Decrease in the ratio of polyreactive IgG titres with IgG concentration is associated with long-term complications of primary Sjögren's syndrome

Sirs,

For almost 100 years, immunologists have known that immunoglobulins in pre-immune sera could bind to foreign antigens. The 'lock and key' hypothesis of antigen-antibody interaction had for a long time dominated in immunological thinking. Recent studies, using hybridoma technology, suggest that the antigen-binding 'pocket' of many antibody molecules is more flexible than previously thought and thus can accommodate different antigenic configurations (1, 2). These antibodies are widely known as polyreactive antibodies (PAbs). PAbs are pre-immune antibodies generated in the absence of exogenous antigenic stimulation, which are non-specific, broadly cross-reactive, low affinity and germline-encoded (3). Most PAbs belong to the IgM class, but some are IgG and IgA and can bind to proteins carbohydrates, lipids and nucleic acids (4,9). Recent evidence suggests the stimulation of Toll-like receptors (TLRs) as a potential key link in the mechanism of polyreactive antibody secretion (5). Tissue damage by any of these means could result in the release of TLR ligands which could stimulate the secretion of PAbs (6).

PAbs can bind to gram-negative and gram-positive bacteria and acting through the classical complement pathway can inhibit bacterial growth by lysis, generate anaphylatoxin C5a, enhance phagocytosis and neutralise the functional activity of endotoxin (7). Beyond protection against infections, PAbs serve a number of additional essential functions in the immune system including regulation of B cell development, selection of B cell repertoire, regulation of B cell responses, clearance of apoptotic debris, vascular homeostasis, protection against atherosclerosis, allergic suppression and protection from cancer (3).

These observations prompted us to investigate PAbs in systemic autoimmune rheumatic diseases, in an attempt to define their prevalence and clinical significance. In a previous report it was found that PAbs are increased in sera of patients with systemic lupus erythematosus (SLE) (6). The present study was initiated to quantify the titre of PAbs in sera of patients with pSS and to investigate associations with particular clinical manifestations. Sera from 38 patients with pSS (45±15 years, 97% females), who fulfilled the 2002 American-European Consensus Criteria for Sjögren's syndrome were studied. The levels of PAbs in these patients were compared with those in healthy individuals (sera from 30 age- and sex matched healthy individuals who served as controls (34±12 years 77% females). Histological (focus score), laboratory (Anti Ro/La, RF, hypergammaglobulinaemia, anaemia, leukopenia, monoclonality) and clinical parameters (*e.g.* Raynaud, purpura, lymphomas, peripheral neuropathy, articular manifestations, vasculitic involvement, renal and parenchymal organ involvement) were recorded. Furthermore, the patients were subdivided, according to risk factors of lymphoma development (10) and PAbs were analysed in the two groups and between independent risk factors for lymphoma development (11).

A surrogate assay was used to measure PAbs. Dinitrophenol (DNP) was chosen as the surrogate substrate, because it is a synthetic molecule, not present in the environment, and individuals are not normally exposed to it. Therefore, if antibodies in sera bind to DNP, these antibodies would almost certainly have to be PAbs (8). In brief, ELISA plates were coated with DNP and serially two-fold dilutions of serum was used to determine the PAb titre and the titre is reported as the reciprocal of highest serum dilution that produced an absorbance above the plate background activity. Serum immunoglobulin concentrations were determined by sandwich ELISA using a standard curve (6, 8). Significant differences in the levels of antibodies among patient groups were evaluated by the non-parametric Tukey's multiple comparison test, whereas differences between patients expressing or not various clinical, histological and serological markers by the non-parametric Mann-Whitney test. Associations with patient features were analysed by Spearman's rank correlation test. GraphPad Prism-5 (GraphPad Software, San Diego, CA, USA) software was used.

The concentration of serum IgG in pSS patients was higher than in normal controls. The levels of IgG PAbs were higher but not significantly higher in pSS patients than in normal controls but were significantly lower than in SLE patients (6). Similarly, the ratio of the polyreactive IgG antibody titre to the IgG concentration was not significantly higher in pSS patients than in normals, but was significantly lower than in SLE patients as described previously (6). This argues that the serum IgG of SLE patients contains more PAbs than the serum of pSS patients and the serum of controls. No significant differences were found between the levels of IgM PAbs in pSS patients compared to normal controls.

