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► **To cite this version:**

Yannick Dieudonné, Aurélien Guffroy, Vincent Poindron, Pauline Soulas-Sprauel, Thierry Martin, et al.. B cells in primary antiphospholipid syndrome: Review and remaining challenges. *Autoimmunity Reviews*, 2021, 20 (5), pp.102798. 10.1016/j.autrev.2021.102798 . hal-03415533

HAL Id: hal-03415533

<https://hal.science/hal-03415533>

Submitted on 24 Apr 2023

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B CELLS IN PRIMARY ANTIPHOSPHOLIPID SYNDROME: REVIEW AND REMAINING CHALLENGES

Section designation: Review

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Running Title: B cells in primary anti-phospholipid syndrome

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TEXT COUNT

Words number: 5721

Figure number: 1

Table number: 2

ABSTRACT

It is now widely accepted that antiphospholipid antibodies (aPL) have direct pathogenic effects and that B cells, notably through aPL production, play a key role in the development of antiphospholipid syndrome (APS). Recent findings strengthened the implication of B cells with the description of specific B cell phenotype abnormalities and inborn errors of immunity involving B cell signaling in APS patients. In addition, it has been shown in preclinical models that cross-reactivity between APS autoantigens and mimotopes expressed by human gut commensals can lead to B cell tolerance breakdown and are sufficient for APS development. However, B cell targeting therapies are surprisingly not as effective as expected in APS compared to other autoimmune diseases. Elucidation of the B cell tolerance breakdown mechanisms in APS patients may help to develop and guide the use of novel therapeutic agents that target B cells or specific immune pathway.

HIGHLIGHTS

- Antiphospholipid syndrome (APS) is characterized by a hypercoagulable state related to pathogenic antiphospholipid antibodies (aPL).
- B cells, notably through aPL production, have a central role in the pathogenesis of APS.
- aPL development/persistence may result from mono/polygenic inborn errors of immunity and specific interactions with commensal hosts.
- Paradoxically, B cell targeting therapies are not as effective as expected in APS considering experiences in other autoimmune diseases.

KEY WORDS

Autoantibody, antiphospholipid syndrome, B cell, tolerance, autoimmunity, thrombosis.

1. Introduction

1 Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of
2 pathogenic autoantibodies directed against membrane phospholipids and/or their associated plasma
3 proteins. Antiphospholipid antibodies (aPL) were first described by Hughes *et al.* in 1983 [1]. Annual
4 incidence and prevalence are estimated at approximately 2 and 50 per 100 000 individuals [2]. Clinical
5 features are related to a hypercoagulable state leading to obstetrical complications and/or venous or
6 arterial thromboembolic events (TEs) affecting almost every organ system [3]. The criteria derived
7 from the Sapporo classification revised in 2006 (Sidney) are quite consensual and require the
8 association of one of these clinical manifestations and the persistence of “classical” aPL, namely
9 lupus anticoagulant (LA), anticardiolipin antibodies (anti-CL) and anti- β 2 glycoprotein I antibodies
10 (anti- β 2GPI) [4]. Patients may encompass non TEs manifestations or “non-classical” aPL (including
11 phosphatidylserine/prothrombin complex, PS/PT, PE, or anti-annexin5A, Anx5A, antibodies) that are
12 excluded from Sydney criteria due to a role that remain elusive [5].

13 Recently coronavirus disease 2019 (COVID-19) and the associated abnormalities in coagulation
14 measures have put the spotlight back on aPL occurrence and their clinical relevance. There are strong
15 evidences that B cells, notably through aPL production, have a central role in APS pathogenesis.
16 Paradoxically, B cell targeting therapies are somehow not as effective as expected considering
17 experiences in other autoimmune diseases. Herein we propose a review that highlights the major role
18 of B cells and autoantibodies in APS, and how they provide a window into the pathogenesis, the
19 clinical features and the risk assessment in APS.

20

21 **2. B cells: the leading role?**

22 *Pathogenesis of aPL*

23 B cells contribute to APS pathogenesis notably through antibody production. The pathogenic
24 mechanisms mediated by aPL have been largely supplied by animal models, especially for β 2GPI-
25 dependent aPL. In 1990 Mc Neil *et al.* found that the binding of aPL to cardiolipin requires the
26 presence of β 2GPI as a cofactor [6]. Passive transfer of purified human anti- β 2GPI IgG, but not anti-

27 β 2GPI depleted IgG or normal human IgG, potentiates thrombosis formation in animal models after
28 vessel injury or pre-treatment with lipopolysaccharide (LPS) [7,8]. Anti- β 2GPI also induce fetal
29 losses or growth retardation in pregnant mice without the need of additional factors [9], and the
30 prominent role of the β 2GPI/anti- β 2GPI complex is highlighted by the absence of thrombosis in
31 β 2GPI deficiencies [10]. The binding of anti- β 2GPI antibodies to their antigen is dependent on β 2GPI
32 conformation. β 2GPI comprised 5 domains, the fifth domain shielding the domain 1 epitope in the
33 circular conformation form in plasma [11]. Autoantibodies do not bind this conformation form but
34 β 2GPI binding to negatively charged structures (such as anionic phospholipids) induce conformational
35 changes [12,13]. As a result, cryptic epitopes in domain I are exposed and the autoantibodies are able
36 to recognize β 2GPI [14]. The type of interaction is also critical since human IgG anti- β 2GPI domain I
37 (anti- β 2GPI-DmI) with high avidity induced significantly larger thrombi *in vivo* compared to anti-
38 β 2GPI-DmI with poor avidity [15].

39 A part of APS pathophysiology is mediated by the disruption of AnxA5 shielding over phospholipid
40 bilayers, making them available for coagulation reactions [16]. Interestingly, anti- β 2GPI-DmI has
41 been associated with significantly reduced annexin A5 anticoagulant activity in adults with aPL [17].
42 Nevertheless, considering β 2GPI-dependent aPL as the only ones with pathological relevance would
43 be too straightforward, since aPL targeting other phospholipid-binding proteins (such as prothrombin)
44 [18,19] or cofactor-independent aPL [20] may also exert thrombogenic effects in mice, but it has
45 become increasingly evident that the phospholipid binding protein β 2GPI is the major antigenic target
46 for thrombogenic aPL antibodies.

