REVIEW

Under-Evaluated or Unassessed Pathogenic Pathways in Autoimmune Hepatitis and Implications for Future Management

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Abstract

Autoimmune hepatitis is a consequence of perturbations in homeostatic mechanisms that maintain self-tolerance but are incompletely understood. The goals of this review are to describe key pathogenic pathways that have been under-evaluated or unassessed in autoimmune hepatitis, describe insights that may shape future therapies, and encourage investigational efforts. The T cell immunoglobulin mucin proteins constitute a family that modulates immune tolerance by limiting the survival of immune effector cells, clearing apoptotic bodies, and expanding the population of granulocytic myeloid-derived suppressor cells. Galectins influence immune cell migration, activation, proliferation, and survival, and T cell exhaustion can be induced and exploited as a possible management strategy. The programmed cell death-1 protein and its ligands comprise an antigen-independent inhibitory axis that can limit the performance of activated T cells by altering their metabolism, and epigenetic changes can silence pro-inflammatory genes or de-repress anti-inflammatory genes that affect disease severity. Changes in the intestinal microbiota and permeability of the intestinal mucosal barrier can be causative or consequential events that affect the occurrence and phenotype of immune-mediated disease, and they may help explain the female propensity for autoimmune hepatitis. Perturbations within these homeostatic mechanisms have been implicated in experimental models and limited clinical experiences, and they have been favorably manipulated by monoclonal antibodies, recombinant molecules, pharmacological agents or dietary supplements. In conclusion, pathogenic mechanisms that have been implicated in other systemic immune-mediated and liver diseases but under-evaluated or unassessed in autoimmune hepatitis warrant consideration and rigorous evaluation.

Keywords Pathogenesis · Immunomodulation · Epigenetic changes · Intestinal microbiome

Introduction

Multiple disturbances in immune regulatory pathways have been described in autoimmune hepatitis, but full understanding and integration of these pathogenic pathways into a cohesive knowledge base are incomplete [1, 2]. Studies have implicated molecular mimicries as a basis for epitope spread and loss of self-tolerance [3–6]. Dysregulated cytokine pathways may favor the differentiation and proliferation of liver-infiltrating cytotoxic CD8+ T lymphocytes [7–15], and over-expression of certain chemokines may orchestrate the migration of inflammatory and immune cells to the injured site [16]. Regulatory T cells (Tregs) may lack the number and function to suppress immune-mediated tissue damage [17–21], and disturbances in counter-regulatory molecules, such as microribonucleic acids (miRNAs) [22, 23], programmed cell death-1 (PD-1) protein [23–28], soluble CD163 [23, 29, 30], macrophage migration inhibitory factor (MIF) [23, 31, 32], and B cell-activating factor (BAFF) [23, 33, 34], may contribute to the homeostatic imbalance.

Genetic predispositions inside and outside the major histocompatibility complex (MHC) have been described [35–41]. Pathogenic processes that influence the survival of hepatocytes and activated lymphocytes (apoptosis) are being scrutinized [42–44]. Oxidative and nitrosative stresses that may damage cellular membranes, disrupt mitochondrial function, and promote hepatocyte loss are being defined [45–49], and efforts to monitor and prevent or reverse hepatic fibrosis are being developed [50–53]. Despite these investigational activities, there are several fronts that are

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being explored in diverse immune-mediated diseases that have been under-evaluated or not considered in autoimmune hepatitis.

The molecular signaling pathway involving the T cell immunoglobulin mucin proteins (Tims) and galectins can modulate T lymphocyte activity and the function of Tregs [54–57]. T cell exhaustion can result in a dysfunctional state characterized by sustained expression of inhibitory receptors, reduced T cell function, and altered outcome of immune-mediated diseases [58–65]. Dysregulation of antigen-independent inhibitory signals may favor autoimmunity [66–68], and alterations in the methylation of deoxyribonucleic acid (DNA) and modifications of the histones within nucleosomes may affect the transcriptional activity of genes that promote or suppress inflammatory activity (epigenetic changes) [69–71]. Furthermore, bacterial products and activated T lymphocytes may translocate from the intestinal microbiome to the peripheral lymph nodes and shape the immune response [72–74]. These elements have been implicated in the occurrence and severity of systemic immune-mediated and diverse liver-specific diseases, and they warrant rigorous evaluation in autoimmune hepatitis. This knowledge might in turn indicate pivotal pathogenic disturbances that could be targeted for therapeutic intervention.

The goals of this review are to describe key pathogenic pathways that have been under-evaluated or not examined in autoimmune hepatitis, indicate the potential role of these pathways in influencing disease occurrence and outcome, describe the insights in pathogenesis that may shape future therapies, and encourage the extension of investigational efforts into these areas.

Methods

English abstracts were identified in PubMed using the search words "pathogenesis of autoimmunity" and "pathogenesis of autoimmune hepatitis." Abstracts judged pertinent to the review were identified; key aspects were recorded; and full-length articles were selected from relevant abstracts. A secondary bibliography was developed from the references cited in the selected full-length articles, and additional PubMed searches were performed to expand the concepts developed in these articles. The discovery process was repeated, and a tertiary bibliography was developed after reviewing selected articles from the secondary bibliography. The number of abstracts cited by PubMed from March 2015 to November 2017 and reviewed for pertinence to this review during the primary search was 850. The number of abstracts reviewed during the secondary and tertiary reviews exceeded 100. The abstracts judged most pertinent to the topic exceeded 300,

and the number of full-length articles reviewed exceeded 100.

Results

Six pathogenic pathways implicated in diverse systemic and liver-specific diseases have had little or no consideration in autoimmune hepatitis (Fig. 1). These pathways can modulate the adaptive immune response [54–57], alter the responsiveness of T lymphocytes after chronic stimulation [59, 61], weaken antigen-independent co-inhibitory signals that check immune activity [66, 67], influence the transcriptional activity of pivotal regulatory genes [69–71], and promote autoimmunity after translocation of gut-derived bacterial antigens and activated immune cells to the systemic circulation [72]. Perturbations within these pathways have possible pathogenic implications in autoimmune hepatitis, and their role in this disease warrants scrutiny.

Pathway Involving T Cell Immunoglobulin Mucin Proteins

The T cell immunoglobulin mucin proteins (Tims) constitute a family of three glycoproteins expressed on the surface of T helper-1 (Th1) lymphocytes, Th2 lymphocytes, and dendritic cells [54, 75, 76] (Table 1). Tim-2 shares 85% nucleotide sequence homology with Tim-1 [77], and Tim-1, Tim-3, and Tim-4 are the family members recognized in humans [78]. All Tim proteins have common structural motifs that characterize the family, and an immunoglobulin variable (IgV) domain constitutes a cleft that can serve as a receptor for ligand binding [79–81]. Each Tim family member influences the immune response, and together they modulate peripheral immune tolerance. Their pathogenic roles in autoimmune hepatitis are speculative.

Tim-1

Tim-1 polarizes the differentiation of activated CD4$^+$ lymphocytes, and it is preferentially expressed on Th2 lymphocytes [82] (Table 1). The secretion of interleukin (IL)-10 by B cells is promoted by Tim-1 [83], and regulatory B cells (Bregs) expressing Tim-1 have broad anti-inflammatory actions. Bregs reduce the secretion of IL-17 and interferon gamma (IFN-γ), and they favor the generation of Tregs [84–88]. Phosphatidylserine is present on apoptotic cells, and the IgV domain of Tim-1 serves as a receptor for phosphatidylserine. Bregs expressing Tim-1 can bind apoptotic bodies, and this ligation increases the production of IL-10 [88] which in turn can suppress immune reactivity [89, 90].

