39 Apoptosis and Antiphospholipid Antibodies

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Introduction

In the early 1980s, a motley crew of itinerants strove to discover the cause of systemic lupus erythematosus (SLE) in Graham Hughes' laboratory at the Hammersmith Hospital, London. The crew included myself (KBE), Azzudin Gharavi, Bernie Colaco, and others. Having presented a stimulating paper by Robert Schwartz at a journal club demonstrating that some murine anti-DNA monoclonal antibodies cross react with phospholipids [1], Aziz, Bernie, and I decided to test the same idea in human SLE, and Graham decided to re-explore the clinical associations of anticardiolipin (aCL) autoantibodies. The resulting publications [2, 3] were a start and led to the subsequent collaboration between Aziz and Nigel Harris, and the development of the quantitative solid-phase immunoassay for antiphospholipid antibodies (aPL) that transformed the field.

In this brief review, we will provide an outline of apoptosis and discuss its relevance to aPL and the development of systemic autoimmunity.

Why Is Apoptosis Clinically Relevant to aPL and Systemic Autoimmunity?

A number of clinical observations have focused attention on the products of apoptotic cells as antigens or immunogens in SLE:

1. An increase in apoptosis of SLE peripheral blood mononuclear cells (PBMC) in vitro has been observed [4] and has been correlated with lymphopenia in the patient [5]. Freshly isolated lymphocytes from patients show high annexin V binding [6] and elevated caspase 3 functional activity [7], suggesting that accelerated apoptosis occurs in vivo.
2. SLE macrophages *may* have a reduced uptake of apoptotic cells in vitro [8].
3. Nucleosomes (histones complexed to DNA), a product of apoptotic cells (see below) are detected in the circulation of SLE patients with active disease [9].
4. Nucleosomes are more strongly antigenic than DNA or histones alone, and antibodies to nucleosomes precede those to DNA and histones [10, 11].

5. Nucleosomes, but not isolated DNA or histones, deposit in the glomeruli, suggesting that it is in situ fixation of nucleosomes, rather than DNA/anti-DNA immune complexes that causes lupus nephritis [12, 13].
6. SLE antigens are redistributed to apoptotic blebs when cells such as keratinocytes undergo programmed cell death [14]. Some, but not all of these antigens undergo modification, including cleavage and phosphorylation. It is possible that this makes them more antigenic.
7. Phosphatidylserine (PS), the negatively charged phospholipids that flips from the inside to outside of the dying cell (see below), may serve as an antigen for aCL [15] and PS (either on activated platelets or apoptotic cells) provides a scaffold for the coagulation cascade [16, 17].

In addition to SLE, aPL have been detected in other autoimmune diseases where apoptosis is abnormal. For example, impaired Fas mediated apoptosis results in massive lymphadenopathy and systemic autoimmunity in humans [18, 19]. It appears that aPL directed at phospholipids and at related proteins such as β_2-glycoprotein I (β_2-GPI), prothrombin, and annexin V are a common feature in these patients [20].

aPL Bind to Many Different Antigens Derived from Apoptotic Cells

The aPL traditionally refer to both aCL and lupus anticoagulant antibodies (LA), identified by in vitro assays that quantify aCL binding or prolongation of coagulation, respectively. Although prototypic aPL bind to negatively charged phospholipids, aPL react with a broad array of cell membrane derived phospholipids and their associated proteins that include not only cardiolipin (CL), but also phosphatidylserine (PS), phosphatidycholine (PC), phosphatidyethanolamine (PE), β_2-GPI, prothrombin, protein C, protein S, kininogen, and annexin V (see other chapters in this volume).

The polyclonal autoantibodies that occur in patients are heterogeneous with respect to their fine binding specificities, affinities, effects on coagulation, and their role in the antiphospholipid syndrome (APS). Although these antibodies could be generated by polyclonal B cell activation or cross-reactivity in an immune response to foreign antigen (for a review of aPL generated via infectious disease-related molecular mimicry see [21]), in this chapter we will provide evidence to support the argument that aPL are generated in response to dead and dying cells. This evidence includes the localization of CL in mitochondrial membranes (a critical site involved in the regulation of apoptosis as discussed below); the translocation of another negatively charged phospholipid, PS, which is normally located on the inner cytoplasmic leaflet of the lipid bilayer to the outer cell surface during apoptosis; and, finally, we review studies showing that aPL can be generated by immunization of animals with dying cells.

What Is Apoptosis?

The term *apoptosis* was coined by Kerr, Wyllie, and Currie in 1972 to describe the form of cell death characterized by shrinkage, nuclear condensation, and cell blebbing [22]. Notably, the process of apoptosis is ATP dependent, and the observable