47 The pathogenic effects of aPL do not depend solely on the immunogenicity of the phospholipid, but
48 also on the downstream effect of the autoantibodies. aPL or the phospholipid/antibody complex can
49 activate multiple cells and factors that finally drive clot formation, which is the key event
50 underpinning vascular manifestations. A non exhaustive list of such mechanisms is listed in **Table 1**.

51 ***B cell phenotype in APS***

52 Despite the central role of aPL, little is known about B cell disturbance and characteristics of the aPL
53 producing clones. Indeed, only few data on small cohorts are available concerning B cell phenotype in

54 APS. A work assessing B cell phenotypic abnormalities in obstetric APS women described reduced
55 memory B cells and increased percentage and absolute count of CD27-IgM⁺ naive B cells in patients
56 with history of TEs. This higher number of naive B cells was associated with lower serum levels of
57 C4, higher levels of anti-CL and anti- β 2GPI IgG and with the development of thrombotic
58 complications during follow-up [22]. The authors assumed that altered peripheral B cell differentiation
59 and activation could bypass normal B cell differentiation leading to a bias toward plasma cell
60 maturation. These results were confirmed on another cohort of 11 patients with thrombotic primary
61 antiphospholipid syndrome (PAPS), which showed expansion of naive B cells as compared to
62 controls, non-APS thrombotic patients, systemic lupus erythematosus (SLE) and rheumatoid arthritis
63 (RA) patients [23]. An increased proportion of transitional B cells, a putative source of autoreactive B
64 cells, was also noticed [23]. However, a recent work heightens the debate, showing a decrease of
65 naive/immature B cell compartment only in obstetric PAPS patients [24]. Increase of CD19⁺CD5⁺ B
66 cells percentage was found in PAPS patients with TEs, which can represent up to 20% of total B cells
67 in this population [25]. CD5, firstly described in T cells, is not a distinctive marker but is highly
68 expressed by B1 cells in humans, a subset producing natural polyreactive IgM with a repertoire biased
69 towards bacterial and self-antigens [26]. An increase of CD5⁺ B cells have been described in several
70 conditions, including SLE, RA, or chronic viral infections [27]. In APS, their number correlates with
71 aPL IgM titers and most of them have reactivity against phospholipids [27]. Recently, Hisada *et al.*
72 reported a decreased frequency of IL-10 producing regulator B cells (B-regs) and an increase of
73 plasmablasts in 26 PAPS and 19 SLE/APS patients. This increase was associated with TLR7 SNP
74 rs38553839 and type I IFN upregulation [28]. Another soluble mediator that could dysregulate B cell
75 survival and development is B-cell activating factor (BAFF), known as a key cytokine in SLE [29]. In
76 APS, soluble BAFF level is increased both in patients with secondary APS and PAPS and correlates
77 with prognostic scores of thrombosis [24,30].

78 ***BCR repertoire in APS***

79 Features of the BCR repertoire have been investigated in several immune-mediated diseases and
80 profound changes have been described in SLE, RA or vasculitides [31]. However, in depth BCR

81 repertoire analysis is lacking in APS. In a systematic analysis of 36 identified aPL sequences, there
82 was no evidence of preferential gene or family usage in any group of aPL studied, but only two aPL
83 were proven to be pathogenic and many of them were derived from asymptomatic patients [32]. Our
84 group performed molecular and functional analysis of anti-CL autoantibodies in a patient with PAPS,
85 by cloning the BCR of autoreactive B cells isolated as single cells [33]. The five different antibodies
86 were highly heterogeneous in terms of V, D or J gene usage and mutations in variable regions.
87 However, the only antibody with LA activity had the highest mutated rate [33]. In accordance, among
88 five selected monoclonal aPL originating from another patient, the only one inducing fetal losses,
89 when passively injected in mice, had the highest rate of mutation in the Vk region [34]. This
90 pathogenic effect was reversible when this autoantibody was *in vitro* reverted to inferred naive
91 germline configuration [34]. In this process of somatic hypermutation, the presence of newly inserted
92 positively charge in CDR3 seems to play a particularly important role, and Pericleous *et al.* showed
93 that new arginine residues enhance the binding to β 2GPI-DmI [35]. In conjunction with studies
94 proposing that aPL belong to the natural antibodies [27], pathogenic aPL clones identified by our
95 group were polyreactive, suggesting that they may be part of the natural repertoire [34]. However, in
96 lupus-prone mice, B cells specific for phospholipid are largely found in germinal centers, whereas few
97 are localized in the extrafollicular compartment, suggesting that follicular class-switch recombination
98 of autoreactive clones is important to drive aPL pathogenicity [36].

99 **3. APS: a “hit(s)-parade” toward B cell maturation**

100 Although aPL are directly implicated in pathophysiology and correlate with the thrombotic risk, TEs
101 occur occasionally, suggesting that other factors are needed to push the thrombotic/haemostatic
102 balance in favor of thrombosis [37]. The “second hit” hypothesis is widely accepted and regarded as
103 an additional procoagulant condition that is needed to precipitate clotting formation in aPL carriers
104 [9]. This theory is strongly supported by several experiments in APS prone mice models. In this view,
105 Pierangeli *et al.* described mouse model of injury-induced thrombosis with surgically induced
106 thrombus formation and showed in this model the importance of complement activation in aPL’s
107 pathogenicity [38]. Similarly, the same group showed that aPL mediated thrombogenic effects were

108 reduced in mice non-responsive to LPS [39]. Hence, a priming factor (e.g. vessel injury or pre-
109 treatment with LPS) is needed for passively transferred human aPL to induce thrombosis [7,8].

110 Among various mechanisms, infection agents or oxidative stress may increase aPL exposure [40],
111 β 2GPI conformation modifications that expose cryptic epitopes [41], or complement activation.
112 Whereas β 2GPI binding to endothelial cells requires priming with pro-inflammatory factors, uterine
113 vessels and trophoblast physiologically express high level of β 2GPI [42]. Thereby, pregnancy
114 increases specific epitope exposure and could act as a second hit *per se* [9]. Other clinical situations,
115 such as traumatism, inflammatory state or surgery can trigger clot formation, leading to clinical events
116 defining APS. However, and as discussed further, such additional hits may, in an alternative or
117 complementary manner, step in earlier in the pathogenic process driving B cells from benign and
118 transient aPL production toward persistent and pathological autoantibodies generation.