Tim-1 is also present on Th1 cells, and it can help regulate the activity of this population (Table 1). Blockade of
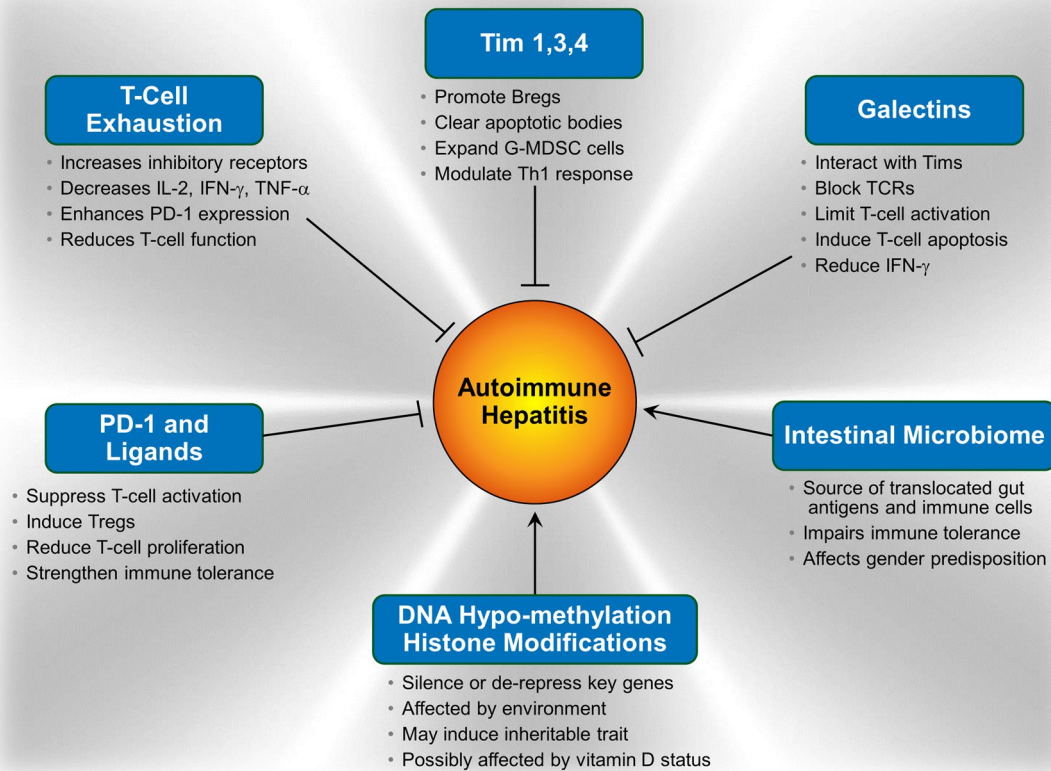
Fig. 1 Under-evaluated or unassessed pathogenic factors in autoimmune hepatitis. The family of T cell immunoglobulin mucin proteins (Tims) promotes the secretion of interleukin (IL)-10 by regulatory B cells (Bregs), clears apoptotic bodies, expands the population of granulocytic myeloid-derived suppressor cells (G-MDSCs), and modulates the response of T helper 1 (Th1) lymphocytes. These combined actions could reduce the severity of autoimmune hepatitis (blunted arrow). The family of galectins can interact with the Tims family and block T cell antigen receptors (TCRs), limit T cell activation, induce T cell apoptosis, and reduce the production of interferon gamma (IFN-γ). These combined actions could also have an inhibitory effect on disease activity (blunted arrow). In contrast, changes in the composition of the intestinal microbiome (dysbiosis) and increased permeability of the intestinal mucosal barrier could enhance disease activity (sharp arrow) by facilitating translocation of gut-derived microbial antigens and activated immune cells into the systemic circulation, impairing immune tolerance, and affecting gender predisposition for the disease. Epigenetic changes manifested by hypomethylation of deoxyribonucleic acid (DNA) and histone modifications could also enhance disease activity by altering the transcriptional activity of key genes. Vitamin D status is one factor that may affect the gene response (sharp arrow). The programmed cell death-1 protein (PD-1) and its ligands and T cell exhaustion might help counter the immune stimulatory effects on disease severity (blunted arrows) by reducing T cell activation, inducing the formation of regulatory T cells (Tregs), and limiting the production of pro-inflammatory cytokines, including IL-2, IFN-γ, and tumor necrosis factor alpha (TNF-α). Perturbations in these interactive regulatory mechanisms could influence the persistence and severity of autoimmune hepatitis, and their investigation could suggest corrective interventions

Tim-1 expression with a Tim-1-specific antibody has inhibited Tim-1-dependent Th1 responses while preserving Th2 responses in a murine model of cardiac transplantation [91]. The net result has been prolonged survival of the fully mismatched cardiac allografts [91]. These findings suggest that Tim-1 may have positive or negative inhibitory effects on different T cell populations.

The avidity with which Tim-1 is engaged on the surface of the T cell is a major determinant of T cell behavior (Table 1). Anti-Tim-1 antibodies with a high infinity for Tim-1 can increase the population of antigen-specific T cells, the secretion of IFN-γ and IL-17, and the severity of experimental autoimmune encephalitis [78]. In contrast, low affinity antibodies can prevent these pro-inflammatory responses and promote a Th2 response [78]. Clarification of the immunomodulatory effects of Tim-1 in autoimmune hepatitis might suggest opportunities for targeted intervention.

Tim-3

Tim-3 is expressed on activated Th1 lymphocytes, Tregs, natural killer (NK) cells, and antigen-presenting dendritic cells [92] (Table 1). The ligation of Tim-3 with galectin-9 modulates the Th1 immune response directly by triggering

the death of Th1 lymphocytes that secrete IFN-γ [55, 93, 94]. IFN-γ stimulates the secretion of galectin-9 by Th1 lymphocytes, and the galectin-9 in turn marks the Th1 lymphocytes for destruction [93, 94].

Tim-3 can also dampen the Th1 response indirectly by expanding the population of granulocytic myeloid-derived suppressor cells (G-MDSCs) that are produced in high levels in cancer, inflammation, and infection [94–98] (Table 1). These cells constitute a population of neutrophils that are close to naïve neutrophils [98], but they are characterized by the surface markers CD11b+Ly6G+ (lymphocyte antigen 6 complex, locus G) [94, 99].

G-MDSCs can suppress T lymphocyte activity by generating reactive oxygen species (ROS) [95], by secreting immunosuppressive molecules such as CD200 that can inhibit monocytes, granulocytes, and T lymphocytes [97, 100, 101], and by promoting the dissociation of antigenic peptides from the T cell antigen receptor (TCR) of CD8+ T lymphocytes [102]. Tim-3 on the surface of macrophages and dendritic cells can also bind apoptotic bodies, and it can reduce autoantibody production by promoting phagocytosis of the apoptotic bodies and eliminating them as potential autoantigens [103]. The role of G-MDSCs in autoimmune hepatitis is unknown.

Tim-4

Tim-4 is present on macrophages and dendritic cells [81], and it is the natural ligand for Tim-1 [104] (Table 1). Tim-4 can also bind to phosphatidylserine on the surface of apoptotic cells in conjunction with Tim-1, and it can promote the phagocytosis and clearance of these potential antigens [81]. The amount of Tim-4 influences the degree of its ligation with Tim-1, and this interaction in turn affects T lymphocyte proliferation. High doses of Tim-4 promote T lymphocyte proliferation, whereas low doses are inhibitory [78, 104]. In this fashion, Tim-4 can modulate the anti-inflammatory effects of Tim-1. Interventions that manipulate Tim-4 levels might be useful in managing immune-mediated diseases, and this possibility is another reason to consider evaluation of the Tim family members in autoimmune hepatitis.

