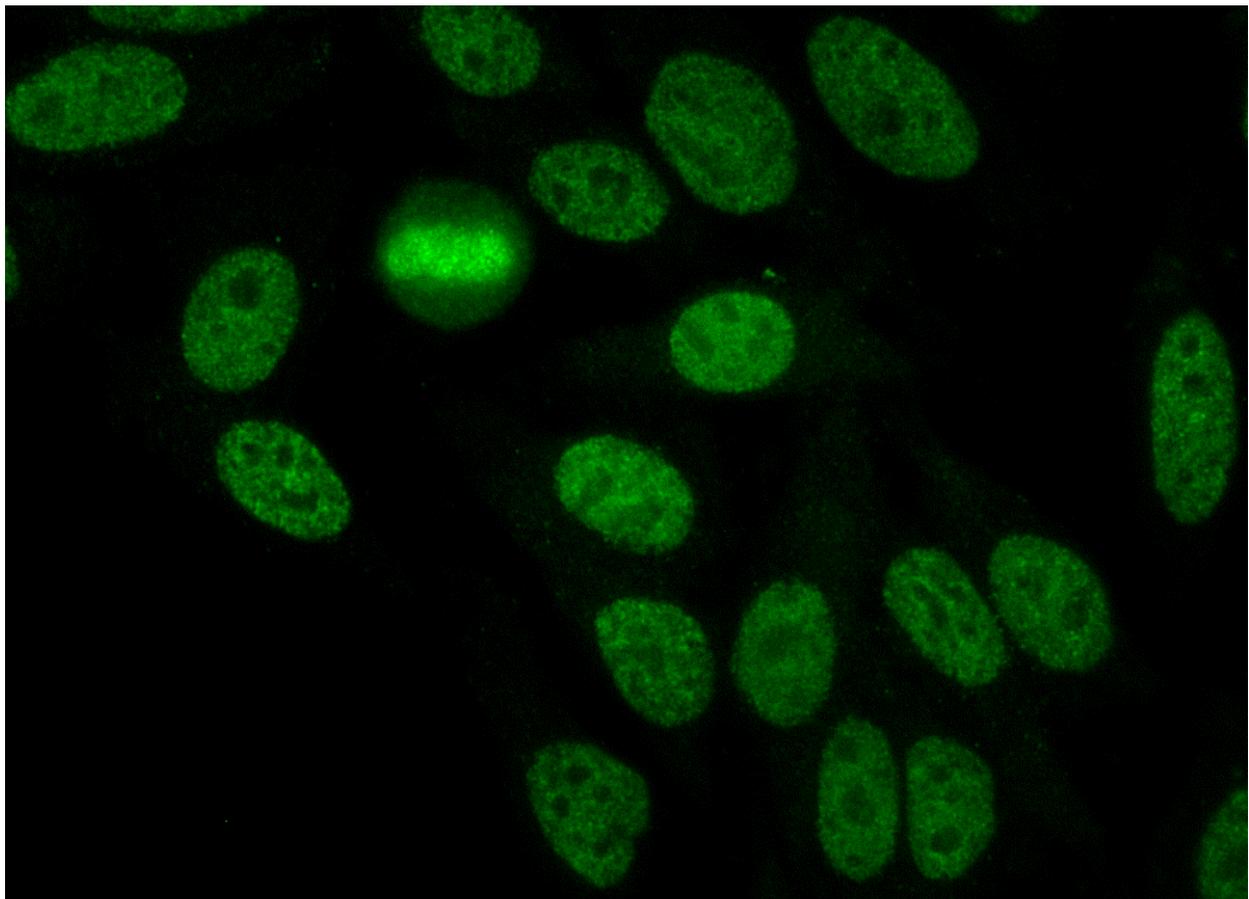


K. Conrad, E.K.L. Chan, M.J. Fritzler, R.L. Humbel, P.L. Meroni, G. Steiner, Y. Shoenfeld (Eds.)

Infection, Tumors and Autoimmunity

Report on the 11th Dresden Symposium on Autoantibodies held in Dresden on September 1-4, 2013



AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY

Volume 9 - 2013

Infection, Tumors and Autoimmunity
Report on the 11th Dresden Symposium on Autoantibodies

K. Conrad, E.K.L. Chan, M.J. Fritzler, R.L. Humbel, P.L. Meroni, G. Steiner, Y. Shoenfeld (Eds.)



Gesellschaft zur Förderung der Immundiagnostik e.V.
Dresden

<http://www.GFID-eV.de>

AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY

Edited by: K. Conrad (Dresden, Germany)

Vol. 9 – 2013

K. Conrad, E.K.L. Chan, M.J. Fritzler,
R.L. Humbel, P.L. Meroni, G. Steiner, Y. Shoenfeld (Eds.)

Infection, Tumors and Autoimmunity

Report on the 11th Dresden Symposium on Autoantibodies held in Dresden
on September 1–4, 2013

AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY
Volume 9 – 2013



PABST SCIENCE PUBLISHERS
Lengerich, Berlin, Bremen, Miami,
Riga, Viernheim, Wien, Zagreb

Bibliographic information published by Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie;
detailed bibliographic data is available in the Internet at
<<http://dnb.ddb.de>>.

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in other ways, and storage in data banks. The use of registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulation and therefore free for general use.

The authors and the publisher of this volume have taken care that the information and recommendations contained herein are accurate and compatible with the standards generally accepted at the time of publication. Nevertheless, it is difficult to ensure that all the information given is entirely accurate for all circumstances. The publisher disclaims any liability, loss, or damage incurred as a consequence, directly or indirectly, of the use and application of any of the contents of this volume.

© 2013 Pabst Science Publishers, 49525 Lengerich

<http://www.pabst-publishers.de>

Manufacturer: Digitale Dokumentationen Jana Schollich

Typesetting: Rico Hiemann, Thiendorf

Cover: Rico Hiemann, Thiendorf

ISBN 978-3-89967-881-9

Contents

Preface XI

Chapter 1

Infections, Tumors and Autoimmunity

- | | | |
|----|---|----|
| 1 | Autoimmune/inflammatory syndrome induced by adjuvants (ASIA)
<i>Carlo Perricone, Yehuda Shoenfeld</i> | 2 |
| 2 | Possible role of infections in the pathogenesis of autoimmune liver diseases
<i>Daniel S. Smyk and Dimitrios P. Bogdanos</i> | 5 |
| 3 | GP2 – The link between innate and acquired immune system in the intestine
<i>Dirk Roggenbuck, Dirk Reinhold, Dimitrios P. Bogdanos, Karsten Conrad</i> | 16 |
| 4 | Vitamin D in infection and autoimmunity
<i>E. Borella, Y. Shoenfeld</i> | 21 |
| 5 | Clinical implications of anti-rods and rings autoantibodies in hepatitis C patients treated with interferon/ribavirin therapy
<i>S. John Calise, Wendy C. Carcamo, and Edward K.L. Chan</i> | 25 |
| 6 | The hyperferritinemic syndrome in infection and autoimmunity
<i>Cristina Rosário, Yehuda Shoenfeld</i> | 33 |
| 7 | The fate of the Ro autoantigen-associated Y RNAs in Coxsackievirus-infected cells
<i>Ger J.M. Pruijn, Dirk den Braanker, Qian Feng, Frank J. van Kuppeveld</i> | 36 |
| 8 | Autoantibodies to tumor-associated antigens (TAAs) as biomarkers in the immunodiagnosis of cancer
<i>Liping Dai, Pengfei Ren, Xinxin Liu, Carlos A. Casiano, Jian-Ying Zhang</i> | 38 |
| 9 | Immunoproteomic profiling of autoantibodies in prostate cancer: a health disparities perspective
<i>Tino W. Sanchez, Anamika Basu, Saied Mirshahidi, Chen Chien-Shing, Susanne Montgomery, Colwick Wilson, Jianying Zhang, Nathan Wall and Carlos A. Casiano</i> | 46 |
| 10 | Characterisation of novel autoantibody biomarkers in oncology and autoimmunity
<i>Will Colón, Colin Wheeler, Ezam Uddin, Nicholas Workman, Pooja Agashe, Tim Vyse and Michael B. McAndrew</i> | 57 |

Chapter 2

Pathogenesis and Therapy of Autoimmune Diseases - Novel Aspects

- 11 The role of neutrophil extracellular traps (NETs) in autoimmunity and generation of autoantibodies 60
Elaine F. Kenny & Arturo Zychlinsky
- 12 Revisiting the cell death paradigm in systemic autoimmune diseases 63
Felipe Andrade
- 13 NETs and ANCA-associated vasculitides 69
Elena Csernok
- 14 Towards understanding the pathogenesis of granulomatosis with polyangiitis: The role of neutrophil extracellular traps 72
C. Lange, E. Csernok, J.U. Holle
- 15 Aggregation of NETs: flipping the switch to resolution of inflammation 73
Christine Schorn, Luis Munoz, Deborah Kienhöfer, Martin Herrmann, and Markus Hoffmann
- 16 Memory plasma cells in autoimmunity - An update 78
Falk Hiepe
- 17 Protective natural autoantibodies in atherogenesis and autoimmunity 82
Gregg J. Silverman and Caroline Grönwall
- 18 The interface between primary immunodeficiency and systemic lupus erythematosus 88
Sandro F. Perazzio, Magda Carneiro-Sampaio, Luis E.C. Andrade
- 19 Inherited defects in nucleic acid metabolism and autoimmunity 94
Stefanie Kretschmer, Nadja König, Min Ae Lee-Kirsch
- 20 Induction of autoimmunity by defects of intracellular nucleases: Mouse models for Aicardi-Goutières syndrome 98
Axel Roers
- 21 Anti-AT₁R and anti-ET_AR autoantibodies from patients with SSc and their agonistic effects 99
A. Kill, Christoph Tabeling, Anja A. Kühn, J. Günther, M.O. Becker, Harald Heidecke, D. Dragun, G.R. Burmester, G. Riemekasten
- 22 The use of Aptamers as a new tool for the treatment of autoantibody associated diseases 101
G. Wallukat, A. Haberland, S. Berg, E.J. Freyse, E. Salzsieder, W. Schulze, I. Schimke
- 23 Harnessing parasitic infection to treat autoimmunity 102
Miri Blank, Lachnish Jordan, Bashi Tomer and Yehuda Shoenfeld

Chapter 3

Immune Reactions against Drugs

- 24 Clinical relevance of anti-drug antibodies 110
Arno Kromminga
- 25 Mycophenolic acid and ribavirin induce cytoplasmic Rods and Rings (RR) structures in vivo, but only ribavirin induces anti-RR autoantibodies 114
Gerson D. Keppeke, Sandro F. Perazzio, Monica S. Prado, Edward K.L. Chan, Luis Eduardo C. Andrade
- 26 Effects of intracellular glutamine levels on the reversible assembly of cytoplasmic rod and ring autoantigenic structures 116
S. John Calise, Wendy C. Carcamo, Claire Krueger, Daniel L. Purich, and Edward K.L. Chan