119 ***aPL occurrence***

120 It is widely accepted that autoimmune diseases, including APS, may arise via an inappropriate
121 immune response towards infections. Apart from chronic infectious, such as the one induced by
122 human immunodeficiency virus (HIV) or hepatitis C virus, in which link between aPL and APS-
123 related features is controversial [43], most “infectious” aPL are transient, cofactor independent, bind
124 neutral or negative PL with low affinity, and clinical APS-related complications remain an
125 epiphenomenon [32,44]. While not frequently reported, cytomegalovirus (CMV), Epstein Barr Virus
126 (EBV), Herpes simplex virus (HSV) and adenovirus infections could lead to anti-CL production and
127 transient LA positivity [45]. Development of pathogenic aPL has been reported during Parvovirus B19
128 infection, with aPL of IgG isotype and cofactor-dependent binding, similar to autoimmune disease
129 [45–47]. Recently, high prevalence of LA have been described in patients with SARS-CoV2 [48].
130 Among 216 patients, 44 (20%) were found to have a prolonged activated partial-thromboplastin time
131 (aPTT), due to LA in 90% of them. Additionally, in a recent series, 15% of 122 infected patients were
132 positive for anti- β 2GPI IgG and about 5% for anti-CL IgG [49]. Similarly to patients with triple
133 positive APS or CAPS, IgG isolated from COVID-19 patients increase thrombus extension in a mouse
134 model of inferior vena cava thrombosis [50]. TEs are frequent during acute SARS-CoV2 infection

135 [48,51], and the severe form of COVID-19 share several clinical and laboratory features with
136 hyperferritinemic syndromes [52,53]. However, no association between thrombosis and aPL is clearly
137 established and, during COVID-19, anti- β 2GPI displayed an epitope specificity different than in PAPS
138 [49]. The role of aPL in this hypercoagulable state should be elucidated, as well as the question of
139 their long-term persistence.

140 Regarding bacterial infections, aPL are most commonly associated with *Coxiella burnetii*,
141 *Mycoplasma pneumoniae*, *streptococci*, and *Mycobacterium tuberculosis* infections. The latter three
142 infections may be associated with persistent autoantibodies and authentic APS [54]. The relatively
143 high prevalence of anti-CL antibodies found in syphilis is largely due to a cross-reactivity of syphilis
144 antibodies against treponemal cardiolipins [43]. Multiple aPL positivity and β 2GPI-dependent binding
145 are found in sera from patients with leprosy [55]. Mice immunized with proteins from *Haemophilus*
146 *influenzae* or *Neisseria gonorrhoeae*, which share sequence homology with β 2GPI-DmIII, led to the
147 production of anti-CL and anti- β 2GPI IgG autoantibodies [56]. Parasite or yeast induced-aPL are very
148 rare and mostly occur after *Plasmodium falciparum* or visceral leishmaniasis infection [57].

149 Finally, several other clinical conditions have been associated with the occurrence of aPL. Drug
150 induced aPL, following antiarrhythmic or antipsychotic agents, are common, but they are usually
151 benign and disappear after the drug withdrawal [44]. Reports documented the appearance of aPL after
152 vaccine administration responses. Indeed, the frequency of aPL production after influenza
153 immunization is higher than after infection [44]. In a prospective study, 8% (7/101) of healthy subjects
154 developed anti-CL but not anti- β 2GPI after seasonal influenza vaccination. Anti-CL positivity was no
155 longer observed at week 12 post-vaccination [58]. Tetanus toxoid vaccination triggers antibodies
156 which are cross-reactive with β 2GPI as shown in animal models [57]. The role of adjuvants as
157 stimulators of innate actors in vaccine associated aPL is not elucidated.

158 ***From aPL to thrombosis***

159 Several potential mechanisms have been evoked to explain the production of aPL during infections,
160 including epitope spreading, bystander activation, and molecular mimicry. However, little is known
161 about mechanisms sustaining chronic autoimmunity in APS. The transient character of aPL after

162 infection might suggest that continuous antigen exposure is needed for the maintenance of chronic
163 autoreactive B lymphocyte activation. The microbiome is a potential source of persistent self-antigens
164 that either trigger or perpetuate an autoreactive B cell response [59]. Depletion of the gut microbiota
165 with antibiotic regimen leads to lower anti- β 2GPI titers and protection from thrombotic events in mice
166 [60]. In a recent work, *Roseburia intestinalis* (*R. int*) was identified as a chronic driver of β 2GPI
167 autoreactivity [61]. Indeed, Ruff *et al.* provided evidence for cross-reactivity between mimotopes of
168 this common human gut commensal and the major T cell (p276-290 in DmV) and B cell (R39-R43 in
169 DmI) autoepitopes of β 2GPI. APS-derived monoclonal antibody with LA activity binds to a mimotope
170 of a *R. int* DNA methyltransferase (DNMT). *R. int.* immunization of BALB/c mice induces
171 autoepitope-specific cross-reactivity to human β 2GPI, and oral gavage of the spontaneous APS mouse
172 model (NZW x BXSB) F1 mice lead to a significantly elevated anti-human β 2GPI IgG autoantibody
173 and TEs. In conjunction, elevated levels of anti-*R. int* DNMT IgG were found in 15 patients with APS,
174 and positively correlated with anti- β 2GPI IgG. The authors suggested that cross-reactivity with
175 commensals is likely to impact mechanisms of tolerance of B cells but also T cells providing help to
176 PL-specific B cells [62]. Could this hypothesis be sufficient to explain how aPL producing B cells
177 bypass tolerance mechanisms? Indeed, autoreactive B cells are detectable in healthy individuals [63],
178 but several checkpoints ensure their counterselection or their immunologic ignorance to thwart
179 autoimmunity in physiological conditions [64]. Hence, pathogenic aPL producing clones may only
180 arise in predisposed individuals.

