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# *Opsonization of Early Apoptotic Cells by IgG from Lupus Nephritis Amplifies Activation of Early Complement Components and Promotes Inflammatory Phagocytosis*

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**Abstract**

**Introduction:** SSA/Ro 60 has been reported to be exposed on the surface of early apoptotic cells and recognized by auto antibodies from lupus. The aim of this study was to explore the subsequent effects of the binding of IgG from anti-SSA positive LN patients on the fate of early apoptotic cells.

**Methods:** IgG fractions were purified from three patients with lupus nephritis. Apoptosis was induced in Jurkat cells by UVC irradiation. Patient IgG induced C3c/C5b-9 deposition was assessed by flow cytometry. Phagocytosis of early apoptotic cells by THP-1 derived macrophages in the presence of patient IgG and/or Normal Human Serum (NHS) was assessed by flow cytometry. Cytokines analysis induced by the phagocytosis of early apoptotic cells was determined by ELISA.

**Results:** IgG which have been confirmed with extensive binding to early apoptotic cells were purified from three anti-SSA positive LN patients. Opsonization of early apoptotic cells by IgG from LN augmented C3c deposition without influence on the assembly of the terminal complement components or cell lysis. IgG from LN enhanced opsonization and phagocytosis of early apoptotic cells by macrophages directly or dependent on complement activation, with massive TNF-a and IL-1β secretions.

**Conclusion:** IgG from anti-SSA positive LN facilitates early apoptotic cell clearance by macrophages and triggers proinflammatory cytokines release, possibly exacerbating underlying pathogenic mechanisms in lupus nephritis.

**Keywords:** SLE; Lupus nephritis; Autoantibodies; Early apoptotic cells; Complements; Phagocytosis.

**Abbreviations:** SLE: Systemic Lupus Erythematosus; LN: Lupus Nephritis; NLS: Neonatal Lupus Syndrome; NHS: Normal Human Sera; PMA: Phorbol 12-Myristate 13-Acetate; CFSE: Carboxyfluorescein Diacetate Succinamidyl Ester; MAC: Membrane Attack Complex; MBL: Mannose-Binding Lectin.

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## Introduction

Systemic Lupus Erythematosus (SLE) is a prototypic autoimmune disease characterized by the production of multiple pathogenic autoantibodies, in combination with diverse clinical manifestations [1]. Plenty of studies have indicated that apoptotic cells with expression of altered or unaltered nuclear substance might be a source of autoantigens in SLE [2-4]. In healthy individuals, dying cells are cleared by macrophages at very early stages with intact cell membrane, inducing anti-inflammatory cytokines, and obviating any inflammation or immune response [5,6]. If they are not promptly cleared, they may progress to late apoptotic cells with disrupted membrane, releasing toxic and immunogenic intracellular contents, and fostering inflammation [7,8].

Esther Reefman et al found that IgG fractions from all of their studied SLE patients could bind to late apoptotic cells and inhibit their uptake by macrophages, indicating that the interference of autoantibodies in the clearance of late apoptotic cells might be a common denominator in the pathogenesis of SLE [9]. However, to date the occurrence of late apoptosis has not been confirmed in vivo, and something must have happened before large numbers of unexpected late apoptotic cells emerged in the body. Early apoptotic cells are more likely to be the target of the autoimmunity in SLE. Manfredi et al showed that affinity purified antiphospholipid antibodies could recognize early apoptotic cells, facilitate apoptotic cell clearance by macrophages and trigger TNF-α release, indicating a pathogenic role in SLE [10]. Autoantibodies against early apoptotic cells have also been detected in 62% anti-Ro60-positive SLE patients, and the specificity of the Ro 60 epitope expressed on apoptotic cells was determined by inhibition experiments with recombinant and native Ro 60 [11]. In our earlier study, we detected autoantibodies against early apoptotic cells in active Lupus Nephritis (LN) patients, whatever anti-SSA autoantibody positive or negative, and found the prevalence rate in the whole study group was 33.3%. Autoantibodies against early apoptotic cells could be detected in anti-SSA negative LN patients, but patients who were double positive for anti-SSA and anti-early apoptotic cell antibody had significantly increased risk of poor short-term outcome. Moreover, in our study, IgG presenting extensive binding capacity to early apoptotic cells were from 3 patients, all of whom were anti-SSA antibody positive and antiphospholipid antibody negative [12]. To the best of our knowledge, the subsequent consequence of the binding of IgG from patients with lupus to auto antigens expressed on early apoptotic cells except phospholipid have not been investigated as yet.