Chapter 4

Autoantibodies in Systemic Autoimmune Rheumatic Diseases

4.1

Connective Tissue Diseases

- 27 Anti-Mup44 autoantibodies in inclusion-body myositis 120
Helma Pluk, Megan Herbert, Judith Stammen-Vogelzangs, Baziel G. van Engelen, Ger J.M. Pruijn
- 28 Autoantibody profiling in idiopathic inflammatory myopathies: Validation of the myositis antigens Mi-2a, MDA5, NXP2 and SAE1 in a line immune assay 127
L. Komorowski, W. Meyer, T. Scheper, W. Schlumberger, A. Ott, P.J. Charles, C. Probst
- 29 Evaluation of recombinant TIF1- γ in a line immune assay for the detection of autoantibodies in idiopathic inflammatory myopathies 129
C. Probst, L. Komorowski, W. Meyer, T. Scheper, A. Ott, P.J. Charles, K. Danko, L. Bodoky, H. Chinoy, W. Schlumberger
- 30 Autoantibodies to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) a novel biomarker for statin associated necrotizing myopathies 131
Makoto Miyara, Olivier Benveniste, Jean-Luc Charuel, Alexander Shikhman, Olivier Boyer, Joe Philips, Lucile Musset, Michael Mahler
- 31 Autoantibody signatures of connective tissue disease patients identified with a bead-based assays approach 133
Angelika Lueking, Petra Budde, Heike Goehler, Jessica Schwermann, Martin Gamer, Klaus Marquardt, Anna Telaar, Daniel Chamrad, Carmen Theek, Matthias Schneider, Peter Schulz-Knappe

- 32 Identification of novel autoantibody signatures reflecting the heterogeneity in systemic lupus erythematosus 135
P. Budde, A. Lueking, S. Vordenbäumen, H. Göhler, M. Gamer, K. Marquardt, A. Telaar, D. Chamrad, C. Theek, B. Ostendorf, P. Schulz-Knappe, M. Schneider
- 33 What is the clinical significance of anti-Sm-antibodies in SLE patients? A comparison with anti-dsDNA-antibodies and C3 137
Alexandra Richartz, Thomas Rose, Cornelia Dähnrich, Anke Rosemann, Fidan Barkhudarova, Wolfgang Schlumberger, Gerd-Rüdiger Burmester, Karl Egerer, Robert Biesen and Falk Hiepe
- 34 Clinical evaluation of total and high avidity anti-dsDNA antibody EIA for the diagnosis of systemic lupus erythematosus 139
Pengjie Wan, Shuxia Zhou, Hua Cao, Weiping Li, Doug Scott, Joel Hardy, Tony Presitigiacomo, Jie Zheng
- 35 Significance of anti-C1q antibody detection in SLE patients with lupus nephritis 140
Ivana Stiborová, Vlastimil Král, Stanislava Blažíčková

4.2

Rheumatoid Arthritis

- 36 Autoantibodies in rheumatoid arthritis: predictors of disease, prognosticators of outcome and pathogenic players 143
Markus Hoffmann, Karin Lundberg and Günter Steiner
- 37 Antibodies against carbamylated proteins in different stages of rheumatoid arthritis 151
Leendert A. Trouw
- 38 Development and initial use of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides in rheumatoid arthritis patients 153
Johan Rönnelid, Linda Mathsson, Mats Nystrand, Lars Klareskog, Monika Hansson
- 39 The anti-CCP2 is the anti-citrullinated protein antibody (ACPA) with highest diagnostic value in rheumatoid arthritis 157
E. Grenmyr and Y. Sommarin
- 40 Occurrence of anti-RA33 antibody in various types of juvenile idiopathic arthritis 159
Gabor Nagy, Barbara Szasz, Diana Garan, Ibolya Szollosi, Sandor Sipka, Tamas Constantin
- 41 MMP-3 is a marker for disease activity in rheumatoid arthritis 160
S. Reuter, T. Matthias
- 42 The conversion of QuantiFERON TB Gold test during biological treatment by anti-TNF preparations 162
E. Zanova, D. Kozakova, I. Rybar, I. Solovic, J. Rovensky
- 43 Interferon-gamma and QuantiFERON-TB Gold test conversion during biological treatment 164
D. Kozakova, E. Zanova, I. Rybar, I. Solovic, J. Rovensky

Chapter 5

Autoantibodies in Organ Specific Diseases

5.1

Neurological Diseases

- | | | |
|----|---|-----|
| 44 | New antibodies in multiple sclerosis
<i>Bernhard Hemmer</i> | 168 |
| 45 | Identification of coronin-1a as a novel antibody target for clinically isolated syndrome and multiple sclerosis
<i>Myrthe Rouwette, Jean-Paul Noben, Jack Van Horsen, Bart Van Wijmeersch, Raymond Hupperts, Peter J. Jongen, Marcel M. Verbeek, Peter P. De Deyn, Piet Stinissen and Veerle Somers</i> | 169 |
| 46 | Diagnostic and pathophysiologic relevance of autoantibodies against aquaporin-4
<i>F. Paul, B. Wildemann, S. Jarius</i> | 186 |
| 47 | Autoantibodies against the neuronal surface antigen DPPX: markers for an emerging neurological disease entity
<i>Bettina Balint, Simon Nagel, Uwe Haberkorn, Christian Probst, Inga-Madeleine Blöcker, Bianca Teegen, Kristin Rentzsch, Ramona Bahtz, Lars Komorowski, Sandra Saschenbrecker, Andreas Kastrup, Martin Kuthe, Hans-Michael Meinck, Winfried Stöcker</i> | 188 |
| 48 | Nonradioactive determination of autoantibodies against acetylcholine receptors in myasthenia gravis: A new enzyme-linked immunoassay for manual and automated processing
<i>J. Westermann, E. Denczyk, C. Probst, L. Komorowski, W. Schlumberger</i> | 190 |
| 49 | Generation of stable HEp-2 cell line M4 expressing human muscle-specific kinase for automatic videoscanner-based immunofluorescence diagnostics of Myasthenia gravis
<i>S. George, S. Paulick, I. Knütter, N. Röber, R. Hiemann, D. Roggenbuck, K. Conrad and J.-H. Küpper</i> | 192 |
| 50 | Clinical validation of a novel immunofluorescence assay for the detection of anti-MuSK antibodies based on MuSK transfected HEp-2 cells
<i>Nadja Röber, Silke Weber, Jochen Schäfer, Sandra George, Ilka Knütter, Dirk Roggenbuck, Jan-Heiner Küpper, Karsten Conrad</i> | 193 |
| 51 | Pleural mesothelioma and anti-MA2-associated encephalitis
<i>Adriano Jiménez-Escrig, Silvia Medina, Ángela Carrasco</i> | 195 |
| 52 | What should be the cut-off for VGKC complex antibodies?
<i>Sylvie Coito, René-Louis Humbel</i> | 197 |
| 53 | Multi-center validation study of DNER as the target of anti-PCA-Tr autoantibodies associated with paraneoplastic neurological syndrome
<i>Kristin Rentzsch, Inga-Madeleine Blöcker, Bianca Teegen, Winfried Stöcker, Francesc Graus, Véronique Rogemond, Jérôme Hônnorat, Esther S.P. Hulsboom, Peter A.E. Sillevs Smitt, Christian Probst, Lars Komorowski</i> | 199 |

- 54 Multi-parametric recombinant cell-based indirect immunofluorescence assay (RC-IFA) for the determination of autoantibodies associated with paraneoplastic neurological syndromes 201
Inga-Madeleine Blöcker, Christiane Radzimski, Mandy Unger, Linda Döring, Winfried Stöcker, Josep Dalmau, Peter Sillevs Smitt, Lars Komorowski, Christian Probst

5.2

Liver and Gastrointestinal Diseases

- 55 Preliminary report: Detection of antibodies against D-3-phosphoglycerate dehydrogenase (PGDH) in autoimmune liver diseases using a line immune assay 204
W. Meyer, C. Probst, L. Komorowski, A. Ott, T. Scheper, W. Schlumberger, W. Stöcker and H. Yan
- 56 Can the HEp-2 cell assay replace the indirect immunofluorescence on rat cryostat section for AMA-M2 screening? 206
Kristin Kühnert, Uta Kießling, Teresa Trimpop, Manuela Rejzek, Andrea Thieme, Nadja Röber, Karsten Conrad
- 57 Prevalence of anti-nuclear antibodies in patients with primary biliary cirrhosis 208
Ana Kozmar, Ivan Padjen, Miroslav Mayer, Mirna Sentic, Branko Malenica
- 58 Evaluation of a high sensitive assay for the diagnosis of celiac disease 209
Lisbeth Witt, Martin Salden, Klas Sjöberg, Fritz Koning and Yngve Sommarin
- 59 CytoBead-assay for the simultaneous detection of celiac disease specific antibodies and IgA deficiency 211
Nadja Röber, Kai Grossmann, Ilka Knütter, Rico Hiemann, Mandy Sowa, Martin W. Laass, Karsten Conrad, Dirk Roggenbuck
- 60 Pancreatic-specific autoantibodies to glycoprotein 2 in pediatric celiac disease 213
Martin W. Laass, Dirk Roggenbuck, Ursula Range, Nadja Röber, Karsten Conrad
- 61 Comparison of two ELISAs for the detection of anti-MZGP2 antibodies as a new serological marker for Crohn`s disease 215
Laura M. Stinton, Gary L. Norman, Zakera Shums, Maria T. Arias-Loste, Marcos Lopez-Hoyos, Michael Mahler

5.3

Endocrine, Kidney and Skin Diseases

- 62 Detection of autoimmunity against the insulin- and insulin-like growth factor-1 receptor and their prevalence in humans 218
T. Welsink, C. Schwiebert, W.B. Minich, L. Schomburg
- 63 Role of autoantibodies anti zinc transporter protein (anti-ZnT8) in the diagnosis and follow-up of type I diabetes mellitus: an Italian experience 219
S. Zago, M. Fabris, P. Melli, M. Zanatta, T. Basso, A. Comici, E. Carlin, G. Zanette, E. Tonutti, A. Rosolen

64	Serological profile of idiopathic membranous nephropathy cohort <i>Astrid Behnert, Beina Teng, Janina Müller-Deile, Andrej Skoberne, Mario Schiffer, Marvin J. Fritzler</i>	222
65	Our experience with anti-phospholipase A2 receptor (PLA2R) indirect immunofluorescence test <i>I. Janatková, M. Hindoš, Z. Hrušková, B. Svobodová, V. Tesař, K. Malíčková, T. Zima</i>	224
66	Evaluation of a new ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy <i>W. Schlumberger, C. Dähnrich, C. Probst, B. Seitz-Polski, V. Esnault, J.F. Wetzels, J.M. Hofstra, E. Hoxha, R.A. Stahl, G. Lambeau, W. Stöcker, L. Komorowski</i>	225
67	Detection of anti-phospholipase A ₂ receptor autoantibodies: A comparison of three different immunoassays <i>Astrid Behnert, Mario Schiffer, Michael Mahler, Marvin J. Fritzler</i>	227
68	Anti-M-type Phospholipase A2 Receptor (PLA2r) antibodies in diagnosis and follow-up of idiopathic membranous nephropathy (IMN) <i>S. Glionna, L. Bianchi, B. Trezzi, M. D'Amico, T. Stellato, D. Santoro, R.A. Sinico and A. Radice</i>	230
69	The antiphospholipid antibodies in psoriasis patients with metabolic syndrome and non-alcoholic fatty liver disease comorbidities <i>N. Virstyuk, M. Volochynovych, M. Nykyforuk</i>	232
70	Development of sensitive and specific assays for serological diagnosis of anti-laminin 332 mucous membrane pemphigoid <i>S. Groth, A. Abadpour, K. Vafia, C. Probst, L. Komorowski, W. Schlumberger, W. Stöcker, D. Zillikens and E. Schmidt</i>	233
71	Autoantibodies to Golgi complex and Ro51 protein in non-specific interstitial pneumonia (NSIP): case report and literature review <i>S. Andrejevic, B. Milenkovic, J. Stojsic, R. Stevic, B. Bonaci-Nikolic</i>	234

