Review article: next-generation transformative advances in the pathogenesis and management of autoimmune hepatitis

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Summary
Background: Advances in autoimmune hepatitis that transform current concepts of pathogenesis and management can be anticipated as products of ongoing investigations driven by unmet clinical needs and an evolving biotechnology.
Aim: To describe the advances that are likely to become transformative in autoimmune hepatitis, based on the direction of current investigations.
Methods: Pertinent abstracts were identified in PubMed by multiple search terms. Full-length articles were selected for review, and a secondary bibliography was developed. The discovery process was repeated, and a tertiary bibliography was identified. The number of abstracts reviewed was 2830, and the number of full-length articles reviewed exceeded 150.
Results: Risk-laden allelic variants outside the major histocompatibility complex (rs3184504, rs36000782) are being identified by genome-wide association studies, and their gene products are potential therapeutic targets. Epigenetic changes associated with environmental cues can enhance the transcriptional activity of genes, and chromatin re-structuring and antagonists of noncoding molecules of ribonucleic acid are feasible interventions. The intestinal microbiome is a discovery field for microbial products and activated immune cells that may translocate to the periphery and respond to manipulation. Epidemiological studies and controlled interview-based surveys may implicate environmental and xenobiotic factors that warrant evidence-based changes in lifestyle, and site-directed molecular and cellular interventions promise to change the paradigm of treatment from one of blanket immunosuppression.
Conclusions: Advances in genetics, epigenetics, pathophysiology, epidemiology, and site-directed molecular and cellular interventions constitute the next generation of transformative advances in autoimmune hepatitis.
1 | INTRODUCTION

Transformative advances in autoimmune hepatitis are milestones in understanding that promise to impact on future management and investigation. They are products of a continuum of clinical and laboratory observations and technological improvements that are defining the antigenic triggers, genetic predispositions and homeostatic disruptions that favour loss of self-tolerance. They are products of population-based epidemiological studies that have suggested associations between the environment and the disease, and they are consequences of animal studies and clinical trials that are changing the paradigm of treatment from one of blanket immunosuppression to one of site-specific molecular and cellular intervention.

Recognition of cytochrome P450 2D6 (CYP2D6) as the principal autoantigen of one form of autoimmune hepatitis has been a transformative advance. It has been critical in developing animal models of the human disease and in understanding mechanisms for breaking the tolerance of self-antigens. Clarification of the cell populations that characterise the innate and adaptive immune responses in autoimmune hepatitis has led to discoveries of homeostatic imbalances that favour autoimmunity, and these insights into specific pathogenic pathways and perturbed cell populations have constituted another transformative advance that continues to fuel efforts at therapeutic manipulation. The future transformative advances can be anticipated as natural progressions of current knowledge and as products of unmet clinical needs and an evolving biotechnology.

The goal of this review was to describe the advances that are likely to be transformative in the future understanding and management of autoimmune hepatitis. The review anticipates investigational progress in autoimmune hepatitis and in autoimmune diseases outside autoimmune hepatitis that will provide applicable insights into pathogenesis and management.

2 | METHODS

English abstracts were identified in PubMed using the search words "advances in autoimmune hepatitis", "epidemiology of autoimmune hepatitis", "genetics of autoimmune hepatitis", "epigenetics in autoimmunity", "intestinal microbiome and autoimmunity", and "novel treatment 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 December 1963 to June 2017 and reviewed for pertinence to this review during the primary, secondary and tertiary searches was 2830. Those judged most pertinent to the topic exceeded 500, and the number of full-length articles reviewed exceeded 150.

3 | RESULTS

3.1 Anticipated advances in epidemiology

Population-based epidemiological studies have indicated that the incidence of autoimmune hepatitis varies widely between countries and between adults and children. The annual incidence of autoimmune hepatitis ranges from 0.67 cases per 100 000 persons in southern Israel to 2.0 cases per 100 000 persons in New Zealand, whereas the annual incidence in children is 0.23 cases per 100 000 persons in Canada and 0.4 cases per 100 000 persons in the United States. The incidence of autoimmune hepatitis has also been increasing in some countries. In Spain, the annual incidence of autoimmune hepatitis has increased from 0.83 cases to 1.07 cases per 100 000 persons from 1990 to 2003, and in Denmark, the annual incidence has increased from 1.37 cases to 2.33 cases per 100 000 persons between 1994 and 2012 (Table 1).