Pathway Involving Galectins

Galectins are a family of guanosine diphosphates that are secreted directly into the extracellular environment without passing through the endoplasmic reticulum and Golgi apparatus [105, 106]. Galectins can bind β-galactosides in glycoproteins, and they can influence molecular interactions at the cell surface of immune cells [107] (Table 1). Alterations in the glycosylation of cell surface molecules can affect immune cell migration, activation of Toll-like receptors

(TLRs), and differentiation of naïve T lymphocytes into Th1, Th2, and Th17 populations [107–110]. Macrophages, dendritic cells, B and T lymphocytes, Kupffer cells, and endothelial cells express galectins, and these molecules are abundant in the liver [106, 111].

Galectin-3 has sequence homology with the anti-apoptotic B cell leukemia/lymphoma-2 molecule (Bcl-2), and it can promote T cell proliferation or hepatocyte survival by preventing apoptosis [112]. Galectin-3 can also form a lattice with glycoprotein which restricts the presentation of antigen by TCRs. Galectin-3 may thereby increase the threshold for T lymphocyte activation and limit the autoreactive response [113].

Galectin-9 is the ligand of Tim-3 [79], and studies are beginning to clarify its role in liver disease [19, 106, 114, 115] (Table 1). The interaction between Tim-3 and galectin-9 increases the flux of calcium into Th1 lymphocytes and selectively induces their aggregation and death. Th1 immune responses are inhibited, and the production of IFN-γ is reduced [55, 116]. Galectin-9 can also modulate signaling pathways that influence cytokine profiles and induce apoptosis independently of Tim-3 [107, 117].

In experimental rheumatoid arthritis, galectin-9 has decreased the production of pro-inflammatory cytokines, IL-12, IL-17, and IFN-γ, in synovial fluid. It has also increased the in vitro differentiation of naïve T lymphocytes into Tregs while suppressing their differentiation into pro-inflammatory Th17 cells [118]. In a murine model of chronic eye infection by herpes simplex virus (HSV), the administration of galectin-9 has reduced the severity of keratitis by increasing apoptosis of effector T cells, reducing the production of pro-inflammatory cytokines, and expanding the population of Tregs [119].

Paradoxically, galectin-9 in the absence of Tim-3 has increased in a dose-dependent fashion the secretion of the pro-inflammatory cytokines IFN-γ and tumor necrosis factor alpha (TNF-α) by cultured T helper lymphocytes [117]. These observations underscore the variable and inverse actions of certain immune modulators depending on their amount and microenvironment. They also indicate the difficulty in developing molecular interventions that are precisely targeted and dosed to minimize unsuspected and unwanted consequences [120].

Tim-3 and Galectin-9 in Non-autoimmune Liver Diseases

The Tim-3/galectin-9 pathway is being investigated in diverse non-autoimmune liver diseases [106]. Plasma levels of galectin-9 are upregulated in chronic hepatitis C, and recombinant galectin-9 can expand Tregs and promote the apoptosis of virus-specific CD8+ cytotoxic T lymphocytes [121]. The reduced immune reactivity may in turn facilitate

the maintenance of chronic infection with the hepatitis C virus (HCV) [106, 122].

Galectin-9 and Tim-3 are upregulated in nonalcoholic fatty liver disease (NAFLD), and the apoptosis of natural killer T (NKT) cells is promoted [106, 123]. The production of IL-15 is also stimulated by Tim-3 and galectin-9, and IL-15 induces proliferation of NKT cells. The counterregulatory actions of NKT cell depletion and stimulation may modulate inflammatory activity in NAFLD [123].

Galectin-9 levels are also increased in the plasma of patients with acute alcoholic hepatitis compared to patients with alcoholic cirrhosis and healthy individuals [106, 124]. High levels of galectin-9 and Tim-3 are expressed by T lymphocytes from these patients, and they may weaken the innate immune response that protects against septic complications [124].

Tim-3 and Galectin-9 in Autoimmune Hepatitis

Concanavalin A induces a severe immune-mediated liver injury that is dependent on activated T lymphocytes, and recombinant galectin-9 has ameliorated this injury in a murine model [114, 115]. Circulating levels of pro-inflammatory cytokines (TNF-α, IFN-γ, and IL-6) are reduced; serum levels of alanine (ALT) and aspartate (AST) aminotransferase improve; concanavalin A-specific $CD4^+$ T cells undergo apoptosis; and the number of Tregs is expanded [114]. Furthermore, survival has been prolonged, and histological features improved in treated mice compared to control mice [115].

In patients with autoimmune hepatitis, the expression of galectin-9 on Tregs is reduced, and the presentation of Tim-3 on the surface of naïve $CD4^+CD25^-$ T cells is downregulated [19]. These changes may diminish the function of the Tregs and render T lymphocytes less able to be regulated. The net effect may be worsening of disease activity as suggested by the inverse correlation between Tim-3 downregulation and serum AST levels [19].

Further insights into the regulatory deficiencies and homeostatic imbalances of the Tims and galectins involved in autoimmune hepatitis may identify factors that can be manipulated to attenuate disease activity.

Pathway Influencing T Cell Exhaustion

$CD4^+$ and $CD8^+$ T lymphocytes are the principal cell populations that influence the occurrence and severity of autoimmune hepatitis [125], and multiple molecular and cellular interactions influence the activation, differentiation, proliferation, and survival of these cells [2, 16]. Evolving management strategies in autoimmune hepatitis have been designed to disrupt or modulate these activation pathways [126], but

none has been formulated to therapeutically induce T cell exhaustion.

T cell exhaustion has been well described in experimental models and patients with persistent antigen stimulation associated with chronic infection (animal models) [58, 127, 128] and malignancy (animal models and patients) [60, 65, 129] (Table 1). It has been recognized as a deleterious consequence of persistent antigen exposure that weakens immune-mediated defense mechanisms, and reversal of T cell exhaustion has been a therapeutic goal in these instances [62, 65]. Intentional induction of T cell exhaustion as a management strategy for autoimmune disease has only recently be considered, and the concept has been justified by the recognized plasticity of the $CD4^+$ and $CD8^+$ T cell populations and the multiplicity of factors that modulate their exhaustion [61, 63, 64].

T cell exhaustion in autoimmune disease may have the beneficial effect of attenuating disease activity and preventing relapse [61, 63, 64]. Studies have already demonstrated that T cell exhaustion can be reversed [130, 131] and induced [61]. This plasticity of the $CD4^+$ and $CD8^+$ T cells suggests that manipulations are possible to adjust effector T cell activity by direct intervention [64]. The mechanisms that induce or prevent T cell exhaustion in autoimmune hepatitis have not been evaluated, but their clarification might direct future management strategies.

Characteristics of T Cell Exhaustion

T cell exhaustion connotes a dysfunctional state in which immune responsiveness becomes progressively diminished during persistent antigen exposure and stimulation of TCRs [59, 61–63, 129, 132, 133]. The exhausted phenotype is characterized by the expression of multiple inhibitory receptors on the cell surface, progressive loss of cytokine production (IL-2, IFN-γ, and TNF-α), and altered metabolic and transcriptional activity [62, 65, 134] (Table 1).

The expression of the programmed cell death-1 (PD-1) protein is a key manifestation of T cell exhaustion [135–137], but 12 other inhibitory receptors have been described to date. These co-inhibitory surface molecules are differentially expressed and associated with the exhausted state, and they include cytotoxic T lymphocyte antigen-4 (CTLA-4), lymphocyte-activated gene-3 (LAG-3), NK cell receptor 2B4 (CD244), Tim-3, B and T lymphocyte activator (BTLA), NK cell receptor BY55 (CD160), and CD200 [58, 65, 134, 138, 139]. The co-expression of multiple inhibitory receptors is the requisite feature of T cell exhaustion, and the number of these inhibitory receptors reflects the severity of the exhaustion [62, 138].