Chapter 6

Methodical Aspects and Diagnostic Strategies

6.1

New Concepts and Novel Assays

72	New Concepts and Approaches to Autoantibody Testing <i>Laura M. Sinton, Marvin J. Fritzler</i>	237
73	PR3-ANCA as a promising biomarker for the diagnosis of primary sclerosing cholangitis (PSC) <i>Laura M. Stinton, Chelsea Bentow, Michael Mahler, Gary L. Norman, Bertus Eksteen, Andrew L. Mason, Gilaad G. Kaplan, Bjorn Lindkvist, Angela Cheung, Harry Janssen, Marvin J. Fritzler</i>	249

74	Standardization of reference material for autoantibody testing: the state of the art <i>Pier Luigi Meroni, Maria Orietta Borghi</i>	251
75	New diagnostics in autoimmunity: a review and our experience <i>Boris Gilburd, Carlo Perricone, Enrica Cipriano, Nancy Agmon-Levin, Yehuda Shoenfeld</i>	258
76	Evaluation of a novel sensitive and reliable Lateral Flow Assay (LFA) for the detection of anti-dsDNA antibodies Anti-dsDNA LFA <i>N. Offermann, M. Petschinka and M. Fooke</i>	267
77	Microtiter plate-based multiparametric assay for detection of non-organ specific autoantibodies <i>Jana Langer, Erik Höchel, Klemens Löster, Martin Eberhardt, Nadja Röber, Karsten Conrad, Silvia Porstmann, Tomas Porstmann</i>	269
78	CytoBead ANCA - a novel indirect immunofluorescence test for the simultaneous detection of anti-neutrophil cytoplasmic antibodies <i>Mandy Sowa, Ilka Knütter, Rico Hiemann, Kai Grossmann, Nadja Röber, Ursula Anderer, Karsten Conrad, Dirk Reinhold, Elena Csernok, Dirk Roggenbuck</i>	271
79	Evaluation of ANCA by automated IFA image analysis on NOVA View <i>Carol E. Buchner, Cassandra C. Bryant, Rachel A. Rosenblum, Marlene Gonzalez, Gabriella Lakos</i>	273
80	Development of a method for multiplex real-time analysis of enzymatic activity on a microbead-chip <i>Stefan Rödiger, Alexander Böhm, Jörg Nitschke, Luba Stavitskaya, Melanie Gruner, Alena V. Kundzer, Margarita V. Volkova, I. Generalov, Carsten Schmidt, Christian Schröder, Dirk Roggenbuck, Peter Schierack</i>	275

6.2

Antinuclear Antibodies – Diagnostic Strategies and Novel Developments

81	CytoBead – A novel platform for antibody multiplex analyses <i>Rico Hiemann, Mandy Sowa, Kai Grossmann, Ilka Knütter, Juliane Scholz, Nadja Röber, Ursula Anderer, Dirk Reinhold, Peter Schierack, Karsten Conrad, Dirk Roggenbuck</i>	281
82	Anti-nuclear antibodies: a long way to harmonization <i>Jan Damoiseaux, Nancy Agmon-Levin</i>	284
83	Standardization of ANA-HEp-2 immunofluorescence pattern nomenclature <i>Wilson M. Cruvinel, Paulo L.C. Francescantonio, Alessandra Dellavance, Luis E.C. Andrade</i>	290
84	ANA measurement by IIF in comparison to other methods in the diagnosis of connective tissue diseases <i>Xavier Bossuyt</i>	299
85	Reading and interpretation of ANA immunofluorescence tests: results of a comparative study with six automated systems <i>Nicola Bizzaro, Stefan Platzgummer, Elio Tonutti, Antonio Antico, Danila Bassetti, Danilo Villalta, Fiorenza Pesente, Francesco Cucchiario, Marilina Tampoia, Renato Tozzoli</i>	300
86	Efficient autoantibody titration using indirect immunofluorescence assays (IIFA) <i>Jan Martinek, Ivo Lochman, Alexandra Lochmanová, Vladimír Krauz</i>	306

87	Autoimmunity diagnostic strategies (ANA, anti-dsDNA, anti-ENA) in Italian clinical laboratories <i>A. Radice, L. Bianchi, S. Glionna, B. Trezzi</i>	310
88	Evaluation of ANA testing results: is there a need for revision of our protocol for antibody specificity testing? <i>Andrea Tesija Kuna, Ines Vukasovic, Nada Vrkic</i>	312
89	Automation in ANA HEp-2 cells microscopy: can the machine substitute the human eye? <i>Cristovao Pitangueira Mangueira, Eliane Aparecida Rosseto</i>	314
90	HELIOS, a bright future for ANA - automated indirect immunofluorescence (IIF) assay <i>B. Gilburd, N. Agmon-Levin, T. Martins, A. Petzold, T. Matthias and Y. Shoenfeld</i>	315
91	Comparability of the AESKUSLIDES ANA-HEp-2 antinuclear antibody (ANA) immunological test system with a commercially available system <i>Torsten Matthias, Sascha Pfeiffer, Trishna Martins, Peter Trinder and Andrea Petzold</i>	317
92	CytoBead ANA - a novel indirect immunofluorescence test for the simultaneous detection of anti-nuclear antibodies <i>Juliane Scholz, Ilka Knütter, Kai Grossmann, Rico Hiemann, Mandy Sowa, Nadja Röber, Karsten Conrad, Peter Schierack, Dirk Roggenbuck</i>	319
93	Evaluation of a novel chemiluminescent assay (QUANTA Flash® CTD Screen Plus) for the rapid detection of autoantibodies in systemic autoimmune rheumatic diseases <i>C. Bentow, A. Seaman, A. Swart, A. Shikhman, N. Goodman, M. Mahler</i>	321
94	Quantitative results of automated indirect immunofluorescence enable value added reporting for antinuclear antibody testing <i>Sofie Schouwers, Myriam Bonnet, Patrick Verschueren, René Westhovens, Daniel Blockmans, Godelieve Mariën, Xavier Bossuyt</i>	323
95	Optimization of the automated HEp-2 cell assay by stepwise improvement of software algorithms <i>Rico Hiemann, Nadja Röber, Dirk Roggenbuck, Karsten Conrad</i>	325
96	Autoantibodies to mitotic cells <i>René-Louis Humbel</i>	327
97	Anti-nematin antibodies in practice <i>J. Goetz, R.L. Humbel, G. Uettwiller, F. Habersetzer, M. Doffoel</i>	340
98	The clinical significance of anti-DFS70 antibodies as part of ANA testing <i>Michael Mahler</i>	342
99	Prevalence and clinical associations of anti-DFS70 antibodies in samples with high titer of ANA but without detectable fine-specificity <i>Michael Mahler, Sofie Schouwers, Roger Albesa, Eric Tena, Nathalie Vandeputte, Doreen Dillaerts, X. Bossuyt</i>	353
100	LEDGF/DFS70 antibodies and DFS pattern in routine diagnostics <i>Uwe Höpner, Tim Fülling, Uta Kießling, Teresa Trimpop, Manuela Rejzek, Andrea Thieme, Nadja Röber, Karsten Conrad</i>	355

- 101 Longitudinal study of anti-DFS70 antibodies in 5 patients without antinuclear antibody associated autoimmune rheumatic disease 357
Sofie Schouwers, Michael Mahler, Doreen Dilaerts, Nathalie Vandeputte, Xavier Bossuyt
- 102 Evaluation of the first dot blot assay for the detection of anti DFS 70 antibodies as part of a 12plex ANA Profile 359
Johannes Schulte-Pelkum, Eric Tena, Alain Vigneron, Nicolas Bodart, Michael Mahler
- 103 Novel autoantibodies showing chromatin binding in the HEp-2 cell assay 361
Angelika Lueking, Heike Goehler, Petra Rengers, Martin Gamer, Klaus Marquardt, Petra Budde, Anna Telaar, Daniel Chamrad, Carmen Theek, Karsten Conrad, and Peter Schulz-Knappe

Preface

Autoimmunity is a complex network and spectrum of immune responses against self ranging from naturally occurring autoreactivity at one end of the spectrum which, in most cases, are harmless or even beneficial, to pathological (induced) autoimmunity that may be harmful by leading to the development of autoimmune diseases. Therefore, autoimmunity may play different roles in healthy and diseased subjects. For improvement of the diagnostics and therapeutics as well as prediction and prevention of autoimmune diseases, it is important to investigate all aspects of these very complex and multifactorial pathological processes. In this regard, infections may have protective or enhancing capabilities in the development of autoimmune diseases. Neutrophil extracellular trap (NET) formation is a novel mechanism that may be involved in autoimmune induction triggered by certain infections. Besides the role of accelerated NETosis, other novel aspects of autoimmune pathogenesis such as defects of intracellular nucleases, agonistic effects of anti-receptor antibodies, the role of protective natural antibodies, vitamin D, adjuvants, memory plasma cells and the innate immune system can be found in this volume. Another important area of autoimmunity is the relevance of tumor associated autoantibodies (TAAB). Regardless of their pathogenic role, TAAB may be important markers in the risk assessment or prediction of tumor development.

A major challenge for the improvement of immune diagnostics is the optimization and standardization of autoantibody determinations combined with standardized evaluation studies. On the backdrop of trying to understand the clinical relevance of autoantibodies in patients, the search for and description of new autoantibody specificities continues. Novel autoantibody biomarkers in tumors, idiopathic myopathies, multiple sclerosis, kidney diseases and rheumatoid arthritis will be presented along with new concepts and novel assays that may further improve the diagnostics of autoimmune and neoplastic diseases.

Hopefully, the data and informations described and discussed in this volume will stimulate novel concepts and research on autoimmune pathogenesis as well as the improvement of immune diagnostics and therapy.