Coincident with the different and changing incidences has been disparity in the age of disease onset between countries. In the Netherlands, the median age of onset is 43 years in men and 48 years in women. In Denmark, the peak age of onset is 70 years, and in New Zealand, it is between 60 and 69 years. These differences suggest that unrecognised factors outside the genotype are affecting disease occurrence.

3.2 Epidemiological implications

Autoimmune hepatitis has had codified diagnostic criteria since 1999, and the population-based epidemiological studies that have demonstrated differences in the incidence of disease between countries have applied these criteria to well-defined populations during similar time frames. Incomplete case detection and misdiagnosis can never be excluded, but unrecognised environmental factors that contribute to the disparities in incidence and peak age of onset must also be considered. These unidentified environmental factors may complement or supersede genetic predispositions for the disease (Table 1). The changing incidence and clinical phenotype of autoimmune hepatitis compels the performance of population-based epidemiological studies that map susceptibility patterns in different countries, ethnic groups and sub-populations (Figure 1).

3.3 Epidemiological precedents

Regional factors, including seasonal variations in sun exposure, activities, diet, infections and toxic (xenobiotic) encounters have already been implicated by epidemiological studies in primary biliary cholangitis (PBC; Table 1). These insights have strengthened general health maintenance recommendations that emphasise the avoidance of...
3.4 Anticipated advances in genetics

The association of autoimmune hepatitis with certain human leucocyte antigens (HLAs) has been recognised for decades in white North American and northern European patients and confirmed recently by genome-wide association studies (GWAS) in northern European (Dutch) patients.56 The next transformative advance in the genetics of autoimmune hepatitis will emerge from interrogations outside the major histocompatibility complex (MHC). The ability to characterise subgroups of patients at risk for disease progression by GWAS and high density genotyping arrays will facilitate the early identification of these patients and the institution of individualised interventions.58 The products of risk-laden genes that contribute to the disruption of key pathogenic pathways might also be identified and considered for therapeutic targeting (Figure 1). Ustekinumab is an example of an agent chosen for clinical trial in refractory PBC because of findings by GWAS.58 Interleukin (IL)-12A and IL-12RB2 were identified as pertinent genes in PBC,56,58-60 and their gene products were targeted by a monoclonal antibody (ustekinumab) directed against the p40 subunit in IL-12.61,62

3.4.1 Explorations outside the MHC in autoimmune hepatitis

Genome-wide association studies in autoimmune hepatitis have implicated the rs3184504*A allele of SH2B3 gene as a variant outside the MHC that is associated with disease risk in northern European (Dutch, German and Swiss) patients (Table 1).63 The gene product of SH2B3 is an adaptor protein, SH2B3(Src homology 2-B adaptor protein 3), also known as LNK (lymphocyte adaptor protein), that provides a molecular platform for the coordination of signalling...
events that affect the regulation of hematopoiesis, cell migration, inflammatory activity and lymphocyte differentiation.\textsuperscript{64-69} SH2B3 inhibits T lymphocyte activation.\textsuperscript{66}

The rs3184504*A risk allele influences protein phosphorylation by the Janus kinase (JAK) family of tyrosine kinases.\textsuperscript{66,70} These effects may in turn affect cytokine production (tumour necrosis factor, interferon-gamma), the transduction of cytokine-mediated signals and the adaptive immune response.\textsuperscript{63,71} The same risk allele has been recognised in other immune-mediated diseases, including primary sclerosing cholangitis (PSC),\textsuperscript{57} coeliac disease,\textsuperscript{72} hypothyroidism,\textsuperscript{73} rheumatoid arthritis\textsuperscript{74} and type 1 diabetes mellitus.\textsuperscript{75}

Recognition and confirmation of the principal variant alleles associated with autoimmune hepatitis will allow functional analyses to proceed and possibly indicate pertinent gene products that could be selectively targeted.\textsuperscript{58,76}