During the process of fetal cardiocytes apoptosis, SSA/SSB antigens translocate to the cell surface, and mediate the phagocytosis of the apoptotic fetal cardiocytes by nearby healthy cardiocytes. In Neonatal Lupus Syndrome (NLS), maternal antibodies to SSA and SSB transport across the placenta, bind to cognate antigen expressed on apoptotic cardiocytes and decrease the clearance, which may contribute to the development of autoimmune associated congenital heart block and fatal cardiomyopathy [13,14]. Since SSA might be the particular autoantigen exposed on early apoptotic cells in non-neonatal SLE as elucidated hereinabove, whether binding of autoantibodies to early apoptotic cells is one of the mechanisms contributing to the pathologic cascade of non-neonatal SLE as they did in NLS is deserved to be studied.

In this study, we aimed to elucidate whether the binding of IgG from LN patients with anti-SSA antibodies to early apoptotic cells enhance complement activation, and affect phagocytosis by macrophages.

## Methods

### IgG isolation

IgG from three LN patients which have been confirmed with extensive binding to early apoptotic cells in our previous study were used for functional assays [12]. Complete clinical data of the three patients were collected upon presentation. They fulfilled the 1997 American College of Rheumatology revised criteria for SLE [15]. The clinical characteristics of the three patients are presented in Table 1. Informed consent was obtained for blood sampling. The research was in compliance of the Declaration of Helsinki. Ethical approval was obtained from hospital ethics committee of Shandong Provincial Hospital affiliated to Shandong University for this study.

**Table 1:** Clinical characteristics.

| | P5 | P16 | P23 |
|---|---|---|---|
| Gender | M | F | M |
| Age (years) | 16 | 14 | 29 |
| Serum creatinine (μmol/L) | 96 | 89 | 187 |
| Proteinuria (g/Day) | 3.23 | 5.64 | 10.17 |
| SLEDAI | 32 | 12 | 28 |
| ANA (titer) | 1:1000 | 1:1000 | 1:1000 |
| Anti-dsDNA (U/mL) | 734 | 210 | 297 |
| Anti-Sm | + | + | + |
| Anti-SSA | + | + | + |
| Anti-SSB | + | — | + |
| Antiphospholipid antibody | — | — | — |
| % IgG binding to early apoptotic cells | 90.83 | 64.88 | 29.50 |
| Outcome after 1-year follow-up | Death | Remission | Death |

[a]**Abbreviation:** SLEDAI: SLE Disease Activity Index.

IgG was purified from patient and control sera on protein G-Sepharose columns (Pharmacia) according to the manufacturer's recommendations. Briefly, IgG from serum was bound to protein G columns, washed with 50-column volumes of Phosphate Buffered Saline (PBS), and eluted with 0.1 M glycine (pH 2.7). Eluted IgG was neutralized by collection in 2M Tris-HCl (pH 9.0) and dialyzed against PBS.

### Cell culture

The human T cell line Jurkat and promonocytic cell line THP-1 (Cell Resource Center, IBMS, CAMS/PUMC, China) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% $CO_2$. THP-1 monocytes ($2\times10^5$ cells/well) were stimulated with 100 nM phorbol 12-myristate 13-acetate (PMA; Sigma, USA) for 48 h in 24-well plates to induce a macrophage phenotype.

### Induction of apoptosis

Apoptosis was induced according to a previously described method [12]. In brief, Jurkat cells were washed twice with serum

free RPMI 1640, and then re suspended at a concentration of $1\times10^6$ cells/ml and irradiated with UVC light for 3 min. After UV irradiation, cells were cultured for 3 h in serum-free RPMI medium, and stained with APC–labeled annexin V and 7-AAD (KeyGen Biotech, Nanjing, China), which were then analyzed by flow cytometry. Since IgG from patients with lupus have been reported to bind to late apoptotic cells extensively and inhibit phagocytosis, we controlled the percentage of late apoptotic cells to a minim level in this study. As described in our previous study, after UVC irradiation and 3 hours of culture, about 50% of Jurkat cells were early apoptotic, whereas less than 5% of Jurkat cells were late apoptotic.

