Absence of anti-β2 glycoprotein I antibodies in giant cell arteritis: A study of 45 biopsy-proven cases

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SUMMARY

Objective. To search for a relationship between serum anti-β2 glycoprotein I (anti-β2GPI) antibodies and the occurrence of ischaemic complications in giant cell arteritis (GCA), since the latter do not correlate with anti-cardiolipin antibodies (ACL), which are frequently observed in GCA.

Methods. IgG and IgM anti-β2GPI antibodies and ACL were measured by enzyme-linked immunosorbent assays in sera, collected before treatment, from 45 unselected patients with biopsy-proven GCA, including 15 patients with ischaemic events.

Results. IgG and IgM anti-β2GPI antibodies were not detected in any of the patients, contrasting with the presence of ACL in 51% of them, without correlation with ischaemia.

Conclusion. Anti-β2GPI antibodies are not detectable in GCA, contrasting with the occurrence of ACL, and ischaemic complications are apparently unrelated to the most frequent anti-phospholipid antibodies.

Key words: Giant cell arteritis, Ischaemia, Anti-phospholipid antibodies, β2 Glycoprotein I, Cardiolipin.

Giant cell arteritis (GCA) predominantly affects medium- and large-sized arteries. The initial lesions are marked by inflammatory infiltrates of the vessel wall. Activated T lymphocytes infiltrate temporal arteries, supporting the hypothesis that the pathogenesis of GCA involves a T-cell response against an unknown arterial component [1]. The course of the disease is characterized by the occurrence of arterial thrombosis which is not foreseeable, and leads to blindness, myocardial or limb ischaemia, or stroke. These complications affect only certain patients and several studies failed to define a predictive biological factor, except for thrombocytosis in a study [2]. Anti-phospholipid antibodies are known to be associated with vascular thrombosis in diseases such as systemic lupus erythematosus, and define the biological criteria of the anti-phospholipid syndrome (APS). Anti-cardiolipin antibodies (ACL) were first described, but antibodies directed against β2 glycoprotein I (β2GPI), which is the cofactor for the main group of anti-phospholipid antibodies (lupus anticoagulant and ACL), appear to be more significant in terms of the occurrence of thrombosis [3–6]. The dependence of ACL on β2GPI seems to be critical in their pathogenesis, β2GPI-independent ACL being observed in the absence of thrombosis in various conditions, such as certain infections or drug-induced manifestations [7]. In addition, the coating of β2GPI alone on irradiated plates allows the detection of antibodies directed against an epitope of β2GPI exposed following its interaction with oxidized plastic surface [8]. These anti-β2GPI antibodies, mainly of the IgG class, were detected in association with other IgG anti-phospholipid antibodies in sera from APS patients with thrombosis [3, 4]. In our experience, such IgG anti-β2GPI antibodies were significantly associated with β2GPI-dependent IgG ACL in APS (82 cases, P = 0.006, Spearman test). In addition, antibodies directed against β2GPI alone, in its native form (detected using non-irradiated plates), were found in APS patients in the absence of other detectable anti-phospholipid antibodies [9]. In GCA, moderate serum levels of ACL, mainly of the IgG class, are frequently detected [10–14]. The correlation of ACL with the occurrence of ischaemic complications is a matter of controversy [10, 11]. Our previous study failed to demonstrate any correlation [14]. There are virtually no data on anti-β2GPI antibodies in GCA, except for a study which included 19 treated and untreated patients and reported ACL and anti-β2GPI antibodies in eight and two cases, respectively [15]. We therefore studied anti-β2GPI antibodies, before treatment, in sera from 45 patients with proven GCA.

METHODS

Patients

Anti-β2GPI antibodies and ACL were searched for prior to therapy in the serum from 45 informed and consenting patients (16 males, 29 females, mean age 74.9 ± 7.3 yr, range 57–93), with characteristic histological features of GCA on temporal biopsies. Revealing symptoms were cranial symptoms without polymyalgia rheumatica (28 cases), both cranial symptoms and polymyalgia rheumatica (12 cases), isolated polymyalgia rheumatica (one case) and systemic symptoms with fever (four cases). Ischaemic complications were diagnosed in 15 patients (33.3%), at presentation in all but three cases. In patients 3, 8 and 15, they occurred 5, 2 or 10 days after diagnosis, respectively, during corticosteroid treatment (oral prednisone 0.7 mg/kg daily for patient 3 or 1 mg/kg daily for patients 8 and 15, associated with pulse methylpredni...
solone in patient 15: 100 mg/8 h during 3 days). They included ocular complications in 12 cases (in association with a delayed myocardial infarction on day 30 after diagnosis in patient 10), upper limb ischaemia (cases 2 and 5) and an extensive vertebrobasilar stroke (patient 3) (Table I). Ocular involvements were revealed, on the one hand, by permanent visual loss, related to an anterior ischaemic optic neuropathy (AION) diagnosed by fundoscopy and retinal angiography findings in six cases (nos 1, 7, 8, 9, 11 and 12) or to an ischaemic retrolubular optic neuritis (IRON) with normal fundoscopy in two patients (nos 10 and 15) and, on the other hand, by transient visual loss in two cases (nos 13 and 14) and blurred vision associated with diplopia (case 6).

