Editorial

The Antiphospholipid Story

Throughout the history of humanity, and except for a few contributions by true pioneers, each bit of new knowledge or new development has been based on the work of many others, often unknown, who have helped build the corresponding edifice. Each milestone takes up so many yards, inches, centimeters, or millimeters according to the relative importance of the apparent new knowledge. However, the lack of even an apparently millimetric step, in this same sense, might prevent reaching the milestone. Stephenson may not have thought about it, but his invention of the railroad steam engine was only possible because throughout history people had learned to harness fire, including the unknown primitive humans who first learned to produce and keep it.

I will attempt to recount the saga of the antiphospholipid syndrome (APS), including my own experiences, with the precision of the scientist, the insight of one who has lived through it, and the emotion of one who sees in this story a wonderful example of the sum of contributions made for the sake of knowledge and helping fellow humans. Interest in this topic should not falter if we are to understand it fully, and with this understanding be able to provide our patients with a definitive solution.

I would like to take you as far back as 1906, to Wassermann and his coworkers[1], who developed serological reactions for the diagnosis of syphilis utilizing phospholipid-rich tissues as antigens, later to be termed cardiolipin by Pangborn (1941)[2]. The finding of individuals who were positive for these reactions, but who were either too pious or too young to be suspected of having acquired syphilis, first brought about the possibility of false positive serological reactions for this disease, and tests were developed to distinguish these from the true positive reactions.

The first milestone resulted from the attempt to determine who, and perhaps why, some individuals had such false positive reactions for syphilis, and the finding that the majority were women who, when followed by Moore and Lutz (1955)[3] for enough time, developed systemic lupus erythematosus (SLE). Interestingly, 3 of their 21 lupus patients with antecedent false positive tests for syphilis had had major unexplained thrombophlebitis. Here we should acknowledge the contributions, albeit indirect, of Hargraves, Richmond, and Morton (1948)[4], who found LE cells that permitted a more ample diagnosis of SLE, and of Edmund Dubois (1953)[5], who helped us recognize its wider clinical spectrum.

The second milestone resulted from the search for the cause of bleeding in a patient by Conley and Hartmann (1952)[6], with the resulting finding of an inhibitor of coagulation that was subsequently related by Sánchez-Medal and Lisker (1959)[7] from our own institute to patients with SLE, most of whom did not bleed.

The paradox encountered by Bowie and his coworkers (1963)[8] of a frequent occurrence of thrombosis, rather than bleeding, in patients with SLE who had this inhibitor of coagulation, subsequently termed lupus anticoagulant by Feinstein and Rapaport (1977)[9], marks the initiation of the concept of APS, particularly when somewhat later Thiagarajan, *et al* (1980)[10], found that at least some of the lupus anticoagulants actually were antiphospholipid antibodies (aPL).

Shortly after the study of Bowie and his coworkers, and stemming from the same institution, Alarcón-Segovia and Osmundson (1965)[11] described 11 patients with SLE who had peripheral vascular syndromes. Some of these are particularly interesting: one had chronic ulcers of the legs and livedo reticularis, clinical manifestations later found to be associated with aPL, and both circulating anticoagulant and false positive serologic tests for syphilis; another patient had had 4 miscarriages, thrombophlebitis, livedo reticularis, thrombosis of the left ulnar artery, convulsions, and long-standing false positive tests for syphilis; a third patient had false positive tests for syphilis, recurrent superficial thrombophlebitis, leg ulcers, intermittent claudication with evidence of popliteal artery occlusion, a vascular lesion of the brain stem, and a terminal occlusion of a basilar artery.

Shortly thereafter (1967) in a letter to *Lancet*, I insisted on the association of a circulating anticoagulant with infarction rather than bleeding[12]. Somewhat later (1974) Johansson and Lassus[13] would indicate the concurrent findings of circulating anticoagulants and false positive tests for syphilis as in our described patient.

In 1980 Soulier and Boffa[14] recorded the occurrence of recurrent abortions, thromboses, and a circulating anticoag-

ulant in patients not having a primary condition, and in 1981 Carreras and his coworkers[15] studied the possible role of inhibition of prostacyclin formation by the lupus anticoagulant in the causation of thrombosis.