**Complement activation assay**

Normal human sera (NHS) used for complement binding and activation were stored at -80°C in small aliquots. Patient or control IgG was incubated with apoptotic cells for binding according to a previously described method [12]. In brief, $1\times10^6$ apoptotic cells were resuspended in 100 µl 3% BSA/PBS, and incubated with 500 µg/ml of patient or control IgG at 4°C for 1 h. Washing was repeated, and complement binding studies were performed by incubation of $10^6$ cells with 100 µl of medium containing 20% human serum in TC buffer (140 mM NaCl, 2 mM $CaCl_2$, 10 mM Tris, pH 8.0 or 7.4, supplemented with 1 mM $Mg^{2+}$ and 1% BSA) at 37°C for 30 min for C3c analyzing, 1h for C5b-9 analyzing, or 3h for 7AAD staining. After incubation, apoptotic cells were washed twice in TC buffer, stained with 1:100 dilutions of FITC-conjugated anti-human C3c (Abcam) at 4°C for 30 min, or incubated with 1:100 rabbit anti-human C5b-9 (Abcam) at 4°C for 30min and then stained with 1:500 dilutions of FITC-conjugated goat anti-rabbit secondary antibodies(Abcam) for flow cytometry analysis.

**Phagocytosis assay**

Prior to the induction of apoptosis, Jurkat cells were fluorescently labeled with carboxyfluorescein diacetate succinamidyl ester (CFSE; Sigma, USA), according to a previously described method with mild modification [16]. In brief, Jurkat cells were washed three times and suspended in PBS at $1\times10^7$ cells/ml and incubated for 30 minutes at 37°C with 5 µM CFSE. Cells were washed and resuspended at $1\times10^6$ cells/ml in serum-free RPMI culture medium, used for apoptosis induction, as described above. THP-1 derived macrophages were washed gently with RPMI 1640 twice. For the phagocytosis assay, UVB-irradiated early apoptotic cells were incubated with 500 µg/ml of patient or control IgG, and the cells were washed twice to remove nonbinding IgG. Subsequently, apoptotic cells ($5\times10^5$ cells/well) were incubated with macrophages ($2\times10^5$ cells/well) for 30 minutes at 37°C in an atmosphere containing 5% $CO_2$, in the presence or absence of 20% NHS. After co-incubation for 30 min, the cells were detached from the surface with accutase (Invitrogen). Macrophages were then stained with APC-conjugated monoclonal antibodies against CD11b (BD Biosciences) and uptake was analyzed by 2-color flow cytometry. CD11b-positive cells which represented the subset of macrophages were under analysis, and the percentage of CD11b-positive cells that stained positive for CFSE was used as a measure for the percentage of macrophages which ingested apoptotic cells.

**Collection of supernatants and analysis of cytokines**

Cytokines analysis induced by the phagocytosis of early apoptotic cells was preformed according to a previous described method [6]. In brief, $5\times10^5$ early apoptotic cells were pre incubated with patient IgG (4°C, 1h) and/or NHS (37°C, 30 min) as above, washed, and then incubated with $2\times10^5$ adherent macrophages for 18 h at in 300 µl fresh RPMI 1640 in 24-well plates [6]. Supernatants were collected, and centrifuged at 2,000 rpm to remove particulate debris, then were stored in aliquots at -80°C. Cytokine concentrations in the culture supernatants were determined by ELISA, using the Quantikine immunoassays manufactured by R & D Systems. The cytokines analyzed were IL-1β and TNF-a. Assays were performed according to the instructions provided with each kit.

**Statistical analysis**

Statistical software SPSS 22.0 (SPSS, Chicago, IL, USA) was employed for statistical analysis. Data were presented as means of parallel measurements. Comparison between groups was performed using one way analysis of variance with Bonferroni's correction for multiple comparisons as appropriate. Statistical significance was considered as $p < 0.05$.