The editors

Chapter 1

Infections, Tumors and Autoimmunity

Autoimmune/inflammatory syndrome induced by adjuvants (ASIA)

Carlo Perricone^{1,2}, Yehuda Shoenfeld^{2,3}

- 1) Reumatologia, Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Italy
- 2) Zabudowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel
- 3) Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Sackler Faculty of Medicine, Tel-Aviv University, Israel

E-mail: shoenfel@post.tau.ac.il

Keywords

Adjuvant, aluminium, *Saccharomyces cerevisiae*, yeast, autoimmune/inflammatory syndrome induced by adjuvants, ASIA, autoantibodies, autoimmunity, vaccine

Abstract

The term "ASIA–Autoimmune/inflammatory Syndrome Induced by Adjuvants" [1] has been recently coined to describe an "umbrella" of clinical conditions namely siliconosis, Gulf War Syndrome (GWS), macrophage myofasciitis syndrome (MMF), sick building syndrome (SBS) and post-vaccination phenomena, which share similar signs or symptoms [2-6]. The most frequently reported symptoms include myalgia, myositis, arthralgia, incapacitating fatigue, neurological manifestations, fever, dry mouth and cognitive alterations. Moreover, really common is the presence of chronic fatigue syndrome [7], often associated with sleep disturbances or non-restful sleep. These shared symptoms suggested the presence of a common denominator which has been subsequently identified in the adjuvant. The adjuvant is defined as "any substance that acts to accelerate, prolong, or enhance antigen-specific immune response" [8]. It is an agent that may stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect in itself. This immune-mediated condition appears following a chronic stimulation of the immune system by agents with adjuvant characteristics. The prevalence of immune-mediated conditions is rising in different geographical areas and these geo-epidemiological changes may be explained by a complex of genetic and environmental factors [9, 10]. Genetic leads to a predisposition in developing an autoimmune or an auto-inflammatory syndrome but, the presence of an external or endogenous environmental factor, recently called "exposome" [11], is essential in triggering the immune response. The presence of a favorable genetic background for the development of such conditions explains why they are so rare [12], and also why physicians should be aware of the possible complications that may occur, for example post-vaccination, in specific individuals [13]. Silicone, alum, pristane and infectious component are some of the environmental factors that comprise an immune adjuvant effect. Also other oil substances, sometimes illegally injected for cosmetic purposes, may have an immune

adjuvant effect and are reported to be possible inducer of ASIA [14]. These adjuvants seem to be able to induce autoimmunity both in animal models and in humans [15, 16]. There are several proposed mechanisms by which an adjuvant may trigger the immune system including polyclonal activation of B cells, effects on cellular immunity, immune regulatory cells and on viral induced antibodies, molecular mimicry, bystander activation, and epitope spreading. Molecular mimicry refers to the concept that host immune response, initially directed to bacterial or virus antigens, can target host molecules that share sequence homology or structural similarities with microbial epitopes [8]. Adjuvants accomplish this task by mimicking specific sets of evolutionarily conserved molecules (liposomes, LPSs, unmethylated CpG dinucleotide-containing DNA etc). On the other hand, other possible involved mechanisms which may induce autoimmunity are the polyclonal activation of B cells [17], the bystander activation which enhances cytokine production and further induces the expansion of auto-reactive T cells [18], and finally the epitope spreading by which invading antigen accelerate the local activation of antigen presenting cells and the over processing of antigen [19]. For the diagnosis of the disease criteria have been proposed and may aid in the diagnosis of ASIA syndrome [1]. These criteria were further validated by Zafrir et al. [20] who studied 93 patients who suffered a constellation of symptoms and fulfilling the proposed criteria of ASIA [20]. Despite the huge efforts invested in vaccine safety there are few observational studies and virtually no randomized clinical trials documenting the effects of existing vaccines on autoimmunity. Thus, there is the need of innovative clinical trial design and the vaccines should be designed in order to be risk-free. To conclude, the accumulation of many reports and the temporal association between the occurrence of autoimmune diseases and vaccination led some authors to hypothesize the presence of a causal link. Such a link may suggest that adjuvants serve as triggers for presentation of an overt disease or exacerbation of a non-symptomatic autoimmune condition, in certain individuals that are probably prone to develop such devastating event [21]. Perhaps, in twenty years physicians will be dueling with better characterized particles of autoimmunity, and the vaccines will be fully safe as well as effective. Still, the available data suggest that the benefits of vaccination outweigh the risks. Nonetheless, the recognition of ASIA has initiated the change to put more efforts in identifying the good, the bad and the ugly of vaccines and in particular of adjuvants as triggers of autoimmunity. Nonetheless, particular attention should be given in order to develop safer vaccines.

References

- (1) Shoenfeld Y, Agmon-Levin N. ASIA—Autoimmune/inflammatory Syndrome Induced by Adjuvants. *J Autoimmun* 2011;36: 4–8
- (2) Shoenfeld Y. Autoimmune (autoinflammatory) syndrome induced by adjuvants provides a diagnostic framework for enigmatic conditions. *The Rheumatologist* 2012;6:26-32
- (3) Carvalho JF, Barros SM, Branci JC, Fonseca JE. ASIA or Shoenfeld's syndrome: highlighting different perspective for diffuse chronic pain. *Acta Rheumatol Port* 2011;36:10-12
- (4) Meroni PL. Autoimmune or auto-inflammatory syndrome induced by adjuvants (ASIA): Old truths and a new syndrome? *J Autoimmun* 2011;36:1-3
- (5) Hughes GRV. Foreword. *Lupus* 2012;21:117
- (6) Shoenfeld Y, Huges GRV, Agmon-Levin N. The spectrum of ASIA: 'autoimmune (auto-inflammatory) syndrome induced by adjuvants'. *Lupus* 2012;21:118-120
- (7) Rosenblum H, Shoenfeld Y, Amital H. The common immunogenic etiology of chronic fatigue syndrome: From infections to vaccines via adjuvants to the ASIA syndrome. *Infect Dis Clin North Am.* 2011;25:851-63
- (8) Israeli E, Agmon-Levin N, Blank M, Shoenfeld Y. Adjuvants and autoimmunity 2009; 18:1217-1225
- (9) Youinou P, Pers JO, Gershwin ME, Shoenfeld Y. Geo-epidemiology and autoimmunity. *J Autoimmun* 2010;34:163-7

- (10) Shapira Y, Agmon-Levin N, Shoenfeld Y. Defining and analyzing geoepidemiology and human autoimmunity. *J Autoimmun* 2010; 34:168-77
- (11) Bogdanos DP, Smyk DS, Invernizzi P, Rigopoulou EI, Blank M, Pouria S, Shoenfeld Y. Infectome: a Platform to trace infectious trigger of autoimmunity. *Autoimmun Rev* 2013;12:726-40
- (12) Toubi E. ASIA – autoimmune syndrome induced by adjuvants: rare, but worth considering. *IMAJ* 2011;14,121-124
- (13) Cervera R. 'ASIA': a new systemic autoimmune syndrome? *Lupus* 2011;20:665–666
- (14) Vera-Lastra O, Medina G, del Pilar Cruz-Dominguez M, Ramirez P, Gayosso-Rivera JA, Anduaga-Dominguez H, Lievana-Toress C, Jara LJ. Human adjuvant disease induced by foreign substances: a new model of ASIA (Shoenfeld's syndrome). *Lupus* 2012;21:128-135
- (15) Israeli E, Agmon-Levin N, Blank M, Shoenfeld Y. Adjuvants and autoimmunity. *Lupus* 2009;18:1217–1225
- (16) Hornung V, Bauernfeind F, Halle A, et al. Silica crystals and aluminium salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat Immunol* 2008;9:847–856
- (17) Barzilai O, Ram M, Shoenfeld Y. Viral infection can induce the production of autantibodies. *Curr Opin Rheumatol* 2007;19:636-643
- (18) Murali-Krishna K, Altman JD, Suresh M et al. Counting antigen specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 1998; 8:177-187
- (19) Lehmann PV, Forthuber T, Miller A, Sercarz EE. Spreading of T cell autoimmunity to cryptic determinants of an antigen. *Nature* 1992;358:155-157
- (20) Zafrir Y, Agmon-Levin N, Paz Z, Shilton T, Shoenfeld Y. Autoimmunity following hepatitis B vaccine as part of the spectrum of 'Autoimmune (Auto-inflammatory) Syndrome induced by Adjuvants' (ASIA): analysis of 93 cases. *Lupus* 2012;21:146-52
- (21) Zafrir Y, Agmon-Levin N, Shoenfeld Y. Post-influenza vaccination vasculitides: a possible new entity. *J Clin Rheumatol* 2009;15:269-70

Possible role of infections in the pathogenesis of autoimmune liver diseases

Daniel S. Smyk¹ and Dimitrios P. Bogdanos^{1,2}

- 1) Institute of Liver Studies, Division of Transplantation Immunology and Mucosal Biology, King's College London School of Medicine at King's College Hospital, London, UK
- 2) Department of Medicine, Faculty of Medicine, School of Health Sciences, University of Thessaly, Thessaly, Greece

E-mail: dimitrios.bogdanos@kcl.ac.uk

Abstract

Autoimmune liver diseases include primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC). Each condition has their own unique clinical and diagnostic features. AIH involves the hepatocytes whereas PBC and PSC involve the biliary epithelial cells. It is believed that a combination of genetic susceptibility and environmental triggers act in concert to induce these conditions. Infectious agents are included among the environmental causes, and have been of interest for several decades. It is believed that bacteria, viruses and parasites may induce autoimmune liver disease by mechanisms such as molecular mimicry and cross reactivity. The evidence underlying these theories varies from one organism to the next, and one disease to the other. The evidence linking infectious agents to PBC and AIH is much stronger than in PSC. In the case of PBC, bacterial causes have been heavily implicated, especially in regards to *E. coli*. This is in comparison to AIH, where mostly viruses have been implicated, although the evidence is not strong. The evidence linking infectious triggers and PSC is even scarcer. This chapter will discuss the current evidence in the literature surrounding the role of infections in the development of autoimmune liver disease.

1. Introduction

Autoimmune liver diseases represent a distinct group of autoimmune disorders affecting the hepatocytes in the case of autoimmune hepatitis (AIH) or the biliary epithelial cells (BEC) as for primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). The clinical features, as well as the management, of these diseases differ significantly amongst each other.

Though the aetiologies of these diseases are poorly understood, infectious agents have been considered important for the induction of immune-mediated pathology.

This chapter discusses current evidence linking infections with autoimmune liver diseases.

2. Primary biliary cirrhosis

PBC is an autoimmune liver disease with a long asymptomatic phase characterized by the destruction of small and medium-size intrahepatic bile ducts [1]. Disease specific antimitochondrial antibodies (AMA) and/or antinuclear antibodies (ANA) are the serological hallmarks of PBC [2]. The diagnosis of PBC is based on biochemical evidence of cholestasis, the presence of detectable AMA (or PBC-specific ANA) and diagnostic histological features on liver biopsy [1].

Infectious agents linked to PBC are numerous [3,4]. An overview of these agents, in addition the mechanisms that have been investigated linking these micro-organisms with PBC, are provided in Table 1. It has become apparent that the evidence linking their involvement in the induction of PBC varies amongst pathogens. The most cited of those is *Escherichia coli* (*E. coli*) [4].

***E. coli* as a PBC trigger**

Several epidemiological studies have been conducted to investigate risk factors for the development of PBC, including risks regarding infection (reviewed in [3]). These studies have been based on questionnaires addressing geographical and lifestyle factors, as well as personal and familial medical and surgical histories, including history of infection. One of the most significant infectious risk factors in PBC was found to be a history of recurrent urinary tract infections (rUTI) [4]. Recent data from British investigators demonstrated that rUTI precedes the diagnosis of PBC [5].