3.4.2 | Investigational challenges

The observed risk of autoimmune hepatitis cannot be fully explained by the findings of GWAS.\textsuperscript{58,77-80} Genetic predispositions for autoimmune hepatitis vary between age groups, genders and ethnicities,\textsuperscript{58,81} and the multiplicity of comparisons in microarray studies can challenge statistical analyses.\textsuperscript{58} Only 53% of northern European patients with autoimmune hepatitis have the rs3184504 allele,\textsuperscript{63} and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Next-generation transformative advances that would satisfy unmet clinical needs in autoimmune hepatitis. The next generation of transformative advances can be predicted by current and anticipated investigational progress in identifying risk-laden allelic variants outside the major histocompatibility complex (MHC); clarifying the epigenetic effects on gene transcriptional activity associated with demethylation of deoxyribonucleic acid (DNA), histone modifications within chromatin and noncoding ribonucleic acid molecules (micro-RNAs); defining the pathogenicity of the intestinal microbiome; recognising the exogenous factors influencing disease behaviour; and developing site-directed molecular and cellular therapeutic interventions, including the expansion and adoptive transfer of regulatory T cells (Tregs). The key pathogenic processes that would be affected by these transformative advances are shown within the ellipse.}
\end{figure}
HLA DRB1*0301 or HLA DRB1*0401 occurs in only 51% and 55% of white North American patients, respectively.82 Children with autoimmune hepatitis may have different class II HLA associations than adults,83 and patients in different countries or with different ethnicities within the same country may have disparate predisposing genetic factors.81,84,85 These findings suggest that genetic analyses will have a limited impact on explaining the risk of autoimmune hepatitis and an applicability restricted to subgroups defined by age, race and location. The challenges are to identify variant alleles alone or in combination that influence disease behaviour by having functional expressions that can be targeted and to characterise the individuals with these genotypes.

Another challenge is to distinguish between differences related to risk-laden genes that differ because of ethnicity and those that differ because of cohort selection, detection method and statistical power. GWAS in northern European patients have suggested a possible association of the rs36000782 allele of the caspase recruitment domain family member 10 (CARD10) gene with autoimmune hepatitis \( (P = 3.0 \times 10^{-9}) \), whereas a multicentre cohort study in Japan based on a polymerase chain reaction-restriction length polymorphism method (PCR-RFLP) disclosed no association \( (P = .38) \).86 Similar discordant observations between ethnically diverse cohorts have been described for polymorphisms of the cytotoxic T lymphocyte antigen-4 (CTLA-4) gene87-91 and the tumour necrosis factor (TNF) gene.92-94

The pre-study hypothesis can also affect the number of comparisons analysed in the study and the statistical validity of the associations. The association between the rs7574865 allele of the signal transducer and activator of transcription (STAT) gene and autoimmune hepatitis in Japanese patients \( (P = .001) \) might be difficult to demonstrate if the loci had not been pre-selected.95

Studies involving microarray technology should have a hypothesis-free approach in which multiple comparisons are made across the genome without areas of pre-defined interest.58 Such studies require large patient and control cohorts of similar age and ethnicity, and international collaborations are required to make a truly transformative advance that applies genetic information to patient management. Importantly, hypothesis-free GWAS can generate data suitable for combination with other similar studies, and meta-analyses can empower the findings of individual studies.58

Transformative advances in the genetics of autoimmune hepatitis will be difficult to achieve, but the biotechnology, study methods, and expectations are well founded and likely to drive these investigations.

3.5 | Anticipated advances in epigenetics

Epigenetic changes modulate the expression of genes without altering the sequence of deoxyribonucleic acid (DNA), and they can account for stable inherited phenotypic features.96-98 Inheritance through cell divisions or pregnancy is a requirement of an epigenetic trait,77 but any structural adaptation of a chromosomal region that affects gene activity is also epigenetic.96,99 Epigenetic modifications of gene activity may explain the difference between the observed risk for autoimmune hepatitis and the genetic factors implicated by GWAS. Epigenetic changes also exhibit plasticity, organ-specificity, and reversibility.100,101

The main epigenetic changes occur in the chromatin structure as a result of modifications in the methylation status of DNA97,102 and in the structure of the histone proteins by acetylation, methylation, phosphorylation, or ubiquitination of amino acid residues103-105 (Table 1). Tightly packed DNA within the nucleosomes of chromatin limits the binding of transcriptional factors and alters gene activity.106 Demethylation of the DNA of gene promoters and modifications of the histone proteins within the nucleosomes can unpack the chromatin and increase the binding of motifs that change gene function. The DNA of gene promoters in autoimmune disease frequently has a reduced number of methylation sites, and gene activity tends to be increased.107 Histone modifications have a less predictable effect on gene activity depending on the specific nature and location of the amino acid residues in the histone code.96,108,109