181 In this way, primary abnormalities of tolerance mechanisms linked to genetical defects could favor the
182 maintenance of aPL reactive B cell clones. *BLK* polymorphism (rs2736340) displayed a strong genetic
183 association with PAPS [65]. Polymorphisms in *IRF5* or *BANK1*, classically reported in SLE, displayed
184 low or no association in PAPS respectively [65]. In line with other autoimmune diseases, several
185 candidate genes especially in HLA locus (eg. *HLA DR4*, *DRw53*) were identified in monozygotic
186 twins and familial clusters of PAPS, and suggested to be involved in the pathogenesis of PAPS
187 through genome wide association studies (GWAS) or next-generation sequencing (NGS) approach
188 [66–69]. Monogenic inborn errors of immunity that affect B cells can be associated with aPL
189 seropositivity or PAPS, and offer a prototypical condition to explore the tolerance breakdown against

190 aPL. We reported recently a family of patients with APS and an heterozygous variant of *IKZF1*
191 causing loss of function for IKAROS, a key transcription factor involved in primary and secondary
192 affinity maturation processes of B cells [70]. Long-term persistence of aPL may also depend on
193 intrinsic characteristics of autoreactive B cells. Indeed, one could speculate that somatic mutations
194 affecting aPL producing clones could provide selective advantage to escape negative selection, in line
195 with findings in autoimmune lymphoproliferative (ALPS) syndrome, where somatic mutations appear
196 relevant for the accumulation of Fas-deficient memory B cells and the development of autoimmunity
197 [71].

198 These data strengthen a model whereby, on a predisposing genetic background, transient or benign
199 phospholipid reactive clones could pass through B cell tolerance checkpoints, expand and mature,
200 leading to aPL-secreting plasma cells accumulation. The development of such persistent and
201 pathogenic clones may be in part sustained by an adaptive immune responses against cross-reactive
202 microbiota, as opposed to acute infections due to pathogens that are cleared by the host [61]. Specific
203 epitope exposure in pregnancy and/or other clinical situations, including vessel injury and/or
204 inflammatory processes, could thereafter provide additional triggers leading to clinical events defining
205 APS (**Figure 1**).

206

207 **4. Autoantibodies and clinical implication**

208 *aPL and diagnosis*

209 The main role of autoreactive B cells is emphasized by the central place of aPL in the APS diagnostic
210 [72]. However, the various association between large panels of serological and clinical phenotypes
211 remains elusive. According to Sydney classification, diagnosis laboratory criteria include LA, anti-CL
212 and anti-β2GPI, of IgM or IgG isotype at medium-to-high titres (generally tested by ELISA), that
213 should be tested repeatedly positive at least 12 weeks apart. Usually, anti-CL and anti-β2GPI from the
214 same isotype are detected together [73]. The positivity for LA reflects the ability of aPL to prolong
215 phospholipid-dependent coagulation assays. The LA activity is often displayed by anti-β2GPI,

216 especially those directed against domain I of β 2GPI. Seropositivity of all three-classification criteria-
217 aPL is linked with a more aggressive APS phenotype, named "triple-positive".

218 Alternative aPL, named "non-classical", have been excluded from Sidney criteria due to an initial lack
219 of clinical implication. However, patients with clinical manifestations compatible with APS, positive
220 for such extra criteria aPL, and negative for classical aPL exist and were named "seronegative APS
221 patients" (SN-APS). This entity introduced in 2003 by Hughes and Khamashta [74] remains
222 controversial and three possible explanations have originally been considered to explain the existence
223 of such SN-APS patients: (i) a wrong diagnosis, (ii) previously positive aPL tests that became
224 negative, or more likely (iii) aPL not detected by the current consensual tests. A retrospective study
225 found no significant differences in clinical manifestations between patients with APS and those with
226 SN-APS [75]. Zohoury *et al.* showed that about 35% of SN-APS patients have detectable non
227 classical aPL [76]. Hence, some consider that SN-APS is a proof that classic aPL tests are not
228 sufficient to diagnose APS, arguing for an update to the current criteria incorporating non-
229 conventional aPL and consensual technical procedures in diagnosis criteria [77]. Recently, four
230 profiles of non-criteria APS were proposed : 1) clinical non-criteria APS, 2) laboratory non-criteria
231 APS, 3) incomplete laboratory APS: patients fulfilling clinical criteria, plus positive aPL, but not
232 fulfilling the classification criteria, and 4) seronegative APS: patients with persistently negative
233 antiphospholipid antibodies despite fulfilling clinical criteria [78].

234 Our actual knowledges on non-classical aPL highlight their importance in the APS physiopathology.
235 Among them, antibodies targeting the PS/PT complex can display LA activity [79–81] and seems to
236 be of strong clinical relevance. Their presence, together with anti- β 2GPI-DmI, correlate with vascular
237 and obstetrical complications, and their combination have 100% predictive value for the diagnosis of
238 APS [82–84]. In 14 triple positive patients also positive for anti-PS/PT (so called "tetra positive APS
239 patients"), from whom different type of aPL were affinity purified, anticoagulant activity was largely
240 attributable to anti-PS/PT antibodies [85]. Anti-PS/PT detection is not influenced by anticoagulant
241 treatment and could be considered to confirm the presence of LA. Concerning other non-classical aPL,
242 among a cohort of patients with thrombosis, anti-PE antibodies were found in 15%, being the sole aPL
243 detected in 67% of the cases [86]. Antibodies targeting phosphatidic acid (PA), or phosphatidylinositol

244 (PI) may be the only one detectable in women with recurrent pregnancy losses [87,88], although their
245 binding to antigen and their pathogenicity may be β 2GPI-dependent. Lastly, IgA against β 2GPI are
246 significantly associated with TEs, especially in SLE patients [89]. Despite the clinical interest of non-
247 conventional aPL, a lack of standardization in the assays used for their detection still remains [90].

248 *Specific aPL for distinct phenotypes?*

249 Vascular occlusions belong to the classical clinical pictures of APS, and include deep venous
250 thrombosis, pulmonary embolism or arterial TEs such as strokes [91]. In addition to this thrombotic
251 APS, some patients present with an “obstetric APS” phenotype and suffer from pregnancy morbidity,
252 including recurrent early miscarriage but also late (after 10 weeks) pregnancy loss and premature birth
253 (<34 weeks) related to preeclampsia or placental insufficiency [92]. Patients with APS may also
254 experience a myriad of additional features, usually branded as “extra criteria”, including neurological
255 (epilepsy, chorea...) and hematological manifestations (thrombocytopenia and hemolytic anemia),
256 livedo reticularis, nephropathy and valvular heart disease [93]. Catastrophic antiphospholipid
257 syndrome (CAPS) is the most severe form of the disease and characterized by widespread small vessel
258 thrombosis with multiorgan failure presenting over a short period of time and is associated with a
259 mortality higher than 30% [91][94]. Despite published association with drugs, malignancies and a
260 wide spectrum of autoimmune diseases [95], secondary APS is mainly associated with SLE, in about
261 20-30% of cases [2,5]. aPL positive patients with SLE have higher rates of arthritis, cytopenia,
262 hypocomplementemia and severe renal disease than those without SLE [96].