Both $CD4^+$ and $CD8^+$ T cells can become progressively exhausted, but their exhausted phenotypes may differ

[134, 139]. The expression of inhibitory molecules and transcriptional factors are similar between the two T cell populations, but CD4$^+$ T cells exhibit more pronounced expression of CTLA-4, BTLA-4, and CD200 [134, 139]. Exhausted CD8$^+$ T cells have reduced expression of the interleukin-7 receptor (IL-7R), and this reduction correlates with the duration of antigen exposure. The reduction of IL-7R is also associated with decreased expression of the anti-apoptotic Bcl-2 and a shortened survival of the exhausted CD8$^+$ T cells [140].

The expression of IL-7R is a feature of memory T cells whose proliferation after clearance of antigen and ability to reactivate with restimulation are maintained by IL-7 and IL-15 [61, 62]. The level of IL-7R expression is a distinguishing feature between exhausted CD8$^+$ T cells and memory T cells, and the cell surface display of IL-7R^{low}PD-1^{high} has been proposed as the phenotype of exhausted CD8$^+$ T cells [61].

Severity of T Cell Exhaustion

T cells do not exhibit uniform responses during chronic antigen exposure, and subsets expressing different co-stimulatory molecules may have different proliferative potentials and propensities for exhaustion [65, 141]. Furthermore, exhausted T cells do not have the same severity of exhaustion or ability to recover. Exhausted T cells that have highly expressed T-bet (T-box transcription factor) and intermediately expressed PD-1 (T-bet^{high}PD-1^{int}) are still able to secrete IFN-γ and TNF-α, proliferate at a reduced level, and recover from exhaustion [65, 142]. In contrast, T cells with the highly expressed transcription factor, eomesodermin (eomes), and inhibitory factor, PD-1, are in a severe state of exhaustion with limited reversibility, and they are defined by the surface markers, eomes^{high}PD-1^{high} [62, 143, 144]. Variations among T cell subsets in their propensity to become exhausted and in the severity and reversibility of the exhaustion that develops can influence the effectiveness of interventions designed to induce or reverse the exhausted state.

Modulators of T Cell Exhaustion

Co-stimulatory signals can override the propensity for CD8$^+$ T cell exhaustion, and activated CD4$^+$ T cells are the main promoters of continuous CD8$^+$ T cell activity [61, 62]. In the absence of the co-stimulatory effect of CD4$^+$ T cells, CD8$^+$ T cells develop an exhausted phenotype during persistent antigen exposure [145] (Table 1). IL-21, produced by activated CD4$^+$ T cells, induces the proliferation of CD8$^+$ T cells, and it thereby preserves their identity as effector

cells [146–151]. CD2 is also instrumental in preventing the exhaustion of CD8$^+$ T cells in vitro [61].

CD2 is an adhesion molecule on the surface of T cells, and it maintains their function [152]. T cells with low levels of CD2 are associated with increased viral loads and disease progression in untreated patients with human immunodeficiency virus (HIV) [153]. Similarly, patients with advanced melanoma and recurrent disease have fewer CD2-positive lymphocytes and shorter survivals than patients without recurrent disease [154]. In these instances of chronic infection and malignancy, low levels of CD2 expression may have contributed to exhaustion of the immune defenses and worsening of the disease.

Other molecules that favor T cell exhaustion are IL-10 and PD-1 [135, 155] (Table 1). The inhibitory effects of IL-10 and PD-1 on CD8$^+$ T cells are independent and synergistic. Simultaneous blockade of both inhibitory pathways invigorates antiviral T cell responses more than blockade of any individual pathway [155]. Transforming growth factor beta (TGF-β) has been associated with increased apoptosis of virus-specific CD8$^+$ T cells, but it has not been essential for functional exhaustion [156], as confirmed by blocking experiments [157, 158].

Therapeutic induction of T cell exhaustion is a fresh concept in the management of autoimmune diseases, and there are multiple modulators within the network that maintains T cell activity [61–64]. Investigations of T cell exhaustion in autoimmune hepatitis may clarify mechanisms that perpetuate effector cell activity and survival despite chronic antigen stimulation. These insights may help explain variations in disease severity and propensities for spontaneous exacerbation or relapse after drug withdrawal, and they may also direct interventional strategies.

Pathway Involving Programmed Cell Death-1 (PD-1) Protein and Its Ligands

PD-1 (CD279) is a 288 amino acid protein that is expressed on the surface of activated T cells, B cells, NK cells, Kupffer cells, and thymocytes [24, 67, 68]. It is an antigen-independent inhibitory receptor that suppresses T cell activation, decreases lymphocyte proliferation, and maintains immune tolerance [66, 159] (Table 1). The actions of PD-1 can also synergize with signals mediated by TGF-β [159], and this synergy can induce naïve T cells to transform into Tregs [160].

The ligands of PD-1 are programmed death ligand-1 (PD-L1) [161, 162] and programmed death ligand-2 (PD-L2) [163]. These ligands are each preferentially expressed on different cell surfaces, including hepatic sinusoidal endothelial cells, Kupffer cells, hepatocytes, and hepatic stellate cells (PD-L1) [164] and dendritic cells and activated macrophages (PD-L2) [165]. The expression of each ligand is enhanced by

pro-inflammatory stimuli (lipopolysaccharide [LPS], IFN-γ, and IL-4) [166], and their ligation with PD-1 dampens the adaptive immune response [136] (Table 1). The principal inhibitory mechanism of the ligation is to change the metabolism of activated T cells from aerobic glycolysis to fatty acid oxidation [167–170]. This metabolic switch to fatty acid oxidation may also induce the formation of Tregs [170, 171].

The expression of PD-1 is the hallmark of T cell exhaustion [59, 61, 63, 135–137], and the PD-1/PD-L1/2 pathway has also been incriminated by preliminary studies in autoimmune hepatitis [24–28]. For these reasons, the PD-1 axis warrants further investigation as a potential target of therapeutic interventions designed to strengthen its immune inhibitory effects.

PD-1 Inhibitory Axis and Autoimmune Hepatitis

Serum antibodies to PD-1 (anti-PD-1) have been present more frequently in patients with autoimmune hepatitis (63%) than in normal individuals (3%) and patients with drug-induced liver injury (8%), acute viral hepatitis (13%), or primary sclerosing cholangitis (18%) [26] (Table 1). The anti-PD-1 levels have also correlated with serum concentrations of bilirubin and ALT, and the presence of anti-PD-1 has been associated with a slow response to corticosteroid treatment and relapse after drug withdrawal [26]. The pathogenic effects of circulating anti-PD-1 in autoimmune hepatitis are unknown, but one possibility is that the disease-related antibodies block the inhibitory signals generated by PD-1 and thereby promote T cell activation and proliferation.

Variant forms of the PD-1 molecule can be expressed by the *PD-1 gene*, and one variant is soluble because the domain encoded by exon 3 that anchors the molecule to the cell membrane has been spliced out [172]. This variant soluble form (PD-1Δex3) has an intact extracellular domain and ligates normally with PD-L1 and PD-L2. Patients with autoimmune hepatitis who have untreated active disease, disease in exacerbation, and incomplete responses to standard corticosteroid therapy have had increased plasma levels of soluble PD-1 [28] (Table 1). These patients have also had a > threefold upregulated expression of PD-1 on CD4$^+$ T cells compared to healthy subjects.