Micro-ribonucleic acids (miRNAs) are also part of the epigenetic machinery that can alter gene function without changing DNA sequence.110 miRNAs are small double-stranded molecules ranging in length from 21 to 25 nucleotides that tend to silence genes.111 They can repress gene activity by pairing with messenger RNA (mRNA) and marking the mRNA for degradation by a RNA-induced silencing complex.112 The gene-silencing effect can also occur without degradation of the mRNA if the nucleotide sequences between the miRNA and the mRNA are imperfectly aligned.113-115

3.5.1 | Epigenetic traits and environmental cues

Epigenetic modifications of gene activity are mechanisms whereby environmental cues can influence genetic code and in turn affect cellular response.96 Pollutants, pharmaceuticals, infectious agents, diet and age have been implicated as environmental factors that can induce epigenetic changes.107,116-122 These changes may in turn influence the propensity for autoimmunity.96,98,123 Tobacco smoke,124 aerosolised particles,125,126 heavy metal,127 and toxins (trichloroethylene)128 are environmental factors that may alter the DNA methylation of immune regulatory genes.107,129 Hydralazine118 and procainamide116,117 are drugs that inhibit DNA methyltransferase activity, and old age has been associated with genomic instability and hypo-methylation of DNA in naive CD4+ T lymphocytes.122

Epigenetic changes have the plasticity to respond to environmental pressures, and their evaluation in autoimmune hepatitis could have transformative implications in understanding pathogenic pathways, mechanisms of susceptibility and therapeutic opportunities (Figure 1). Unlike genetic determinants, disease-associated epigenetic changes can be modified by selective therapies that favourably skew gene activity.97,98,130,131 The epigenetic changes that have been described in obesity and NAFLD have been reversed by bariatric surgery.101 Diet-associated weight reduction100 and dietary supplementation with methyl group donors132,133
3.5.2 | Epigenetic traits and autoimmune diseases

Epigenetic changes have been described in systemic immune-mediated diseases, including systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis, and they have been evaluated in liver diseases, including NAFLD and PBC (Table 1). A common feature has been the hypo-methylation of CD4+ T lymphocytes.

In PBC, demethylation of the promoter gene for the chemokine receptor, CXCR3, in CD4+ T lymphocytes has been associated with increased expression of this pro-inflammatory molecule. In a murine model of liver fibrosis, the differentiation of hepatic stellate cells (HSCs) into myofibroblasts has been associated with an epigenetic mechanism influenced by methyl-DNA binding proteins that participate in a relay pathway involved in the down-regulation of miR-21. Genetic and pharmacological disruptions of this pathway have inhibited myofibroblasts and reduced fibrogenesis.

In a mouse model of SLE, the histone proteins 3 and 4 are hypomethylated, and the disease can be ameliorated by administering inhibitors of histone deacetylase. miRNAs also contribute to immune reactivity in lupus models by targeting DNA methyltransferases, impairing maintenance methylation of DNA, and increasing the transcription activity of methylation-sensitive genes associated with autoimmunity.

There is ample precedent in studies already performed to indicate the importance of epigenetic changes in systemic immune-mediated diseases and primary liver diseases to justify their robust evaluation in autoimmune hepatitis.

3.5.3 | Epigenetic traits and autoimmune hepatitis

miRNAs 21 and 122 are increased in the circulation of patients with autoimmune hepatitis (Table 1). The miRNA levels correlate with serum alanine aminotransferase concentrations, and the miR-21 level is associated with histological grades of inflammatory activity. In contrast, circulating levels of both miR-21 and miR-122 are inversely associated with the stages of hepatic fibrosis. miR-21 and miR-122 could be biomarkers of inflammatory activity in autoimmune hepatitis, but they could also have epigenetic effects that promote the inflammatory and immune cell responses.

miR-21 is strongly expressed in lymphocytes, and miR-122 is abundant in hepatocytes. miR-21 down-regulates the gene that modulates the apoptosis and proliferation of activated T lymphocytes, and its over-expression could suppress apoptosis of activated immune cells and increase the production of pro-inflammatory cytokines. Similarly, miR-122 could increase the production of pro-inflammatory type 1 interferon by blocking the suppressor of cytokine signalling pathway. Anti-sense oligonucleotides have been used to suppress the production of miRNAs and improve disease manifestations in murine-models of the metabolic syndrome (Table 2). Manipulation of noncoding miRNAs that favourably modulate inflammatory and immune responses would constitute a transformative advance in managing autoimmune hepatitis.

