Deletion 13q14 in Plasma Cells of a Patient with Lupus Erythematosus and Autoimmune Hepatitis

Sebastian Tuve1, Ulrich Sommer2, Heike Kostka1, Brigitte Mohr3, Uta Oelschlägel1, Jan Moritz Middeke1, Marco Berning1, Julia Fantana1, Anne Erlert1, Christiane Jakob1, Martin Aringer2, Gustavo Baretton2, Marc Schmitz2, Martin Bornhäuser1 and Jana Babatz1

1 Medical Clinic I, University Hospital Dresden, Germany
2 Institute of Pathology, University Hospital Dresden, Germany
3 Institute of Laboratory Medicine, University Hospital Dresden, Germany
4 Medical Clinic III, University Hospital Dresden, Germany
5 Institute of Immunology, Medical Faculty, TU Dresden, Germany

Abstract

The molecular mechanisms that lead to autoantibody production and autoimmune disease remain poorly defined. Based on a clinical observation in a patient with lupus erythematosus and autoimmune hepatitis we here provide the first evidence that (i) a particular cytogenetic aberration (deletion (13)(q14)) could be detected in a proportion of plasma cells and that (ii) the surface marker CD56 could be used to enrich for plasma cells with this cytogenetic aberration.

Case Report

A 50 year old female with an unremarkable previous history was referred to the hospital in December 2012 because of elevated liver enzymes and hyperbilirubinemia (Table 1). She also complained about intermittent neck and lower limb joint pain, and intermittent eczema (retroauricular, palmar, plantar), currently only located at her right foot. Viral hepatitis (A, B, C, D or E) was excluded by screening for the presence of the respective IgM antibodies and by PCR for hepatitis B and C genomes (data not shown). Table 1 shows the results of routine laboratory chemistry and autoantibody screening. Serum protein electrophoresis (SPEP) showed polyclonal hypergammaglobulinemia (Figure 1A). Laboratory testing found Coombs positive hemolytic anemia with a hemoglobin of 7.3 mmol/l and decreased serum haptoglobin. To screen for multiple myeloma or lymphoma, bone marrow biopsy, conventional X-ray films of the spine, and abdominal ultrasound were performed. Radiography was unremarkable with exception of osteochondrosis of the upper cervical spine. Hematologic ultrasound showed no pathologies in liver parenchyma. However, two pathological enlarged lymph nodes were detectable near the portal vein (34 x11 mm) and a third one close to the celiac trunk (23 x 8 mm). Bone marrow aspiration showed no elevated proportion of dysplastic plasma cells or pathologies of other cellular compartments. Histologic staining of the bone marrow showed plasma cells producing both kappa and lambda light chains and no evidence for a kappa or lambda restriction, as it would be expected in plasma cell dyscrasia (Supplementary Figure 1). In accordance, no monoclonal protein, but polyclonal bands were found in serum (Figure 1A) and urine (not shown) on serum immunofixation electrophoresis (IFE). The serum free light chain (FLC) kappa/lambda ratio was 1.1 (normal range: 0.26-1.65, Table 1), indicating no predominant production of kappa or lambda FLC. Autoantibody screening revealed the presence of high-titer anti-dsDNA and anti-SMA antibodies (Table 1). In view of these data, the patient was diagnosed with systemic lupus erythematosus (SLE) with autoimmune hemolysis and autoimmune hepatitis (type I). Type II autoimmune hepatitis was excluded by screening for anti-LKM antibodies (Table 1). To confirm autoimmune hepatitis, a liver biopsy sample was taken, which showed the characteristic features of this disease and excluded infiltration with lymphoma or other malignant cells (Figure 1B).

Keywords: Autoimmune Hepatitis; CD56, del(13)(q14); Heavy/Light Chain Assay; Lupus Erythematosus; Plasma Cells

Treatment

Treatment with prednisolone (1mg/kg per day, reduction 5mg per week) was started, which led to a complete remission of clinical symptoms and normalization of liver function tests within 42 days (Table 1). Importantly, immunoglobulin and whole serum protein showed normal concentrations (with the exception of IgM, Table 1) and the SPEP and IFE did not show M-spike or monoclonal bands (Figure 1A). Using high sensitive subdifferentiation of immunoglobulin according to kappa and lambda-restriction (HLC), we did also not detect evidence for monoclonal immunoglobulin production (Table 1). In accordance, no kappa- and lambda restriction was detected using light chain-RNA-expression immunohistochemical analysis of the bone marrow biopsy (data not shown). Hydroxychloroquine (200 mg/d) and azathioprine (75 mg/d) were added to the medication starting 42 days and 60 days after the start of steroid treatment, respectively. One month after initiation of azathioprine treatment nausea occurred. Therefore, azathioprine was changed to 6-mercaptopurine (50mg/d). Prednisolone was tapered and discontinued after 12 month and the patient remained in clinical remission for the entire follow-up of 21 month after diagnosis. Anti-dsDNA and anti-SMA antibodies became negative (Table 1), and ultrasound showed complete regression lymph nodes near the portal vein and celiac trunk within 3 months of immunosuppressive treatment.

