrane expression, indicated as MFI, was comparable between patients and controls at any time point (Table 4). However, isolation of the mononuclear cell fraction resulted in a significant decrease in CD14 expression. After 7 days of culture, a further reduction in CD14 expression, and a significant decrease in the expression of CD18 and CD36 could be demonstrated on MDM both from patients and controls (Table 4). CD83 expression remained negative, indicating that no differentiation into dendritic cells had occurred during the culture period.

**Figure 2: Influence of serum on phagocytosis of apoptotic cells.** MDM from healthy controls were incubated with apoptotic Jurkat cells in the presence of 30%, 20%, 10%, 5% and 1% pooled serum (NHS) of normal healthy individuals (□). MDM were incubated simultaneously with serum samples obtained from 20 SLE patients at the moment of either active (■) or inactive disease (≡). Phagocytosis index was calculated as the number of apoptotic Jurkat cells internalized by 100 MDM. The data shown represent the mean ± SEM. * P<0.05, ** P<0.01, *** P<0.001, compared to NHS.

**Effect of serum**

To test whether constituents present in patients’ and control serum influence the phagocytic capacity of MDM, phagocytosis experiments were performed using different serum samples. Incubation of control MDM with apoptotic Jurkat cells was now performed in the presence of serum derived from patients with active disease (n=20), from the same patients with inactive disease (n=20) (Table 2), and with NHS (n=20), in decreasing concentrations. The phagocytic capacity of control MDM was dependent on serum concentration as demonstrated by the positive relation between serum concentration and phagocytosis index (Fig 2). Furthermore, compared to NHS, a significant decrease in phagocytosis index was observed when 30, 20 or 10% serum from patients with active disease was added during incubation of apoptotic Jurkat cells with MDM from healthy controls (p<0.001, p<0.05 and p<0.01, respectively)).
Addition of normal human serum was able to restore phagocytosis when mixed 1:1 with serum of active SLE patients (Figure 3). Except for complement factor B, all complement levels were decreased during active disease (Table 2). We analyzed whether decrease of complement levels was related to the decrease in phagocytosis index as found in the presence of serum from active lupus patients. A significant correlation could be demonstrated between serum levels of complement C1q, C4 and C3 and the phagocytosis index (Figure 4). No correlations were found between phagocytosis index and complement factor B (p=0.99), levels of CRP (p=0.82), and levels of anti-dsDNA antibodies (p=0.85) or presence of anti phospholipids antibodies.
DISCUSSION

This study demonstrates that the intrinsic capacity of MDM from SLE patients to internalize apoptotic cells is unaltered compared to controls. In addition, the expression of membrane receptors on monocytes as well as on MDM relevant for the uptake of apoptotic cells did not differ between patients and controls. However, when phagocytosis experiments were performed with MDM from healthy controls incubated with sera derived from active SLE patients, a significant decrease in phagocytosis of apoptotic cells was observed. This decrease in phagocytosis could be restored by the addition of normal human serum to serum from active patients. The lowered capacity to clear apoptotic cells was associated with decreased levels of complement factors C1q, C4, and C3 which confirms, in part, results of others (22).

In recent years the role of apoptotic cells in the development of autoimmunity has been highlighted (23;24). Apoptotic cells display intracellular auto-antigens on their cell membrane (25). These membrane expressed auto-antigens might be altered through posttranslational modification processes (26;27). It has been suggested that persistent exposure to these cells can break tolerance (28;29). A diminished capacity to phagocytose apoptotic cells is supposed to be one of the major mechanisms that might underlie this persistent exposure.

Figure 4: Correlation between levels of complement components C1q (A), C3 (B), C4 (C) and factor B (D) and phagocytosis index. MDM from healthy controls were incubated with apoptotic Jurkat cells in the presence of 30% serum derived from healthy controls (n=20, ○) or SLE patients (n=20). Paired samples were used obtained during active disease (●) and as during inactive disease (▲). Correlation was calculated using data from all experiments (n=60).
In the uptake of apoptotic cells, membrane receptors on phagocytes and opsonizing serum constituents are involved. In SLE patients, membrane expression of these relevant receptors on monocytes/macrophages has been addressed in several studies. CD44 expression was reported to be reduced on monocytes of SLE patients (30). One study showed a reduction in the expression of CD14 on monocytes of SLE patients after 7 days of culture (8). In contrast, Steinbach et al. found a reduction in CD14 expression on freshly isolated monocytes of SLE patients (31). In the present study we could not demonstrate any difference between healthy controls and SLE patients in membrane expression of CD14, CD18, CD36, or CD51/61 immediately after isolation, nor after differentiation into MDM. However, we did show that isolation of PBMC as well as culture of MDM significantly influence membrane expression of several receptors. Differences in methodology might therefore, account for the discrepancies in results found between studies.