3.5.4 | Treatment opportunities for autoimmune hepatitis

Key therapeutic targets in autoimmune disease would be regions in the modified chromatin that enhance the activity of pro-inflammatory genes and the miRNAs that silence immune suppressor genes. Most autoimmune diseases have hypo-methylated DNA, and these hypo-methylated states are difficult to reverse by targeted interventions. The histone proteins comprising the chromatin core of the CD4+ T cells and the over-expression of noncoding miRNAs have been the preferred therapeutic targets in diverse immune-mediated diseases.

Inhibitors of the bromodomain and extra-terminal (BET) family of proteins may emerge as agents that dampen the pro-inflammatory effects of modified histones within nucleosomes. The BET proteins recognise acetylated lysine residues in the N-terminal tails of histones, and they promote gene activity. Inhibition of the BET proteins have histone acetyltransferase activity that contributes to re-modeling of the chromatin structure and recruitment of transcription factors. Molecular inhibitors of the BET proteins have reduced the production of pro-inflammatory cytokines and impaired the differentiation of CD4+ T cells into effector cells (Table 2).

Inhibitors of the methyl-DNA binding domain protein 2 (MBD2) are feasible molecular interventions for reversing DNA hypo-methylation in autoimmune diseases. MBD2 is a nuclear protein that binds specifically to methylated DNA and exhibits DNA demethylase activity. Levels of MBD2 in T lymphocytes from patients with SLE have been directly associated with the degree of genomic hypo-methylation and increase in transcriptional activity. Anti-sense oligonucleotides have inhibited MBD2, and they have decreased tumorigenesis in human cancer cell lines and in a nude mouse model. Inhibition of MBD2 activity has the potential to reverse DNA hypo-methylation, the principal epigenetic change in autoimmune disease.

Other molecular interventions of interest in animal models of immune-mediated diseases are inhibitors of histone deacetylase and antagonists of noncoding miRNAs. Clarification of the epigenetic changes associated with autoimmune hepatitis could herald a fresh era of therapeutics that includes evidence-based dietary and environmental changes and targeted molecular interventions that alter gene activity (Figure 1).

3.5.5 | Treatment challenges in autoimmune hepatitis

The main obstacles are the lack of momentum for epigenetic research in autoimmune hepatitis, the difficulty in developing interventions with highly selective actions and the uncertain long-term consequences of manipulated epigenetic marks. The modification of complex and interactive mechanisms of gene activity could have unsuspected and unwanted consequences, including the transgenerational passage of an undesired trait. Each obstacle can be surmounted by investigations that continue to elucidate and manipulate
TABLE 2  Next-generation transformative molecular and cellular interventions