The apparent paradoxical association of increased plasma levels of soluble PD-1 with disease activity in autoimmune hepatitis is unexplained, but the findings suggest that the variant soluble form interferes with the binding of full-length PD-1 with its ligands. In patients with rheumatoid arthritis, the levels of soluble PD-1 in synovial fluid have correlated with synovial levels of TNF-α, and the PD-1Δex3 splice variant has blocked the inhibitory effect of membrane-bound PD-1 on T cell proliferation [173].

Additional evidence of a disturbed PD-1 axis in autoimmune hepatitis has been evident in assessments of liver tissue. Messenger ribonucleic acid (mRNA) for PD-L2 has been less abundant in liver tissue from patients with autoimmune hepatitis than in liver samples from patients with primary biliary cholangitis (PBC) and chronic hepatitis C [24] (Table 1). Furthermore, IL-4, the principal inducer of PD-L2, has been absent in the liver tissue of patients with autoimmune hepatitis. These findings suggest that patients with autoimmune hepatitis have a deficiency of one of the key ligands for PD-1 in liver tissue and that the absence of PD-L2 may compromise the inhibitory effects of PD-1 on the adaptive immune response.

Implications in Autoimmune Hepatitis

The PD-1 axis has been implicated as an inhibitory mechanism by which to induce T cell exhaustion and suppress T cell activation and proliferation. These attributes may be deficient in autoimmune hepatitis as a result of antibodies to PD-1 that interfere with its inhibitory function [26], over-expression of variant soluble PD-1 molecules that block PD-1 signaling [28], or deficient expression of ligands that are essential for PD-1 activity [24]. The results of the preliminary studies disclosing these deficiencies in autoimmune hepatitis warrant further clarification to translate the correlative findings into pathogenic mechanisms. These investigations might also support future efforts to bolster the PD-1 axis [61, 64].

Pathway Involving Epigenetic Changes

Changes in chromatin structure can alter the transcriptional activity of promoter genes without altering the sequence of deoxyribonucleic acid (DNA) [69–71, 174, 175]. These epigenetic changes involve alterations in the methylation status of DNA and modifications of the histones that constitute the chromatin (Table 1). Such changes can silence pro-inflammatory genes or de-repress anti-inflammatory genes and alter the clinical manifestations and outcome of immune-mediated diseases. The changes can be induced by cues from the environment [69–71], and this interaction can strengthen hypotheses that link the occurrence and outcome of autoimmune disease with exogenous factors such as drugs, pollutants, infections, and diet [176–179].

Epigenetic changes can also occur as a natural consequence of aging. Genomic instabilities can be induced by repeated cycles of DNA replication and the cumulative effects of protracted exposure to toxic environmental elements [180–182] (Table 1). A stable inheritable trait may be a consequence of these epigenetic perturbations and influence disease risk in progeny [69, 174, 175]. Individual variations in phenotype, disparities in disease occurrence, and dissimilarities in outcome may also be attributable in part to epigenetic alterations [178].

Table 1 Under-evaluated or unassessed regulatory pathways in autoimmune hepatitis

Under-evaluated pathways	Pathogenic significance	Supportive evidence
Tim network	Tims modulate lymphocyte differentiation and immune tolerance [76] Tim-1 promotes B cell secretion of IL-10 and generation of Bregs and Tregs [88] Tim-3 expands G-MDSCs [94, 97, 98] Tim-4 ligates with Tim-1 and promotes phagocytosis of apoptotic bodies [81] Degree of Tim-1/Tim-4 ligation has pro- and anti-inflammatory effects [78, 104]	Tim-1-deficient mice have reduced IL-10 [83] Anti-Tim-1 increases T cells, IL-17, and IFN-γ in murine encephalitis [78] Tim-1 blockade prolongs survival of mismatched allografts [91] Immune regulatory effects of Tim-1 modulated by Tim-4 ligation [78, 104] Tim-3 upregulated in NAFLD and limits NKT cells [123]
Galectin network	Galectins influence T cell migration, TLR activation, and T cell differentiation [106] Galectin-3 prevents apoptosis, restricts TCRs, and limits T cell activation [113] Galectin-9 ligated with Tim-3 induces T cell apoptosis and reduces cytokines [107]	Galectin-9 reduces IL-17 and IFN-γ and increases Tregs in RA [118] Galectin-9 increases T cell apoptosis and reduces severity of keratitis [119] Galectin-9 and Tim-3 reduced in AIH [19]
T cell exhaustion	Characterized by inhibitory receptors on effector T cells, less cytokine production, and impaired immune response [59, 61, 62]	Therapeutic induction possible [61, 64] Alefacept induces exhaustion [273, 275] Chimeric PD-L1 may be effective [61]
PD-1 axis	PD-1 axis suppresses T cell activation, limits lymphocyte proliferation, expands Tregs [66]	Antibodies to PD-1 in AIH [26] Soluble PD-1 associated with AIH [28] Deficient PD-L2 in liver tissue of AIH [24]
Epigenetic changes	DNA hypomethylation and histone modifications increase gene activity [69, 71] VDRE alters gene function [224, 230]	Present in immune diseases [193, 196] Antagonized by MBDs and enzymes [207] Vitamin D deficiency common in AIH [46]
Dysbiosis and altered intestinal barrier	Gut-derived antigens translocate [262] Microbial products appear in blood [251] Skews autoimmunity to females [266–268]	Dysbiosis associated with AIH [72] Disrupted duodenal mucosa in AIH [72] Gender affects dysbiosis and risk [266–268]

Numbers in brackets are references

AIH autoimmune hepatitis, *Bregs* regulatory B cells, *G-MDSCs* granulocytic myeloid-derived suppressor cells, *IFN-γ* interferon gamma, *IL-10* interleukin-10, *MBDs* methyl-DNA-binding domain proteins, *NAFLD* nonalcoholic fatty liver disease, *NKT* cells, natural killer T cells, *PD-1* programmed cell death-1 protein, *PD-L1* programmed death ligand-1, *PD-L2* programmed death ligand-2, *Th1* T helper 1, *Tims* T cell immunoglobulin mucin proteins, *TLR* Toll-like receptor, *Tregs* regulatory T cells, *VDRE* vitamin D response element

Interventions designed to promote the expression of tumor suppressor genes and upregulate the expression of tumor rejection antigens are being evaluated in malignancy [183–188]. These efforts at epigenetic manipulation suggest that therapeutic interventions that modulate the transcriptional activity of pro- and anti-inflammatory genes in autoimmune disease may be possible [189–191]. Epigenetic changes have been described in systemic lupus erythematosus (SLE) [192, 193], rheumatoid arthritis [194], and systemic sclerosis [195], and they have also been recognized in primary biliary cholangitis (PBC) [196] and NAFLD [197]. Similar investigations are warranted in autoimmune hepatitis to clarify pathogenic mechanisms, risk factors, and management possibilities.

DNA Demethylation

DNA within chromatin is methylated at the site of a cytosine nucleotide that is separated by a phosphate from a guanine nucleotide (CpG site) [69]. These CpG sites are in a linear sequence along the DNA, and their methylation inhibits the binding of transcription factors to the gene [69, 198] (Table 1). Gene activity relates to the density of DNA methylation. Heavily methylated DNA inhibits the binding of transcription factors to the gene promoter and reduces production of gene product. In contrast, hypomethylated DNA accommodates the transcription factors and sustains or promotes gene activity. The gene promoters implicated in SLE, rheumatoid arthritis, and multiple sclerosis are hypomethylated [176, 199].

