Differential Activation of Complement by Apoptotic Cells Opsonized with Anti-Ro60 and Anti-La Autoantibodies

To the Editor:

Human anti-Ro60 and anti-La autoantibodies bind to B-cell epitopes on the surface of late apoptotic cells [1, 2]. La epitopes have been mapped to the NH₂ terminal and central regions of the protein, while an immunodominant Ro60 epitope has been mapped recently to a region at the apical tip of the Ro60 protein [3, 4]. The binding of anti-Ro60 and anti-La autoantibodies to late apoptotic cells is virtually mutually exclusive [3]. IgG-apoptotic cell immune complexes are thought to contribute to the pathogenesis of congenital heart

block (CHB) by impairing the clearance of apoptotic cardiocytes by neighbouring cardiocytes, resulting in a diversion towards macrophage uptake with subsequent inflammation and scarring [5]. The role of complement in the pathogenesis of CHB has not been formally studied. Here, we use flow cytometry analysis of C1q and C3 deposition on late apoptotic cells to compare the potential of anti-Ro60 and anti-La IgGs to activate complement.

IgG was purified from the sera of six patients with systemic lupus erythematosus with anti-Ro60 epitope

Figure 1 Flow cytometric analysis of IgG, C1q, and C3c binding to late apoptotic (annexin V, PI-positive) Jurkat cells opsonised with either healthy control, anti-Ro60, or anti-La IgGs. (A) Anti-Ro60 and anti-La IgGs show equivalent binding to late apoptotic Jurkat cells ($P > 0.05$, Mann–Whitney) however, anti-Ro60 IgG preferentially activates complement ($P < 0.0005$). Values are mean and SEM of healthy control, anti-Ro60 apopto-positive and anti-La apopto-positive IgGs, each of which was prepared from six separate patients. MFI, mean fluorescence intensity. (B) Representative flow cytometry histograms depicting the binding of IgG, C1q, or C3c to apoptotic cells opsonised with healthy control (black line), anti-Ro60 (red line) and anti-La (blue line) IgGs.

reactivity; six patients with primary Sjögren's syndrome with anti-La apotope reactivity; and six healthy controls. Late apoptotic Jurkat cells were incubated with individual IgGs (500 µg/ml) for 30 min at room temperature, washed three times in PBS with 1% foetal calf serum (FCS) and IgG binding assessed by multi-parameter flow cytometry as detailed previously [6]. Parallel aliquots of apoptotic cells preopsonized with either anti-Ro60 IgG, anti-La IgG or healthy control IgG were incubated with 20% normal human serum as a source of complement for 30 min at 37 °C and washed two times in PBS with 1% FCS. Apoptotic cell surface-bound C1q and C3b/iC3b were detected with rabbit anti-human C1q and C3c antibodies, respectively, on flow cytometry of gated late apoptotic cells (annexin V/PI positive).

Similar levels of cell surface-bound IgG were detected by flow cytometric analysis of late apoptotic cells incubated with IgGs specific for either Ro60 or La apotopes (Fig. 1). In contrast, a high level of C1q and C3 deposition was only observed on apoptotic cells coated with anti-Ro60 IgGs, whilst little or no C1q or C3 was detected on apoptotic cells opsonized with anti-La or healthy control IgGs (Fig. 1). This differential activation of complement by the two populations of IgG-apoptotic cell immune complexes cannot be explained by differences in the levels of anti-Ro60 and anti-La IgG membrane binding. The binding of C1q and C3 on the anti-Ro60 IgG-apoptotic cell complexes is consistent with activation of the classical pathway of complement (Fig. 1).

The differential activation of complement by anti-Ro60-opsonized apoptotic cells was unexpected, given similar levels of anti-Ro60 and anti-La IgG binding to their respective apotopes. The discordance between opsonization and complement activation cannot be explained by antibody isotype, because both autoantibodies comprise complement-activating IgG1 (anti-Ro60) and IgG1/IgG3 (anti-La) subclasses [7]. We hypothesize that IgG autoantibodies that bind to Ro60 apotopes are able to form aggregates on the apoptotic cell surface that favour the binding of C1q and subsequent activation of complement. A precedent comes from studies with different anti-CD20 antibodies, whose differential abilities to activate complement were found to correlate with the formation of antibody complexes in lipid rafts [8]. The mechanism by which Ro60 autoantigen translocates to the apoptotic cell surface and exposes an apotope is unknown. A possible role of apoptotic cell lipid rafts in this process and their effect on complement activation is worth further investigation. The pathogenic role of complement in CHB, if any, may depend on the balance between C1q-mediated uptake of apoptotic debris and complement-mediated pro-inflammatory activity.

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J. H. Reed*, L. Thurgood†, D. L. Gordon‡
& T. P. Gordon†

*Department of Medicine, Division of Rheumatology,
New York University School of Medicine, New York,
NY, USA;

†Department of Immunology, Flinders Medical Centre,
Bedford Park, SA, Australia; and

‡Department of Microbiology and Infectious Diseases,
Flinders Medical Centre, Bedford Park, SA, Australia

Correspondence to: J. H. Reed, PhD,
Department of Medicine, Division of Rheumatology,
New York University School of Medicine,
560 First Avenue, New York,
NY 10016, USA.
E-mail: joanne.reed@flinders.edu.au