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Type 2 autoimmune hepatitis murine model: The influence of genetic background in disease development

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Abstract

Genetic predisposition is recognized as an important factor for the development of autoimmune hepatitis (AIH). To assess the potential contribution of MHC and non-MHC genes, type 2 AIH was reproduced in three mice strains, taking advantage of their different genetic makeup with regard to MHC and non-MHC genes. Mice (C57BL/6, 129/Sv and BALB/c) were DNA vaccinated with a pCMV-CTLA4-CYP2D6-FTCD plasmid coding for the extracellular region of CTLA-4 and for the antigenic region of the CYP2D6 and FTCD, and with pCMV-IL12. ALT and total IgG levels, liver histology, FACS analysis and liver T-cell cytotoxicity assays were monitored up to 8 months post-injection. C57BL/6 mice showed elevated serum ALT levels, autoantibodies, antigen-specific cytotoxic T-cells and lobular and periportal inflammatory infiltrate. The 129/Sv mice showed slightly elevated ALT levels, sparse liver lobular infiltrate and cytotoxic T-cells. The BALB/c mice showed no liver inflammation. All mice had elevated total serum IgG levels. This murine model of type 2 AIH shows that MHC and non-MHC genes contribute to the susceptibility to autoimmune hepatitis. The understanding of the genetic determinants implicated in AIH development will be a major advance in the study of its pathogenesis and could lead to a better diagnostic approach and preventive strategies.

Keywords: Autoimmune hepatitis; MHC; Animal model; Autoantibody

1. Introduction

Autoimmune hepatitis (AIH) is a disease of unknown aetiology characterized by a progressive destruction of the hepatic parenchyma by the immune system [1–3]. Progression to cirrhosis and end stage liver disease may occur in 10–20% of cases and liver transplantation may be necessary [1,3], even when the appropriate available treatment is administered. Particular characteristics of this disease are the presence of a hypergammaglobulinemia and circulating autoantibodies. These autoantibodies allowed the classification of AIH into two types. Type 1 is characterized by the presence of anti-smooth muscle and/or anti-nuclear antibodies, whereas type 2 shows anti-liver kidney microsomal type 1 and/or anti-liver cytosol type 1 antibodies [4–6]. Previous work has shown that the targets of LKM1 and LC1 antibodies are cytochrome P-450 2D6 (CYP2D6) [7], and formiminotransferase cyclodeaminase (FTCD) [8], respectively; both autoantigens are mainly expressed in hepatocytes [9,10].

A model of type 2 autoimmune hepatitis using xenoimmunization with human CYP2D6 and FTCD antigens of wild-type C57BL/6 mice was recently developed in our laboratory [11]. These mice showed an increase of serum alanine aminotransferase (ALT) levels, circulating anti-LKM1 and anti-LC1 autoantibodies associated with massive infiltration of the liver by lymphocytes. This model is particularly appropriate for the study of the influence of the genetic background on the development of the disease. Susceptibility to autoimmune hepatitis in humans has been shown to be related to both the MHC haplotype and to non-MHC genes [12–16]. Further studies have

Abbreviations: AIH, autoimmune hepatitis; LCI, liver cytosol type 1; FTCD, formiminotransferase cyclodeaminase; LKM1, anti-liver kidney microsome type 1.

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implicated different loci with either type 1 or type 2 autoimmune hepatitis [17–20].

To evaluate the importance of the genetic background on the development of AIH and to further validate our animal model of type 2 AIH, we have compared the susceptibility of three mouse strains displaying similar or different MHC and non-MHC genes to develop a type 2 AIH.

2. Materials and methods

2.1. DNA vaccination

The DNA vaccination was done using the pRc/CMV-CTLA-4-CYP2D6-FTCD vector as described [11] along with pVR-IL12 plasmid (kindly provided by G. Prud'homme, Montreal, Canada). All the plasmids were propagated in *E. coli* by standard techniques and purified using QIAGEN Endofree Plasmid Giga Kit (QIAGEN, Santa Clarita, CA), according to the manufacturer's guidelines.