The methylation status of DNA is modulated by methyl-DNA binding domain proteins (MBDs) which adhere to the methylated CpG site [200, 201] and by the ten eleven translocation oxygenases (TET1-3) [202–205] (Table 1). Some MBDs have DNA demethylase activity which promotes the reversal of methylation and an increase in transcriptional activity, whereas other members of the same family suppress gene activity [206–208]. The TET family of enzymes catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, or 5-carboxylcytosine, and

they can also reverse methylation and increase gene activity [202–205].

Countering the stimulatory actions of certain MBDs and the TET enzymes are the DNA methyltransferases that methylate DNA using S-adenosylmethionine as the principal methyl donor [176, 209, 210] (Table 1). Perturbations in this counter-regulatory interactive network can upset homeostatic balance and contribute to the development and severity of autoimmune disease. The counter-regulatory proteins affecting DNA methylation are logical targets for therapeutic manipulation [189].

Histone Modifications

Four histones arranged as an octamer constitute a nucleosome around which the DNA molecule within the chromatin is wrapped [71, 211]. These histones can be modified by processes (phosphorylation, methylation, acetylation, and ubiquitination) which alter the amino acids residing in the tail of the molecule [212–214]. Counter-regulatory enzymes shape the histone modifications, and their cumulative effects influence the transcriptional activity of the gene [69, 214].

Histone acetyltransferase can transfer an acetyl group to an N-terminal lysine [215], and histone deacetylase can remove the acetyl group [216, 217] (Table 1). Histone methyltransferases can methylate arginine residues [213], and the peptidylarginine deiminases (PADs) can convert histone methylarginine to citrulline [218]. The amino acid alterations induced by the interactive enzymes do not have a predictable effect on gene activity, and the suppression or promotion of transcriptional activity can depend on the nature and location of the amino acid transformations [70, 217, 219]. Strategies to modulate specific enzymes and favorably alter histone structure may have a less certain effect on gene activity than altering the methylation status of DNA [189].

Considerations in Autoimmune Hepatitis

Vitamin D deficiency (serum 25-hydroxyvitamin D3 level, < 50 nmol/L) has been found in 51–68% of patients with non-cholestatic chronic liver disease [220, 221], and the vitamin D level is < 30 μg/L in 81% of Turkish patients with autoimmune hepatitis [46]. The liver hydroxylates the natural skin-derived vitamin D3 to the metabolically inactive circulating form of 25-hydroxyvitamin D3. This circulating form can then be activated in the kidney to 1, 25-dihydroxyvitamin D3 [222, 223]. The active metabolite is a ligand for the vitamin D receptor (VDR) which can form a heterodimer with the retinoid X receptor (RXR) and regulate gene expression by binding to the vitamin D response element (VDRE) located in introns [224, 225]. The binding of VDR to VDRE attracts co-activators and enzymes that can induce histone acetylation, alter chromatin structure, and promote gene transcriptional activity [224]. Genetic polymorphisms can influence the structure of the VDR, and the association of these polymorphisms with autoimmune hepatitis suggests that individuals may have different inherited VDR binding avidity and different epigenetic effects on gene activity [226, 227].

Vitamin D deficiency has been associated mainly with cirrhosis, and it may reflect an inability of the liver to hydroxylate vitamin D3 [220, 221]. Other factors, including diet, malabsorption, and medications that inhibit the cytochrome P450 enzymes involved with vitamin D metabolism, may also contribute [220, 228]. 1, 25-Dihydroxyvitamin D3 has been ascribed diverse immune, inflammatory, and anti-fibrotic effects in chronic liver disease [229, 230], and future investigations of epigenetic changes in autoimmune hepatitis must examine the role of vitamin D deficiency in modulating gene function and promoting disease activity and progression.

Requisites for Therapeutic Intervention

Therapeutic interventions must be directed at the principal epigenetic change that contributes to the disease, and they must be designed for precision targeting and trans-generational tolerance [71]. Induced undirected epigenetic changes may disrupt homeostatic pathways and lead to unwanted consequences and an undesired inheritable trait. Regions near the binding sites for gene regulation are the preferred targets, and most epigenetic variations in the immune-mediated diseases have been in these areas [231] (Table 1). Multiple areas that are collectively arrayed in highly expressed genes are super-enhancer regions, and they are likely to modulate disease-relevant gene function [232–234]. The super-enhancer regions are prized targets for manipulation.

The enzymes that modify the DNA and histones within the chromatin are active at certain sites because of particular genomic sequences and sequence-specific binding factors [189] (Table 1). Different isotypes of the same enzyme family have cell and site specificity because of these genomic sequences and the binding factors that characterize the cell's microenvironment [235, 236]. Interventions can be designed to modulate the particular isotype of the enzyme contributing to the undesired gene effect. In this fashion, the cell type and culpable genomic sequence can be precisely targeted [189].

Progress in designing epigenetic interventions for autoimmune hepatitis requires delineation of the changes that are affecting the disease-pertinent genes and recognition of the enzymatic isotypes and binding proteins shaping those changes. This knowledge will help explain disparities in disease risk between different populations and clarify pathogenic mechanisms.

Pathway Involving Dysbiosis and Translocation of Gut-Derived Antigens and Immune Cells

The human intestinal tract harbors 10–11 trillion bacteria representing 500–1500 species that can be organized in four main phyla (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*) [74, 237–239]. This vast microbial population constitutes the intestinal microbiome, and it remains relatively stable from late childhood through adulthood becoming less diverse and more variable over short intervals in the elderly [239–242]. Multiple environmental factors can influence the composition of the microbiome, including diet, antibiotics, and socioeconomic status [243], but the commensal populations are generally well tolerated because of an acquired immune tolerance [244].

Changes in the composition of the intestinal microflora (dysbiosis) have been associated with diverse autoimmune diseases (type 1 diabetes, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease) [245–249], and these findings have suggested that changes in the intestinal microbiome may overcome intestinal immune tolerance and become a pathogenic factor. The intestinal microbiome has also been implicated in several liver diseases, including autoimmune hepatitis [72, 73, 250, 251] (Table 1).

An animal model of NAFLD has demonstrated the presence of microbial products in the portal circulation and the release of pro-inflammatory cytokines that sustain the disease [252–254]. TLRs in biliary epithelial cells (BECs) respond to lipopolysaccharide (LPS), a major component of gram-negative bacteria, by producing IFN-γ in a murine model of primary sclerosing cholangitis (PSC) [255]. BECs in PBC express TLRs that respond to gut-derived bacterial products (LPS, flagellin, and cytosine-phosphorothioate-guanine oligonucleotide), and the BECs produce the pro-inflammatory cytokines, IL-1β, IL-6, IL-8, and TNF-α [256–258]. Commensal intestinal microflora have had a protective effect in a model of PSC [259], and LPS-stimulated NK cells have contributed to bile duct destruction and disease progression in a model of PBC [260, 261].

The intestinal microbiome is now recognized as a reservoir of immunogenic substrates and activated immune cells that can shape systemic innate and adaptive immune responses by mechanisms of translocation [74]. Gut-derived activated immune cells have been demonstrated in peripheral lymph nodes [262], and bacterial products have been found in the systemic circulation of patients and experimental models with liver disease [72, 254, 263, 264]. In autoimmune hepatitis, a pathogenic role for the intestinal microbiome has been proposed [72, 73] and this possibility warrants further evaluation [265].

Intestinal Microbiome and Autoimmune Hepatitis

Dysbiosis has been described in an animal model and in patients with autoimmune hepatitis (Table 1). The diversity and total load of intestinal bacteria have been reduced in a murine model of autoimmune hepatitis, and the dysbiosis has been associated with disease activity, Th1 response, and reduced number of Tregs in the liver tissue [73]. Dysbiosis, manifested by a reduced number of intestinal anaerobes (*Bifidobacterium* and *Lactobacillus*), has also distinguished patients with autoimmune hepatitis from healthy subjects [72].