263 Relying these homogenous clinical phenotypes to serological characteristics remains challenging.
264 Patients with single obstetrical APS tend to have lower rate of multiple aPL positivity [9,97]. In series
265 on the Serbian national cohort, patients with secondary APS had more frequent multiple aPL positivity
266 than those with PAPS, whereas LA alone was more frequently reported in the latter [98]. However,
267 these results were not found in the large Euro-Phospholipid cohort. Additionally, in this cohort of
268 1000 European patients with APS, no differences were found in the clinical presentation of PAPS, at
269 its onset and during a 10-year follow-up period, according to the presence or absence of anti-CL or LA
270 [5,91]. In 2016 data from the CAPS Registry, LA was present in 83% of episodes, anti-CL IgG in

271 81%, IgG anti- β 2GPI in 78% whereas anti-CL and anti- β 2GPI of IgM isotype were less frequent (50%
272 and 58% respectively) [99]. Recently, three studies on large cohort aimed to aggregate patients into
273 subgroups sharing common clinical and specific classical aPL profiles [97,100,101]. The main results
274 are depicted in **Table 2**. Nonetheless, these studies do not consider extra criteria aPL. Using multiplex
275 line immunoassay, Volkov *et al.* evaluated the presence of non-criteria aPL in a cohort of 130 APS
276 patients. Whereas arterial thrombosis was associated with multiple aPL positivity (>7), venous TE was
277 not linked to any specific aPL and inversely correlated with anti-phosphatidic acid IgM detection. The
278 presence of anti-CL IgG and anti-PS IgG was linked with pregnancy morbidity [102]. These results
279 suggest that evaluating a broad spectrum of aPL may enable to better define APS phenotype.

280 *Prognostic markers and risk assessment*

281 Identifying the risk of clinical events in patients with APS is challenging due to the clinical
282 heterogeneity, the different sets of aPL and their fluctuation in the disease course. Multiple positivity
283 for anti-CL, anti- β 2GPI or LA is associated with higher rate of TEs than single test positivity
284 [103,104]. Subjects with triple positivity for these criteria aPL have an annual incidence of TEs of
285 5,3%, with a cumulative risk of 37% after ten years [105]. LA positivity is associated with the highest
286 risk for both arterial and venous TEs [106] whereas results are controversial for anti-CL [106–108].
287 β 2GPI antibodies seems to confer a moderate increase of the risk, with an odd ratio from 1.6 to 2.4 for
288 venous TEs and of 2.3 for ischemic strokes [109–111]. β 2GPI-dependent LA activity assay correlated
289 two times better with TEs than the classic LA assay [112]. The relevance of anti-CL/ β 2GPI IgM
290 antibodies has been debated. Most studies were unable to demonstrate a correlation between IgM
291 positivity and TEs, but Chayoua *et al.* recently provided evidence for an independent association with
292 pregnancy morbidity [113]. Among non-criteria aPL, anti- β 2GPI-DmI positively correlates with the
293 Global Antiphospholipid Score (GAPSS) [114,115], a validated thrombotic risk score [116]. Hence,
294 anti- β 2GPI-DmI seems to represent a robust biological criterion, in association with classical aPL, to
295 better apprehend the thrombotic risk. Other works showed diverse clinical association with reactivity
296 to different epitopes on β 2GPI and suggested that classification of anti- β 2GPI into subgroups
297 regarding epitope specificity would be of clinical relevance [117–120]. As an example, in a cohort of

298 135 women with persistently positive anti- β 2GPI IgG, anti- β 2GPI-DmI positivity was associated with
299 triple positivity, thrombosis and predictive of late pregnancy morbidity, whereas anti-domain IV/V
300 (anti- β 2GPI-DmIV/V) antibodies were not associated with clinical APS manifestations [118].
301 Conversely, DmIV/V are preferentially recognized by anti- β 2GPI antibodies from one-year-old
302 healthy children born to mothers with systemic autoimmune diseases and from subjects with non-APS
303 conditions [119], and the ratio anti- β 2GPI-DmI to anti- β 2GPI-DmIV/V IgG has been proposed as a
304 helpful tool to discriminate patients with systemic autoimmunity from those carrying less pathogenic
305 anti- β 2GPI antibodies [120].

306 Regarding other non-classical aPL, it is widely accepted that prothrombin is a common antigenic
307 target for aPL, but assays may detect distinct populations of autoantibodies which have different
308 clinical implications. Positivity for anti-PT, detected by directly coating prothrombin on ELISA plates,
309 does not seem to be such a strong risk factor for APS [121]. However, the cumulative incidence rate of
310 thrombotic events is significantly higher when anti-PS/PT, which recognize a conformational epitope,
311 are detected among aPL carrier patients [122]. Additionally, the risk of pregnancy morbidity seems to
312 be associated with the presence of anti-PE, PA, PS and PI [90].

313 Based on the global aPL profile, different models, such as the Antiphospholipid Score, have been
314 tested to evaluate the risk of having APS and for predicting thrombosis [123]. The use of antibody
315 profiles as prognostic markers is however delicate for several reasons. High inter-laboratory variability
316 and the widespread of retrospective designs may explain the large discrepancies between the finding
317 of clinical studies. Moreover, conventional cardiovascular risk factors should be considered to assess
318 the thrombotic risk. The GAPSS proposed by Sciascia *et al.*, based on the profile of four aPL and the
319 presence of dyslipidemia or arterial hypertension, has been proven to represent the probability of
320 having TEs or pregnancy loss in patients with SLE and is an example of risk model that takes in
321 account such factors [116]. EUREKA is another algorithm that predicts the risk of pregnancy
322 morbidity considering aPL titres and profiles [124].

