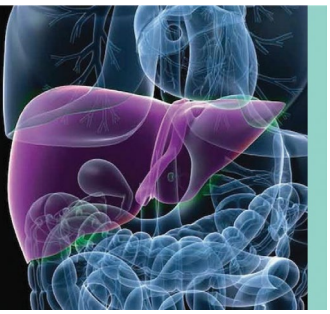

Immunopathogenesis of Autoimmune Hepatitis*

David N. Assis, M.D.

The pathogenesis of autoimmune hepatitis (AIH) is complex and reflects unique interactions between an otherwise tolerant liver, environmental stimuli, and dysregulated immunological mechanisms that break tolerance and leads to clinical manifestations of the disease. A deeper understanding of the immunopathogenesis can inform key areas of unmet need and promote much-needed innovations in therapeutic strategies to treat patients with AIH.

TOLERANCE AND THE LIVER

Central tolerance plays an important role through the interaction of T cells in the thymus with thymic epithelial cell (TEC) presentation of self-antigens. Cortical TECs help generate tolerant T cells, whereas medullary TECs eliminate autoreactive T cells and thereby regulate the production of central regulatory T cells (Tregs).[1] A mutation of the autoimmune regulator gene (*AIRE-1*), a key regulator of autoreactive T cell–negative selection, results in a syndrome of polyendocrinopathy-candidiasis-ectodermal dystrophy, with reduced Tregs and that frequently includes AIH. The implication of this mutation can be studied through a mouse model characterized by depletion of the thymic medulla resulting in features of AIH.[2] Peripheral tolerance is generated principally through the dynamic local cytokine environment and results in the development of inducible Tregs within the liver.

The liver itself is a highly tolerogenic organ. It is continually exposed, through the portal vein, to environmental stimuli including toxins, commensal and potentially pathogenic organisms and their DNA products, and a multitude of antigens. To prevent a pathological inflammatory response to these heterogeneous molecules, the liver critically serves as a functional vascular firewall, both combating potential invasive organisms and promoting a symbiosis between

Abbreviations: AIH, autoimmune hepatitis; *AIRE-1*, autoimmune regulator gene; anti-LKM, Liver-Kidney Microsomal antibody; BAFF, B cell–activating factor; CYP2D6, cytochrome P450 2D6; IFNγ, interferon-gamma; IL, interleukin; MAIT, mucosal-associated invariant T; MIF, macrophage migration inhibitory factor; NK, natural killer; TEC, thymic epithelial cell; TGF-β, transforming growth factor β; T_h1, T helper 1; TNF, tumor necrosis factor; Treg, regulatory T cell.

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commensal microbes and the body.[3] As such, the hepatic immunological environment must be at once tolerant of external antigen while also responsive to truly pathogenic attacks.

The liver can promote systemic immune tolerance, a remarkable feature first observed in the context of transplantation.[4] Acting as a secondary lymphoid organ, in which CD8+ T cells can be directly primed, the liver also promotes local tolerance.[5] Mechanisms responsible for this effect include stunning, in which reactive T cells are inactivated by immunosuppressive molecules, and exhaustion, in which overwhelming and sustained antigenic stimulation leads to T cell dysfunction. This strong tolerogenic immunological environment can prevent unnecessary inflammation but can also facilitate persistence of infections, such as chronic viral hepatitis, and persistent dysregulated inflammation in the context of autoimmunity.

