Development of auto-antibodies towards β2-glycoprotein I in the antiphospholipid syndrome

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CHAPTER 1

Current insights into laboratory diagnosis and pathophysiology of the antiphospholipid syndrome

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INTRODUCTION

The antiphospholipid syndrome (APS) is a non-inflammatory autoimmune disease characterized by the presence of antiphospholipid antibodies (aPL) in the plasma of patients with venous and/or arterial thrombosis and/or recurrent complications of pregnancy. The presence of aPL in plasma of patients can be detected by either a prolongation of phospholipid dependent coagulation test (lupus anticoagulant, LAC), or by solid phase immune assays against the protein $\beta_2$-glycoprotein I ($\beta_2$GPI) or the phospholipid cardiolipin (anti-$\beta_2$GPI antibody ELISA and anti-cardiolipin antibody ELISA, respectively). For a long time there was a lot of confusion on who had the syndrome and who not. To solve this dispute, an international consensus meeting was organized in Sapporo in 1999 to formulate classification criteria for patients with the antiphospholipid syndrome. These criteria have been updated in 2004 at another international consensus meeting in Sydney. The classification criteria were defined for scientific purposes and were aimed to be used as inclusion criteria in patient related studies. They were not defined for diagnostic purposes. The actual practice is that these criteria are now used as a diagnostic tool. This is very unfortunate because the specificity of the different aPL assays to detect the clinical manifestations that characterize APS are disputable and one of the aims of defining the criteria was the validation as biomarker of the different assays used to detect the presence of thrombosis and pregnancy morbidity. The progress made recently on this important topic will be discussed in the next chapter.

LABORATORY DIAGNOSTICS

APS is an exceptional syndrome because the clinical symptoms such as thrombosis occur relatively often but are in most cases not due to the presence of antiphospholipid antibodies. As a consequence, the detection of the presence of aPL in plasma of a patient with thrombosis or complications of pregnancy is the essential step to define the syndrome. aPL is a generic term that describes a collection of closely related but not identical antibodies: LAC activity, anti-cardiolipin antibodies (aCL) or anti-$\beta_2$GPI antibodies. The fact that the three assays do not measure the same population of antibodies immediately raises two fundamental questions: what are the differences between the different types of antibodies detected with the three assays and which of these three assays is the most relevant one. Meta analyses, case-control cohort studies and prospective studies on the predictive value of the different types of aPL have shown that the antibodies that induce LAC activity correlate by far the best with a history of thrombo-embolic complications. Apparently, an assay that measures a functional activity, inhibition of a clotting reaction, better predicts a thrombotic risk than assays that measure the presence of a heterogeneous population of auto-antibodies that comprise both those that influence a functional activity and those that do not. Another possible reason why the ELISAs developed to detect the presence of
anti-cardiolipin or anti-\(\beta_2\)GPI antibodies perform so badly in these association studies is that they are poorly standardized\(^{11-13}\). A plasma sample that scored positive in one laboratory can score negative in another. Even between laboratories with extensive experience in the detection of aPL antibodies, discordant findings with samples with low titre antibodies are more a rule than an exception. Reliable detection of low titre aCL and anti-\(\beta_2\)GPI antibodies is not possible until now. Based on these observations, a number of researchers including one of us, expressed serious doubts whether the aCL ELISA, as it is performed today with the available commercial kits, is specific enough to detect the antiphospholipid syndrome\(^{14}\).

From 1990 on it is known that a subpopulation of aCL is directed against \(\beta_2\)GPI and there are many indications that the anti-\(\beta_2\)GPI antibodies are in fact the pathological antibodies. However, anti-\(\beta_2\)GPI antibodies are also a heterogeneous group of antibodies. Antibodies were found directed against all five domains of the protein. A number of studies from different laboratories have suggested that antibodies directed against an epitope around amino acids Arg39 and Arg43 within domain I of \(\beta_2\)GPI correlates best with the observed clinical manifestation of APS\(^{15-17}\). Moreover, addition of isolated domain I to plasma of mice inhibits thrombus formation in a murine model of the antiphospholipid syndrome\(^{18}\). Apparently, antibodies directed against domain I of \(\beta_2\)GPI are more relevant antibodies to measure than antibodies against whole \(\beta_2\)GPI, although we cannot exclude that besides antibodies against domain I other pathological subpopulations of auto-antibodies circulate in blood of APS patients.