Differential Diagnosis

As a part of the initial myeloma screening, we sorted bone marrow plasma cells (using antibodies against CD38 and CD138) and screened for cytogenetic changes occurring in multiple myeloma.

*Corresponding authors: Sebastian Tuve, Medical Clinic I, University Hospital Carl Gustav Carus, Fetscherstr. 74, 01307 Dresden, Germany, Tel: +49-351-458-4186; Fax: +49-351-458-5362; E-mail: Sebastian.Tuve@uniklinikum-dresden.de

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Figure 1A: Electropherogram of the serum protein electrophoresis (SPEP, upper panel) and serum immunofixation electrophoresis (IFE, lower panel) prior prednisolone treatment and after 42-days of prednisolone treatment (starting 1mg prednisolone per kg BW).
Unexpectedly, deletion (13)(q14) was detected in 19% of plasma cells using iFISH analysis. No other chromosomal abnormalities were detectable in the iFISH analysis including del(17p) and t(4; 14)p16; q32. Deletion (13)(q14) has so far been reported as an early event in several hematologic malignancies, in particular MGUS (monoclonal gammopathy of undetermined significance), multiple myeloma, or chronic lymphatic leukemia [1,2]. During malignant transformation of plasma cells into MGUS and multiple myeloma, this deletion is thought to enable proliferation due to loss of the expression of MIR15A/MIR16A microRNAs, which are considered the main candidate tumor suppressors involved in del(13)(q14). These two microRNAs are known to inhibit the expression of multiple genes, including BCL2 [3], the cyclins CCND1 and CCND3, and cyclin-dependent kinase 6 (CDK6) [1,4]. Hence, deletion of MIR15A/MIR16A abrogates this inhibitory effect and promotes the constitutive cycling of plasma cells. Furthermore miR-15a/miR-16a has also been shown to participate in a microRNA/tumor protein p53 (microRNA/TP53) feedback circuitry associated with pathogenesis and prognosis of chronic lymphocytic leukemia (CLL) [5]. The second surprising finding was the detection of abnormal marker expression within the CD38+CD138+ plasma cell population. We detected aberrant CD56 (NCAM – Neuronal cell adhesion molecule) expression on 43% of plasma cells at diagnosis, using FACS analysis (Figure 1C). In addition, CD19 was expressed at low levels in 57% of plasma cells (Figure 1C). Immunohistochemical analysis of the bone marrow biopsy confirmed the presence of plasma cells exhibiting CD56 expression (Figure 1D). CD56 is physiologically expressed on NK and NK-T cells, but not on plasma cells. In normal embryogenesis CD56 mediates cell-cell and cell-matrix interactions and is mainly involved in axon growth and guidance. Aberrant CD56 expression on plasma cells has been considered a marker for plasma cell proliferative disorders (e.g. MGUS and multiple myeloma) [6]. It has
been hypothesized that CD56 could mediate homotypic interactions between myeloma cells themselves or with osteoblastic cells [7].

After 3 months of treatment the patient underwent a second bone marrow biopsy. This time the percentage of plasma cells in the bone marrow was 0.2% of total cells (before treatment: 0.5%, (Figure 1C) as detected via flow cytometry. Phenotyping of plasma cells with flow cytometry revealed the persistence of aberrant CD56 expression and low CD19 expression in plasma cell subsets. To test, in which plasma cell compartment the deletion (13)(q14) was present, we subjected plasma cells to FACS-sorting according to their CD56 expression. To test, in which plasma cell compartment the deletion (13)(q14) was present, we subjected plasma cells to FACS-sorting according to their CD56 expression (CD56-positive and CD56-negative CD38+CD138+ plasma cells) using a FACS-Aria II (BD Biosciences). Cytogenetic analysis revealed that del(13)(q14) was only present in plasma cells with aberrant CD56 expression, but not in normal plasma cells that lack CD56 expression (Figure 1E).

