

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

C Janko, S André, LE Munoz, JP Briand, C Schorn, S Winkler, M Schiller, L Andreoli, AA Manfredi, DA Isenberg, G Schett, HJ Gabius, S Muller and M Herrmann
Lupus 2012 21: 781
DOI: 10.1177/0961203312443422

The online version of this article can be found at:
<http://lup.sagepub.com/content/21/7/781>

Published by:



<http://www.sagepublications.com>

Additional services and information for *Lupus* can be found at:

Email Alerts: <http://lup.sagepub.com/cgi/alerts>

Subscriptions: <http://lup.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

>> [Version of Record](#) - May 25, 2012

[What is This?](#)

SPECIAL ARTICLE**Autoantibodies against galectin-2 peptides as biomarkers for the antiphospholipid syndrome**C Janko^{1,*}, S André^{2,*}, LE Munoz¹, JP Briand³, C Schorn¹, S Winkler¹, M Schiller⁴, L Andreoli⁵, AA Manfredi⁶, DA Isenberg⁷, G Schett¹, HJ Gabius^{2,#}, S Muller^{3,#} and M Herrmann^{1,#}

¹Department of Internal Medicine 3, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany; ²Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Munich, Germany; ³Centre National de la Recherche Scientifique, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France; ⁴Department of Medicine V, University of Heidelberg, Heidelberg, Germany; ⁵Rheumatology and Clinical Immunology, Spedali Civili, University of Brescia, Brescia, Italy; ⁶Hospital San Raffaele Scientific Institute and University Vita-Salute San Raffaele, Milano, Italy; and ⁷Department of Medicine, Centre for Rheumatology, University College London, London, UK

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 to 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

*These authors contributed equally to the work.

#These authors contributed equally to the senior authorship.

Correspondence to: Professor Martin Herrmann, Krankenhausstraße 12, 91054 Erlangen, Germany
Email: martin.herrmann@uk-erlangen.de

Table 1 Clinical manifestations and serological parameters of the patients

Diagnosis	Number of patients	sex [female %]	age [years; mean ± standard deviation]	Clinical manifestations				Serological parameters			category I; (more than one laboratory criteria present)
				thrombotic event & pulmonary embolism	obstetric complications	cerebral vascular event	Raynaud-Syndrome	Lupus anti-coagulant	anti-CL (IgG or IgM)	anti-β2GPI (IgG or IgM)	
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

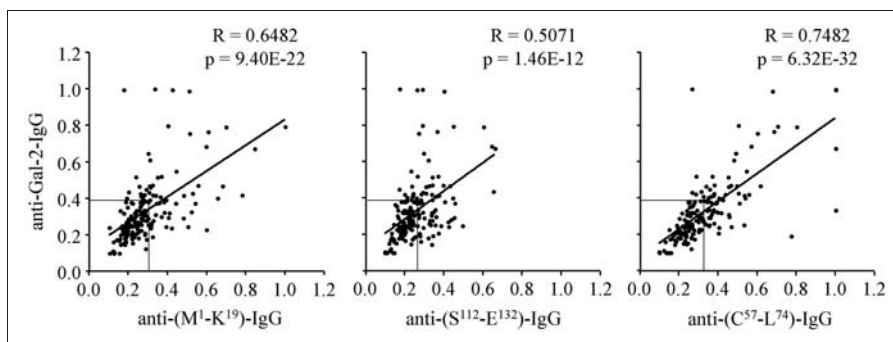


Figure 1 Autoantibodies against Gal-2 and the corresponding Gal-2-derived peptides correlate in sera of patients with SLE and APS. *R* and *p*-values of the Spearman-rho correlations are indicated. The cut-off levels (99th percentile of the HBD) were inserted as boxes into the graphs. Amino acid sequences are shown in http://www.ncbi.nlm.nih.gov/protein/NP_006489.1. The sequences of the Gal-2 peptides are: MTGELEVKNMDMKPGSTLK (M¹-K¹⁹), CNSLDGSNWGQEQRDHL (C⁵⁷-L⁷⁴), and SHLSYLSVRGGFNMSFKLKE (S¹¹²-E¹³²).

