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The influence of genetic predisposition and autoimmune hepatitis inducing antigens in disease development

Matthias Hardtke-Wolenski [a, *, 1], Janine Dywicki [a, 1], Katja Fischer [a], Martin Hapke [a], Maren Sievers [a], Jerome Schlue [b], Mark S. Anderson [c], Richard Taubert [a, d], Fatih Noyan [a, d], Michael P. Manns [a], Elmar Jaeckel [a, d]

[a] Dept. of Gastroenterology, Hepatology & Endocrinology, Hannover Medical School, Germany
[b] Inst. of Pathology, Hannover Medical School, Germany
[c] Diabetes Center, University of California—San Francisco, San Francisco, United States
[d] Integrated Research and Treatment Center Transplantation (IFB-Tx), Hannover Medical School, Germany

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ABSTRACT

Autoimmune hepatitis (AIH) is defined as a chronic liver inflammation with loss of tolerance against hepatocytes. The etiology and pathophysiology of AIH are still poorly understood because reliable animal models are limited. Therefore, we recently introduced a model of experimental murine AIH by a self-limited adenoviral infection with the AIH type 2 antigen formiminotransferase cyclodeaminase (FTCD).

We could demonstrate that break of humoral tolerance towards liver specific autoantigens like FTCD and cytochrome P450 2D6 (CYP2D6) is not dependent on the genetic background. However, the development of AIH in autoantibody positive animals is determined by genetic background genes. We could also show that the break of humoral tolerance is necessary but not sufficient for the development of AIH. In contrast the break of tolerance against the ubiquitously expressed nuclear antigens (ANAs) is strictly dependent on genetic predisposition. Priming with the UGA suppressor tRNA-associated protein (soluble liver antigen; SLA) is a strong inducer of ANA reactivity, but not sufficient to cause AIH development thereby questioning the importance of anti-SLA immune response as an important driver in AIH. Monogenetic mutations such as Aire-deficiency can cause AIH in otherwise genetically resistant strains.

Conclusion: The results have important implications for our understanding of the pathophysiology of AIH development and for the interpretation of humoral antibody responses in AIH.

1. Introduction

Autoimmune hepatitis (AIH) is a chronic, progressive liver disease of unknown etiology characterized by hepatocellular inflammations often leading to cirrhosis. Most patients are diagnosed late in the disease course. Therefore it is difficult to study potential infectious or environmental triggers of AIH. In any case there seems to be a long lag-period between initiation of autoimmunity and diagnosis of symptomatic disease as almost 40% of patients showed liver cirrhosis at time of diagnosis [1,2]. Despite of these difficulties, the concept of environmental agents that may resemble self-antigens (i.e. molecular mimicry or altered-self) or infectious agents triggering autoimmunity has emerged in the late 1980-ies and since then guided research in autoimmunity and AIH [3].

Genetic evidence suggests that distinct MHC II alleles [4] and specific small nucleotide polymorphisms within regulatory genes are associated with increased risk for development of AIH, e.g. CTLA-4 and TNF-α [5,6]. Most of these findings usually followed reports of genetic predispositions in other autoimmune diseases as type 1 diabetes (T1D) [7] and the contribution to liver related autoimmunity remained unclear. Nonetheless, individuals with the HLA haplotypes A2, B8, C7, DR3, DR4, and DQ2 are genetically predisposed to develop AIH [8,9]. A recent genome-wide association study (GWAS) has shown that HLA-DR3 and HLA-DR4 are best associated with AIH [10]. In this GWAS, other genetic

Abbreviations: Ad, adenovirus; AIH, autoimmune hepatitis; CYP2D6, cytochrome P450 2D6; emAIH, experimental murine autoimmune hepatitis; FTCD, formiminotransferase cyclodeaminase; SLA/LP, soluble liver antigen; T1D, type 1 diabetes.

* Corresponding author. Dept. of Gastroenterology, Hepatology & Endocrinology, Hannover Medical School, Carl Neuberg Str. 1, 30625, Hannover, Germany.
E-mail address: Wolenski.Matthias@mh-hannover.de (M. Hardtke-Wolenski).

[1] Equally contributed first authors.

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predispositions were identified as likely risk factors e.g. variants of SH2B3 and CARD10 [10].

Autoantibodies are a valuable tool for diagnosing of autoimmune diseases. While LC-1 against formiminotransferase cyclodeaminase (FTCD), LKM-1 against cytochrome P450 2D6 (CYP2D6) and SLA/LP against UGA suppressor tRNA-associated protein are highly specific for AIH, the more prevalent anti-nuclear antibodies (ANA) and smooth muscle antibodies (SMA) are far less disease specific and are also seen in other inflammatory liver diseases than AIH, i.e. like PBC, PSC, drug-induced, viral and (non-alcoholic) steatohepatitis. ANA also appear in type 1 diabetes (T1D), systemic lupus erythematosus, Sjögren's syndrome, cancer and other inflammatory conditions.

In the experimental murine AIH (emAIH) model break of immune tolerance could be induced by a self-limited infection with adenovirus (Ad) expressing heterologous human FTCD in genetically predisposed NOD/Ltj mice [11]. The induced hepatitis is chronic and leads to a progressive fibrosis in mice. FTCD was chosen because of its liver-specific expression. However, autoantibodies against FTCD are just observed in a small group of mainly pediatric patients with AIH type 2 while most adult patients do not develop antibodies to FTCD. The majority of adult AIH patients just show anti-nuclear and/or anti-smooth muscle antibodies, which are neither liver-disease- nor liver-specific. However, reactivities to SLA/LP and CYP2D6 are more specific and can also be observed in a subgroup of AIH patients. Christen and co-workers already tested CYP2D6 as an autoantigen in elegant studies in FVB/N mice: They induced hepatitis with the adenovirus expressing human CYP2D6 (Ad-CYP2D6) in wild-type FVB/N, while the same approach resulted in milder hepatitis in transgenic FVB/N mice expressing human CYP2D6 [12]. Most probably, the expression of the identical transgene in the mice might have led to negative selection of autoreactive T cells in the thymus. However, this result was still not tested in mice being genetically susceptible to liver-autoimmunity like the NOD mice.