## Results

**IgG enhances early complement activation on early apoptotic cells**

Complements have previously been reported to assemble on the surface of early apoptotic cells. According to Gershov's study, evidence of C3 activation was detected on early apoptotic cells by 30 min post incubation with NHS, relatively obvious Membrane Attack Complex (MAC) assembly was detected by 1h, and MAC mediated cell lysis was observed over time [17]. Then in this study, we analyzed C3c binding on early apoptotic cells at 30min post incubation with NHS, 1h for C5b-9 binding, and 3h for 7AAD staining.

As shown in Figure 1A(b), 1B(b), incubating apoptotic cells with 20% NHS in TC buffer, 46.2% of early apoptotic cells were C3c positive at 30min, and 7.5% of early apoptotic cells were C5b-9 positive at 1h. As shown in Figure 1C(a-b), the percentage of 7AAD positive cells was higher when incubating apoptotic cells with NHS compared with incubating apoptotic cells in TC buffer only. These findings are consistent with Gershov's results.

As shown in Figure 1A(c-d), 1B(c-d), 1C(c-d), when apoptotic cells were pre incubated with IgG from patient P5, deposition of C3c on the surface of early apoptotic cells was substantially amplified compared with IgG from healthy control (86.9 ± 4.0% vs. 50.7 ± 12.7%, $p<0.05$), but no more C5b-9 deposition was induced by IgG from patient P5 compared with control IgG. Opsonization of early apoptotic cells by IgG from patient P5 didn't increase the percentage of 7AAD positive cells in the presence of NHS compared with control IgG. These findings indicated that autoantibodies from LN activate complement on early apoptotic cells, but autoantibody-dependent complement activation are not efficiently to induce cell lysis.

IgG from the other two patients P16 and P23 had no interference with complement activation on the surface of early apoptotic cells (data not shown).

**IgG enchances phagocytosis of early apoptotic cells**

THP-1-derived macrophages were used to assess the uptake of early apoptotic cells opsonized with IgG fractions from LN patients. As shown in Figure 2, in the absence of NHS, pre incubation of early apoptotic cells with control IgG fractions resulted in a phagocytosis index comparable to that of cells pre incubated with PBS alone (22.0 ± 3.6% vs. 26.5 ± 1.6%), opsonization of early apoptotic cells with IgG fractions from patient P5 significantly enhanced the phagocytosis index to 41.0 ± 4.7%.

When the phagocytosis took place in the presence of 20% NHS, uptake of early apoptotic cells was markedly increased, which is consistent with the results of previous reports that complement activation on early apoptotic cells facilitates phagocytosis. Compared with IgG control, opsonization of IgG from patient P5 significantly enhanced the phagocytosis in the presence of NHS (64.3 ± 6.6% vs. 44.2 ± 6.1%, $p<0.01$), probably due to amplification of complement activation on the surface of the apoptotic cells. Our results demonstrated that IgG from LN patients could enhance phagocytosis of early apoptotic cells directly or dependent on complement activation.

IgG from the other two patients P16 and P23 had no interference with phagocytosis (data not shown).

**IgG induces secretion of proinflammatory cytokines by macrophages**

It has been shown that phagocytosis of apoptotic cells does not stimulate the production of proinflammatory cytokines by macrophages. As shown in Figure 3, uptake of early apoptotic cells, opsonized by NHS or not, induced limited amounts of TNF-a and IL-1β secretion by macrophages. Opsonization of early apoptotic cells by IgG from patient P5 stimulated TNF-a and IL-1β secretion apparently compared with IgG control. When early apoptotic cells coated with IgG from patient P5 were subsequently opsonized by NHS, secretions of TNF-a and IL-1β were inhibited to some extent, which was probably due to anti-inflammatory property of complement opsonized apoptotic cells. However, phagocytosis of early apoptotic cells opsonized by IgG from patient P5 and NHS still induced significantly higher TNF-a and IL-1β release compared with cells opsonized by control IgG and NHS.

IgG from the other two patients P16 and P23 had no interference with proinflammatory cytokines release (data not shown).