**Antibody detection**

Anti-β2GPI antibodies were characterized by enzyme-linked immunosorbent assay (ELISA) [16] with purified human β2GPI (Stago, Asnière, France) controlled by polyacrylamide gel electrophoresis. Polystyrene irradiated microplates (Maxisorp, Nunc, Roskilde, Denmark) were coated for 18 h at 4°C with 200 ng/well of β2GPI diluted in phosphate-buffered saline solution (PBS; 50 μl/well). After saturation with PBS–0.1% Tween 20 (Sigma, St Louis, MO, USA) containing 1% bovine serum albumin (BSA; Dianed, Cressier/Morat, Switzerland), sera diluted 1/100 in the latter solution were incubated for 1 h at 37°C in triplicate in coated and uncoated (blank) wells. After six washes with PBS–0.05% Tween 20, biotin-coupled monospecific anti-human IgG (γ chain specific) or IgM (μ chain specific) antibodies (Amersham, Les Ulis, France) were added at a 1/1000 dilution and incubated for 1 h. After washing, the streptavidin–peroxidase complex (Amersham) diluted 1/1000 in PBS was incubated for 30 min at 37°C. The reaction was revealed with o-phenylenediamine (Sigma) and optical densities (OD) were read at 492 nm. Only corrected OD (after background subtraction) were taken into account. Antibody levels were expressed in arbitrary units by reference to positive and negative sera used as internal standards in every plate. The cut-off value for positive levels (28 IgG units and 10 IgM units) was determined from the study of 82 sera with known anti-phospholipid antibodies (from primary APS and lupus erythematosus patients) and 107 sera from healthy subjects, according to Griner et al. [17], with a specificity of 84% and a sensitivity of 56%.

ACL were also searched for by ELISA [18], following the same procedure as above, except for microtitration plates (Immunosorb, Nunc) coated with cardiolipin purified from bovine heart (Sigma) and saturated with 1% BSA in PBS. The same solution was also used for serum dilutions (1/100). ACL levels were expressed in IgM and IgG units calculated using sera calibrated with Harris’ standards [19] included in every plate, with positive levels ≥ 20 U.

**RESULTS**

Anti-β2GPI antibodies were not detected at positive levels in any untreated patients with GCA, either of the IgG (mean level 3 ± 5, range 0–26 U) or IgM (mean level 3 ± 2, range 0–10 U) classes. In contrast, confirming previous results [14], ACL were found to be positive in 51% (23/45) of sera (mean level 52 ± 29, range 21–110 U). These ACL belonged mainly to the IgG class, except for a patient without ischaemic complication, who only had a low level of IgM ACL (34 U). ACL did not correlate significantly with the occurrence of ischaemia (P = 0.83, χ² test; P = 0.93, Mann–Whitney U-test). The mean platelet level was 438.5 ± 133 × 10⁹/l (range 216–913 × 10⁹/l) without

<table>
<thead>
<tr>
<th>Patient</th>
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<th>IgG anti-β2GPI</th>
<th>IgM anti-β2GPI</th>
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PVL, permanent visual loss; AION, anterior ischaemic optic neuropathy; ULI, upper limb ischaemia; TVL, transient visual loss; IRON, ischaemic retrolubular optic neuritis; MI, myocardial infarction.

*IgG ACL: IgG anti-cardiolipin antibodies, positive value 20 U.
†IgG anti-β2GPI: IgG anti-β2 glycoprotein I, positive value 28 U.
‡IgM anti-β2GPI: IgM anti-β2 glycoprotein I, positive value 10 U.
any correlation with the occurrence of ischaemia ($P = 0.12$, Mann–Whitney $U$-test).

**DISCUSSION**

The present study failed to find evidence of anti-$\beta$2GPI antibodies in proven GCA, in contrast with ACL which were demonstrated in half of the patients. These ACL were not $\beta$2GPI dependent and did not correlate with thrombotic events, confirming that $\beta$2GPI-independent ACL are usually not involved in thrombosis. Hence, these anti-phospholipid antibodies fail to be predictive for the occurrence of ischaemic complications in GCA. The origin of ACL in GCA is as yet unknown. They could be induced by the exposure of anionic phospholipids at the outer leaflet membrane following the rearrangement of membrane phospholipids due to endothelial cell stimulation during inflammation. The pathogenic mechanism of GCA seems to relate to activated T cells reactive with an uncharacterized antigen expressed locally in the arterial walls [1]. During the T-cell response, cytokine (especially interferon-$\gamma$) production may play a role in the formation of the inflammatory infiltrates [20] and activation of endothelial cells. Therefore, ACL could be merely secondary and induced by local inflammation. This hypothesis is supported by the dramatic decrease in ACL levels following corticosteroid administration [12–14]. Understanding of the mechanisms underlying arterial ischaemia in GCA clearly needs further investigations.

**References**