Despite strong peripheral and central tolerogenic mechanisms, loss of tolerance in AIH can occur through a combination of genetic predisposition, environmental stimulus (e.g., microbial products, drugs metabolites, and associated haptens), and an imbalance in immunological regulatory mechanisms. The resulting loss of tolerance leads to cytotoxic T cell–mediated hepatocellular injury with important participation of multiple T cell subsets and B cells.[6,7]

IMMUNOLOGICAL DISEASE PATHWAYS IN AIH

Presentation of self-antigen to naive T cells in the presence of costimulation can lead to development of T helper 1 (T_h1), T_h2, and T_h17 pathogenic pathways, and the relative predominance of key stimulatory cytokines can favor one pathway over another (Table 1). Importantly, cytokines produced by the local innate immune response can direct polarization of the adaptive immune response toward autoantigens. In the presence of interleukin-12 (IL-12), the naive T cell can differentiate to a Th1 phenotype, characterized by secretion of interferon-gamma (IFNγ), IL-2, and macrophage migration inhibitory factor (MIF) with development of activated macrophages and effector CD8+ T cells that cause direct cytotoxic damage to antigen-expressing hepatocytes. The presence of IL-4 can promote a $CD4^+$-based Th2 phenotype, characterized by IL-10, IL-4, and IL-13 secretion, which promotes B cell differentiation to antibody-producing plasma cells and complement activation. The combined presence of transforming growth factor β (TGF-β) and IL-6 results in promotion of a Th17 pathway, with subsequent Th17 cell–mediated induced liver damage and release of inflammation cytokines including IL-6, IL-22, and IL-23. At the same time, a predominance of TGF-β results in the development of antigen-specific Tregs. Therefore, a dynamic balance between proinflammatory Th17 and immunosuppressive Tregs can critically determine the immunopathology of AIH. Further illustrating this modifiable and fluid balance, a combination of IL-1B, IL-6, IL-23, and TGF-β can result in transformation of an immunosuppressive Treg into a proinflammatory, antigen-specific Th17 cell. Additional T cell subsets that likely also participate in the pathogenesis of AIH include both γδ T cells and mucosal-associated invariant T (MAIT) cells, with the latter expressing retinoic acid–related orphan receptor γt, which promotes increased IL-17 production.

TABLE 1. KEY INFLAMMATORY PATHWAYS IN THE DEVELOPMENT OF AIH

Phenotype	Key Stimuli	Cytokines Secreted	Effects on Pathogenesis
Th1	IL-12	• IFNγ • IL-2 • MIF • IL-1β	• CD8+ effector T cell recognition of self-antigen on major histocompatibility complex class I and class II on hepatocytes, cytotoxic damage • NK cell activation
Th2	IL-4	• IL-4 • IL-10 • IL-13 • IL-21	• $CD4^+$ Th cell • B cell differentiation to antibody-producing plasma cells • Complement activation, antibody-mediated cytotoxicity • NK recognition of Fc on hepatocytes
Th17	TGF-β, IL-1β, IL-6	• IL-17 • IL-22 • IL-23 • TNF-α	• Secretion of IL-6 by hepatocytes • Th17 effector T cell–mediated hepatocyte damage

The role of γδ T cells, follicular T cells, and MAIT cells in AIH is under investigation.

One illustration of the relationship between these aberrant T cell phenotypes and clinical challenges in AIH was recently reported by Renand et al.[8] The high incidence of relapse in AIH despite prolonged biochemical remission is well known, and the authors demonstrate a reduction of the peripheral Th1/Th17 cytokine profile of memory CD4 T cells and elevation of granzyme B–producing MAIT cells in newly diagnosed patients with AIH compared with control subjects. Furthermore, patients with AIH who achieved biochemical remission also failed to correct these Th1/Th17 and MAIT cell imbalances, demonstrating that a deeper immunological restoration of tolerance does not occur despite satisfactory resolution of hepatitis using standard immunosuppression.

The role of B cells in promoting AIH has historically been underemphasized in the context of this T cell–mediated disease. However, B cells play an important role in AIH both by generating autoantibodies and by inducing T cell responses through antigen presentation and cytokine production.[9] Autoantibodies in AIH are clinically useful as diagnostic biomarkers for categorizing disease subtypes. In addition, type 2 AIH, which has a specific autoantibody (anti-LKM [Liver-Kidney Microsomal antibody]), is characterized by an immunodominant epitope on the hepatocyte membrane (cytochrome P450 2D6 [CYP2D6]) that is the actual disease-specific autoantigen and plays a direct pathogenic role. However, the antigens in type 1 AIH, which is more common in adults, are unknown and may well comprise a multitude of heterogeneous antigens generating variant immunological responses over time.