The next milestones in the antiphospholipid story resulted from the development of more sensitive tests for aPL: in 1980 radioimmunoassays by Smolarsky[16], by Harris and coworkers in 1983[17], and more practical, an ELISA developed by Loizou and his coworkers, using cardiolipin as antigen (1985)[18]. The more ample use of these tests permitted Hughes[19] to propose the occurrence of an anticardiolipin syndrome in patients with SLE (1985). In some of his writings at that time Hughes mentioned that there were some patients with this syndrome who had no lupus, but he did not actually describe such patients.

The notion that within SLE there might be patients with a set of manifestations caused by one of their multiple autoantibodies and thus have a specific syndrome seemed interesting enough to me to try to confirm it. I therefore wrote Graham Hughes requesting some specific instructions on their anticardiolipin ELISA. I obtained a prompt response from Nigel Harris with instructions as well as anticardiolipin positive sera. Carmen Oria, a Venezuelan chemist working in our laboratory, was then able to reproduce the method, with modifications (1988)[20]. Meanwhile, with the help of Margarita Delezé, an enthusiastic rheumatologist who had come to our department after her hospital was destroyed in the 1985 Mexico City earthquake, we had started a cohort of consecutive lupus patients who were to be repeatedly tested for anticardiolipin antibodies. When the cohort reached 500 patients with a mean followup of more than 7 months and at least 5 antiphospholipid antibody tests of IgG, IgM, and/or IgA isotypes, we published our results[21] confirming the existence of an APS in patients with SLE and documenting the clinical manifestations associated with high levels of aPL (1989). We would subsequently extend our cohort to 667 lupus patients, and after 3.5 years of followup, we proposed classification criteria for the APS (1992)[22]. Consensus criteria for the APS were later proposed (1999)[23].

Analyzing our findings in the initial cohort of the 500 lupus patients, it became clear to me that there were patients who had the same clinical manifestations and high concentrations of anticardiolipin antibodies but who had no other clinical or serologic evidence of SLE. A search of the literature did not reveal any descriptions of this, although there were isolated reports of patients with some of these manifestations and those who had a circulating anticoagulant, as also described by Soulier and Boffa, whom we cited[14].

With the help of Jorge Sánchez-Guerrero I prepared a description of 9 patients with what we termed primary antiphospholipid syndrome. Eventually, after some unexpected delays, on September 12, 1988, we submitted our manuscript to *The Journal of Rheumatology*; it was ultimately published early in 1989, and became one of its most highly cited articles[24]. At the end of 1988, however, Asherson published an editorial on the same topic in *The Journal*[25]. It was supposed to have accompanied our paper.

About 3 months later a paper by Mackworth-Young and coworkers appeared describing a series of patients with the same syndrome[26], and later in the same year a larger multicenter series coordinated by Asherson was also published[27]. Our paper[24] was already cited in their publication[27]. On July 5, 1993, Graham Hughes and I shared the Ciba-Geigy–International League Against Rheumatism Award, given to us at its international congress in Barcelona, for our contributions to knowledge on the APS.

A further milestone was reached in 1990 at the antiphospholipid meeting in Sirmione, Italy, when Monica Galli proposed that antibodies detected by ELISA tests with cardiolipin as antigen were not directed to the phospholipid but to a protein cofactor present in the bovine serum used to block the plates. This was supported by Steven Krilis, who had actually determined that the protein cofactor was $ß_2$-glycoprotein-I ($ß_2$-GPI), which had natural anticoagulant properties and high affinity for anionic molecules such as cardiolipin. Moreover, as noted by Takao Koike, pathogenic antibodies can be distinguished from those present in infectious disease (e.g., syphilis). The pathogenic antibodies require the presence of bovine serum with such cofactor, the infectious antibodies do not, he said. The corresponding articles were published that same year (1990)[28-30].

At a $ß_2$-GPI symposium in Milan in 1992, Cabral and his coworkers described the presence of anti-$ß_2$-GPI antibodies in a group of patients with primary APS[31]; this finding was also published by Viard, *et al* that year[32]. That these antibodies may have the most important role in the causation of the APS, particularly its thrombotic component, was evidenced by their systematic fall at the time of thrombosis, as found by Gómez-Pacheco and coworkers[33] in 1999.