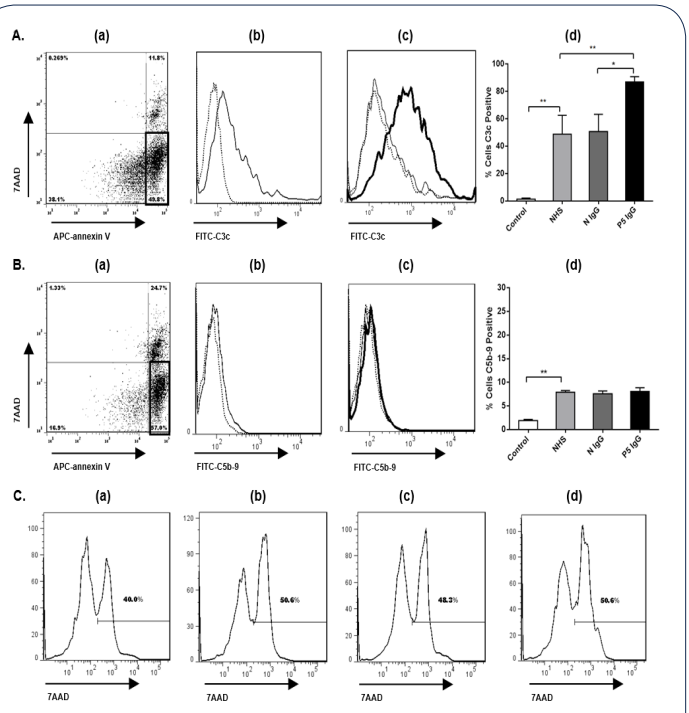


**Figure 1: IgG enhances early complement activation on early apoptotic cells.** Apoptosis was induced in Jurkat cells with UV irradiation, preincubated with 500 μg/ml IgG fractions or PBS only, then incubated with 20% normal human sera (NHS) and analyzed by flow cytometry for binding of complement components in early apoptotic cells (EA) (annexin V+/7AAD-) as well as cell permeability to 7AAD at different time points as described in Methods. In A, cells were analyzed after 30 min for C3c binding. A(b) showed C3c deposition on EA after incubation with NHS in the absence of IgG opsonization (solid line). The dotted line represented the fluorescence background stained with FITC-isotype control. A(c) showed C3c deposition induced by opsonization of control IgG (N IgG) (thin line), patient P5 IgG (thick line), and PBS only (dotted line). The results of 5 experiments are summarized in A(d) (*$p<0.05$,**$p<0.001$). In B, cells were analyzed after 60 min for C5b-9 binding. B(b) showed C5b-9 deposition on EA after incubation with NHS in the absence of IgG opsonization (solid line). The dotted line represented the fluorescence background stained with FITC-secondary antibodies only. B(c) showed C5b-9 deposition induced by opsonization of NIgG (thin line), patient P5 IgG (thick line), and PBS only (dotted line). The results of 5 experiments are summarized in B(d) (**$p<0.001$). In C, EA opsonized by patient P5 IgG (d), N IgG (c), PBS only (b) were incubated with NHS for 3 hours for 7AAD staining. In C(a), EA opsonized by PBS only were then incubated with TC buffer in the absence of NHS for 3 hours for 7AAD staining. The results shown in C are representative of three experiments.

