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SPECIAL ARTICLE

Autoantibodies against galectin-2 peptides as biomarkers for the antiphospholipid syndrome

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Autoantibodies against opsonins of dying and dead cells mediate Fcγ receptor-dependent phagocytosis of autologous apoptotic and necrotic cells and hereby tend to elicit inflammation instead of silent clearance. We analysed sera of patients with chronic autoimmune diseases for the occurrence of IgG autoantibodies recognizing galectins. These pluripotent effectors can also bind to apoptotic or necrotic cells. Patients with antiphospholipid syndrome (APS; $n = 104$) and systemic lupus erythematosus (SLE; $n = 62$) were examined, healthy donors ($n = 31$) served as controls. Selected peptides of galectin (Gal)-2 were employed for peptide-based ELISAs. Levels of anti-Gal-2^{PEP}-IgG were significantly increased in SLE and APS when compared with controls. In addition, patients with APS showed significantly higher levels of anti-Gal-2^{PEP}-IgG compared with patients with SLE. Anti-Gal-2^{PEP}-IgG may, therefore, be considered novel biomarkers for APS. *Lupus* (2012) **21,** 781–783.

Key words: antiphospholipid syndrome; autoantibodies; lectins; systemic lupus erythematosus

Introduction

A fast and effective clearance of dying and dead cells is essential to prevent secondary necrosis, accompanied by leakage of autoantigens and inflammation.[1] Compounds from uncleared cells have been discussed to elicit production of autoantibodies in chronic inflammatory diseases.[2] To ensure silent clearance of apoptotic cells, a plethora of soluble adaptor molecules assists in bridging dying cells to phagocytes. Galectins (Gal), a family of endogenous lectins sharing galactoside specificity and sequence signature/folding,[3, 4] bind apoptotic and necrotic cells and an involvement in clearance for Gal-3 is suggested.[5, 6] For interest, opsonins (e.g. Gal) can often become targets of an adaptive humoral immune response followed by autoimmune disease.[7, 8] To address this issue for Gal we analysed anti-Gal-IgG in patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) employing peptide-based ELISAs.

Patients and methods

Sera were processed from 104 patients with primary or secondary APS, 62 patients with SLE, and 31 healthy blood donors (HBDs) who served as controls. Patients were diagnosed as APS according the revised classification criteria for APS.[9] Patients classified as SLE met the classification criteria of the American College of Rheumatology for SLE.[10] Clinical manifestations and serological parameters of the patients are compiled in Table 1. Three chemically synthesized Gal-2 derived

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Table 1 Clinical manifestations and serological parameters of the patients

				Clinical manifestations				*Serological parameters*			
Diagnosis	*Number of patients*	*sex [female %]*	*age [years; mean ± standard deviation]*	*thrombotic event & pulmonary embolism*	*obstetric complications*	*cerebral vascular event*	*Raynaud-Syndrome*	*Lupus anti-coagulant*	*anti-CL (IgG or IgM)*	*anti-β2GP1 (IgG or IgM)*	*category I; (more than one laboratory criteria present)*
APS	104	80.8	46.0 ± 12.0	59	32	13	13	56	72	47	49
SLE	62	83.9	40.4 ± 14.3	0	0	0	0	0	15	0	0

APS patients met the revised classification criteria for APS,[9] SLE patients met the classification criteria of the American College of Rheumatology for SLE.[10]

