Case Report

JOURNAL OF HEPATOLOGY

Single-gene association between *GATA-2* and autoimmune hepatitis: A novel genetic insight highlighting immunologic pathways to disease

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Abstract

Background & Aims: Autoimmune hepatitis (AIH), an immune-mediated liver disease, originates as a consequence of interacting genetic and environmental risk factors. Treatment remains non-specific and prone to side effects. Deficiencies in regulatory T cell (Treg) function are hypothesized to contribute to the pathogenesis of AIH.

Methods: We describe an adult patient who presented with AIH in the context of monocytopenia. The patient was characterized by *GATA2* gene sequencing, flow cytometry of peripheral blood for leucocyte subsets, ELISA for serum Flt-3 ligand, and immunohistochemistry of liver biopsy tissue.

Results: Sequencing confirmed a *GATA2* mutation. Peripheral Treg were absent in the context of a preserved total T cell count. Immunostaining for the Treg transcription factor FOXP3 was reduced in liver tissue as compared to a control AIH specimen. There were marked deficiencies in multiple antigen-presenting cell subsets and Flt-3 ligand was elevated. These findings are consistent with previous reports of *GATA2* dysfunction.

Conclusions: The association of a *GATA2* mutation with AIH is previously unrecognized. *GATA2* encodes a hematopoietic cell transcription factor, and mutations may manifest as monocytopenia, dendritic and B cell deficiencies, myelodysplasia, and immunodeficiency. Tregs may be depleted as in this case. Our findings provide support for the role of Tregs in AIH, complement reports of other deficiencies in T cell regulation causing AIH-like syndromes, and support the rationale of attempting to modulate the Treg axis for the therapeutic benefit of AIH patients.

Keywords: Regulatory T cell; Pathogenesis; Immune-mediated liver disease.
Received 13 August 2015; received in revised form 15 January 2016; accepted 19 January 2016

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Introduction

Autoimmune hepatitis (AIH) is an immune-mediated liver disease with environmental and genetic risk factors. Deficits in immunoregulation, most notably regulatory T cell (Treg) function, are associated with etiopathogenesis [1].

HLA associations with AIH represent the strongest genetic risk factors, implying specific immune presentation of triggering antigens [2]. Rare Mendelian genetic variation is additionally mechanistically informative, with strong association between AIH and recessive mutations in the autoimmune regulator gene, *AIRE* [3]. Such mutations prevent the thymic medulla presenting tissue-restricted antigens to developing T cells, impairing both negative selection of autoreactive cells and generation of self-specific Tregs. Furthermore, murine immune-mediated hepatitis can be generated by medullary thymic epithelial cell depletion through deletion of *TRAF6* and Treg deficiency causes periportal inflammation [4,5].

We report and characterize AIH associated with a mutation in *GATA2*, a novel observation with mechanistic and therapeutic insights.

Patient

Our patient, of European Caucasian ancestry and without family history of note, presented at age 27, but gave a history of lymphedema, possible porphyria cutanea tarda, and intermittent superficial skin infections as a teenager. Aged 21 she developed trilineage cytopenias including monocytopenia initially diagnosed as myelodysplastic syndrome (MDS). Bone marrow aspiration revealed absolute loss of multi-lymphoid and granulocyte-macrophage progenitors in a hypocellular marrow. She developed an erythrocyte transfusion requirement and her infections became more frequent.

Aged 27, she developed elevated liver biochemistry consistent with hepatitis, which resolved spontaneously. Aged 28, whilst free of medical immunosuppression, she developed hepatitis with ascites. Ultrasound and magnetic resonance cholangiopancreatography showed hepatosplenomegaly and showed no evidence of biliary disease. Total IgG was elevated peaking at

41.05 g/L, polyclonal and predominantly IgG1. Anti-nuclear antibodies were positive at 1:100 in a speckled pattern; other autoantibodies including anti-mitochondrial antibody were negative (Supplementary Tables 1 and 2). Liver biopsy revealed plasma cells and interface hepatitis consistent with AIH (Fig. 1). There was mild macrovesicular steatosis, moderate iron deposition consistent with previous repeated transfusions and moderate fibrosis; staining suggestive of alternate etiologies was negative, including specific staining for Epstein-Barr virus (EBV; Supplementary materials). An EBV viral load of 10^4–10^5 copies/ml was present throughout; no other viral factors were identified including negative PCR for hepatitis B, C, E and cytomegalovirus. Human leukocyte antigen (HLA) genotyping revealed non-AIH risk alleles: *HLA-DPB1*03:01* and *HLA-DPB1*10:01* (Supplementary Table 3). Functional antibody testing confirmed preserved ability to generate antigen-specific responses (Supplementary Table 4).

Corticosteroids were commenced as prednisolone 40 mg/day, and her ascites and liver biochemistry tests resolved. Shortly afterwards, she developed JC/polyoma virus-positive progressive multifocal leukoencephalopathy (PML). Corticosteroids were discontinued, PML treatment commenced, and she regained the ability to walk. On discontinuing corticosteroids, her liver biochemistry again deteriorated. Over subsequent years she received variable corticosteroid-tacrolimus immunosuppression without recurrence of PML but with varying elevations in transaminases.