A key question in the study of AIH pathogenesis is what leads to perpetuation of T cell–mediated inflammation itself long after the presumed absence of the inciting antigenic stimulus. The initial environmental stimuli and antigen/T cell contact may in fact cease, but inflammation can persist through molecular mimicry and perpetuation of cytokine-driven injury. One murine study tested varying levels of homology to the human CYP2D6 antigen and, surprisingly, found that T cell reactivity was highest to intermediate homology, suggesting that molecular mimicry for a similar antigenic target may more strongly induce reactivity compared with a fully identical target.[10] This inducible response to CYP2D6 also highlights the possibility for drug metabolites and haptens to result in an autoimmune recognition and response to the epitope.

APPLYING THE STUDY OF IMMUNOPATHOGENESIS TO NOVEL THERAPEUTIC APPROACHES

The lack of innovative therapies to treat AIH is a major area of unmet need, and novel approaches under development are based on a translational understanding of key pathogenic mechanisms (Table 2). The role of Tregs in the pathogenesis of AIH remains an important topic that has been actively explored in recent years. Whereas some authors have reported numerical and functional deficiency of peripheral Tregs in AIH,[11] other studies have suggested normal functionality and numbers in the liver compartments itself,[12] potentially because of methodological differences.[13] The ability of Tregs to suppress autoimmune inflammation, in both antigen-specific and -independent manners, highlights the potential role of Treg expansion in the restoration of tolerance in AIH. Indeed, Treg-directed therapy, through *ex vivo* expansion or IL-2 administration, is increasingly tested in the context of posttransplant tolerance,[14] and studies in AIH are anticipated shortly. Furthermore, improvement in Treg functionality with reduction in hepatic inflammation may be possible through inhibition of the IL-17 pathway,[15] and further research is needed in this area.

There is new interest in reducing T cell–induced liver injury through reduction of pathogenic B cells. Specifically, recent case reports have highlighted the potential role of

TABLE 2. APPLICATIONS OF IMMUNOPATHOGENESIS INSIGHTS TO PROPOSED NOVEL TREATMENTS IN AIH

Approach	Agent(s)	Goal
Treg adoptive transfer	• *Ex vivo* generation and expansion of antigen-specific and -independent Tregs	Suppression of inflammation, long-term restoration of tolerance
Treg expansion	• Infusion of low-dose IL-2	Expansion of suppressive Treg populations in the liver, long-term restoration of tolerance
CD20 depletion	• Anti-CD20	Reduction in plasma cell activity and cross-presentation of self-antigen from B cells to T cells
BAFF receptor antagonism	• BAFF receptor antibody	Reduction in B cell survival and cross-presentation of self-antigen from B cells to T cells

B cell depletion therapy (anti-CD20) in refractory cases of AIH.[16] Furthermore, the tumor necrosis factor (TNF) superfamily cytokine receptor B cell–activating factor (BAFF), produced by T cells and critical for B cell survival, is increasingly a targeted focus for autoimmune disorders to reduce T cell–mediated damage through B cell modulation.[17] Indeed, BAFF receptor inhibition is increasingly evaluated as a novel therapeutic approach, and a new clinical trial in AIH is underway (NCT03217422).

In summary, the liver is a highly tolerant organ, although AIH can occur through a variety of T and B cell mediated mechanisms, and is refractory to restoration of tolerance by currently available treatment strategies. The importance of understanding the most relevant immunological pathways is made clear by recently proposed innovative therapeutic approaches, which are critically necessary to successfully advance the management of AIH in the 21st century.

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