The potential pathophysiological mechanisms linking *E. coli* infection (and rUTI) with the development of PBC have been reviewed elsewhere [4]. Briefly, immunological data have also demonstrated B- and T-cell cross-reactive responses involving the major AMA-specific autoepitopic region, namely human PDC-E2, and its *E.coli* mimics. Gershwin's lab in collaboration with Japanese co-workers subsequently demonstrated that *E.coli* and human PDC-E2 cross-react at the cellular level (CD4 T-cell) [6]. Our own group has provided data suggesting that mimics of the human PDC-E2 autoepitope originated from *E. coli* proteins irrelevant to PDC-E2 are also targeted by cross-reactive responses [7]. Also, some evidence linking *E.coli* infection with PBC has come from an animal model which demonstrated that recurrent UTI is capable of inducing histological features resembling those seen in PBC [4].

***Novosphingobium aromaticivorans*: an unexpected trigger of PBC**

In search of microbial triggers of PBC, Gershwin's group reported that two bacterial proteins from *N. aromaticivorans* were highly homologous to human PDC-E2 [8]. Also, patients with PBC demonstrated antibody responses against the lipoylated *Novosphingobium aromaticivorans* proteins. The investigators have found that *N. aromaticivorans* was isolated in faecal samples from patients with PBC, and obtained data demonstrating the existence of immune responses against lipoylated proteins of *N. aromaticivorans* in PBC patients and their first degree relatives [8]. Based on these data, an animal model of the human disease was developed in which infection of mice with *N. aromaticivorans* led to serological and histological evidence of PBC.

The role of *Lactobacillus delbrueckii* in PBC

Beta-galactosidase of *L. delbrueckii subsp. bulgaricus* is one of the very few lactobacilli proteins that have remarkable similarity with the core CD4, CD8 and B-cell epitope of human PDC-E2, the major AMA antigenic determinant. Experimental testing has demonstrated evidence of IgG antibodies against the homologous peptides being present in patients and controls. However, IgG3 antibodies to the lactobacillus

mimic are highly specific for PBC [9, 10]. Also, IgG3 antibodies to the lactobacillus mimics cross-react with the human autoepitope PDC-E2, suggesting a potential involvement of this pathogen in the pathogenesis of PBC. In fact, one study has reported the case of an AMA positive female who developed PBC after being vaccinated with lactobacillus for recurrent vaginitis [9]. Also, serological testing has provided evidence of human and lactobacilli immunological cross-reactivity. We formulated a hypothesis based on the assumption that alterations in normal vaginal flora leading to bacterial vaginosis may provide the impetus for bacterial colonisation important for the pathogenesis of PBC [9, 10]. It is known that UTI is more frequently reported in females with alterations in the normal vaginal flora. This may allow for the colonisation of uropathogenic *E.coli* and subsequent development of cross-reactive immune responses involving lactobacilli mimics but this needs to be determined at the experimental level.

Mycobacterial-induced PBC: facts and fictions

Granulomata can be seen in liver biopsy specimens from patients with PBC, and this has led investigators to consider mycobacteria as potential causative agents of PBC [11]. Early studies reported the presence of PBC-specific AMA in patients with active pulmonary tuberculosis and leprosy and the presence of antibody reactive specifically targeting mycobacterial antigens in sera from patients with PBC. However, mycobacterial DNA has not been detected in livers from patients with PBC [11].

Nevertheless, a Spanish group reported the presence of antibodies against the 65-kDa heat shock protein (hsp65) of *Mycobacterium gordonae* extract in all patients with PBC [12], and our group has demonstrated that cross-recognition of *M. gordonae* hsp65 and human PDC-E2 was due to amino acid homologies between the human PDC-E2 mitochondrial autoepitope, and an hsp65 *M. gordonae* antigenic determinant [13].

Chlamydia pneumoniae as a cause of PBC

A single study has found that *C. pneumoniae* antigens are detectable in all the explanted liver tissues from patients with PBC but these data have not been confirmed by Gershwin's group [14]. A serological study has shown that anti-chlamydial antibodies are present in the great majority (91%) of AMA-positive patients with PBC and just 21% AMA-negative patients with PBC. These antibodies were undetectable in the relevant pathological and normal controls. However, *C. pneumoniae* DNA could not be detected in liver tissues obtained from patients with PBC suggesting that chlamydial infection is not directly involved in the pathogenesis of PBC.

Helicobacter pylori and PBC: current evidence

Reported data have failed to provide evidence in support of a causative link between *H. pylori* infection and development of PBC. In search of microbial mimics of the human PBC autoepitope, we identified significant amino acid similarity between the major mitochondrial autoepitopic region and *H. pylori* urease beta [15], but we were unable to provide evidence of cross-reactive immunity at the B- cell or T-cell level. We have also looked for molecular mimicry between between *H. pylori* VacA antigen and human PDC-E2, but again we reported lack of cross-reactive immunity of the homologous sequences. Early data suggesting that anti-helicobacter pylori antibodies are present at comparable frequencies in patients with PBC and controls have been challenged by the results of a recent study that antibody reactivity against *H. pylori* are more frequently present in patients with PBC than in controls (54% vs 31%).

Betaretroviruses: the viral triggers of PBC

Several studies have attempted to incriminate EBV and CMV in the pathogenesis of PBC but the data have been inconclusive. However, infection, with the mouse mammary tumour virus (MMTV) has been linked with PBC. Data from the group of Mason in Canada suggest that beta-retroviruses may indeed infect biliary epithelial cells, leading to increased PDC-E2 expression on the cell surface [16]. These authors were the first to show that anti-retroviral antibodies are frequently detectable in patients with PBC, and have also provided evidence for the appearance of viral particles in biliary epithelial cells of affected individuals. Mason has cloned a human betaretroviral sequence highly homologous to MMTV. As well, retroviral sequences were identified in a murine model of experimental cholangiopathy with features of PBC. Increased MMTV gag and env expression was found in the liver tissue of mice with immunological dysfunction. Also, titres of AMA correlated with anti-MMTV antibodies in these mice. All these findings have come from the same group, and external validation from two independent groups has failed to replicate these data. For example, Gershwin and co-workers have found MMTV less frequently in livers from PBC patients than in livers from patients with other chronic liver diseases.

Parasitic infections and PBC: the link with *Toxoplasma gondii*

Toxoplasmosis is caused by *Toxoplasma gondii*, a parasite within the genus *Toxoplasma*. Cats are the primary host of *T. gondii*. So far, this parasite has not been reported as a risk factor for PBC. However, a recent study has reported that antibodies against *Toxoplasma gondii* are more prevalent in patients with PBC compared to healthy controls (71% vs 40%, $p < 0.0001$).

Table 1.

Major findings involving infectious agents in the induction of primary biliary cirrhosis (PBC). Features vary amongst studies and therefore their strength of support differs amongst studies. None of them is conclusive of a decisive role of an infectious agent as a cause of PBC.

Infectious Agent	Type of study	Finding	In support or against a causative link
<i>E. coli</i>	Clinical observation	Bacteriuria is more frequent in PBC than controls	In support
	Clinical observation	Bacteriuria is equally prevalent in PBC patients and controls	Against
	Epidemiological	History of urinary tract infection more frequently reported in patients than in controls	In support
		History of infection precedes disease	
	Immunological	Women with rUTI have PBC-specific AMA and ANA	In support
		Evidence of cross-reactive antibodies and T-lymphocytes	In support
Experimental animal model	Infection induces AMA and causes PBC-like disease	In support	
	Data have been reported in the form of an abstract, and other convincing animal models have not been developed	In support	
<i>N. amoraticivorans</i>	Immunological	Presence of anti-microbial antibodies	In support
		Evidence of microbial/self cross-reactive antibodies	In support
	Microbiological Experimental animal model	Positive faecal cultures	In support
		Infection induces AMA and causes PBC-like disease Disease is transferable	In support
<i>L. delbrueckii</i>	Immunological	Evidence of cross-reactive antibodies	<i>In support</i>
		A woman develops PBC after lactobacilli vaccination	<i>In support</i>
		Antibodies are also found in controls	<i>Against</i>
<i>Betaretroviruses</i>	Immunological	Anti-viral antibodies are present in PBC patients	<i>In support</i>
		Cloning of MMTV from PBC patients	
		Lack of molecular evidence	<i>In support</i>
	Virological	Virus is less prevalent in liver of PBC than in other liver diseases	<i>Against</i>
		Identification of retroviral sequences in a murine model of PBC	<i>In support</i>
	Experimental Animal Model	Inconclusive data regarding the efficacy of anti-viral treatment	<i>Against</i>
Clinical			

<i>M. gordonae</i>	Microbiological Immunological	Molecular evidence of the mycobacterium is not limited to PBC Presence of cross-reactive antibodies Anti-mycobacterial antibodies are specific for <i>M. gordonae</i> Anti-mycobacterial antibodies are not specific for <i>M. gordonae</i>	<i>Against</i> <i>In support</i> <i>In support</i> <i>Against</i>
<i>Helicobacter pylori</i>	Immunological	Lack of cross-reactive B- and T-cell responses Prevalence of anti-helicobacter antibodies is similar in patients and controls Prevalence is higher in patients than in controls	<i>Against</i> <i>Against</i> <i>In support</i>
<i>Toxoplasma gondii</i>	Immunological	Increased prevalence of antibodies in patients compared to controls	<i>In support</i>
Epstein-Barr Virus	Virological Immunological	Increased EBV DNA levels in patients than in controls Prevalence of EBV early antigen-specific antibodies higher in PBC patients	<i>In support</i> <i>In support</i>
<i>P. aeruginosa</i>	Immunological	Evidence of T-cell cross-reactivity	<i>In support</i>
Human cytomegalovirus	Immunological	Lack of cross-reactive antibodies	<i>Against</i>
<i>Haemophilus influenzae</i>	Immunological	Lack of cross-reactive antibodies	<i>Against</i>
Infectious Agent	Type of study	Finding	In support or against a causative link
<i>E. coli</i>	Clinical observation Clinical observation Epidemiological Immunological Experimental animal model	Bacteriuria is more frequent in PBC than controls Bacteriuria is equally prevalent in PBC patients and controls History of urinary tract infection more frequently reported in patients than in controls History of infection precedes disease Women with rUTI have PBC-specific AMA and ANA Evidence of cross-reactive antibodies and T-lymphocytes Infection induces AMA and causes PBC-like disease Data have been reported in the form of an abstract, and other convincing animal models have not been developed	<i>In support</i> <i>Against</i> <i>In support</i> <i>In support</i> <i>In support</i> <i>In support</i> <i>In support</i> <i>Against</i>
<i>N. amoraticivorans</i>	Immunological Microbiological Experimental animal model	Presence of anti-microbial antibodies Evidence of microbial/self cross-reactive antibodies Positive faecal cultures Infection induces AMA and causes PBC-like disease Disease is transferable	<i>In support</i> <i>In support</i> <i>In support</i> <i>In support</i> <i>In support</i>
<i>L. delbrueckii</i>	Immunological	Evidence of cross-reactive antibodies A woman develops PBC after lactobacilli vaccination Antibodies are also found in controls	<i>In support</i> <i>In support</i> <i>Against</i>