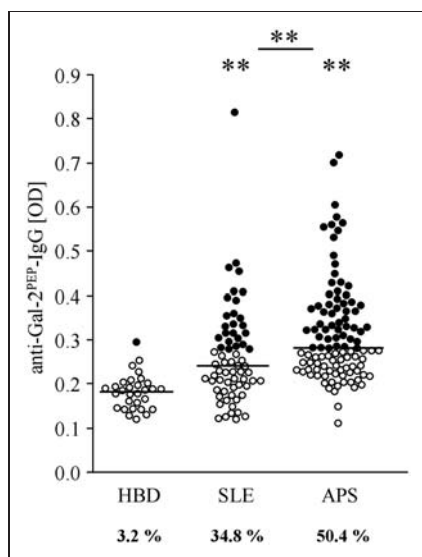


Figure 2 Patients with APS and SLE show increased levels of anti-Gal-2^{PEP}-IgG. Anti-Gal-2^{PEP}-IgG were measured by ELISA, displayed are the mean values of the three anti-Gal-2^{PEP}-IgG. The 99th percentile of the HBD was set as cut-off value (open circles <99th; closed circles >99th percentile 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 (M¹-K¹⁹), a central 18mer (C⁵⁷-L⁷⁴) and a C-terminal 21mer (S¹¹²-E¹³²) 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.

Funding

This work was supported by the Masterswitch project of the EU, the DFG (training grant SFB 643), the Interdisciplinary Center of Clinical Research (IZKF, grant number TP A41) at the University Hospital of the University of Erlangen-Nuremberg, the K&R Wucherpennig Stiftung, the EC (GlycoHIT, grant number 260600), and CNRS (to JPB and SM).

Conflict of interest

The authors declare no conflict of interest.

References

- 1 Schulze C, Munoz LE, Franz S, *et al.* Clearance deficiency—a potential link between infections and autoimmunity. *Autoimmun Rev* 2008; 8: 5–8.
- 2 Munoz LE, Lauber K, Schiller M, *et al.* The role of defective clearance of apoptotic cells in systemic autoimmunity. *Nat Rev Rheumatol* 2010; 6: 280–289.
- 3 Gabius HJ, Andre S, Jimenez-Barbero J, *et al.* From lectin structure to functional glycomics: principles of the sugar code. *Trends Biochem Sci* 2011; 36: 298–313.
- 4 Kaltner H, Gabius H. A toolbox of lectins for translating the sugar code: the galectin network in phylogenesis and tumors. *Histol Histopathol* 2012; in press.
- 5 Beer A, Andre S, Kaltner H, *et al.* Human galectins as sensors for apoptosis/necrosis-associated surface changes of granulocytes and lymphocytes. *Cytometry A* 2008; 73: 139–147.
- 6 Karlsson A, Christenson K, Matlak M, *et al.* Galectin-3 functions as an opsonin and enhances the macrophage clearance of apoptotic neutrophils. *Glycobiology* 2009; 19: 16–20.
- 7 Massardo L, Metz C, Pardo E, *et al.* Autoantibodies against galectin-8: their specificity, association with lymphopenia in systemic lupus erythematosus and detection in rheumatoid arthritis and acute inflammation. *Lupus* 2009; 8: 539–546.
- 8 Janko C, Franz S, Munoz L, *et al.* CRP/anti-CRP antibodies assembly on the surfaces of cell remnants switches their phagocytic clearance toward inflammation. *Front Immun* 2011; 2.
- 9 Miyakis S, Lockshin MD, Atsumi T, *et al.* International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
- 10 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
- 11 Neimark J, Briand JP. Development of a fully automated multi-channel peptide synthesizer with integrated TFA cleavage capability. *Pept Res* 1993; 6: 219–228.
- 12 Andre S, Kaltner H, Lensch M, *et al.* Determination of structural and functional overlap/divergence of five proto-type galectins by analysis of the growth-regulatory interaction with ganglioside GM1 in silico and in vitro on human neuroblastoma cells. *Int J Cancer* 2005; 114: 46–57.