C57BL/6, BALB/c and 129/Sv mice were used due to their different genetic makeup (Table 1). In mouse, the H-2 denomination refers to both class I and class II genes. The C57BL/6 mice have been previously found to be susceptible to develop an AIH following vaccination with pRc/CMV-CTLA-4-CYP2D6-FTCD and pVR-IL12 [11]. The 129/Sv mice were used because they share the same MHC class I and II genes with the C57BL/6 but have different non-MHC genes (Table 1). The BALB/c mice were used because they have different MHC and non-MHC genes (Table 1). By testing these strains, it will be possible to evaluate the importance of MHC and non-MHC genes in AIH development.

Six to eight week old females (20) C57BL/6, BALB/c, 129/Sv mice were injected under general anaesthesia in the internal tibial muscle with 100 μg (50 μl) of both plasmids (pRc/CMV-CTLA-4-CYP2D6-FTCD, pVR-IL12) dissolved in saline buffer. Mice were injected three times, at 2-week intervals. Control mice of each strain were injected with the pVR-IL12 plasmid only following the same protocol (100 μg, three times).

2.2. Serum alanine aminotransferase (ALT) activity

Serum ALT levels were measured in a Beckman-Synchron CX9 apparatus, from blood samples taken every month after the last plasmid injection.

2.3. Total serum IgG measurement

Mouse total serum IgG was measured by ELISA. Briefly, sera from non-injected (controls) and injected mice (control and experimental) were applied to a 96-well ELISA plate in 0.1 M $NaHCO_3$, pH 8.6, overnight at 4 °C. After blocking with 5 mg/ml BSA in 0.1 M $NaHCO_3$ for 2 h at 4 °C, 100 μl of anti-mouse IgG conjugated to alkaline phosphatase (1/3000) (Biosource International, Camarillo, CA) was added and incubated for 2 h at room temperature. The plates were washed and the alkaline phosphatase activity was revealed by incubation with *p*-nitrophenyl phosphate; the results were read at 405 nm. Results were expressed as times above normal serum levels.

2.4. Histopathology and immunohistochemistry

Vaccinated mice were sacrificed 8 months after the last plasmid injection, and their livers were dehydrated, embedded in paraffin, sectioned, and stained with H&E. Characterization of the lymphocyte infiltrate was made by immunochemistry using specific antibodies against murine CD3, CD4, CD8 and CD23 (Santa Cruz Biotechnology, Santa Cruz, CA) according to published protocols [21]. Histopathological examination of various organs (heart, kidney, brain, small intestine) was also performed to evaluate possible extra-hepatic inflammations.

2.5. ELISA

ELISA was performed as described [22]. Briefly, the fusion protein produced by the pMAL-cR1-CYP2D6-FTCD plasmid was purified and used as antigen in this ELISA (0.2 μg/well). An antiserum was considered positive if its specific OD was at least two times higher than the mean OD of the preimmune mice sera. The same technique was applied to establish the Ig subclass using anti-IgG1, anti-IgG2a, anti-IgG2b, anti-IgG3, anti-IgA and anti-IgM secondary antibodies (Sigma-Aldrich Canada Ltd., Canada).

2.6. Preparation of mouse liver microsomal and cytosolic fractions

Normal mouse livers were homogenized with a Potter-Elvehjem in lysis buffer (1% NP-40, 1% DOC, 0.1% SDS, 150 mM NaCl, 10 mM Tris–HCl, pH 7.4) at 4 °C. The crude homogenate was centrifuged at 500 × g for 10 min, and the supernatant was centrifuged at 10,000 × *g* for 20 min. The pellet was discarded and the supernatant was fractioned into cytosolic fraction (supernatant) and microsomal fraction (pellet) by a 40 min period of centrifugation at 100,000 × *g*. To ensure fraction purity, the supernatant was centrifuged again for 40 min at 100,000 × *g*.

2.7. Western blot analysis

Proteins from the cytosolic and microsomal fractions were separated by electrophoresis on 10% SDS-PAGE and transferred onto nitrocellulose filters (Amersham Life Sciences, Oakville, Canada). The western blot technique was carried out as previously described [11]. Briefly, the membrane was blocked with 5% powder milk then incubated with the mouse sera (1/200) for 2 h. After washing, the filter was incubated with peroxidase-conjugated anti-mouse IgG (Biosource International, Camarillo, CA). Bound peroxidase was revealed by chemiluminescent blotting substrate (Boehringer Mannheim, Germany).