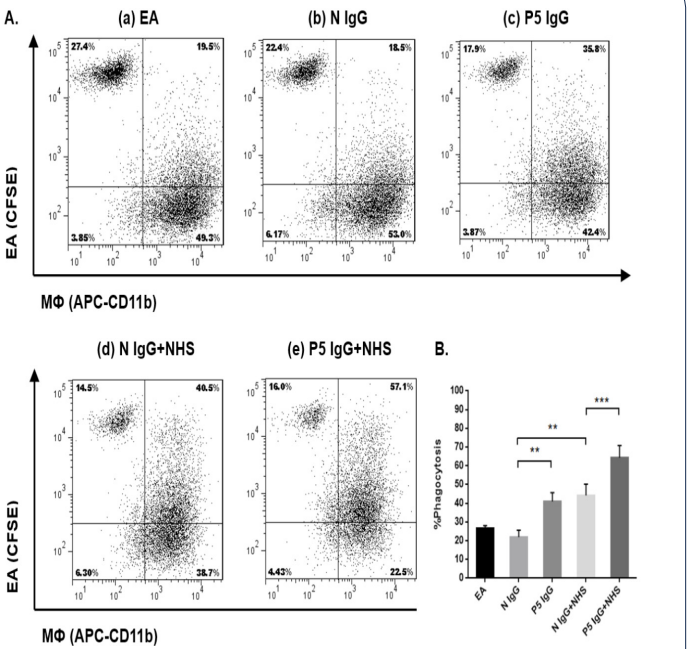


**Figure 2: IgG enchances phagocytosis of early apoptotic cells.** A(a-e) showed the results of early apoptotic cells (EA) and THP-1-derived macrophages co-incubated under different condition. Quadrants were placed on the basis of appropriate negative controls, and the percentage of CD11b-positive cells that stained positive for CFSE represented macrophages which ingested apoptotic cells. The results of 5 experiments are summarized in B, IgG from patient P5 could enhance phagocytosis of early apoptotic cells directly or in the presence of NHS compared with control IgG (**$p<0.001$).

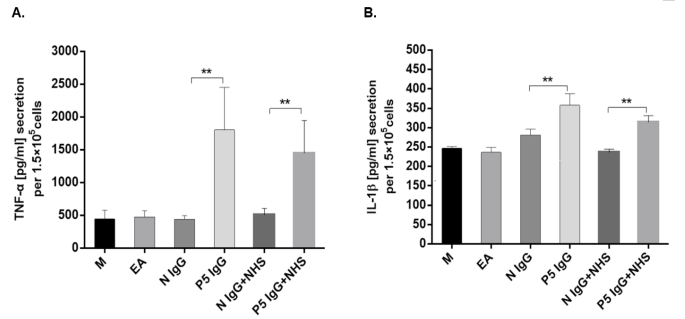


**Figure 3: IgG induces secretion of proinflammatory cytokines by macrophages.** Early Apoptotic cells (EA) or opsonized EA by IgG or/and NHS were added to THP-1-derived macrophages and supernatants were collected 18 h later. Cytokine concentrations were determined by ELISA. As a control, macrophages were incubated for 18 h with no stimulus. Data are displayed as the mean cytokine production±SEM for 5 experiments (**$p$<0.001).

## Discussion

Phospholipids and Ro 60 have been reported to be exposed on the surface of early apoptotic cells and recognized by autoantibodies from SLE. Opsonization of early apoptotic cells by affinity purified antiphospholipid antibodies enhanced recognition and phagocytosis by macrophages, with massive TNF-α secretion. In this study, we explored the subsequent effect of binding of IgG from anti-SSA positive and antiphospholipid negative LN patients on the fate of early apoptotic cells.

The interaction of complement proteins with apoptotic cells has been studied in many papers. Apoptotic cells can bind C1q [18,19], Mannose-Binding Lectin (MBL) [20], surfactant proteins A and D [21] and C-reactive protein [17]. These complement proteins are opsonins marking apoptotic cells for uptake by phagocytes. Following the binding of the recognition molecules C1q and MBL to their specific target, complement cascade can be slightly activated, resulting in complement cleavage products deposition, which are also efficient opsonins for phagocytosis [22]. In normal circumstance, opsonized apoptotic cells are cleared quickly by macrophages at the early stage, and they have little chance to undergo complement-mediated cell lysis to induce inflammation. When the phagocytosis was delayed, early apoptotic cells progressed, then complement activated extensively on the surface of the apoptotic cells. At the meantime, cells acquired fluid phase complement inhibitors to protect against excessive complement activation and lysis [23]. Immune complexes are the prominent initiators of complement classical pathway. Attali et al showed the binding of rabbit anti-Jurkat antibodies to early apoptotic Jurkat cells enhanced C3 and C9 deposition, which indicated early apoptotic cells were sensitive to antibody-dependent complement-mediated lysis [24]. To the best of our knowledge, this is the first study to explore the interference of the binding of IgG from lupus with complement activation on early apoptotic cells. We observed limited deposition of C3b on early apoptotic cells when incubated with NHS, and to a much lesser extent, C5b-9 on cells after a lag phase, which was consistent with the general findings of previous studies. The binding of patient IgG substantially enhanced and sustained deposition of C3c on early apoptotic cells, but unlike rabbit anti-Jurkat antibodies, patient IgG had no obvious interference on the formation of MAC and complement-mediated cell lysis, which was probably due to the recruitment of fluid phase complement inhibitors on apoptotic cells surface. The reason for the difference between rabbit anti-Jurkat antibodies and patient IgG might be attributed to variations in complement activation capacity.