An important study by Matsuura, Koike, and their coworkers[34] (1995) revealed that the so-called anticardiolipin antibodies could bind to $ß_2$-GPI in the absence of cardiolipin if the ELISA plates were oxidized by irradiation. This procedure was considered by Roubey and coworkers in 1995[35] to cause higher antigen density and permit bivalent binding. In 1995 Cabiedes, *et al*[36] found that manifestations of APS associated more strongly with antibodies to $ß_2$-GPI determined in non-irradiated plates versus those with anticardiolipin, and Cabral, myself, and our respective coworkers subsequently (1996, 1997)[37] described groups of patients with clinical manifestations of APS who had persistently negative anticardiolipin antibodies when studied in conventional assays, but persistently positive anti-$ß_2$-GPI antibodies determined in non-irradiated plates.

In 1988, early in the antiphospholipid story, I began inquiring about the pathogenic potential of aPL in an editorial[38], and participated in a series of studies on their role in

deficiencies of natural anticoagulants, headed by Guillermo and Alejandro Ruiz-Argüelles (1989)[39], in a study by Vázquez-Mellado, *et al* (1994)[40] on their role in thrombocytopenia by recognizing $ß_2$-GPI that had bound to anionic phospholipids switched to the outer leaflet of platelets upon their activation or aggregation, as well as in a study by Cabral, *et al*[41], where we found a role for an IgM natural autoantibody to phosphatidylcholine in the causation of hemolytic anemia (1990).

The synergism between aPL and antiendothelial cell antibodies in the causation of vascular damage in the APS began to be explored by Meroni and coworkers (1992)[42], and a potential role of aPL in the causation of atherosclerosis stemmed from the seminal work by Vaarala and coworkers on the crossreactivity between anticardiolipin antibodies with oxidized low density lipoprotein (1993)[43].

But perhaps the most important milestone for determining the pathogenicity of antiphospholipid antibodies came with the elegant series of studies by Shoenfeld and his group (1991)[44,45], i.e., animal models of the APS induced both by passive and by active immunization, as well as their therapeutic manipulation (1999)[46]. In addition, these workers identified a hexapeptide that is recognized specifically by pathogenic anti-$ß_2$-GPI antibodies and by antibodies produced on immunization of mice with microbial preparations. Naive mice infused with the antipeptide antibody produced by these immunizations developed clinical evidence of an antiphospholipid syndrome, indicating that molecular mimicry elicited by infections may lead to the development of pathogenic anti-$ß_2$-GPI antibodies reactive with this peptide (2002)[47].

The presence of autoreactive interleukin 6 producing CD4+ T cell clones to $ß_2$-GPI in patients with APS was recently identified by Kuwana and his group (2001). These cells recognized at least 4 different epitopes, but the majority recognized a 15 amino acid peptide in the phospholipid-binding fifth domain. Most of these T cells stimulated autologous blood B cells to promote anti-$ß_2$-GPI production in the presence of recombinant $ß_2$-GPI. In a subsequent study they found that such autoreactive T cells had restricted T cell receptor ß-chain usage (Vß7 and Vß8) (2002)[48].

Some of these findings may have implications regarding potential forms of treatment. Other avenues of treatment reside in the induction of tolerance of anti-$ß_2$-GPI-producing B cells or in anionic blockade of the phospholipid-binding sites of $ß_2$-GPI by means of heparin or other compounds, as proposed by Guerin and coworkers (2002)[49].

Before the concept of an antiphospholipid syndrome originated, lupus patients with venous occlusions and particularly those with arterial occlusions were treated mainly with corticosteroids and immunosuppressives. In addition, patients with primary APS were often diagnosed as lupus and met classification criteria for this disease. This could have been considered reasonable were it not for the unnecessary steroid treatment they received instead of merely anticoagulant and/or platelet antiaggregant treatment.

We have come a long way in our understanding of the antiphospholipid syndrome. In looking back, I cannot help but feel that the wheels of the antiphospholipid story are turning more slowly, and at times even seem to stop. It might be only natural that the previous large scope of studies would now tend to be reduced. However, many unknowns await our renewed vigor. Only with vigor can we attain a brighter future for our patients.

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