Using this emAIH model, we could show in this study the importance of the environmental trigger since FTCD alone as heterologous antigen is able to trigger emAIH, while CYP2D6 and SLA/LP were only able to break humoral tolerance. Since Ad-CYP2D6 and Ad-SLA/LP are breaking humoral tolerance by induction of ANA and other autoantibodies without triggering AIH on NOD/Ltj background, autoantibodies including ANA seem to be not sufficient to cause AIH. Nonetheless, the differential induction of autoantibodies and the absence of AIH in other strains highlights the importance of background genes.

2. Material and methods

2.1. Ethics statement

Animal care and experiments were performed in accordance with institutional and national guidelines. All animal experiments were executed according to protocols approved by the animal welfare commission of the Hannover Medical School and local Ethics Animal Review Board (Niedersaechsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit/LAVES, Oldenburg, Germany). The grant numbers covering the experiments are 06/1137, 11/0342 and 15/1855.

2.2. Mice

All animal experiments were performed under S2-conditions in the central animal facility of Hannover Medical School. NOD/Ltj (NOD), C; 129S-Cd1^{tm1}Gru/J (BALB/c CD1$^{-/-}$), C; 129S2(B6)-Aire$^{tm1.1}$Doi (BALB/c AIRE$^{-/-}$) and NOD; 129S2(B6)-Aire$^{tm1.1}$Doi (NOD Aire$^{-/-}$) mice were bred in specific pathogen–free facilities at Hannover Medical School. C57Bl/6J, BALB/cJ and FVB/N were purchased at Charles River, Sulzfeld, Germany. All mice were sacrificed 12 weeks post adenoviral infection. Since some NOD/Ltj are developing T1D, diabetic mice were excluded from the study.

2.3. PCR

cDNA from human blood cells or liver was used as target for the amplification of human CYP2D6 and human SLA/LP, respectively. For the preparation of cDNA random hexamers were hybridized to total RNA and a complementary DNA copy was synthesized by a RNA-dependent DNA-Polymerase (Roche). CYP2D6 and SLA/LP were amplified from cDNA with proof-reading Turbo pfu polymerase (Stratagene) using specific primers as follows for the generation of adenoviruses: CYP2D6 Fw (5′- ACGTCTCGA GCCACCATGgggctagaagcactggt-3′), CYP2D6 Rv (5′- ACGTAAG CTTCTAGCGGGGCACAGCACAAAG-3′); SLA/LP Fw (5′-ACGTG CGGCCGCCCACCATGaaccgcgagagcttcgc -3′), SLA/LP Rv (5′-ACG-TAAGCTTTCATGAAGAAGCATCCTG -3′).

2.4. Construction, generation, titering and application of adenovirus

The generation of Ad-FTCD and Ad-eGFP were described before [11]. The generation of Ad-SLA/LP and Ad-CYP2D6 expressing the human antigens cloned in section 2.3 were done accordingly to the published method. In brief, CYP2D6 and SLA/LP were amplified by PCR from cDNA generated from human liver cells; its sequence was verified by sequencing of both DNA strands. The constructs were fused to the Ad transfer vector pShuttle-CMV (Stratagene). By homologous recombination this shuttle vector was recombined with pAdEasy-1 that carries deletions in the E1-and E3 region (Stratagene). The generated adenovirus genome can be amplified only within the HEK 293 packaging cell line, complementing the essential regions. Purification of recombinant adenovirus was done by caesium chloride gradient and the adenoviral stocks were quantified using an Adeno-X™ Rapid Titer Kit (Clontech). Mice were injected intravenously with a total of 1×10^{10} infectious particles in PBS of Ad-FTCD, Ad-CYP2D6, Ad-SLA/LP or Ad-eGFP.

2.5. Western blot

Liver cells were lysed in Triton X-100 containing lysis buffer (120 mM NaCl, 40 mM Tris pH 7.5, 0.5% Triton X-100, 0.3% SDS), heat denaturated and samples were run on 10% SDS–PAGE gels (BioRad) and transferred to PVDF membranes (Biorad). Blots were cut into stripes and blocked with 5% non-fat dry milk (Sigma) in TBS-T buffer. Western blot stripes were incubated with patient sera containing anti-LKM1 or anti-SLA/LP autoantibodies, diluted 1:320 in TBS-T buffer and probed with secondary anti-human-HRP (Jackson Immunoresearch).

2.6. Immunofluorescence

Indirect immunofluorescence staining for the detection of autoantibodies was performed on rat sections of stomach, kidney and liver, normally used for diagnostics, kindly provided by the autoantibody laboratory of Hannover Medical School. Sections were blocked with goat serum (Sigma) in TBS-T buffer. Sera of Ad-FTCD, Ad-CYP2D6, Ad-SLA/LP and Ad-eGFP treated mice were diluted 1:300 in TBS-T buffer and detected with anti-mouse-DyLight488 (Jackson Immunoresearch). All sections were analyzed with a fluorescence microscope, AxioImager M1 (Zeiss) using AxioVision 4.8 software.