<i>Betaretroviruses</i>	Immunological	Anti-viral antibodies are present in PBC patients Cloning of MMTV from PBC patients	<i>In support</i>
	Virological	Lack of molecular evidence	<i>In support</i>
	Virological	Virus is less prevalent in liver of PBC than in other liver diseases	<i>Against</i>
	Virological	Identification of retroviral sequences in a murine model of PBC	<i>Against</i>
	Experimental Animal Model	Inconclusive data regarding the efficacy of anti-viral treatment	<i>n support</i>
	Clinical		<i>Against</i>
<i>M. gordonae</i>	Microbiological	Molecular evidence of the mycobacterium is not limited to PBC Presence of cross-reactive antibodies	<i>Against</i>
	Immunological	Anti-mycobacterial antibodies are specific for <i>M. gordonae</i> Anti-mycobacterial antibodies are not specific for <i>M. gordonae</i>	<i>In support</i> <i>In support</i> <i>Against</i>
<i>Helicobacter pylori</i>	Immunological	Lack of cross-reactive B- and T-cell responses Prevalence of anti-helicobacter antibodies is similar in patients and controls Prevalence is higher in patients than in controls	<i>Against</i> <i>Against</i> <i>In support</i>
<i>Toxoplasma gondii</i>	Immunological	Increased prevalence of antibodies in patients compared to controls	<i>In support</i>
<i>Epstein-Barr Virus</i>	Virological	Increased EBV DNA levels in patients than in controls	<i>In support</i>
	Immunological	Prevalence of EBV early antigen-specific antibodies higher in PBC patients	<i>In support</i>
<i>P. aeruginosa</i>	Immunological	Evidence of T-cell cross-reactivity	<i>In support</i>
<i>Human cytomegalovirus</i>	Immunological	Lack of cross-reactive antibodies	<i>Against</i>
<i>Haemophilus influenzae</i>	Immunological	Lack of cross-reactive antibodies	<i>Against</i>

3. Autoimmune Hepatitis

AIH is an autoimmune liver disease characterised by elevated transaminases, the presence of specific autoantibodies, raised IgG, and interface hepatitis on histology. AIH can be divided into AIH type 1 or type 2, which is largely based on distinct autoantibody profiles [17]. ANA and smooth muscle antibody (SMA) characterise type 1, with type 2 being characterised by liver kidney microsomal antigen type 1 (anti-LKM1) and anti-liver cytosol type 1 (anti-LC1) antibodies [17]. Immunosuppressive treatment is with prednisolone, with or without azathioprine.

The mechanisms underlying the development of AIH are not well defined, but there is some evidence to suggest a numerical and functional impairment of T regulatory cells may be involved. Genetic and environmental factors are also believed to be involved in the development of the disease, mostly viruses (Figure 1).

Hepatitis C Virus and AIH

HCV has been investigated as a potential inducer of AIH, which has been based on the observation that AIH-related autoantibodies are frequently present in chronically infected patients with HCV. In fact, ANA, SMA, as well as anti-LKM1 antibodies can be found in up to 40% of patients with HCV infection. Several research groups, including our own, have shown that HCV and cytochrome P450 2D6, the autoantigen of anti-LKM1 antibodies, are targets of cross-reactive responses and that molecular mimicry operates at the B and T cell levels [18, 19]. We have also demonstrated that molecular mimicry and immunological cross-reactivity involving HCV, and AIH-1 related smooth muscle, nuclear autoantigens may explain the presence of AIH-related autoantibodies during viral infection [19].

As more than 350 million people world-wide are infected with HCV, it could be expected that more cases with AIH could be induced as a result of viral infection with this hepatotropic virus but this does not appear to be the case. Thus, as it stands, the current evidence linking HCV with AIH is not strong.

Other Hepatotropic Viruses: Hepatitis A and B

Several case reports have noted the development of AIH after HAV infection [20]. As well, the histological appearance of acute HAV induced liver damage and AIH may be indistinguishable. Patients infected with HAV and HBV appear to be seropositive for AIH-related autoantibodies such as ANA, SMA and anti-asialoglycoprotein receptor antibodies. This suggested that HAV/HBV infection might trigger a humoral immune response to liver antigens, leading to the subsequent induction of the liver disease. A group of researchers prospectively followed a cohort of relatives of AIH patients, and obtained regular serum samples for the detection of serological markers of AIH. In three of these cases, the relative developed AIH type 1 (with anti-ASGPR positivity) following infection with HAV. It should also be noted that AIH has been reported following HAV vaccinations (see Vaccination section below). Despite these reports, larger molecular studies are needed to determine whether a link between hepatotropic viruses and AIH exist.

Epstein-Barr Virus: Case Reports in AIH

Several cases report the development of AIH following EBV infection [20]. A group of investigators followed relatives of AIH patients, and regularly assessed them for evidence of AIH [21]. Two individuals developed AIH following EBV infection and infectious mononucleosis [21].

It should be noted that EBV infection of the liver might resemble AIH, which should be differentiated histologically. For example, one report describes the case of fatal chronic active EBV infection mimicking AIH [20].

Currently, no large studies have been conducted investigating the role of EBV in the development of AIH. Unlike HCV, the molecular mechanisms that may be involved in the development of AIH following EBV infection (including molecular mimicry and cross reactivity) have not been explored. It currently appears that EBV may induce AIH in individual cases, but there is no conclusive evidence linking EBV with AIH. As well, the prevalence of EBV would infer a higher rate of AIH (if a link was present), and this is not the case.

Herpes Simplex Virus 1

As noted previously in the case of HCV, a study indicated that the immunodominant regions of CYP2D6 share homologous regions with HCV and HSV-1, and suggested that HCV-negative AIH type 2 cases may be related to HSV-1 [19]. We have shown that the ICP4 protein from HSV-1 shares antigenic determinants

with the HCV RNA polymerase and that the mimicking sequences are targets of cross-reactive responses. However, rates of anti-HSV-1 antibodies are comparable between patients with AIH and demographically matched controls [19].

Vaccinations preceding AIH

Vaccinations to prevent from infectious agents have been used as models to study the induction of autoimmunity triggered by foreign pathogens. Case reports comprise most of the evidence linking vaccinations and AIH [20]. One case report describes a case of AIH developing one week after vaccinations for typhoid, hepatitis A, diphtheria/tetanus, oral polio, mumps, measles and rubella (reviewed in [20]). Although liver biopsy and clinical data was suggestive of AIH, it is not known if any autoantibodies were present. That patient responded well to immunosuppressive therapy. Another case reports of a 31-year-old female who presented with a ten day history of nausea, vomiting, fever, and dark urine, which began eleven days after receiving a vaccination for hepatitis A and yellow fever (reviewed in [20]). She was diagnosed with AIH, and responded well to steroid therapy. It should be noted that this patient was serologically negative for autoantibodies associated with AIH, and also notes the development of symptoms whilst abroad in Nigeria. Apart from case reports, there is no direct evidence or larger studies linking vaccination to AIH. Further investigations into liver disease following vaccinations are warranted, as vaccinations have been linked with other autoimmune conditions.

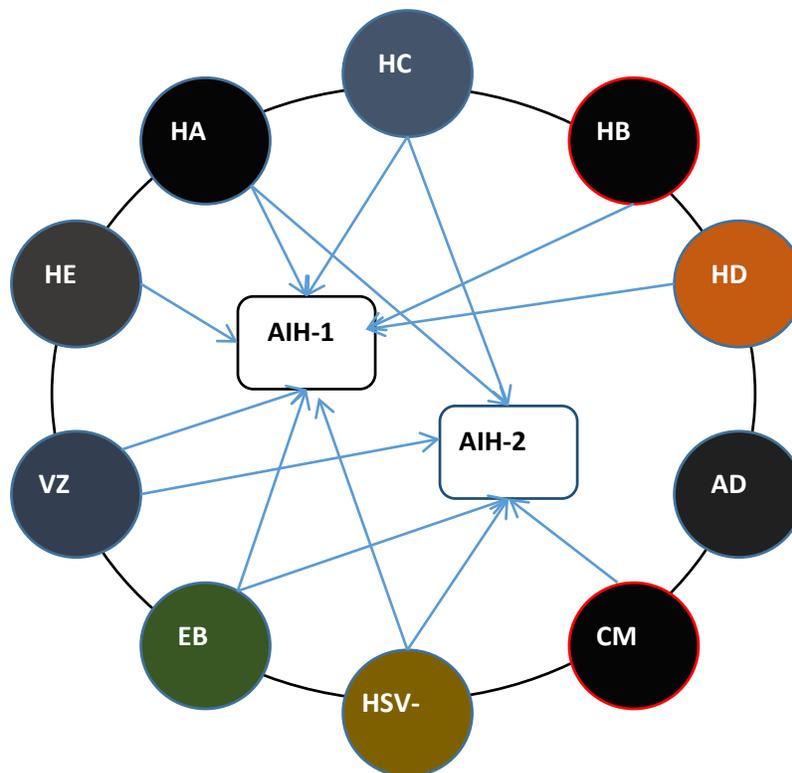


Figure 1. Viruses involved in the pathogenesis of autoimmune hepatitis (AIH) type 1 and type 2.

Arrows indicate links between a virus and a specific type of AIH. HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; EBV, Epstein-Barr Virus; CMV, cytomegalovirus; HSV-1, human herpes virus-1; ADV, adenovirus; VZV, varicella zoster virus.

4. Primary sclerosing cholangitis

PSC is a chronic progressive cholestatic disease, characterized by immune-mediated destruction of the biliary epithelial cells leading to fibrosis and biliary cirrhosis. Unlike most autoimmune conditions, PSC has a higher male predominance. PSC is largely diagnosed based on endoscopic cholangiography and/or magnetic resonance imaging, as well as biochemical indices of cholestasis and disease-related perinuclear anti-neutrophilic antibodies (p-ANCA) [2]. Treatment of PSC includes administration of ursodeoxycholic acid, although curative treatment consists of liver transplantation. Both PBC and PSC are autoimmune disorders characterized by the immune-destruction of the biliary epithelial cells, but the diseases are quite distinct and there are no patients with concomitant overlapping features of PBC and PSC. In fact, PBC can co-exist with AIH, and the same appears to be the case for AIH and PSC.

The link between PSC and infectious triggers is far from clear.

Epstein-Barr Virus and Primary Sclerosing Cholangitis

To date, the only evidence of an infectious trigger in PSC is in regards to EBV [22], which is related to ulcerative colitis (UC), an inflammatory bowel disease which is often concurrent with PSC. Some studies have found an increased number of EBV-infected B lymphocytes in intestinal mucosal samples from patients with ulcerative colitis compared to healthy controls [22]. However, it is unclear as to how many of those patients also had concomitant PSC. To date, there does not appear to be strong or direct evidence linking EBV and PSC.

5. Conclusion

Evidence linking autoimmune liver diseases to infectious agents varies from one condition to the next. *E. coli* appears to be strongly linked to PBC development, based on epidemiological as well as experimental and molecular studies. It also appears likely that other infectious agents may be involved. The evidence is not as strong in AIH and PSC. Although several viruses (namely the hepadnaviridae and HSV) have been implicated in the form of case reports, stronger molecular evidence is needed to make definitive conclusions regarding infection and AIH. So far, very little evidence exists in regards to infection and PSC.