323

324 **5. Management of APS and B cells: paradox and remaining challenges**

325 Despite the widespread of targeted therapies in autoimmune diseases, anticoagulation remains the
326 corner stone of management in patients with APS. Briefly, low dose aspirin (LDA) is recommended
327 for asymptomatic patients with high risk aPL profiles whereas patients with thrombotic APS should
328 have long term treatment with vitamin K antagonists. The use of direct oral anticoagulants (DOACs) is
329 highly debated [92,125]. Patients with obstetric APS only should receive prophylactic dose low-
330 molecular-weight heparin (LMWH) and LDA throughout pregnancy. The European League Against
331 Rheumatism (EULAR) recently developed evidence-based recommendations for the prevention and
332 treatment of adults with APS [126]. Considering the crucial role of autoantibodies in the definition and
333 the pathophysiology of the disease, data on the use of therapies directed towards B cells are lacking
334 and somehow disappointing in APS.

335 ***B cell targeting therapies***

336 The use of anti-CD20 therapies has been supported by strong evidences in other autoimmune
337 diseases[127], but also by clinical and/or serological beneficial effects in case reports concerning
338 PAPS, secondary APS, or CAPS [128]. In the retrospective literature review by Erre *et al.*, eight out of
339 12 patients with classical aPL treated with rituximab, a chimeric monoclonal antibody targeting CD20,
340 showed normalization or reduction of aPL titers. Some of them were however concurrently treated
341 with immunosuppressors [129]. Results on TEs recurrence are debated and limited to small series or
342 case reports [130]. In a phase II trial, safety and efficacy of rituximab was evaluated in 19 patients
343 with APS criteria [131]. Rituximab was efficient for some non-criteria manifestations, especially
344 thrombocytopenia (response rate of 50%) cognitive dysfunction (80%) and skin ulcers (100%), but
345 failed to change significantly aPL profiles [131]. Such effect on extra thrombotic manifestations,
346 including thrombocytopenia, was confirmed in other studies [133,134]. There are concerns about
347 thrombosis exacerbation since severe acute thrombotic events were described in two APS/SLE
348 patients receiving rituximab [132]. In the study from Erkan *et al.*, 12 serious adverse events were
349 recorded including neurological complications (n = 5), infections (n = 3) and recurrence of TEs (n = 1)
350 [131]. According to recommendations, rituximab may be considered in CAPS, where aPL production
351 is associated with systemic inflammatory response syndrome [94]. In a retrospective review, 13 of 20

352 patients with severe/relapsed CAPS treated with rituximab recovered. aPL became negative in fifty
353 percent of those with follow-up (4/8). Comparing 30 patients treated with rituximab versus
354 contemporaneous patients in the CAPS registry, an *ad hoc* analysis provided an odd's ratio for
355 survival of 0.41 (95% CI 0.15, 1.11) [133]. However, evidences are not strong enough to use
356 rituximab as a first line therapy in CAPS and it is considered as an alternative treatment in severe or
357 refractory cases [92]. Novel anti-CD20 therapies such as obinutuzumab, a human monoclonal anti-
358 CD20 antibody, induce different cellular response and stronger antibody-dependent cell-mediated
359 cytotoxicity (ADCC) [134]. Their uses in autoimmune diseases are under evaluation and they could
360 provide a second life to anti-CD20 therapy in APS.

361 Because anti-CD20 treatment does not affect plasma cells, the insufficient therapeutic outcome may be
362 caused by the survival of aPL producing plasma cells. *Ex vivo* experiments showed that aPL producing
363 CD20 negative B cells circulate in peripheral blood of APS patients [28]. This rational is sustained by
364 the successful use of bortezomib on TEs recurrence and aPL titers in case reports [135,136]. As an
365 alternative, anti-CD38 monoclonal antibodies, such as daratumumab, enter the field of autoimmune
366 diseases therapy with impressive response in post-transplant patient with autoimmune cytopenia
367 [137,138] and at least in 2 patients with SLE, in association with belimumab [139].

368 *Cytokines*

369 BAFF blockade has shown therapeutic potential in preclinical models. BAFF antagonists reduced
370 renal and cardiac infarcts and prolonged survival in NZW×BXSB F1 mice although it did not prevent
371 anti-CL development [140]. Interestingly, in human, BAFF level is increased in PAPS and secondary
372 APS [24,30]. Belimumab, a monoclonal antibody against BAFF, was found to reduce anti-CL and
373 anti-β2GPI IgM and IgG titers treatment in 12 SLE/APS patients independently of
374 hydroxychloroquine treatment [141]. However, in a recent post-hoc analysis on two large randomized
375 SLE-controlled trials focusing on anti-CL titers, intravenous belimumab showed a significant
376 reduction of anti-CL IgA and IgG only in patients with concomitant antimalarial treatment [142].
377 Similarly, Bettioli *et al.*, in another recent post hoc analysis of 249 patients with SLE from the BLISS-
378 SC trial, observed a significant reduction of anti-β2GPI IgA and anti-CL IgM titres in the

379 belimumab+antimalarials group as compared with the placebo+antimalarials group, suggesting that
380 the two treatments may exert beneficial synergistic effect [143]. The independent role of belimumab
381 on aPL titre reduction needs to be further evaluated, and, so far, such data are lacking in PAPS.

382 Alternative therapeutic approaches might consider inflammatory mediators inhibitors such as type I
383 IFN antagonists. Anifrolumab, that blocks the type I interferon receptor subunit 1, has proven
384 efficiency on disease activity in SLE patients [144]. The high type I IFN signature observed in about
385 40% of patients with PAPS [145], together with the positive correlation between plasma cells
386 expansion and type I IFN upregulation in APS [28], support the evaluation of such antagonists.

387 ***aPL targeting***

388 Plasmapheresis and intravenous immunoglobulins (IVIg) could thwart aPL pathogenic effects by
389 increasing their clearance, inhibiting their action or through the control of anti-idiotypic antibodies. In
390 CAPS, they are recommended as first line treatment in combination with steroids and heparin [126].
391 Agostinis *et al.* identified a single-chain fragment variable directed against β 2GPI-DmI that induced,
392 in mice, thrombosis and fetal losses in a complement dependent manner. Interestingly, a variant of this
393 antibody lacking the CH2 domain, required for C1q binding and complement activation, compete with
394 human anti- β 2GPI IgG and prevent blood clot formation and fetal loss [146].