The analysis of associations of PAbs with various clinical manifestations of Sjögren's

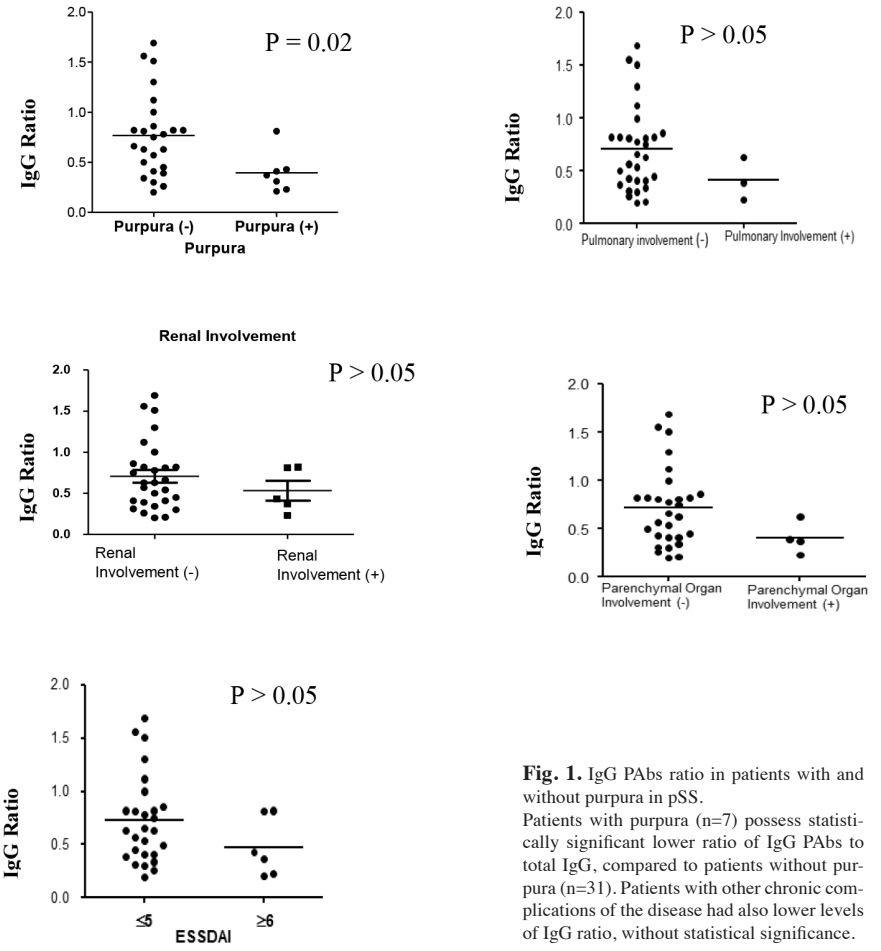

Fig. 1. IgG PAbs ratio in patients with and without purpura in pSS.
Patients with purpura (n=7) possess statistically significant lower ratio of IgG PAbs to total IgG, compared to patients without purpura (n=31). Patients with other chronic complications of the disease had also lower levels of IgG ratio, without statistical significance.

syndrome showed that the IgG ratio of polyreactive to total IgG is statistically significantly lower in patients with vasculitic lesions (purpura p=0.02). PAbs IgG/total IgG ratio was also lower in patients with renal involvement, pulmonary involvement and disease activity as measured by the ESSDAI score but the differences did not reach statistical significance (Fig. 1). No associations were found with focus score, laboratory and clinical parameters. IgA and IgM levels of PAbs did not disclose any significant association. Moreover, levels of PAbs did not differ between high risk patients for lymphoma development (type I pSS) and uncomplicated patients (type II [low risk] pSS) (10) and between independent risk factors for lymphoma development (11).

The fact that PAbs are elevated in the serum of these patients may represent a no specific part of hypergammaglobulinaemia found in these patients. The association of PAbs with vasculitis in these patients is of particular interest, since small vessel vasculitis in pSS is a typical immune complex-mediated disease. Patients with such a pattern of clinical picture constitute around 15–20% of total pSS population and are considered as having a systemic disease. Moreover, patients who will develop lymphoma in the future, usually belong to this group. This association could be explained by two ways. First, PAbs play a protective role for vasculitic development by neutralising active molecules (*e.g.* TLRs, complement receptors) in the local tissue injury. Second, they may participate in the tissue injury by forming low-affinity immune complexes thus, contributing in tissue damage. In any way, elution of immunoglobulins from the damaged tissue in SS and detection of PAbs will address this question. The clinical utility of PAbs as a useful laboratory marker for vasculitis in patients with pSS remains to be determined in large number of patients and well defined disease controls.

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O.D. ARGYROPOULOU[1]*
S. GUNTI[2]*
E.K. KAPSOGEORGOU[1]
A.L. NOTKINS[2]
A.G. TZIOUFAS[1]

**These authors equally contributed to this manuscript.*
[1]Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Greece;
[2]Experimental Medicine Section, Laboratory of Sensory Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA.

Please address correspondence to:
Prof. A.G. Tzioufas,
Department of Pathophysiology,
School of Medicine, National and Kapodistrian University of Athens,
75 Mikras Asias str.,
11527 Athens, Greece.
E-mail: agtzi@med.uoa.gr

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