Repeat liver biopsy at the age of 32 showed similar features with progressing fibrosis. She later developed human papilloma virus-associated vulval carcinoma, which was treated with radiotherapy. At this point investigations were initiated for suspected *GATA2* mutation.

After investigations confirmed *GATA2* mutation, hematopoietic stem cell transplantation was performed. The allograft was unsuccessful and the patient ultimately died from complications of vulval carcinoma.

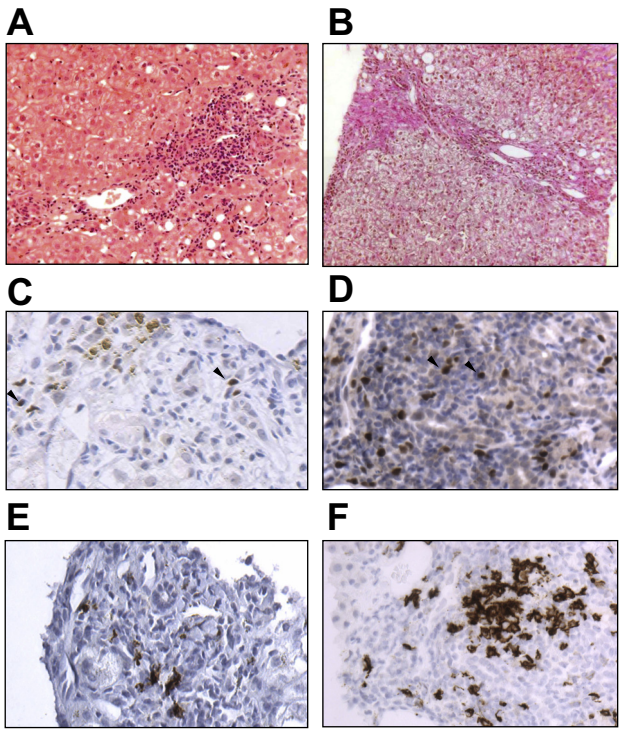

Fig. 1. Liver histopathology. (A) Transjugular liver biopsy sample demonstrating dense lymphocytic infiltrate with interface hepatitis consistent with AIH (haematoxylin and eosin; 20×). (B) Fibrosis bridging between portal areas (Van Gieson; 20×). (C and D) Cells positive for the Treg transcription factor FOXP3 were scant in the inflammatory infiltrate of patient liver (C), but more frequent in a control AIH sample (D) (both 32×; example positive staining denoted by arrowheads). (E and F) CD20-positive B cells were present in patient liver biopsy specimen (E) in contrast to peripheral blood but at a reduced frequency to a control AIH sample (F; 32×). (This figure appears in colour on the web.)

Table 1. Leucocyte subtypes.

Parameter	Normal range	This patient
Leucocyte subtypes (×10⁴/ml)		
T cells	91.9-201.9	343.3
CD4+ T cells	30-140	80
CD8+ T cells	20-90	130
B cells	3.3-28.9	0.6
NK cells	7.9-52.5	0.05
Antigen presenting cells (×10⁴/ml)		
CD14+ monocytes	31.5-59.7	7.2
CD16+ monocytes	2.1-8.2	0.18
CD1c+ dendritic cells	0.7-2.0	0.006
CD141+ dendritic cells	0.05-0.19	0.000
Plasmacytoid dendritic cells	0.8-2.2	0.003
CD34+ progenitors	0.1-0.5	0.46

Results

DNA sequencing revealed a coding 1081C>T R361C abnormality in exon 7 of *GATA2*. Serum Fms-like tyrosine kinase 3 ligand (Flt-3L) was markedly elevated at 1267.2 pg/ml (normal 48.3–173.8 pg/ml); sequencing for Flt-3 receptor mutations was negative. Flow cytometry demonstrated marked reductions in numbers of dendritic cells, monocytes, B and natural killer (NK) cells in keeping with 'DCML deficiency' [6] (Table 1). There was a maintained T cell population but near absence of FOXP3+ Treg (Fig. 2; Supplementary Fig. 1). Sparse FOXP3+ cells were seen in the hepatic inflammatory infiltrate and fewer than when compared to control AIH, or prior reports (Fig. 1) [7]. *In situ* hybridization staining was negative for EBV (Supplementary Fig. 2).

The calculated AIH score according to the International autoimmune hepatitis group revised diagnostic scoring system was 21 with a score >17 suggesting definite AIH (Table 2).

Discussion

Specific therapy in AIH is limited by our understanding of disease etiopathogenesis. Here we demonstrate the association of a missense mutation in *GATA2*, a hematopoietic transcription factor, with classical AIH and systemic Treg deficiency. This new molecular insight in AIH supports the relevance of developing novel therapies focused on reconstituting regulatory balance.

GATA2 dysfunction may present in the first two decades with lymphedema, deafness and myelodysplasia (Emberger syndrome), or acute myeloid leukemia without preceding immunodeficiency [8]. Presentation may also be with non-tuberculous mycobacterial infections and monocytopenia, DCML deficiency