395 The use of peptide therapies recently provided another approach to inhibit aPL pathogenicity. The
396 rationale is to block binding to target cells or receptors using small synthetic peptides. No clinical trial
397 has been developed to date but such molecules, including synthetic analogs of β 2GPI-DmI or A1
398 (ApoER2 ligand-binding domain) reduced thrombus formation in animal models [147,148]. Peptides
399 therapy, as well as CH2-deleted antibody, could represent an innovative approach potentially useful to
400 treat refractory APS patients.

401 ***Beyond B cells***

402 Other conventional or targeted therapies could overcome the apparent limited effectiveness of B
403 therapies and work synergistically to counterbalance pro-coagulation state. Some of them may exert
404 indirect action on aPL or autoreactive B cells development. *In vitro*, hydroxychloroquine reverses the

405 disruption of AnxA5 shielding induced by aPL, and thereby restore the binding of AnxA5 to
406 phospholipids [16]. Besides decreasing aPL titers in patients with secondary APS, hydroxychloroquine
407 reduces LA activity [142,149]. Sirolimus may also contribute to lower aPL titers in SLE patients
408 [130]. Statins exert multiple anti-inflammatory effects, including reduction on tissue factor expression
409 by monocytes and cytokine secretion, but little is known about B cell implication. In accordance with
410 the hypocomplementemia and increased levels of complement activation in APS patients [150], anti-
411 C5 antibody prevents the prothrombotic activity of aPL *in vitro*, and precludes aPL-mediated
412 thrombosis in some murine models [38]. This therapy showed promising results as adjunction therapy
413 in CAPS, but results are limited to cases reports and do not enable conclusion on aPL titers [130,151].
414 Further studies should elucidate the place of these therapies in the APS therapeutic plan and their
415 specific effect on B cells and autoantibodies.

416

417 **6. CONCLUSION**

418 The central role of B cells in APS is highlighted by strong evidence of antibody mediated
419 pathogenesis, specific B cell phenotype abnormalities, and by the development of the disease in
420 patients with inborn errors of immunity involving B cell ontogeny. Recent findings questioned the
421 links between B cell tolerance breakdown, genetic defects, and commensal microorganisms. However,
422 the origin and characteristics of pathogenic aPL producing clones remain largely unknown. Despite
423 the discrepancies in clinical trial results, therapies targeting B cells should remain an important
424 therapeutic approach in APS. A broad range of immunomodulatory drugs has recently emerged with
425 serious rationale to modulate the inflammatory response and vascular damages in APS. Further studies
426 should determine if this new therapeutic area could provide a second wind to the field of B cell
427 targeting in APS and which homogeneous groups of patients should benefit from it.

428

429 **FUNDING**

430 No funding sources.

431

432 **ACKNOWLEDGEMENTS**

433 We thank the Fondation pour la Recherche Médicale, the European reference Networks (ERNs) RITA
434 and ReCONNET, and the INTERREG V program (PERSONALIS project, co-financed by
435 the European regional development fund (ERDF).

436

437 **CONFLICTS OF INTEREST**

438 Declarations of interests: none.

439

440 **AUTHORSHIP**

441 Y.D, A.G., A-S.K. and V.G. wrote the manuscript.

442 Y.D, A.G., V.P., P.S.L, T.M., A-S.K. and V.G. reviewed or edited the manuscript before submission.

443 Y.D, A.G., V.P., P.S.L, T.M., A-S.K. and V.G. contributed to researching data for the article and

444 discussions of the article content.

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Table 1: aPL and/or phospholipid/aPL complex-mediated prothrombotic and proinflammatory changes
[9,12,19,21].

Actors	Binding	Consequences
Anticoagulant mediators		Inhibition of protein C activation inhibition of the tissue factor pathway inhibitor Inhibition of β 2GPI anticoagulant activity Inhibition of antithrombin activity Disruption of Anx5A
Endothelial cells	β 2GPI AnxA2 TLR2 TLR4	Expression of adhesion molecules (E-selectin, ICAM-1, VCAM-1) Increase of tissue factor expression PP2A activation, decreased NO production inhibition of the TF pathway inhibitor and of the C reactive protein activity Proliferation through mTOR pathway activation IL-1, IL-6, IL-8, and TNF- α release
Platelets	ApoER2 GpIbA	Platelet activation Increase of GPIIb-IIIa expression Thromboxan A2 synthesis
Monocytes	AnxA2 TLR2 TLR4	IL-1, IL-6, TNF α secretion Increase of tissue factor expression Increased intracellular ROS
Neutrophils	TLR4 C5a receptor	Increased NET release IL-8 secretion
Complement		Classical complement pathway activation Increased C5b-9 deposition on the cell surface
Trophoblast	β 2GPI	Increased trophoblast apoptosis Decreased trophoblast proliferation and migration Decreased production of hCG defective secretion of growth factors
Decidua		Inflammatory response in decidual cells Defective angiogenesis and spiral artery development

1 Anx, Annexin; β 2GPI, β 2 glycoprotein I; GP, Glycoprotein; hCG, Human chorionic gonadotropin; ICAM, Intercellular adhesion
2 molecule; IL, Interleukine; NET, Neutrophil extracellular traps; NO, Nitric oxide; ROS, reactive oxygen species; TF, Tissue factor; TLR,
3 Toll-like receptor; VCAM, Vascular cell adhesion molecule.

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Table 2: Summary of aPL profiles according to identified clusters.

