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.

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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 peri-portal 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 porphryia 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 $10^4$–$10^7$ 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.

### 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 mis-sense 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.
or familial MDS/acute myeloid leukemia [9,10]. Related carriers may remain healthy for decades. The cause of the variable age of onset may be unidentified environmental exposures or additional genetic risk, for example AXL1 mutation [5]. Most individuals with GATA2 dysfunction develop MDS; elevated serum Fli-3 ligand is almost universal [11]. Monocytopenia is a vital clue to this rare diagnosis; chronic neutropenia and NK deficiency may also be suggestive [9]. This is the first characterization of associated AIH, but autoimmune phenomena including arthritis and hypothyroidism are recognized [11].

Several features support the diagnosis of AIH: plasma cells and interface hepatitis on liver biopsy, steroid responsiveness with relapse on withdrawal, the presence of anti-nuclear antibody and elevated serum IgG. IgG is usually normal in GATA2 dysfunction, despite the near absence of peripheral B cells [5]. In our case, B cells were present in the hepatic inflammatory infiltrate but not peripherally, suggesting local induction. The differential diagnosis of EBV-driven hepatitis was made unlikely by steroid responsiveness, stable viremia and negative in situ hybridization assay. We note that EBV viremia was reported amongst 11% of one cohort of patients with monoMAC but hepatitis was not reported [8].

The increase in absolute and relative numbers of CD8 T cells in the peripheral blood of this patient is of interest. CD8 cells have been implicated in the pathogenesis of AIH: they are present in the inflammatory infiltrate, cells isolated from those with active disease may be resistant to regulation but more resistant than usual to regulation by Treg [1]. In addition, several animal models of AIH demonstrate increases in both peripheral and hepatic CD8+ T cell numbers [1]. Serum soluble CD8, which is increased in CD8 T cell activation, is also reported to be elevated in AIH. Although it is tempting to link the elevation in CD8+ T cells to AIH, it is notable that accumulation of CD8+ T cells is a feature of progressing GATA2 dysfunction without AIH [11].

Of particular interest in this patient is the Treg deficiency despite preserved numbers of total CD4+ and CD8+ T cells. Tregs may be depleted in association with dendritic cell deficiency, including in GATA2 dysfunction [12,13]. We speculate that this deficiency may have contributed to a loss of immunological tolerance. Sparse FOXP3 staining on liver biopsy may reflect activated T cells transiently expressing FOXP3 or alternatively induced/transdifferentiated Treg in an inflammatory environment [14]. Reduced CD127 expression on peripheral T cells was consistent with impaired memory cell induction as reported in GATA2 dysfunction [11]. This may have contributed to a susceptibility to viral infections.

The association of a functional mutation in GATA2 with AIH and Treg deficiency informs efforts to further examine regulatory
mechanisms in AIH to better understand pathogenesis and guide rational therapy.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

GW drafted the manuscript and analyzed patient samples; YC, KL and DN assisted with sample analysis; YO, AR, VB, MC, DA and GMH were involved with patient care and VB also analyzed patient samples; GMH conceived the study, finalized the manuscript and acts as guarantor. All authors approved submission.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.01.017.

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