References

- (1) Smyk DS, Rigopoulou EI, Lleo A, Abeles RD, Mavropoulos A, Billinis C, et al. Immunopathogenesis of primary biliary cirrhosis: an old wives tale. *histochemistry* 2011;2:35.
- (2) Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. *World journal of gastroenterology : WJG* 2008;14:3374-3387.
- (3) Smyk D, Rigopoulou EI, Baum H, Burroughs AK, Vergani D, Bogdanos DP. Autoimmunity and Environment: Am I at risk? *Clin Rev Allergy Immunol* 2011.
- (4) Smyk DS, Bogdanos DP, Kriese S, Billinis C, Burroughs AK, Rigopoulou EI. Urinary tract infection as a risk factor for autoimmune liver disease: From bench to bedside. *Clin Res Hepatol Gastroenterol* 2011.
- (5) Varyani FK, West J, Card TR. An increased risk of urinary tract infection precedes development of primary biliary cirrhosis. *BMC gastroenterology* 2011;11:95.
- (6) Tanimoto H, Shimoda S, Nakamura M, Ishibashi H, Kawano A, Kamihira T, et al. Promiscuous T cells selected by *Escherichia coli*: OGDC-E2 in primary biliary cirrhosis. *Journal of autoimmunity* 2003;20:255-263.
- (7) Bogdanos DP, Baum H, Vergani D, Burroughs AK. The role of *E. coli* infection in the pathogenesis of primary biliary cirrhosis. *Dis Markers* 2010;29:301-311.

- (8) Padgett KA, Selmi C, Kenny TP, Leung PS, Balkwill DL, Ansari AA, et al. Phylogenetic and immunological definition of four lipoylated proteins from *Novosphingobium aromaticivorans*, implications for primary biliary cirrhosis. *Journal of autoimmunity* 2005;24:209-219.
- (9) Bogdanos D, Pusi T, Rust C, Vergani D, Beuers U. Primary biliary cirrhosis following *Lactobacillus* vaccination for recurrent vaginitis. *Journal of hepatology* 2008;49:466-473.
- (10) Bogdanos DP, Baum H, Okamoto M, Montalto P, Sharma UC, Rigopoulou EI, et al. Primary biliary cirrhosis is characterized by IgG3 antibodies cross-reactive with the major mitochondrial autoepitope and its *Lactobacillus* mimic. *Hepatology* 2005;42:458-465.
- (11) Smyk DS, Bogdanos DP, Pares A, Liaskos C, Billinis C, Burroughs AK, et al. Tuberculosis is not a risk factor for primary biliary cirrhosis: a review of the literature. *Tuberculosis research and treatment* 2012;2012:218183.
- (12) Vilagut L, Pares A, Vinas O, Vila J, Jimenez de Anta MT, Rodes J. Antibodies to mycobacterial 65-kD heat shock protein cross-react with the main mitochondrial antigens in patients with primary biliary cirrhosis. *European journal of clinical investigation* 1997;27:667-672.
- (13) Bogdanos DP, Pares A, Baum H, Caballeria L, Rigopoulou EI, Ma Y, et al. Disease-specific cross-reactivity between mimicking peptides of heat shock protein of *Mycobacterium gordonae* and dominant epitope of E2 subunit of pyruvate dehydrogenase is common in Spanish but not British patients with primary biliary cirrhosis. *Journal of autoimmunity* 2004;22:353-362.
- (14) Leung PS, Park O, Matsumura S, Ansari AA, Coppel RL, Gershwin ME. Is there a relation between *Chlamydia* infection and primary biliary cirrhosis? *Clinical & developmental immunology* 2003;10:227-233.
- (15) Bogdanos DP, Baum H, Grasso A, Okamoto M, Butler P, Ma Y, et al. Microbial mimics are major targets of crossreactivity with human pyruvate dehydrogenase in primary biliary cirrhosis. *J Hepatol* 2004;40:31-39.
- (16) Mason A, Xu L, Shen Z, Fodera B, Joplin R, Neuberger J, et al. Patients with primary biliary cirrhosis make anti-viral and anti-mitochondrial antibodies to mouse mammary tumor virus. *Gastroenterology* 2004;127:1863-1864; author reply 1864-1865.
- (17) Bogdanos DP, Mieli-Vergani G, Vergani D. Autoantibodies and their antigens in autoimmune hepatitis. *Semin Liver Dis* 2009;29:241-253.
- (18) Kammer AR, van der Burg SH, Grabscheid B, Hunziker IP, Kwappenberg KM, Reichen J, et al. Molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytotoxic T cell recognition. *The Journal of experimental medicine* 1999;190:169-176.
- (19) Bogdanos DP, Lenzi M, Okamoto M, Rigopoulou EI, Muratori P, Ma Y, et al. Multiple viral/self immunological cross-reactivity in liver kidney microsomal antibody positive hepatitis C virus infected patients is associated with the possession of HLA B51. *International journal of immunopathology and pharmacology* 2004;17:83-92.
- (20) Daniel S, Smyk DPB, Eirini I, Rigopoulou. Risk Factors for Autoimmune Hepatitis: From Genes and Pregnancy to Vaccinations and Pollutants. *Immuno Gastroenterology* 2013;2:29-37.
- (21) Vento S, Guella L, Miranda F, Cainelli F, Di Perri G, Solbiati M, et al. Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. *Lancet* 1995;346:608-609.
- (22) Rigopoulou EI, Smyk DS, Matthews CE, Billinis C, Burroughs AK, Lenzi M, et al. Epstein-barr virus as a trigger of autoimmune liver diseases. *Advances in virology* 2012;2012:987471.

GP2 – The link between innate and acquired immune system in the intestine

Dirk Roggenbuck^{1,2}, Dirk Reinhold³, Dimitrios P. Bogdanos⁴, Karsten Conrad⁵

- 1) Faculty of Natural Sciences, Brandenburg Technical University Cottbus-Senftenberg, Senftenberg, Germany
- 2) GA Generic Assays GmbH, Dahlewitz, Germany
- 3) Institute of Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany
- 4) Institute of Liver Studies, Division of Transplantation Immunology and Mucosal Biology, King's College London School of Medicine at King's College Hospital, UK
- 5) Institute of Immunology, Technical University Dresden, Dresden, Germany

E-mail: dirk.roggenbuck@hs-lausitz.de

Keywords

Inflammatory bowel disease, Crohn's disease, Glycoprotein 2, Innate and acquired immunity

Abbreviations

CD, Crohn's disease; GP2, glycoprotein 2; IBD, inflammatory bowel disease; M cell, microfold cell; PAB, pancreatic autoantibodies; PP, Peyer's patches; SREC-I, scavenger receptor expressed on endothelial cells I; THP, Tamm-Horsfall protein; UC, ulcerative colitis

Autoantibodies against glycoprotein 2 in inflammatory bowel diseases

Glycoprotein 2 has been identified as a major autoantigenic target of pancreatic antibodies (PAB) found in patients with Crohn's disease (CD).[1-3] The loss of tolerance in inflammatory bowel diseases (IBD) has been reported first in the late 1970s by demonstrating the occurrence of autoimmunity in ulcerative colitis (UC), the other main IBD along with CD.[4] First reports on humoral autoimmunity in CD can be traced back to the late 1980s.[5] Ever since, the pathophysiology of both IBDs and the respective role of immune processes in particular autoimmunity remains poorly understood. The incidence of IBD is steadily increasing in developed and developing countries, which amounts approximately up to 37.1 per 100,000 individuals each year in Europe currently.[6] In the United States alone, IBD is one of the five most prevalent gastrointestinal disorders, accounting for more than 700,000 physician visits and 100,000 hospitalizations with an overall healthcare cost of more than \$1.7 billion (CDC data). From a prognostic point of view, up to 75% of patients suffering from CD and 25% of those with UC will require surgery. There is a common belief that pediatric entities of IBD are distinct diseases in comparison with the ones seen in adults. Apart from common susceptibility loci, genome-wide association studies (GWAS) have identified new loci associated with early onset of IBD in children which were not found in adults.

Apart from autoimmunity, genetic predisposition and environmental factors play a pivotal role in the pathophysiology of IBD. By means of GWAS, IBD5 locus (5q31-33 region) has been demonstrated to be strongly associated with UC, whereas the NOD2 (16q12) and major histocompatibility complex (MHC) (6p21) locus appears to be linked to CD.[7] The inflammation observed in CD which penetrates all layers of the bowel wall and adventitia in contrast to the one seen in UC can manifest throughout the whole digestive tract. Tissue lesions such as fissures, abscesses, strictures, and fistulas can develop in the course of CD and the incidence of carcinoma is particularly elevated in patients with Crohn's colitis after extended disease.

The "western" or "westernized" diet is obviously one of the most critical environmental components to be taken into account in terms of the pathophysiology of IBD. Whereas an increased intake of refined carbohydrates, fatty acids, and proteins have been associated with CD and/or UC, the consumption of dietary fiber in the form of vegetables and fruits seems to be correlated with a reduced risk of IBD development. The eating habit may contribute to the triggering of IBDs by a direct effect of dietary antigens, alterations in gene expression, or an impact on the composition of the enteric flora. In this context, gastrointestinal permeability appears to play a critical role.

Notably, autoimmunity is believed to play a major role in IBD inflammation leading to overt disease. In CD, approximately 30% of patients and less than 8% of patients with UC develop loss of tolerance to exocrine pancreas.[8] As a matter of fact, GP2 the major target of PAB is the most abundant membrane protein in pancreatic acinar cells and is not found in endocrine pancreas tissue. The previously unaddressed contradiction of pancreatic autoreactivity and intestinal inflammation in CD was elucidated by demonstrating the expression of GP2 at the site of CD inflammation.[1;9] Notably, GP2 has been found on the surface of microfold (M) cells of intestinal Peyer's patches (PP),[10] which are believed to represent the origin of CD inflammation.[11] Apparently, GP2 seems to support the transcytosis of FimH positive bacteria by M cells through the PP to the underlying gut associated lymphoid tissue. The binding of a subset of commensal and pathogenic enterobacteria, including *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (*Salmonella typhimurium*) has been demonstrated to be mediated by FimH, a component of type I pili on the bacterial outer membrane.

The development of immunoassays for the detection of antibodies to GP2 has paved the way to investigate the association of such antibodies with the clinical phenotype in CD.[12-16] Thus, patients suffering from CD with ileocolonic location revealed a significantly higher prevalence of anti-GP2.[17-19] Even more interestingly, CD patients with stricturing behaviour and perianal disease demonstrated a higher prevalence of anti-GP2. These observations support the assumption that the loss of tolerance to GP2 is correlated with the phenotype of patients with CD. Therefore, it is tempting to speculate about an association of autoreactivity to GP2 with fibrostenosis in CD and anti-GP2 antibodies could be an interesting candidate for a fibrostenosis marker in CD. This may provide the basis for further stratification of CD patients with regard to the type and outcome of inflammation.[20-22]

GP2 and GP2 autoantibodies in the pathogenesis of Crohn's disease

Mucosal inflammation in CD appears to be brought about by dysregulation of the immune system due to an imbalance between tolerance to commensal microbiota or food-derived antigens and immunity to pathogens.[23] In this context it is quite remarkable that the expression of GP2 is elevated in the inflamed tissue of patients with CD compared to patients with UC.[1] This hints to a possible involvement of GP2 in the immune processes leading to inflammation apart from its antimicrobial receptor function. Interestingly, the homolog of GP2 in the urinary tract, the Tamm-Horsfall protein (THP), has been reported to be a regulatory factor of innate and adaptive immunity.[24] Thus, it was not surprising, that GP2 apparently executes a similar effect like THP in modulating innate and adaptive immunity in the intestine.[25] Recently, GP2 has been identified as a binding partner of the scavenger receptor expressed on endothelial cells I (SREC-I), which is also located on dendritic cells.[26] Monocyte-derived dendritic cells bear SREC-I and can interact with GP2 and internalize GP2 or complexes thereof. It is well accepted that innate and adaptive immune responses in the intestinal mucosa are linked by dendritic cells. Such dendritic cells may induce tolerance by releasing the antiinflammatory cytokine IL10 or promote inflammation through the shedding of IL12. Genetic polymorphisms of IL10 and IL12 are associated with the risk to develop IBDs underscoring the assumption adaptive immunity plays a critical role in the pathophysiology of IBD. It could be demonstrated that GP2 distinctly modulates cytokine secretion. Using organ culture assays, GP2 reduced pro-inflammatory CXCL8 secretion in freshly resected mucosal specimens and up-regulated TGF β 1.[25] These data suggests an anti-inflammatory role of GP2 in the mucosal immune system.