Study	Subjects	Ethnicity	Thrombosis	Pregnancy morbidity	APS with SLE	Healthy aPL carriers	Comments
Sciascia (97) 2019	n = 486 - 257 PAPS - 96 SLE/APS - 133 aPL carriers	Caucasian	- high prevalence of anti-CL IgG/M (69%) 85% with multiple aPL positivity - high rate of triple positivity (35%)	- low rate of anti-β2GPI (23%) - low rate of multiple aPL positivity (30%) - low rate of triple positivity (4%)	- high rate of multiple aPL positivity (84%) - 22% with triple positivity	- high prevalence of anti-CL IgG/M (61%) - multiple aPL positivity in 57% of patients - no patient with triple positivity	<u>One additional cluster:</u> - defined APS (42%) and high rate of cytopenia (42%) - low rate of triple positivity (5%) No data on extra criteria aPL
Zuily (100) 2020	n = 497 - 324 PAPS - 173 aPL/APS associated with other autoimmune disease	Various	<u>In females with frequent VTE:</u> - high rate of anti-CL (93%) - high rate of β2GPI (77%) - high rate of triple positivity (55%) <u>In men with frequent ATE, heart valve disease, CV risk factors:</u> - anti-β2GPI :53% - triple positivity: 40%	<u>In females with isolated PM:</u> - high frequency of LA (80%) - high frequency of anti-CL (68%) - lower frequency of anti-β2GPI (40%)	- high prevalence of LA (85%) - low rate of anti-anti-β2GPI (14%) - low rate of triple positivity (7%)	<u>Asymptomatic aPL-positive females:</u> - high prevalence of anti-CL (85%) or anti-β2GPI (64%)	No data on extra criteria aPL
Alijotas-Reig (101) 2019	n = 1000 - 1000 OAPS - 76 OAPS with SLE	Caucasian	/	- high frequency of single LA positivity (35%) - low rate of double (18%) and triple positivity (11%)	/	/	<u>Recurrent miscarriage:</u> - high rate of single anti-CL IgG/M positivity (27%) - 26% with multiple aPL positivity <u>Fetal loss:</u> - high rate of single LA positivity (31%) No data on extra criteria aPL

anti-CL, Anticardiolipin antibody; aPL, Antiphospholipid antibody; ATE, Arterial thromboembolic event; CV, Cardiovascular; LA, Lupus anticoagulant; OAPS, Obstetric antiphospholipid syndrome; PAPS, Primary antiphospholipid syndrome; PM, Pregnancy morbidity; SLE, Systemic lupus erythematosus; VTE, venous thromboembolic events.

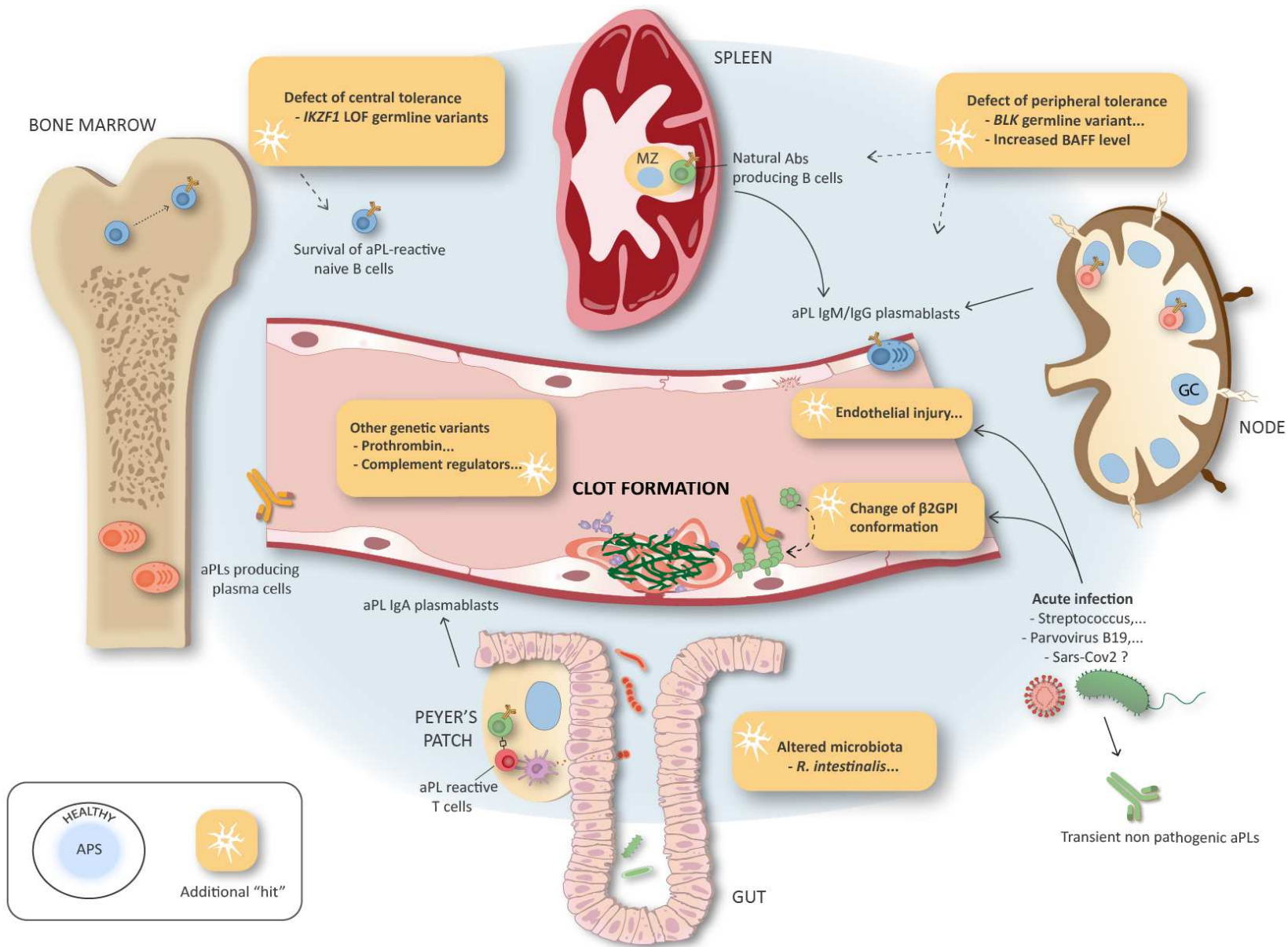


FIGURE LEGEND

Figure 1: From B-cell tolerance breakdown to thrombosis in APS : a multistep process toward B cell maturation. Predisposal genetic background, chronic inflammation or continuous antigen exposure through host-commensal interactions represent crucial “hits” that could favor the escape of transient or anergic aPL reactive clones to tolerance checkpoints, their expansion and maturation, and lead to aPL-secreting plasma cells accumulation. Specific epitope exposure due to additional factors (infection, inflammation...) thereafter provide the trigger leading to clinical events, such as thrombosis, defining APS.

Abs, antibodies; aPL, Antiphospholipid antibody; APS, Antiphospholipid syndrome; BAFF, B-cell activating factor; CMV, cytomegalovirus; GC, Germinal center; IgA/G/M, Immunoglobulin A/G/M; LOF, Loss of function; MZ, Marginal zone.