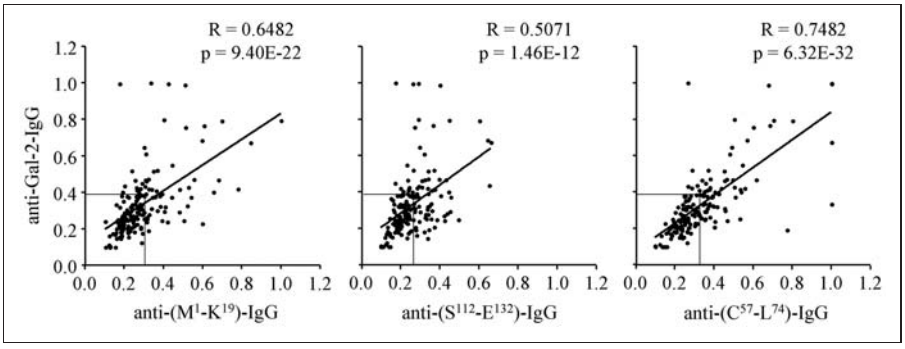

Figure 1 Autoantibodies against Gal-2 and the corresponding Gal-2-derived peptides correlate in sera of patients with SLE and APS. *R* and *p*-values of the Spearman-rho correlations are indicated. The cut-off levels (99th percentile of the HBD) were inserted as boxes into the graphs. Amino acid sequences are shown in http://www.ncbi.nlm.nih.gov/protein/NP_006489.1. The sequences of the Gal-2 peptides are: MTGELEVKNMDMKPGSTLK (M^1-K^{19}), CNSLDGSNWGQEQREDHL (C^{57}-L^{74}), and SHLSYLSVR GGFNMSSFKLKE (S^{112}-E^{132}).

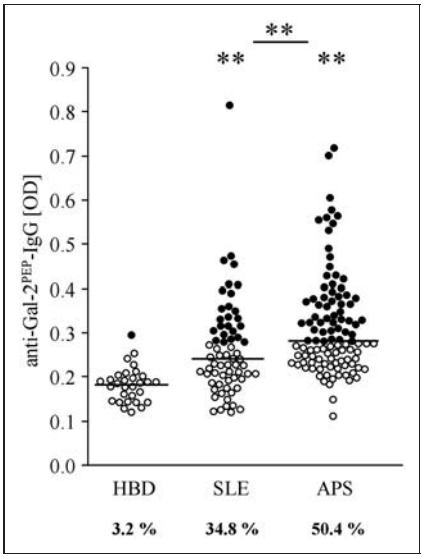

Figure 2 Patients with APS and SLE show increased levels of anti-Gal-2^{PEP}-IgG. Anti-Gal-2^{PEP}-IgG were measured by ELISA, displayed are the mean values of the three anti-Gal-2^{PEP}-IgG. The 99th percentile of the HBD was set as cut-off value (open circles <99th; closed circles >99th percentile of the HBD). Indicated are the percentages of the patients with Gal-2^{PEP}-IgG > cut-off value. The horizontal bar represents the median values of one cohort. HBD, healthy blood donor; SLE, systemic lupus erythematosus; APS, antiphospholipid syndrome. Significances were calculated by Mann–Whitney *U* tests. $^{**}p < 0.01$.

peptides, namely a N-terminal 19mer (M^1-K^{19}), a central 18mer (C^{57}-L^{74}) and a C-terminal 21mer (S^{112}-E^{132}) were tested.[11] The full-size Gal-2 was obtained by recombinant production and purified by affinity chromatography.[12] Anti-Gal-2 IgG in sera of autoimmune patients were detected by ELISAs. The cut-off level was defined as the 99th percentile of the optical density (OD) values of HBD. Experimental values are expressed as mean OD of the duplicates of each serum. SPSS 17.0 software was used for statistic calculations.

Results and discussion

We compared the reactivity of IgG autoantibodies against the three Gal-2 peptides in sera of patients with SLE and APS with the reactivity against the recombinant protein. A significant correlation (Spearman) of the peptide reactivity with that of the corresponding full-size protein was obtained (Figure 1). Compared with HBD controls significantly increased IgG-antibody levels against Gal-2^{PEP} were found in patients with APS and SLE (Figure 2). Interestingly, the anti-Gal-2^{PEP} IgG in the APS cohort was significantly increased

when compared with the SLE cohort containing no patients with APS. Anti-Gal-2^{PEP}-IgG may, therefore, be considered as novel biomarkers for APS.

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Conflict of interest

The authors declare no conflict of interest.

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