Noteworthy, the expression of GP2 is up-regulated on activated human T cells and can be modulated by pharmaceutical TNF α inhibitors. GP2 significantly reduces proliferation, apoptosis, and activation of human intestinal epithelial cells as well mucosal and peripheral T cells.[25] Remarkably, the immunosuppressive effects of GP2 were shown to be mediated by regulatory T cells. It has been shown that intraepithelial lymphocytes mediate the primary response to foreign antigens, but can be also autoreactive. Thus, GP2 seems to partake in keeping the balance of the intestinal immune system fulfilling the enormous task of differentiating between pathogenic and commensal microbiota.[13]

Given the recently discovered immunomodulating role of GP2 in innate and acquired intestinal immunity, this association can shed further light on the pathophysiology of IBD which is a complex interaction of susceptibility genes, environmental factors, and innate as well as adaptive immunity.

Competing interests

Dirk Roggenbuck is a shareholder of GA Generic Assays GmbH and Medipan GmbH. Both companies are diagnostic manufacturers. All other authors declare that they have no competing financial interests.

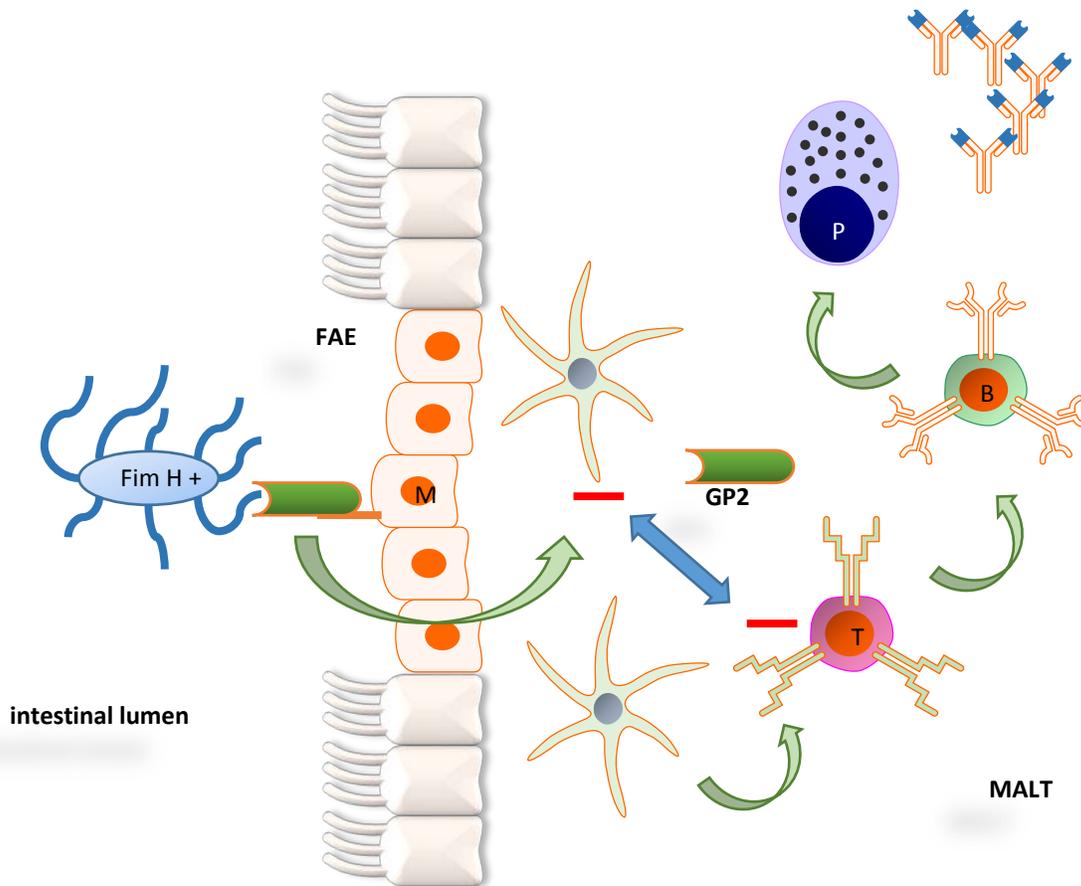


Figure 1. Glycoprotein 2 (GP2) in the pathophysiology of Crohn's disease

GP2 located on the surface of M cells (M) of the follicle-associated epithelium (FAE) can interact with FimH positive bacteria and present these potential pathogens to the underlying mucosa associated lymphoid tissue (MALT) by transcytosis. After having presented the microbial antigens to dendritic cells, T cells (T) will be activated by these potent antigen-presenting cells and B cells (B) will be transformed into plasma cells (P) producing antimicrobial antibodies.

Elevated expression of GP2 seen in CD can interfere with GP2's interaction with dendritic cells and reduce proliferation and apoptosis of T cells.

The loss of tolerance to GP2 observed in CD may lead to the production of anti-GP2 antibodies. These autoantibodies may in turn neutralize the suppressive effect of GP2 in the MALT.

References

- (1) Roggenbuck D, Hausdorf G, Martinez-Gamboa L, Reinhold D, Buttner T, Jungblut PR et al. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut* 2009;58:1620-8.
- (2) Komorowski L, Teegen B, Probst C, Aulinger-Stocker K, Sina C, Fellermann K et al. Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: The glycoproteins CUZD1 and GP2. *J Crohns Colitis* 2012;pii: S1873-9946(12)00433-3. doi: 10.1016/j.crohns.2012.10.011. [Epub ahead of print].
- (3) Roggenbuck D, Bogdanos D, Conrad K. Loss of tolerance to one or two major targets in Crohn's disease or just cross-reactivity? *J Crohns Colitis* 2013;7:e273-e274.

- (4) Lagercrantz R, Hammerström S, Perlmann P, Gustafsson BE. Immunological studies in ulcerative colitis. 1968;1339-52.
- (5) Stöcker W, Otte M, Ulrich S, Normann D, Stöcker K, Jantschek G. Autoantikörper gegen exokrines Pankreas und gegen intestinale Becherzellen in der Diagnostik des Morbus Crohn und der Colitis ulcerosa. *Dtsch Med Wochenschr* 1984;109:1963-9.
- (6) Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;142:46-54.
- (7) Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010;42:1118-25.
- (8) Bogdanos DP, Rigopoulou EI, Smyk DS, Roggenbuck D, Reinhold D, Forbes A et al. Diagnostic value, clinical utility and pathogenic significance of reactivity to the molecular targets of Crohn's disease specific-pancreatic autoantibodies. *Autoimmun Rev* 2011;11:143-8.
- (9) Werner L, Sturm A, Roggenbuck D, Yahav L, Zion T, Meiriowitz E et al. Antibodies against glycoprotein 2 are novel markers of intestinal inflammation in patients with an ileal pouch. *J Crohns Colitis* 2013;pii: S1873-9946(13)00133-5. doi: 10.1016/j.crohns.2013.03.009. [Epub ahead of print].
- (10) Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature* 2009;462:226-30.
- (11) Gullberg E, Soderholm JD. Peyer's patches and M cells as potential sites of the inflammatory onset in Crohn's disease. *Ann N Y Acad Sci* 2006;1072:218-32.
- (12) Roggenbuck D, Reinhold D, Wex T, Gohl A, von Arnim U, Malfertheiner P et al. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta* 2011;412:718-24.
- (13) Roggenbuck D, Reinhold D, Werner L, Schierack P, Bogdanos DP, Conrad K. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem* 2013;60:187-208.
- (14) Roggenbuck D, Humbel RL, Reinhold D, Bogdanos DP, Conrad K, Laass MW. Glycoprotein 2 antibodies in inflammatory bowel disease - no association with disease phenotype? *J Pediatr Gastroenterol Nutr* 2012;56:e5.
- (15) Kovacs M, Lakatos PL, Papp M, Jacobsen S, Nemes E, Polgar M et al. Pancreatic autoantibodies and autoantibodies against goblet cells in pediatric patients with inflammatory bowel disease (IBD). *J Pediatr Gastroenterol Nutr* 2012;55:429-35.
- (16) Op de Beeck K, Vermeire S, Rutgeerts P, Bossuyt X. Antibodies to GP2, the major zymogen granule membrane glycoprotein, in inflammatory bowel diseases. *Gut* 2010;61:162-4.
- (17) Bogdanos DP, Roggenbuck D, Reinhold D, Wex T, Pavlidis P, von Arnim U et al. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. *BMC Gastroenterol* 2012;12:102.
- (18) Pavlidis P, Romanidou O, Roggenbuck D, Mytilinaiou M, Al-Sulttan F, Liaskos C et al. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with crohn's disease. *Clin Dev Immunol* 2012;2012:640835.
- (19) Somma V, Ababneh H, Ababneh A, Gatti S, Romagnoli V, Bendia E et al. The Novel Crohn's Disease Marker Anti-GP2 Antibody Is Associated with Ileocolonic Location of Disease. *Gastroenterol Res Pract* 2013;2013:683824.
- (20) Rieder F, Lawrance IC, Leite A, Sans M. Predictors of fibrostenotic Crohn's disease. *Inflamm Bowel Dis* 2011;17:2000-7.
- (21) Rieder F, Zimmermann EM, Remzi FH, Sandborn WJ. Crohn's disease complicated by strictures: a systematic review. *Gut* 2013;62:1072-84.
- (22) Rieder F, Franke A, Dirmeier A, Lopez R, Lang S, Roggenbuck D et al. Mo1247 Serologic Anti-GP2 Antibodies Are Associated With Strictures and Need for Surgical Resection in Crohn's Disease. *Gastroenterology* 2013;144:S617.
- (23) Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;369:1627-40.
- (24) Säemann MD, Weichhart T, Zeyda M, Staffler G, Schunn M, Stuhlmeier KM et al. Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4-dependent mechanism. *J Clin Invest* 2005;115:468-75.
- (25) Werner L, Paclik D, Fritz C, Reinhold D, Roggenbuck D, Sturm A. Identification of pancreatic Glycoprotein 2 as an endogenous immunomodulator of innate and adaptive immune responses. *J Immunol* 2012;189:2774-83.
- (26) Holzl MA, Hofer J, Kovarik JJ, Roggenbuck D, Reinhold D, Gohl A et al. The zymogen granule protein 2 (GP2) binds to scavenger receptor expressed on endothelial cells I (SREC-I). *Cell Immunol* 2011;267:88-93.