From the studies published so far it is evident that LAC is the assay of choice to measure clinically relevant aPL. However, patients that are not only positive for LAC but also positive for anti-\(\beta_2\)GPI antibodies have a higher risk for recurrent thrombosis than patients positive in only one assay\(^{19}\). This is not really a surprise because LAC can not only be caused by antibodies directed against \(\beta_2\)GPI but also by antibodies against prothrombin\(^{20}\). There is consensus that the anti-prothrombin antibodies are passive bystanders in the syndrome\(^5\), however, not everybody agrees on this point\(^21\). Nevertheless, the combination LAC and anti-\(\beta_2\)GPI antibodies identify those anti-\(\beta_2\)GPI antibodies that are able to induce LAC, a subpopulation of aPL that is thought to be responsible for the pathophysiology of APS. In the next paragraph we will discuss how these antibodies could induce a deregulation of the hemostatic balance.

**PATHOPHYSIOLOGY**

Initially it was thought that aPL were directed against anionic phospholipids. We now know that the antibodies are directed against the glycoprotein \(\beta_2\)GPI bound to anionic...
surfaces. \( \beta_2 \)GPI is a plasma protein with no obvious function and persons or mice lacking this protein seem to be completely healthy. However, animal studies have produced ample evidence that the presence of anti-\( \beta_2 \)GPI antibodies increased thrombus formation after the introduction of a vascular injury. Also, the presence of anti-\( \beta_2 \)GPI antibodies results in pregnancy loss in a mice model. Clearly, the antibodies are gain-of-function antibodies that induce an additional function in \( \beta_2 \)GPI that is responsible for the increased thrombotic risk. \( \beta_2 \)GPI seems to be the playmaker of the antiphospholipid syndrome.

\( \beta_2 \)GPI is a glycoprotein with a molecular weight of approximately 45 kDa. It is present in high concentration in plasma (about 200 mg/mL, 3 mM). Although mRNA of \( \beta_2 \)GPI has been found in endothelial cells, astrocytes, neurons and in the extravillous cytotrophoblast and syncytiotrophoblast of the placenta, its major site of synthesis is the liver. Originally it was thought that a part of \( \beta_2 \)GPI in the circulation was associated with lipoproteins and, as a consequence, \( \beta_2 \)GPI is also known under the pseudonym apolipoprotein H. Recent evidence, however, showed that the term apolipoprotein H is a misnomer and that \( \beta_2 \)GPI is not associated with lipoprotein fractions. \( \beta_2 \)GPI consists of 5 short consensus repeats or sushi domains, domains that are present in many proteins that function in the complement system. The structure of these conserved domains revealed a common globular fold stabilised by two disulfide bridges. The fifth domain is an exception as it has a 6 amino acid residues insertion and a 19 residue C-terminal extension and a third disulphide bridge which includes a cysteine present at the C-terminal end of the protein. The extra amino acids are responsible for the formation of a large positive patch within domain V that forms the binding site for anionic phospholipids. In the middle of this positive loop there is a flexible hydrophobic loop with a classic Trp-Lys motive, often observed in proteins at the site of insertion into cellular membranes.

LAC and anti-\( \beta_2 \)GPI antibodies are exceptional biomarkers for thrombotic complications because they are correlated with an increased risk for both venous- and arterial thrombosis. In general, risk markers related to coagulation factors result in venous thrombosis while risk markers related to platelets correlate with arterial thrombosis. We cannot exclude that the risk for arterial thrombosis and the risk of venous thrombosis are the consequence of two separate actions of the \( \beta_2 \)GPI/antibody complexes. However, the observations that \( \beta_2 \)GPI after interaction with its auto-antibodies can bind and activate different cells, have strongly fueled the idea that the cause of the observed thrombotic and pregnancy complications is the deregulation of different cells involved in the maintenance of the hemostatic balance.

