Aus der Medizinischen Klinik mit Schwerpunkt
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DISSERTATION

Autoantibodies from long-lived 'memory' plasma cells of NZB/W mice drive immune complex nephritis

zur Erlangung des akademischen Grades
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Charité – Universitätsmedizin Berlin

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Contents

Abstrakt

Einleitung: Unsere Arbeitsgruppe konnte erstmalig im Maus-Modell der Lupus-Nephritis demonstrieren, dass sowohl kurzlebige als auch langlebige Plasmazellen zur Autoantikörperproduktion beitragen. In der vorliegenden Arbeit sollte die Rolle der autoreaktiven langlebigen Gedächtnis-Plasmazellen, die refraktär auf Immunsuppressiva und B-Zell-depletierende Therapien sind, in der Pathogenese des systemischen Lupus erythematodes untersucht werden.

Methodik: CD138+ Antikörper-sezernierende Plasmablasten und Plasmazellen wurden aus der Milz von NZB/W Mäusen (Alter >6 Monate), die hohe Anti-dsDNA-Antikörper-Titer aufwiesen, und von Balb/c-Mäusen 5 Tage nach sekundärer Immunisierung mit Ovalbumin (OVA) isoliert und in immundefiziente $Rag1^{-/-}$ Mäuse transferiert. In den $Rag1^{-/-}$ Empfängermäusen wurde regelmäßig die Proteinurie als Zeichen einer Nephritis bestimmt. Die Bestimmung der Gesamt-IgG und -IgM-Spiegel sowie der Anti-dsDNA- und Anti-OVA-Antikörper erfolgte mittels ELISA. 21 Wochen nach dem Plasmazell-Transfer wurden die Mäuse getötet, um die Plasmazellen in Milz und Knochenmark mittels ELISPOT und Zytofluometrie zu analysieren und die Nieren immunhistologisch zu untersuchen.

Ergebnisse: Der adoptive Transfer von Plasmablasten und Plasmazellen aus der NZB/W-Maus und aus der Balb/c-Maus 5 Tage nach sekundärer Immunisierung mit OVA führte zu einer kontinuierlichen Produktion von Anti-dsDNA- bzw. Anti-OVA-Antikörpern ausschließlich durch langlebige Plasmazellen, die in Milz und Knochenmark der Empfänger-$Rag1^{-/-}$ Mäuse nachgewiesen wurden. $Rag1^{-/-}$ Mäuse, die Anti-dsDNA-Autoantikörper produzierten, wiesen eine vermindertes Überleben, Proteinurie und Immunkomplexnephritis mit Ablagerung von C1q, C3, IgG und IgM auf.

Schlussfolgerungen: Diese Untersuchungen zeigen erstmalig, dass Autoantikörper, die ausschließlich von langlebigen Gedächtnis-Plasmazellen sezerniert werden, zur Autoimmunpathologie beitragen. Langlebige Gedächtnis-Plasmazellen stellen deshalb bei (Auto)antikörper-vermittelten Erkrankungen wichtige Ziele für zukünftige Therapiestrategien dar.

Abstract

Introduction: We have previously shown that both short and long-lived plasma cells (PCs) significantly contribute to autoantibody production in NZB/W mice as a model of lupus nephritis. The aim of this study was to determine the role of autoreactive long-lived (memory) PCs refractory to immunosuppression and B cell depletion in the pathogenesis of systemic lupus erythematosus.

Methodology: Splenic CD138+ antibody-secreting cells (ASCs) from >6-month-old NZB/W mice with high titers of anti-dsDNA autoantibodies or from Balb/c mice 5 days after secondary immunization with ovalbumin (OVA) were adoptively transferred to immunodeficient $Rag1^{-/-}$ mice, in which the development of nephritis was investigated by measuring proteinuria. Total IgG and IgM as well as anti-dsDNA and anti-OVA antibody levels were followed up by ELISA. After 21 weeks the recipient mice were sacrificed so that PCs in spleen and bone marrow could be analyzed using ELISPOT and flow cytometry and renal immunohistology performed.

