33 Interaction of Antiphospholipid Antibodies with Endothelial Cells

Pier Luigi Meroni, Elena Raschi, Cinzia Testoni, Arianna Parisio, Maria Gerosa, and Maria Orietta Borghi

Anti-phospholipid Antibody Reactivity with Endothelial Cells

Antiphospholipid antibodies (aPL) are the formal laboratory diagnostic tools for the antiphospholipid syndrome (APS) and important players in its pathogenesis at the same time [1]. Different pathogenic mechanisms have been reported, suggesting that aPL can cause thrombosis or fetal loss in several ways [2].

Owing to its emerging role in hemostasis, inflammation, and immune response regulation, endothelium has been deeply investigated as one of the "most likely" target for aPL. Perturbed endothelium actually displays a procoagulant phenotype that might be pivotal in sustaining the APS vasculopathy [3].

The first report that found a relationship between aPL and endothelial cells (EC) showed that lupus anticoagulant (LA)-positive plasmas suppressed prostacyclin (PGI_2) release by vascular endothelium and the consequent unbalance between endothelial prostacyclin $(PGI)_2$ and platelet thromboxane (TXA_2) was suggested to support the in vivo thrombotic diathesis [4].

Since the first report by Carreras and Vermylen, several authors clearly demonstrated the aPL reactivity with EC and the antibody ability to affect endothelium functions in different in vitro experimental models (Table 33.1).

Interestingly, a direct EC involvement is also evident in some in vivo experimental models of aPL induced thrombosis. Pierangeli et al demonstrated that passive infu-

Table 33.1. Pleiotropic effect of aPL on endothelial cells.

- Induction of a pro-adhesive and a pro-inflammatory phenotype [5–11]
- Tissue factor upregulation [12–15, Vega-Ostertag et al, submitted]
- Interaction with the protein C/S system [16–20]
- Annexin V displacement from the endothelial cell membrane [21]
- Interaction with the eicosanoid metabolism: inhibition of PGI_2 synthesis [4]
- Induction of pre-proET-1 synthesis [22]
- Induction of apoptosis [23]
- Interaction with the late endosomes [24]

PGI_2 = prostacyclin 2; ET-1 = endothelin-1.

sion of aPL in naive mice might increase the leukocyte adherence to the vessel walls of the cremaster muscle microcirculation [25]. The same group also reported that E-selectin expression – the adhesion molecule specific for EC – is essential for the clot formation in their experimental pinch model after the passive infusion of aPL [26]. We have recently described an additional experimental in vivo model, in which the infusion of IgG fractions with strong anti–β_2-glycoprotein I (β_2-GPI) activity, but not control IgG, can induce leukocyte aggregation and endothelial adhesion in the mesenteric rat microcirculation when small amounts of lipopolysaccharide (LPS) are also injected at the same time (Fischetti et al, submitted).

β_2-GPI as the Main aPL Antigenic Target on EC

Several authors described the presence of an anti-endothelial binding activity in sera from both primary and secondary APS, however such a reactivity was not always related to anti-cardiolipin (aCL) or LA activity [27–32]. On the other hand, APS sera were found to react with constitutive EC membrane components whose exact nature was not identified [33].

β_2-GPI – the major plasma protein co-factor for aPL – was then found to represent the bridge for targeting circulating aPL to EC membranes [5–8, 10].

In fact, sera positive for aCL (and anti–β_2-GPI) antibodies displayed anti-endothelial cell activity only when the cells were grown in the presence of bovine serum. Cell starvation in serum-free medium abolished the reactivity that was in turn restored after the addition of purified human β_2-GPI. It was suggested that the fetal calf serum of the culturing media could be the source of β_2–GPI. The molecule, in fact, is apparently able to adhere to EC monolayers and then to be recognized by anti–β_2-GPI antibodies cross-reacting with β_2-GPI of different species, including the bovine one [34]. Studies carried out with affinity purified polyclonal IgG fractions as well as with human IgM anti–β_2-GPI monoclonal antibodies supported the hypothesis [8].

As a whole these findings suggest that most of the reactivity against EC in sera of APS patients is sustained by antibodies that recognize β_2-GPI, but autoantibodies directed against constitutive EC membrane proteins can be also detectable.

Endothelium is a heterogenic tissue that displays phenotypic and functional differences depending on the anatomical localization [3]. Such a heterogeneity has been claimed to explain why thrombotic events are generally episodic and often localized, being frequently determined by local/regional pathologic processes besides systemic risk factors. Anti–β_2-GPI antibodies have been found to recognize β_2-GPI on EC monolayers obtained from both large venous vessels and from the microcirculation, in line with the widespread anatomical distribution of thrombosis in APS [35]. However, a reactivity with brain and dermal microvascular EC higher than that with human umbilical cord vein EC (HUVEC) has been recently reported.

Altogether these data suggest that although β_2-GPI endothelial expression is apparently shared in common by the whole endothelium, different binding/expression characteristics on definite anatomical sites could be responsible for some of the APS clinical manifestations (i.e., the high frequency of skin and central nervous system involvement).

Endothelial Cell Receptor(s) for β_2-GPI

It has been demonstrated that plasma proteins can adhere to in vitro unfixed EC monolayer cultures, so endothelial adhesion of β_2-GPI might be simply the result of