The antibody/\( \beta_2 \)GPI complex has been reported to bind to several cell types, amongst others endothelial cells, monocytes and platelets, all of which play an important
role in hemostasis. The list of potential binding sites on cells for the $\beta_2$GPI/antibody complex is ever increasing and includes annexin A2, LRP8 (low density lipoprotein receptor 8 = apolipoprotein E receptor 2'), glycoprotein Ibα (GPIbα), low density lipoprotein receptor related protein (LRP), megalin, toll-like receptor 2 (TLR2), toll-like receptor 4 (TLR4), the very low density lipoprotein (VLDL) receptor and PSGL-1 (for an overview see reference 2). Most of these receptors are expressed on a number of cell types at various levels in different combinations. The role of these receptors in the activation of different cell types has been studied by several groups with conflicting results. Based on in-vivo experiments with a mouse model for APS, all these receptors seems to be involved in the anti-$\beta_2$GPI antibody-induced thrombotic complications34-37, which seems very unlikely. Our group has identified LRP8 (ApoER2') as the signaling receptor for anti-$\beta_2$GPI antibody/\(\beta_2\)GPI complexes on platelets and endothelial cells, both in in-vitro studies and in-vivo studies38. Moreover, we have shown that thrombus formation in a mouse model of APS can be inhibited with a recombinant fragment of LRP8 and thrombus formation was strongly reduced when anti-$\beta_2$GPI antibodies were injected in LRP8 null mice (submitted). Additionally, we have shown that $\beta_2$GPI/anti-$\beta_2$GPI antibody complexes can bind with high affinity to purified LRP839. Altogether we propose that LRP8 is an important player in the pathophysiology of APS but we cannot exclude a role of other receptors for $\beta_2$GPI.

FUTURE DIRECTIONS

No physician or researcher can state that our current knowledge of APS is adequate enough for proper diagnosis and treatment. There are a number of important questions begging for an answer. The knowledge on APS that we have gathered till now strongly suggests that the secret of APS is hidden in the remarkable molecular and cell biology of the protein $\beta_2$GPI. We need to know what the critical elements are in the recognition of $\beta_2$GPI by the anti-$\beta_2$GPI antibodies. Is domain I the only relevant epitope? Why is $\beta_2$GPI only recognized when it is bound to an anionic surface? What is the physiological function of this abundantly present plasma protein? Why do we so often find auto-antibodies against this specific plasma protein? Is $\beta_2$GPI the only playmaker of the syndrome or are there also other plasma proteins and auto-antibodies involved? We have to identify the receptors on the cells responsible for antibody/$\beta_2$GPI complex interaction, and answer the question which cell (platelets, endothelial cells, trophoblasts, monocytes, or others) is the major target for the complexes. Besides thrombosis and fetal loss, many patients suffer from additional clinical manifestations also observed in other microangiopathies, such as thrombocytopenia and hemolysis. Is there a connection? The list of questions is large. We need to answer these questions in the laboratory and confirm the answers in large patient-related studies.
AIM OF THIS THESIS

There are major discrepancies in our understanding of the antiphospholipid syndrome (APS). This autoimmune disease is diagnosed when a patient suffers from thrombosis or pregnancy morbidity and has persistent circulating antiphospholipid antibodies. Despite its name the antibodies are not directed towards phospholipids rather to phospholipid binding proteins. The antiphospholipid antibodies are a heterogeneous group and have many antigens. In the early nineties the dominant antigen was identified as $\beta_2$GPI. Despite this major breakthrough the understanding and treatment of APS did not alter.

This thesis aims to gain a better understanding of the etiology and function of antibodies towards $\beta_2$GPI. To achieve this first the different conformations of $\beta_2$GPI was studied (Chapter 2). The observed conformational switch provided insight in a mechanism for which anti-$\beta_2$GPI antibodies arise. In chapter 3 we study etiology of the autoantibodies towards $\beta_2$GPI. Furthermore, the apparent paradox of patients with the antiphospholipid syndrome who have a prolonged coagulation time although they are at higher risk for thrombotic complications was examined in chapter 4. For chapter 5 we did not study the anti-$\beta_2$GPI antibodies in disease but $\beta_2$GPI itself and its role in thrombotic thrombocytopenic purpura. Last the effect of new medication on plasmas positive for anti-$\beta_2$GPI antibodies was studied (Chapter 6). In chapter 7 the implications of the findings described in this thesis are connected and discussed in a broader context.
REFERENCES

22. McNeil H, Simpson R, Chesterman C, Krulis S. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: $\beta_2$-glycoprotein I. Proc Natl Acad Sci USA. 1990; 87: 4120-4124
24. Hoeg J, Segal P, Gregg R et al. Characterization of plasma lipids and lipoproteins in patients with beta $\gamma$-glycoprotein I (apolipoprotein H) deficiency. The fasting plasma lipids, lipoproteins, and apolipoproteins were evaluated in 5 subjects. Atherosclerosis. 1985; 55: 25-34