Lupus anticoagulants and anticardiolipin antibodies: personal reminiscences, a little history, and some random thoughts

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I want to thank Dr Harold Roberts for so kindly asking me to write this ‘Reminiscence’ piece, even if it is a gentle reminder of which side of the hill I am on.

My first contact with research in hemostasis came in 1954 when Don McKay – then in the Harvard Medical School pathology department, and later to become chairman at Columbia – took pity on a poor unseasoned second year medical student and agreed, despite my lack of previous experience, to let me work in his lab during the summer. He was studying the Schwartzman phenomenon – what would now be called DIC – induced in rabbits by endotoxin. One thing led to another, and I found myself continuing in his lab on a regular basis during my junior and senior years. By the time I graduated we had published three papers in the Journal of Experimental Medicine, on one of which – the demonstration that we could prevent the phenomenon with warfarin – Don was generous enough to make me first author, perhaps in recognition of the hundreds of venipunctures I had undergone. In the course of our work I had made the acquaintance of the Thorndike Laboratory’s resident hemophiliac patient, Russell White, whose blood we used in several in vitro studies and who had been the first recipient of a corrective plasma fraction, Edwin Cohn’s Fraction I, thus shown to contain the aptly named ‘antihemophilic factor’ (AHF). In 1958, after my internship, I fell into an opportunity to set up a coagulation laboratory in the NIH’s Division of Biologic Standards (now the FDA), where I had my second contact with a hemophiliac when, in the course of establishing coagulation factor normal ranges, I found that a strapping young technician, who had played football in high school and spent two active years in the US Army, had mild hemophilia, with a factor VIII level of 12%. In 1964, shortly after Allan Erslev recruited me to the Cardeza Foundation for Hematologic Research and the Division of Hematology at Jefferson Medical College, I found myself involved in the care of patients with hemophilia after the tragic death in a plane crash of Philip Geisler, who was on his way to a scientific meeting. Phil had been the director of a hemophilia center started through the interest and determination of Leandro Tocantins, Allan’s predecessor as Director. Tocantins believed that hemophilia might be due to the presence of an inhibitor to FVIII, and had presented a substantial amount of experimental evidence consistent, he thought, with that theory. Despite the incorrectness of the theory, I was thus introduced to the concept of coagulation factor inhibitors and, shortly thereafter, found myself involved in the diagnosis and treatment of three patients with inhibitors – one a hemophiliac, the second a postpartum woman, and the third a young man with ulcerative colitis. We showed that all three inhibitors were IgG immunoglobulins, confirming a previously held strong suspicion, and that the antibodies were probably of moderately restricted heterogeneity. With the impetus of this series of studies, in 1975 Mae Hultin and I wrote an ‘all-inclusive’ review of inhibitors to coagulation factors and in the period 1975–80 I directed an NIH-sponsored national co-operative study on the natural history of FVIII inhibitors.

Early during the course of our studies, I had become aware of the existence of the strange phenomenon first described by Mueller et al. in 1951 and by Conley & Hartmann in 1952 (reviewed in [1]), that Don Feinstein and Sam Rapaport, in 1972 [2], dubbed the ‘lupus anticoagulant’ (LAC). This peculiar entity, originally associated with SLE, was characterized by prolongation of almost all tests involving the use of a lipid reagent but, strangely enough, was not accompanied by a bleeding tendency. As a matter of fact, as early as 1963 Walt Bowie, at the Mayo Clinic, had published his experience with a small set of such individuals who not only did not bleed but, to the contrary, actually also had clotting problems [3]. In addition, Laurell and Nilsson, as early as 1957, had suggested that this peculiar laboratory entity might be associated with a
syndrome of recurrent abortions, and even suggested the possibility that the prolonged tests might be associated with inhibitory activity against phospholipids [4]. Nevertheless, it is fair to say that most of us working in hemostasis and thrombosis thought of these observations – if we thought of them at all – as strange, rare, and decidedly odd.

For me, this state of affairs took a decided turn when, in 1978, Scott Murphy brought to our attention a new patient of his with Waldenstrom’s macroglobulinemia whose PT and PTT were nearly infinite, and several of whose clotting factors were unmeasurable by the usual tests. After some ‘playing around’ with the sample and a variety of tests, it occurred to Perumal Thiagarajan (‘Rajan’), Luigi deMarco, and me that the patient’s findings might be due to the presence of a potent lupus anticoagulant, which could well be the macroglobulin itself, giving us an unusual opportunity to study the mechanism of action of this strange inhibitor. We easily purified the homogeneous IgM and were able to demonstrate that the isolated immunoglobulin and a derived monovalent tryptic Fabγ fragment (i) were capable of inducing in normal plasma all the abnormalities seen in the patient’s plasma, (ii) completely inhibited Ca++-dependent binding of radiolabeled prothrombin and factor X to mixed phospholipid micelles but not of FXa to thrombin-treated platelets, and (iii) reacted in immuno-diffusion with anionic phospholipids, but not with phosphatidylcholine or phosphatidylethanolamine [5]. Over time, we developed a method for affinity-purifying the more common IgG LAC activity from SLE and non-SLE patient plasmas and demonstrated a similar phenomenology [6,7]. In addition, we found that very small amounts of such activity could be detected even in plasmas from normal individuals. In order to follow the activity through various fractionation procedures, Rajan, Vittorio Pengo, who was then in the lab, and I developed a test for LAC activity using a variation of the ‘Styven Time’, a test making use of Russell Viper venom, and in 1986 published the test, the dRVVT, in Blood [8]. I remember remarking to Rajan that it would probably be his most quoted publication, a premonition that proved accurate, now that the dRVVT has become the most commonly used test for detection of lupus anticoagulants. What a shame for our financial futures that it did not occur to us at the time to patent the procedure!

