Clinical and Experimental Rheumatology 2000; 18: 431-432.

The relationship of anti-phospholipid antibodies to infections - Do they bind to infecting agents or may they even be induced by them?

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Antiphospholipid antibodies had already been detected in human serum at the beginning of the 20th century, when Wasserman introduced his complement fixation test (WR-CFT) into routine clinical practice for the diagnosis of syphilis (1). Simultaneously, the Venereal Disease Research Laboratory (VDRL) test for syphilis was described (2). The tissue extracts used in the WR and VDRL tests were subsequently shown to be an acidic phospholipid (PL) which was called cardiolipin (CL), as it was present in an alcoholic extract of bovine heart (3). These early experiments showed that the WR and VDRL tests were measuring anti-PL antibodies, which were not entirely specific for the disease (3). The discovery and characterization of CL have advanced in a little over 40 years from its original recognition as the antigen responsible for the detection of reagins in the serological tests for syphilis to its use as the antigen in solid phase aPL-specific assays, and to being employed in the detection of aCL and other aPL (4).

PL molecules are ubiquitous in nature, presenting in every living cell, be it on the inner or outer surface of the cell membrane or intracellular organelles. It is therefore quite conceivable that these molecules could by some unknown mechanism that causes the disruption of cellular membranes and cell destruction - as in the case of a large numbers of infectious diseases, become antigenic and induce the production of aPL. Indeed, aPL have been detected in a large number of infectious diseases, including viral (e.g. HIV, EBV, CMV, adenoviruses), bacterial (e.g. bacterial endocarditis, tuberculosis, mycoplasma pneumonia) spirochetal (syphilis, leptospirosis, lyme disease) and parazitic (malaria) infections (5).

The direct pathogenic role of the aPL was established in 1991 when, following passive infusion of monoclonal and polyclonal mouse and human aCL to naive BALB/c mice, the fully expressed syndrome was induced (6, 7). In 1990, three groups reported on a new classification of aPL- the "pathogenic" antibodies (autoimmune type) whose binding to their respective PL is enhanced by a cofactor, while the "infectious" type and the non-thrombogenic aPL do not require a cofactor (8-10). The aPL cofactor was soon defined as β2-glycoprotein-I (β2-GPI), a 50 Kd serum glycoprotein which has anticoagulant properties (11-13). Many authors have suggested that anti-β2GPI Abs are a better clinical indicator of APS (14, 15). It has been postulated that anti-(2GPI Abs exert a direct pathogenic effect by interfering with homeostatic reactions occurring on the surface of platelets or vascular endothelial cells, passive transfer of these Abs into naive mice resulted in induction of experimental APS (16, 17).

The study of Elbeialy *et al.* published in this issue (18) advocates the conception that infection-associated aPL do not possess anti-β2GPI activity and are not associated with thrombotic manifestations of the APS. In a group of 35 lepromatous leprosy patients, 13 (37%) were found to have aCL Abs but only 1 patient also had anti-β2GPI activity and none of the patients had thrombotic complications.

The dogmatic distinction, however, between infectious-aPL Abs (i.e. β2GPI-independent and non thrombosis-associated) and autoimmune aPL Abs (i.e. β2GPI-dependent and APS associated) has been recently challenged by several reports. With respect to leprosy, Hojnik *et al.* found increased levels of anti-β2-GPI Abs in a significant proportion of leprotic patients (19). Their observation was confirmed by other investigators who indicated that these β2GPI-dependent aPL were associated with thrombosis in leprosy (20). In another study, parvovirus B19-associated aCL Abs were shown to be β2GPI-dependent and to behave in a similar fashion as the autoimmune aPL (21). Furthermore, some cases of viral hepatitis C-associated aPL have been reported to be complicated with thrombosis (22).

The relationship between the full form of APS, even in its most severe presentation ("catastrophic APS") and infectious processes was further strengthened by recent reports on the association between APS and several bacterial and viral pathogens (23-25). Although the incidence and clinical significance of β2GPI-dependent and anti-β2GPI Abs in infectious diseases remain largely unknown, it is possible that infections may

be a trigger for the development of pathogenic aPL Abs and therefore promote the development of APS, particularly in predisposed individuals. It could be hypothesized that aPL Abs may be induced by the *in vivo* binding of foreign (infectious?) β2GPI to self-PL, thus forming an immunogenic complex against which aPL Abs are produced.

Indeed, employing the shotgun phage display technique, Zhang *et al.* (26) identified a *Staphylococcus aureus* protein Sbi, which binds β2GPI and serves as a target molecule for IgG binding. Gharavi *et al.* demonstrated that synthetic peptides which share structural similarity with the putative phospholipid binding region of the β2GPI molecule and also share high homology to cytomegalovirus (CMV), induce the generation of aPL and anti-β2GPI Abs in NIH/Swiss mice (27). These findings demonstrated that some phospholipid-binding viral and bacterial proteins function like β2GPI in inducing aPL and anti-β2GPI production, and are consistent with a role for such viral and bacterial proteins in inducing aPL Abs production in humans.

Using a hexapeptide phage display library, Blank *et al.* have identified 3 hexapeptides that react specifically with anti-β2GPI mAbs, and which cause endothelial cell activation and induce experimental APS (28). *In vivo* infusion of each of the anti-β2GPI mAbs into BALB/c mice, followed by administration of the corresponding specific peptides, prevented the peptide-treated mice from developing experimental APS (28). Employing the Swiss Protein database, a high homology between the hexapeptides and various bacteria, viruses and parasites was found (Blak *et al.*, unpublished data). It is therefore possible that occasional immunization during an infectious process with specific microbial pathogens may trigger, possibly via molecular mimicry, the production of pathogenic aPL Abs followed by the emergence of the full blown APS.

It is only now, nearly a century since the description of the complement fixation test for syphilis by Wasserman, that we have begun to discover the secrets of APS etiology and pathogenesis, in which infection plays a considerable role.

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