Along with the absence of changes in receptor expression on MDM from SLE patients, the normal phagocytic capacity of MDM from SLE patients found in the current study strongly favors the absence of intrinsic abnormalities in their MDM. Also, experiments in lupus-prone mouse strains suggest that the capacity of macrophages to internalize apoptotic cells is normal (32). Licht et al. investigated the efficacy of phagocytosis of apoptotic thymocytes in vitro in the presence of fetal calf serum by resident peritoneal macrophages in control and MRL/+ and MRL/lpr lupus prone-mouse strains (33). They did not detect changes in efficacy between these mouse strains. However, a decrease in phagocytosis was detected in lupus prone-mouse strains in the presence of autologous serum (32). The decreased in vivo phagocytosis in MRL/Mp and NZB/W lupus prone-mouse strains found by others might therefore, be a consequence of differences in levels of serum factors in these mice (34).

The study by Herrmann et al. showed that clearance of apoptotic cells by MDM from SLE patients is reduced in the presence of normal serum (8). This might be explained by differences in methodology. Firstly, monocytes in this study were collected from patients with inactive disease as well as active disease. Furthermore, a previous report have demonstrated that incubation of rat bone marrow-derived macrophages (BMDM) with apoptotic neutrophils for 30 minutes reduced the uptake of apoptotic neutrophils at a re-challenge after 48 hours by 50% compared with previously unchallenged BMDM (35). In lupus patients an increased level of circulating apoptotic neutrophils can be found in the peripheral blood. This level is positively correlated with disease activity (36). Phagocytosis of these cells by macrophages might therefore negatively influence the ability of these macrophages to ingest other apoptotic cells. This might explain the decreased phagocytosis by MDM of SLE patients found by Herrmann et al., who incubated apoptotic cells with MDM for long periods of time (8). Finally, the ability to internalize apoptotic cells has also been shown to be dependent on contacts between macrophages themselves, called the community effect (37). Shoshan et al. demonstrated that during maturation of monocytes from SLE patients into macrophages, accelerated apoptosis occurred, which resulted in a lower density of macrophages and a reduced ability to internalize.
apoptotic cells. Phagocytosis was restored to control levels by restoring cell density (38). Cell densities were not taken into account in the study of Herrmann et al. Differences in cell density may constitute another explanation for the discrepancies found between our study and that of Herrmann et al. In the present study, effects of cell density could be ignored as macrophage density did not differ between patients and controls.

The role of complement factors in the uptake of apoptotic cells has clearly been demonstrated (39). The presence of the classical pathway complement proteins seems essential for phagocytosis of apoptotic cells. In mice, disruption of the C1q gene results in spontaneous autoimmunity (4). Also, C4 deficiency causes spontaneous, lupus-like autoimmunity with glomerulonephritis and glomerular deposition of immune complexes (39). Recently, it was shown using single and double-knockout C3(null)C4(null) lpr (Fas-deficient) mouse models, that the absence of C4, and not the presence of C3, is critical in development of SLE (40). Thus, next to C1q, complement C4 provides an important protective role against the development of SLE. In accordance with these data we demonstrate that the uptake of apoptotic cells through MDM is reduced in association with reduced levels of C1q as well as C4 in sera used in the phagocytosis experiments. In a recent study by our group no correlations could be detected between serum complement levels and phagocytic capacity using sera from inactive SLE patients (41). Although a moderate decrease in complement factors was detected in these inactive SLE patients, this might not be sufficient to affect phagocytosis of apoptotic cells. In our current study only sera from SLE patients in an active state of the disease have significantly decreased phagocytic capacity which correlated with extremely low complement levels seen in sera from these active SLE patients. Furthermore, reconstituting complement deficient sera from active lupus patients with normal human serum restores phagocytic capacity. These findings argue against an inhibitor of phagocytosis present in complement deficient sera. No significant correlation was found between phagocytosis index and levels of complement factor B. In the system used we were not able to analyze the specific influence of the separate complement factors independently of each other. The defect in clearance of apoptotic cells in vivo as demonstrated by others might be explained by (relative) deficiency of complement C1q and C4 (42). It should be mentioned that other serum proteins are involved in uptake of apoptotic cells as well. Serum contains many other constituents as the pentraxins CRP, SAP and PTX3, phospholipase A2, surfactant, all of which influence the capacity to internalize apoptotic cells (21;43-47). In addition, the spectrum of autoantibodies present in the serum of lupus patients might influence the uptake of apoptotic cells. In this respect, it should be noted that autoantibodies, when they opsonize apoptotic cells, seem to facilitate the internalization of these cells via different receptors (48). The absence of a relation between CRP levels, levels of antibodies to dsDNA or presence of anti-phospholipid antibodies and phagocytosis index, argues against a strong inhibitory influence of these serum factors in the uptake of apoptotic cells in SLE patients.
In conclusion, we confirm that phagocytosis of apoptotic cells in SLE patients is reduced. This is however not due to an intrinsic defect of MDM from SLE patients but associated with serum factors involved in the opsonization of apoptotic cells. The association we found between phagocytic capacity of apoptotic cells and serum complement levels of C1q, C4, and (to a minor extend) C3, as found in this study, suggests that the previously reported phagocytic defects in lupus patients are the result of decreased complement levels which are commonly seen in this disease.
Reference List


