38 Lessons from Sequence Analysis of Monoclonal Antiphospholipid Antibodies

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Introduction

The identification of antiphospholipid antibodies (aPL) is central to the diagnosis of the antiphospholipid syndrome (APS) [1]. A great deal of evidence exists from both clinical and laboratory studies to support the idea that aPL are directly pathogenic and not merely an epiphenomenon. Not all aPL, however, are associated with pathogenicity. aPL can occur in healthy adults and in some patients with infectious, malignant, or drug-related disorders, but do not cause thrombosis or fetal loss in those people. These non-pathogenic aPL mostly bind both neutral and negative phospholipid (PL) with low affinity and lack co-factor dependence [2]. In contrast, aPL found in patients with APS, whether primary or secondary, are generally IgG in isotype [3, 4], target predominantly negative PL, and require the presence of serum co-factors in order to bind PL [5–8]. The reason for this co-factor dependence is that the majority of pathogenic aPL are in fact not directed against PL at all but target serum proteins that bind PL. These proteins include protein C [9], protein S [9], prothrombin [10], and β2-glycoprotein I (β_2-GPI) [5–7]. β_2-GPI is the most extensively studied of these proteins and appears to be the most relevant clinically [11–13].

A wealth of evidence implicating aPL in the pathogenesis of thrombosis and pregnancy morbidity and suggesting potential mechanisms of action has been generated from the study of monoclonal aPL. These antibodies (Ab) are ideal for investigating the relationship between the structural properties that are important in determining the ability of pathogenic aPL to bind PL and/or β_2-GPI and the properties that determine their pathogenicity. This chapter will review the origin, binding characteristics, molecular properties, and biological function of all human monoclonal aPL for which sequence analysis has been published and the lessons learned from this with regard to the relationship between aPL structure and function.

Monoclonal Antibodies

The usefulness of monoclonal antibodies (mAb) stems from 4 characteristics: their specificity of binding; their homogeneity; their ability to be produced in unlimited

quantities; and the relative ease with which their antigen binding site may be manipulated. In 1975, a method was devised that allowed Ab secreting cells isolated from an immunized mouse to be fused with a myeloma cell [14]. These hybrid cells (called *hybridomas*) have the immortal growth properties of the myeloma cell and the Ab producing properties of the B cell. The resultant cell lines can be maintained in vitro indefinitely and will continue to secrete Ab with a defined specificity, known as monoclonal Ab. This method was later adapted to produce human mAb from splenic and peripheral blood lymphocytes (PBL) [15, 16].

Hybridoma cells contain large quantities of mRNA encoding the light and heavy chains of the secreted mAb, which can be used to make and amplify cDNA. Sequence analysis of this cDNA gives the nucleotide and amino acid sequence of the mAb. This analysis is a powerful tool to gain greater understanding of features at the molecular level which distinguish natural (non-pathogenic) autoantibodies found in healthy individuals from pathogenic autoantibodies found in patients with autoimmune disease. It has been suggested that the properties of IgG isotype, specificity for negatively charged PL, and ability to bind β_2-GPI may define a population of pathogenic aPL which are particularly likely to cause APS. To investigate the factors that determine these properties at the molecular level, it is important to study the sequences and molecular structures of pathogenic aPL, and thus identify features which are common to those antibodies but are less commonly found in non-pathogenic aPL.

Analysis of Antibody Sequences to Identify Genes of Origin

An antibody molecule is composed of 2 identical heavy chains and 2 identical light chains which can be either κ or λ. The carboxyl terminal domains of both the heavy and light chains are highly conserved and are called the constant (C) regions. The amino terminal domains are much more variable in sequence and are responsible for binding antigen. These domains are known as the heavy chain variable region (V_H) and light chain variable region (V_L).

Antigen binding sites usually comprise 3 polypeptide loops from the V_H domain and 3 polypeptide loops from the V_L domain [17]. These 6 polypeptide loops are regions of high variability (in both sequence and length) known as the complementary determining regions (CDRs). The CDRs are separated by more conserved regions which perform a structural role and are hence called the framework regions (FRs) [18]. In analyzing those sequence features of aPL which are responsible for their binding properties, therefore, it is only necessary to consider the variable regions. Within these regions, the CDRs are likely to be of particular interest.

In humans, functional immunoglobulin (Ig) heavy, κ, and λ genes are found upon separate chromosomes. Sequences encoding the variable regions are assembled during early B cell development by the site specific recombination of three segments, the V_H, D_H, and J_H genes, to form the V_H domain and 2 segments, V_L and J_L, which then encode the V_L domain [19]. The genes in these loci have been sequenced and mapped [20–23]. The availability of a relatively large number of potentially functional variable region genes for recombination contributes to the diversity of possible Ab sequences (and therefore possible antigen binding sites) that can be produced. Diversity is amplified by imprecision at the joining of V_L– J_L, V_H–D_H, and D_H–J_H segments with deletion or insertion of nucleotides [19].