Plasma levels of LPS have been higher in patients with autoimmune hepatitis than in healthy individuals, and these findings have justified speculation that translocation of gut-derived microbial products and activated immune cells may be instrumental in the disease [72]. This possibility has been supported by evidence that the intestinal mucosal barrier may be disrupted in these patients [72]. Histological examination of duodenal mucosa has disclosed small, irregularly arranged, duodenal villi with inflammatory cells in the lamina propria and damaged tight junctions (Table 1). Immunohistochemical analyses of the proteins that maintain tight junctions within the duodenal mucosa have also indicated decreased expression of zona occludens-1 and occludin [72]. These findings cannot be extrapolated to the colonic mucosa, but they should encourage comparable assessments of the colonic mucosa and the integrity of its barrier function.

Intestinal Microbiome and Gender Bias

The composition of the intestinal microbiome may influence the female bias for autoimmune disease, and it is a consideration that has not been evaluated in autoimmune hepatitis (Table 1). Female non-obese diabetic (NOD) mice have a 1.3–4.4-fold greater incidence of type 1 diabetes than male NOD mice, and this difference in susceptibility is absent in germ-free animals [266]. Differences in the composition of the intestinal microflora between genders of NOD mice have been eliminated by male castration [266], and transfer of intestinal microbiota from adult male mice to immature female mice has resulted in elevated serum testosterone levels in the females and protection against type 1 diabetes [267]. Furthermore, blockade of the androgen receptor with flutamide has attenuated the changes associated with the male-specific microbiome in the female NOD mice [267]. The transfer of T cells from male mice to flutamide-treated female mice has not protected against the development of diabetes [267].

These demonstrations in genetically susceptible murine models of autoimmune disease indicate that the composition of the intestinal microbiome can vary between genders, be influenced by male hormones, and also produce

testosterone that can alter disease risk in females [266–268]. Similar observations in experimental models and patients with autoimmune hepatitis could not only transform current pathogenic concepts of autoimmune hepatitis and its female propensity but also prove pivotal in developing new management strategies [74, 265].

Therapeutic Implications

The integration of previously under-evaluated or unassessed pathogenic mechanisms into the knowledge base of autoimmune hepatitis could suggest new management strategies (Table 2). The challenges are to identify the predominant mechanism to target in an individual patient, design interventions that are directed with precision, anticipate and minimize side effects, and institute the intervention at the appropriate time and for the optimal duration.

Pathogenic mechanisms may be variably active at different stages of the disease. Inhibitory receptors may not be uniformly expressed on activated T cells or displayed consistently [62]. The numbers and functions of CD4+, CD8+, and regulatory T cells may change during the inflammatory process. Effector cells may enter or reverse exhaustion, and apoptotic activity may fluctuate [62]. Inhibitory molecules may paradoxically have stimulatory effects in certain microenvironments [57, 78], and interventions may be efficacious only when the pathogenic mechanisms are robust [61]. Most importantly, infection and malignancy may be consequences of the disease or its prospective intervention [189]. These factors complicate the emergence of novel therapies, and they must be considered in evaluating the performance of any new management strategy.

Prospective Interventions that Modulate the Tim Proteins

Tim-1 can inhibit or stimulate T cell proliferation and the production of pro-inflammatory cytokines depending on the affinity with which Tim-1 is engaged with its Tim-4 ligand [78, 269]. Two monoclonal antibodies specific for Tim-1 have opposite effects on T cell proliferation, production of

Table 2 Therapeutic considerations based on under-evaluated or unassessed pathways

Interventions	Possible mechanism	Supportive evidence
Blocking antibodies to Tim-1	Inhibition of Tim-1 decreases T cell proliferation and pro-inflammatory cytokines [78, 269]	Inhibitory antibodies characterized [78] Survival of mismatched allografts prolonged by anti-Tim-1 [91]
Recombinant galectin-9	Ligation with Tim-3 increases apoptosis of Th1 cells, impairs Th1 response, and reduces IFN-γ [55, 116]	Murine herpes keratitis improved [119] RA better in patients [118] Treg population expanded [118, 119] Fulminant hepatitis improved [115]
Induced T cell exhaustion	Effector T cells inhibited by targeted modulation of activated CD4+ T cells, IL-21, CD2, IL-10, or PD-1 [61, 64]	PD-L1 induces exhaustion in cultures [61] Alefacept improves psoriasis and type 1 diabetes in patients [273, 275]
Modulation of PD-1 axis	Strengthening of PD-1 signaling suppresses T cell activation and induces formation of Tregs [66, 159, 160]	PD-L1 induces exhaustion in cultures [61] T cell exhaustion upregulates expression of PD-1 [59]
Reversal of epigenetic changes	Reversal of DNA hypomethylation and histone modifications inhibits pro-inflammatory genes or de-represses anti-inflammatory genes [71, 189] Vitamin D supplementation induces histone acetylation that inhibits cytokine production [229]	MBD2 associated with SLE [281] Anti-MBD2 may de-repress genes [282] BET inhibition improved JIA and autoimmune encephalitis [279, 283] S-adenosylmethionine inhibits DNA demethylase activity [285] Vitamin D prevents liver fibrosis [286, 287]
Modulation of intestinal microbiome or permeability of intestinal mucosal barrier	Correct intestinal dysbiosis associated with AIH [72, 73] Strengthen intestinal mucosal barrier to prevent translocation of gut-derived antigens and effector cells [72, 292] Reduce female bias for AIH [266–268]	Re-colonization improves colitis [288] Probiotics expand Tregs and prevent experimental diabetes [289, 290] Blocked TLRs improve murine SLE [291] Gelatin tannate lowers blood LPS [292] Re-colonization and flutamide alter female bias in murine diabetes [266–268] Antibiotics useful in RA and PSC [293, 294]

Numbers in brackets are references

AIH autoimmune hepatitis, *BET* bromodomain and extra-terminal proteins, *DNA* deoxyribonucleic acid, *IFN-γ* interferon gamma, *IL* interleukin, *LPS* lipopolysaccharide, *MBD2* methyl-DNA-binding domain protein 2, *NAFLD* nonalcoholic fatty liver disease, *PD-1* programmed cell death-1 protein, *PD-L1/L2* programmed death ligand-1/2, *PBC* primary biliary cholangitis, *PSC* primary sclerosing cholangitis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *Tim* T cell immunoglobulin mucin protein, *TLRs* Toll-like receptors, *Tregs* regulatory T cells

IFN-γ and IL-17, and the severity of experimental autoimmune encephalomyelitis [78] (Table 2). Both antibodies have closely related epitopes in the IgV domain of the Tim-1 molecule, but the activating antibody has an avidity for the molecule that is 17 times greater than the inhibitory antibody. These findings suggest that blocking antibodies can be designed with an avidity that promotes the desired action of the Tim-1 molecule. A blocking anti-Tim-1 monoclonal antibody has prolonged allograft survival by downregulating Th1 responses, promoting Th2 responses, and enhancing the regulatory activity of Tregs in a murine model of mismatched cardiac transplantation [91].

Prospective Interventions that Modulate Galectin Levels

Galectin-9 administered to murine models has improved the keratitis associated with HSV infection [119] and the histological features and survival of concanavalin-induced fulminant hepatitis [115], and it has reduced the pro-inflammatory cytokines associated with rheumatoid arthritis [118] (Table 2). These findings in experimental models of immune-mediated disease suggest that galectin-9 may evolve as a therapeutic intervention in autoimmune hepatitis [55, 57, 118]. Countering this prospect is an awareness that galectin-9 can act independently of Tim-3 and increase the production of the pro-inflammatory cytokines INF-γ in Th1 cells and TNF-α in Th2 cells in a dose-dependent fashion [107, 117].