In summary, we have found the (13)(q14) deletion and aberrant CD56 adhesion molecule expression of a subset of bone marrow plasma cells in a patient with newly diagnosed lupus erythematosus with autoimmune hepatitis. Only CD56 expressing plasma cells contained the recurrent aberration del(13)(q14). Hence, CD56 which might be used to enrich for plasma cells with this cytogenetic aberration in subsequent studies. Although a significant proportion of plasma cells contained del(13)(q14) no evidence for a monoclonal proliferative/malignant plasma cell disorder or monoclonal immunoglobulin production was found in the patient, using highly-sensitive methods including HLC (Heavy/light chain assay). Plasma cells in the patient showed polyclonal immunoglobulin expression, in contrast to monoclonal plasma cell disorders that are characterized by kappa or lambda restriction. However, we cannot completely rule out the coexistence of autoimmunity and an early stage of MGUS in the patient. Treatment with prednisolone, azathioprine/6-mercaptopurine and hydroxychloroquine led to a reduction in total numbers of these plasma cells and in inhibition of the polyclonal production of (auto)antibodies leading to clinical remission. However, the immunosuppressive treatment did not lead to an eradication of del(13)(q14) containing plasma cells since plasma cells with this cytogenetic aberration were still detectable while the patients was clinically in remission. It is known that autoimmune diseases like lupus erythematosus require maintenance therapy, and a possible rationale for this phenomenon is the persistence of autoreactive plasma cell clones after induction therapy.

**Comment**

Autoimmunity is a continuously evolving process. Autoimmune responses diversify with time not only to other epitopes in the original antigen (epitope spreading) but also to other related and sometimes to unrelated antigens (antigen spreading) [8]. Polymorphisms in susceptibility genes together with environmental factors and epigenetic changes are a focus of investigation in autoimmune diseases [9]. Here, we demonstrated for the first time that a proportion of plasma cells derived from a patient with SLE and autoimmune hepatitis contain the del(13)(q14). So far, this deletion has been detected in plasma cells of patients with MGUS or multiple myeloma and has been shown to promote cell proliferation. We also observed that del(13)(q14) could only be detected in plasma cells expressing CD56, which allow the enrichment of such plasma cells. Our findings provide first evidence for a potential link between del(13)(q14)-containing plasma cells and antibody-mediated autoimmunity. However, further studies are needed to investigate whether such plasma cells are able to produce autoantibodies and play a role in the immunopathogenesis of autoimmune diseases such as SLE.

**Materials and Methods**

**Histopathology, chromogenic in situ hybridization (CiSH)**

Formalin fixed and paraffin-embedded tissues were cut into 5 μm sections. Subsequently, these sections were deparaffinized in xylene (2 × 15 min, VWR International, Fontenay-sous-Bois, France) and hydrated by washes of graded ethanol and water (B. Braun, Melsungen, Germany) to water (B. Braun, Melsungen, Germany). The CiSH-probe was preheated in the incubator. After drying sections were pre-treated with the enzyme pepsine (20 min) (Zytomed, Berlin, Germany) followed by washes of water (3 × 1 min) and pure alcohol (2 × 1 min). Subsequently, tissue sections were incubated with the CiSH-probe (Kappa and Lambda, Zytomed, Berlin, Germany) for 90 min at 60°C. Another...
washing with TBS-buffer followed (3 × 5 min). For visualization the DAB UltraView-Kit (Roche-Ventana, Mannheim, Germany) was used in the Benchmark XT (Roche, Mannheim, Germany) according to the manufacturer’s instructions. All sections were counterstained with Mayer’s hematoxylin (Roche-Ventana, Mannheim, Germany).

**Fluorescence-activated-Sorting (FACS)**

First, mononuclear cells were stained with CD138 FITC (IQP, Groningen, The Netherlands), CD38 PerCP5.5 (BD Sciences), CD56 PE-cy7 (BD Sciences), and CD19 PacificBlue (DAKO Cytomation, Glostrup, Denmark). Second, CD138+CD38++ plasma cells were sorted in two subfractions: CD56+ and CD56-. The yield after sorting was 15000 and 23800 cells, respectively. Fluorescence activated cell sorting was performed using a FACS Aria II cell sorter and FACS DiVa software (BD Sciences, Heidelberg, Germany). Automated instrument setting with FACS DiVa compensation set up was employed. Flow cytometer performance and drop delay were checked using CS & T and ACCUdrop beads (BD Sciences).

**Serum Protein Electrophoresis (SPEP) and immunofixation electrophoresis (IFE)** were carried out using a Hydrasys instrument (Sebia, Lisses, France) according to the manufacturer’s instructions.

**Immunoglobulin heavy/light chain assay** (HLC; Hevylite TM, The Binding Site GmbH, Schwetzingen, Germany). Antibodies in this assay separately identify the different light chain types of each immunoglobulin class, that is, IgG kappa and IgG lambda. HLC ratios outside of the reference range were considered to indicate a monoclonal process.

**FISH analysis:** The LSI (13q34) Spectrum Green DNA probe as well as the LSI 13(RB1) (13)(q14) Spectrum Orange DNA probe (both from Abbott Molecular Europe, Wiesbaden, Germany) were used for FISH analysis according to the manufacturer’s instructions.

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**References**