Based on our studies, in 1983, Nigel Harris, in Graham Hughes’ rheumatology laboratory in London, devised a radioimmunoassay [9], and later an ELISA, for the detection of antibodies reacting against anionic phospholipids, using cardiolipin as the target phospholipid. This test led to a burst of activity in the field and a realization that ‘antiphospholipid’ antibodies were much more common than any of us had appreciated. The test led directly to the major discovery (reviewed in [10]), made in 1990 in three laboratories independently, that reactivity in the cardiolipin ELISA depended almost completely on the presence of a then (and still) mysterious blood protein known as β2-glycoprotein I (β2GPI), a.k.a. apolipoprotein H. By now, the accepted view is that ‘anticardiolipin’ or ‘antiphospholipid’ antibodies comprise a heterogeneous group of antibodies directed against neoeptopes on proteins capable of binding to anionic phospholipids, of which β2GPI is the most common and seems most related to the risk of venous and arterial thrombosis and pregnancy loss.

Although we were able to convince ourselves without any difficulty that all the IgG antibodies that we had isolated from SLE patients depended for their ELISA reactivity on the presence of β2GPI, it was hard to see how β2GPI could have found its way into both the purified IgM and the Fabγ tryptic fragment from our original patient. To our great disappointment, when we searched our freezer for the IgM paraprotein, we found that we no longer had any material left to test! Thus, I cannot avoid a suspicion that, although giving us an explanation for an in vitro anticoagulant effect in the absence of a bleeding tendency, this patient’s paraprotein would not have given us insight into the mechanism of the LAC-associated thrombotic risk.

By the mid-1990s, my interests, which had been split between lupus anticoagulants and the GpIb complex, had taken another turn when we identified a new intracellular GpIb-binding protein, now termed filamin B. I soon found myself immersed in this area and gave up research activities, but not my interest, in the LAC/ACA field. It has been very exciting to watch the accretion of new clinical and basic laboratory observations over the past few years. Yet the origins of what are still called, for lack of a better term, ‘antiphospholipid’ antibodies, and the mechanism(s) giving rise to the manifestations of the ‘anti-phospholipid syndrome’ are still frustratingly beyond reach. Even the question of which tests are most predictive of thrombotic risk has been difficult to answer, partly because of the variability in how tests are done, and partly because so few rigorous comparative studies have been carried out. Nevertheless, it seems a consensus is now emerging that coagulation tests, and particularly the dRVVT, are more closely associated with arterial and venous thrombotic risk than ELISA assays for ‘anticardiolipin’, β2GPI or prothrombin antibodies [11], and may even delineate a risk of myocardial infarction in SLE patients [12]. Furthermore, it appears that β2GPI-dependent coagulation abnormalities are most specifically associated with these manifestations [13].

But we still are unsure of the mechanistic elements of this vexing syndrome. What causes the appearance of the multiplicity of antibodies against phospholipid-binding proteins? Will a single pathway explain all manifestations, or are arterial thrombosis, venous thrombosis, and pregnancy loss – to name only the major manifestations – due to (slightly?) different mechanisms? For example, are annexins of importance [14] only in pregnancy loss? And why should β2GPI-dependent coagulation tests be most relevant when it seems clear that in vivo physiologic coagulation activation occurs on the surface of platelets and perhaps other cells, and genetic absence of β2GPI, whether in humans [15] or induced in knockout mice [16], is not associated with a thrombotic risk? Are we missing some essential aspect of a prothrombotic pathway?

Increased levels of apoptosis, particularly of endothelial cells, or perhaps decreased clearance of apoptotic cells, have been
suggested as an initiating mechanism [17], and infusion of apoptotic cells into experimental animals [18], like infusion of β2GPI [19], can induce formation of ‘antiphospholipid’ and other antibodies. It appears to me that the whole field is entering an extraordinarily creative and exciting period. Some of the things I would like to know are: (i) Can one detect in the blood of patients evidence of increased apoptosis and does that correlate with symptoms? (ii) Do patients have increased circulating levels of endothelial-derived microparticles [20] and does that correlate with thrombotic manifestations? (iii) Using currently available experimental models of venous [21] and arterial [22] thrombosis, does infusion of apoptotic cells (or acute triggering of apoptosis in some way), followed by infusion of β2GPI or other antibodies cause thrombosis? (iv) Does inhibition of apoptosis blunt the ability to induce the ‘antiphospholipid’ syndrome? (v) Will the very recent and exciting clinical observations, implicating genetic polymorphisms of the FcγRIIa receptor [23], be confirmed in larger studies?

Answers to these questions will undoubtedly lead to new mechanistic insights, which, in turn, will almost certainly lead to new therapeutic approaches or perhaps even preventive strategies. Were I younger, I would dive right back in!

References