Prospective Interventions that Induce T Cell Exhaustion

The known factors that promote the activity of CD8+ T cells and prevent their exhaustion (activated CD4+ T cells, IL-21, and CD2) [61, 146–152] and those that favor exhaustion (IL-10 and PD-l) [135, 155] are potential targets for interventions that induce T cell exhaustion. Preliminary studies have indicated that chimeric PD-L1 can skew the differentiation of non-exhausted robust CD8+ T cells with IL-7R^{high}PD-1^{low} surface markers toward the exhausted phenotype of IL-7R^{low}PD-1^{high} [61] (Table 2). Furthermore, alefacept, a recombinant fusion molecule consisting of lymphocyte function-associated antigen-3 (LFA-3) and immunoglobulin-1 (IgG1), has been engineered to block the CD2 molecule on the surface of T cells [270–275]. Alefacept has already been used clinically to induce histological remission in patients with psoriasis [273] and reduce insulin use and the frequency of hypoglycemic events in patients with recent onset type 1 diabetes [275]. IL-21, a product of activated human CD4+ T cells [146, 147, 276], is another potential therapeutic target as mice that are genetically deficient in IL-21 experience severe exhaustion of CD8+ T cells during chronic infection [149–151].

Prospective Interventions that Strengthen the PD-1 Axis

PD-1 is the hallmark of T cell exhaustion, and advances in promoting T cell exhaustion by suppression of CD4+ T cells, blockage of CD2, or inhibition of IL-21 may be the most promising methods to enhance PD-1 expression and improve immune tolerance [61, 62, 64]. The addition of PD-L1 to cell cultures has moved CD8+ T cells toward exhaustion [61] (Table 2).

Prospective Interventions that Reverse Epigenetic Changes

Investigational efforts to reverse epigenetic changes that suppress anti-inflammatory gene activity or promote the expression of pro-inflammatory gene products have centered mainly on manipulations of the MBDs [208, 277] and the bromodomain and extra-terminal (BET) family of proteins [278–280] (Table 2). DNA hypomethylation and increased transcriptional activity have been associated with MBD2 in patients with SLE [281], and anti-sense oligonucleotides that inhibit MBD2 have limited the growth of human cancer cells in a murine model, possibly by de-repressing tumor suppressor genes [282].

The BET family has histone acetyltransferase activity, and it can increase gene activity by acetylating lysine residues in the histone proteins [278, 280]. Molecular inhibitors of the BET proteins have reduced the activity of super-enhancer regions near gene promoters [279], and they have decreased disease-associated gene expression in patients with juvenile idiopathic arthritis [283], protected against endotoxic shock in animal models [284], and improved experimental autoimmune encephalomyelitis [279].

Powerful methyl donors, such as S-adenosylmethionine, may inhibit DNA demethylase activity and help restore immune homeostasis [285]. These supplements are available, and their evaluation in a well-designed, placebo-controlled, clinical trial would constitute an epigenetic intervention that might be applied to autoimmune hepatitis [285].

The administration of 1, 25-dihydroxyvitamin D3 for 4 weeks to a murine model of cholestatic liver disease has inhibited the activation and proliferation of hepatic stellate cells, increased mRNA levels of metalloproteinase, and reduced serum ALT levels [286]. Similarly, the administration of 1, 25-dihydroxyvitamin D3 to a murine model of toxin-induced liver injury has prevented hepatic fibrosis, inhibited the production of TGF-β, and suppressed the expression of tissue inhibitors of metalloproteinases [287]. In each study, treatment had preventive rather than remedial effects as short-term therapy failed to reduce overall hepatic fibrosis or reverse cirrhosis [286, 287]. Clarification of the role of 1, 25-dihydroxyvitamin D3 in the pathogenesis of autoimmune hepatitis could lead to the evaluation of

preventive and remedial management strategies based on vitamin D supplementation.

Prospective Interventions that Manipulate the Intestinal Microbiome or Prevent Translocation

Modification of the intestinal microbiome has already been evaluated as a therapeutic intervention in multiple experimental models and small clinical trials, and it awaits study in autoimmune hepatitis (Table 2). Allergic diarrhea and colitis have improved in a murine model after intestinal recolonization with 17 strains of human-derived Clostridia selected by their ability to expand Tregs [288]. Two strains of Bifidobacterium have been used as a probiotic supplement to increase the secretion of soluble factors that favor the generation of Tregs in cultures of intestinal epithelial cells [289], and a probiotic enriched in Lactobacillaceae has promoted the generation of tolerogenic dendritic cells, reduced the expansion of Th1 and Th17 cells, and prevented diabetes in NOD mice [290].

Blockade of TLRs that recognize molecular patterns associated with intestinal microbes has attenuated the renal and pulmonary manifestations of experimental SLE [291], and the administration of gelatin tannate, a protector of the intestinal mucosal barrier, has lowered blood levels of LPS and decreased the activity of experimental acute colitis [292]. A meta-analysis of 10 randomized clinical trials in patients with rheumatoid arthritis has supported the use of tetracycline or minocycline [293], and a randomized pilot study of 35 patients with PSC has demonstrated that the administration of vancomycin or metronidazole for 12 weeks reduced serum alkaline phosphatase and bilirubin levels, improved risk scores, and ameliorated pruritus [294].

Dietary adjustments, probiotic preparations, antibiotics, intestinal recolonization, TLR blockade, and pharmacological agents that strengthen the intestinal mucosal barrier have all been mustered to modify the effects of the intestinal microbiome on systemic immune responses and the occurrence of immune-mediated disease [74]. Progress in the management of autoimmune hepatitis by altering the intestinal microbiome must begin by defining the dysbiosis, determining its gender specificity, and establishing dysbiosis as a cause rather than consequence of the disease.

Overview

Autoimmune hepatitis is a consequence of perturbations in homeostatic mechanisms that maintain self-tolerance [2]. Recognition of these regulatory deficiencies is essential in understanding the key mechanisms that induce, sustain, and advance the disease. Identification of pivotal aberrations is also essential in designing corrective strategies [126], and it may generate international collaborations

like the multicenter treatment trial of antibodies to BAFF receptor that is about to launch in autoimmune hepatitis (NCT03217422).

Assessments of the Tim, galectin, and PD-1 pathways are opportunities to discover and manipulate deficiencies in the signaling networks that modulate the adaptive immune response [67, 76, 105, 106]. Assessments of the mechanisms that prevent or induce T cell exhaustion may suggest therapeutic interventions that can modify disease severity or terminate the disease [61, 64]. Assessments of epigenetic changes may identify unsuspected inheritable traits for autoimmune hepatitis, clarify the burden of risk for this disease, and generate studies that discover environmental cues for its occurrence, including acquired vitamin D deficiency [69–71]. Assessments of the intestinal microbiome and the permeability of the intestinal mucosal barrier may help explain loss of tolerance for self-antigens, female propensity for autoimmune hepatitis, and varied susceptibility patterns among different age groups, genders, ethnicities, and geographical regions [74, 265].

Autoimmune hepatitis should be fully integrated into the family of immune-mediated diseases, and pathogenic insights made within this family should be recognized as opportunities to expand disease-specific concepts. Shared insights from studies and experiences within the family of autoimmune diseases can contribute to a positive feedback loop that expedites investigational progress, enhances understanding, and enriches management options for each family member.

Compliance with ethical standards

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