Results: The adoptive transfer of NZB/W and anti-OVA ASCs resulted in the continuous generation of anti-dsDNA antibodies and anti-OVA antibodies, respectively, exclusively by long-lived PCs that had homed to the spleen and bone marrow of recipient $Rag1^{-/-}$ mice. $Rag1^{-/-}$ mice generating autoantibodies including anti-dsDNA had reduced survival, proteinuria and immune complex nephritis with C1q, C3, IgG and IgM deposits 21 weeks after transfer.

Conclusions: These findings demonstrate that autoantibodies exclusively secreted by long-lived (memory) PCs contribute to autoimmune pathology and should be considered as candidate targets for future therapeutic strategies.

Affidavit

I, Qingyu Cheng certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "Autoantibodies from long-lived 'memory' plasma cells of NZB/W mice drive immune complex nephritis". I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

30.10.2013 _____

Date Signature

Detailed Declaration of Contribution

Qingyu Cheng had the following share in the following publication:

Publication: Qingyu Cheng, Imtiaz M. Mumtaz, Laleh Khodadadi, Andreas Radbruch, Bimba F. Hoyer, Falk Hiepe, Autoantibodies from long-lived 'memory' plasma cells of NZB/W mice drive immune complex nephritis, Ann Rheum Dis. 2013 Oct 10

Contribution in detail:

Qingyu Cheng was substantially involved in designing and planning the experiments, and he independently performed all experiments. In large experiments, he was sometimes assisted by Laleh Khodadadi to ensure timely completion and cell survival. Of note, Qingyu Cheng established the method of adoptive plasma cell transfer, the essential technology used in this work He learned other applied methods, such as plasma cell isolation from murine spleen, flow cytometric analysis of cells, and ELISPOT, from Imtiaz M. Mumtaz and Bimba Hoyer. He thoroughly discussed the results with the co-authors. Qingyu Cheng wrote and revised the manuscript after discussion with the co-authors. Consequently, he meets the criteria for being first author of this publication.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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Qingyu Cheng, Imtiaz M. Mumtaz, Laleh Khodadadi, Andreas Radbruch, Bimba F. Hoyer, Falk Hiepe.

Autoantibodies from long-lived ‘memory’ plasma cells of NZB/W mice drive immune complex nephritis.

Ann Rheum Dis. 2013 Oct 10 - DOI:
http://dx.doi.org/10.1136/annrheumdis-2013-203455

Curriculum vitae

My curriculum vitae is not published in the electronic version of my thesis due to data privacy regulations.

Complete list of publications

1. **Qingyu Cheng**, Imtiaz M. Mumtaz, Laleh Khodadadi, Andreas Radbruch, Bimba F. Hoyer, Falk Hiepe, Autoantibodies from long-lived 'memory' plasma cells of NZB/W mice drive immune complex nephritis, **Ann Rheum Dis**. 2013 Oct 10. **Impact factor: 9.111**

2. Tobias Alexander, Sandra Schneider, Bimba Hoyer, **Qingyu Cheng**, Andreas Thiel, Sabine Ziemer, Gerd-Rüdiger Burmester, Renate Arnold, Andreas Radbruch, Falk Hiepe. Development and resolution of secondary autoimmunity after autologous haematopoietic stem cell transplantation for systemic lupus erythematosus: competition of plasma cells for survival niches? **Ann Rheum Dis** 72, 1102-1104 (2013). **Impact factor: 9.111**

3. Imtiaz M. Mumtaz, Bimba F. Hoyer, Daniel Panne, Katrin Moser, Oliver Winter, **Qingyu Cheng**, Taketoshi Yoshida, Gerd-R. Burmester, Andreas Radbruch, Rudolf A. Manz, Falk Hiepe. Bone marrow of NZB/W mice is the major site for plasma cells resistant to dexamethasone and cyclophosphamide: implications for the treatment of autoimmunity. **J Autoimmun** 39, 180-188 (2012). **Impact factor: 8.145**

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