In the case of self antigens exposed on apoptotic cells, recognition by autoantibodies may have different and even conflicting interference with the phagocytosis by macrophages. Opsonization of autoantibodies from SLE patients to late apoptotic cells inhibits their uptake by macrophages via an inhibitory FcγRIIb-dependent mechanism [9]. SLE IgG might decrease the uptake by blocking antigen molecules on the surface of apoptotic cells that are necessary for recognition and facilitation of the phagocytosis, such as C1q [25]. In NLS, binding of maternal anti-SSA antibodies to apoptotic cardiocytes results in increased uPAR expression, which is a kind of “don’t eat me” signals, then impairs the efferocytosis [14]. Previous studies have demonstrated serum complement facilitates the uptake of apoptotic cells by phagocytes [22], which was also observed in our study. In our study, patient IgG could substantially enhance the clearance of early apoptotic cells in the presence of NHS, which was probably due to amplified deposition of complement components. Furthermore, patient IgG facilitated the phagocytosis of early apoptotic cells independent of NHS. Whether IgG promoted the phagocytosis through FcγR or regulation of molecule expression during early apoptosis process needs to be investigated in future studies. If SSA was indeed the major target antigen of our patient IgG exposed on early apoptotic cells, our results suggested different roles of anti-SSA antibody played in NLS and non-neonatal lupus.

Unlike the clearance of external pathogens, phagocytosis of early apoptotic cells is a silent process inducing no secretion of pro inflammatory cytokines to avoid inflammation. In our study, we found few TNF-α and IL-1β secretion in the supernatants after the phagocytosis of either early apoptotic cells or complement-opsonized early apoptotic cells. However, phagocytosis of patient IgG opsonized early apoptotic cells induced massive TNF-α and IL-1β secretion, even in the presence of NHS. Our results indicated that opsonization of IgG from lupus promoted rapid clearance of early apoptotic cells, but it changed early apoptotic cells from anti-inflammatory to pro inflammatory properties, which indicated a contributor in the pathogenesis of lupus. Sophia et al demonstrated that anti-C1q from lupus induced a pro inflammatory phenotype in macrophages reversing the effects of C1q alone [26]. In a recent research, the Ro 60 autoantigen could bind endogenous retro elements and regulate inflammatory gene expression [27], which might provide a direction to investigate the underling mechanisms of pro inflammatory property of patient IgG.

Among the three patients involved in our study, only IgG from one patient (P5) exhibited functional effects, IgG from the other two patients (P16 and P23) had no interference with either complement activation or phagocytosis. Considering IgG from P5 having the highest binding capacity to early apoptotic cells, we thought the amount of binding IgG on the surface of apoptotic cells might determine the subsequent functional effects. Then we incubated apoptotic cells with higher concentrations of IgG from P16 and P23 and performed the functional assays. We found that in the higher concentration, more IgG bound to early apoptotic cells, but still with no interference with either complement activa-

tion or phagocytosis (data not shown). One probable explanation for the variation was that IgG from the three patients might have different isotype profiles with difference in their ability to activate complement. Lupus is a highly heterogeneous and complex disease, in our previous study exploring the functional role of anti modified C-reactive protein autoantibodies, we also observed this discrepancy [16].Further work will be necessary to verify the prevalence rate of IgG with functional effects on early apoptosis in lupus, and whether the binding of IgG to early apoptotic cells has other functional consequences besides interference with complement activation and phagocytosis.

### Limitations

Our study has a limitation in the small number of patients with lupus nephritis, more patients are needed to validate our results in future studies. Another limitation of our study is that although we used IgG from anti-SSA positive and antiphospholipid negative LN patients for the assays, the exact target antigen exposed on early apoptotic cells is unknown, which needs to be confirmed in further studies.

### Conclusion

In conclusion, although IgG from a subset of anti-SSA positive LN patients enhanced early complement activation without aggravating complement-mediated cell lysis on early apoptotic cells and facilitated rapid phagocytosis, it seemed to change early apoptotic cells from self to non self and fuel inflammation from engulfing macrophages. This effect might exacerbate underlying pathogenic mechanisms in lupus nephritis.

### Declaratios

**Competing interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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