<table>
<thead>
<tr>
<th>Anticipated Intervention</th>
<th>Target(s)</th>
<th>Supportive investigational evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies against protein products of risk-laden genes</td>
<td>Gene product of risk-laden variant that influences AIH (possibly SH2B3)</td>
<td>Ustekinumab against IL-12, IL-23 in PBC(^{58,62})</td>
</tr>
<tr>
<td>Molecular inhibitors of BET proteins</td>
<td>Epigenetic changes induced by histone acetyltransferase activity</td>
<td>Cytokine secretion and CD4(^+) T cell differentiation impaired in mice(^{160,161})</td>
</tr>
<tr>
<td>Anti-sense oligonucleotides against noncoding miRNAs</td>
<td>miRNAs that inhibit DNA methyltransferases and increase activity of pro-inflammatory genes</td>
<td>NAFLD improved(^{154}); DNA hypo-methylation less in SLE(^{146,147}); miR-21 and miR-122 increased in AIH(^{148})</td>
</tr>
<tr>
<td>Manipulation of intestinal microbiome</td>
<td>Intestinal dysbiosis(^{191,192}); Permeable intestinal mucosal barrier(^{192})</td>
<td>PSC improved by antibiotics(^{302}); Barrier strengthening by gelatin tannate(^{233}); Colitis improved by re-colonisation(^{234}); SLE improved by TLR blockade(^{235})</td>
</tr>
<tr>
<td>TIPE2 agonists</td>
<td>Low TIPE2 immunosuppressive and anti-inflammatory activity(^{254,257})</td>
<td>TIPE2 low in murine AIH(^{258}); AIH worse in TIPE2-deficient animals(^{258})</td>
</tr>
<tr>
<td>Chemokine inhibitors</td>
<td>Signals orchestrating inflammatory and immune cell migrations(^{253,259})</td>
<td>Neutralising antibodies and drugs block chemokine receptor ligation(^{260,264})</td>
</tr>
<tr>
<td>Caspase inhibitors</td>
<td>Hepatocyte apoptosis(^{303})</td>
<td>Less activity and fibrosis in NAFLD(^{265})</td>
</tr>
<tr>
<td>Antioxidant and anti-nitrosative interventions</td>
<td>Reactive species that promote apoptosis, organelle dysfunction, fibrosis(^{360})</td>
<td>NOX inhibitors reduce murine fibrosis(^{248}); Nrf2 agonists reduce fibrosis in NAFLD(^{270})</td>
</tr>
<tr>
<td>Expansion of Tregs</td>
<td>Deficient Tregs(^{14,16})</td>
<td>Transfer of expanded Tregs in AIH(^{271,273})</td>
</tr>
<tr>
<td>Anti-fibrotic interventions</td>
<td>Hepatic stellate cell(^{304}); Extracellular matrix(^{304})</td>
<td>Angiotensin II inhibitors reduce fibrosis(^{278}); Simtuzumab limits cross-linked collagen(^{283})</td>
</tr>
<tr>
<td>Endocannabinoid agonists</td>
<td>Cannabinoid receptors(^{287-291})</td>
<td>Expand Tregs in experimental AIH(^{288}); Immunosuppression in murine AIH(^{288})</td>
</tr>
</tbody>
</table>

AHI, autoimmune hepatitis; BET, bromodomain and extra-terminal proteins; DNA, deoxyribonucleic acid; IL, interleukin; miRNA, micro ribonucleic acid; miR, individual miRNA signature; NAFLD, non-alcoholic fatty liver disease; NOX, nicotinamide adenine dinucleotide phosphate oxidase; Nrf2, nuclear factor-erythroid 2-related factor 2; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; SH2B3, Src homology 2-B adaptor protein 3 gene; SLE, systemic lupus erythematosus; TIPE2, tumour necrosis factor-alpha-induced protein-8 like-2; TLR, toll-like receptors; Tregs, regulatory T cells.

Epigenetic mechanisms in systemic immune-mediated and primary liver-related diseases\(^{105,155,163,167,169-172}\). Continued progress in understanding the role of epigenetic factors in modulating the immune response will drive their evaluation in autoimmune hepatitis.

3.6 | Anticipated advances in pathogenic mechanisms

Microbial antigens and activated immune cells within the intestine can influence systemic immune responses, and they may contribute to the development of immune-mediated diseases (Figure 1)\(^{173}\). Toll-like receptors (TLRs) can react to commensal bacteria, pathogens, and distress signals within the intestine to maintain antigenic tolerance, generate an innate immune response, and shape subsets of T lymphocytes\(^{174-177}\). The microbial antigens and activated immune cells may then translocate to the systemic circulation where microbial products may mimic normal self-proteins and the sensitised immune cells may promote tissue damage\(^{178,179}\).

Alterations in the composition of the intestinal microflora (dysbiosis) have been associated with diverse systemic immune-mediated diseases, including rheumatoid arthritis,\(^{180}\) type 1 diabetes\(^{181,184}\) and inflammatory bowel disease,\(^{185-187}\) and they have been implicated in liver diseases, including PSC,\(^{188}\) NASH\(^{189,190}\) and autoimmune hepatitis (Table 1)\(^{191,192}\). Investigations that strengthen the association of the intestinal microbiome with autoimmune hepatitis would transform current perceptions of pathogenesis and management.

3.6.1 | Intestinal microbiome and autoimmunity

Changes in the composition of the microflora by antibiotics, diet or disease can circumvent the normal tolerogenic responses to commensal bacteria and generate innate and adaptive immune responses that are pro-inflammatory\(^{193}\). Genetic factors can also influence the nature of the intestinal microbiome and character of the immune responses\(^{194,195}\). Structural proteins (zona occludens 1 and occludin) that bind intestinal epithelial cells are reduced in autoimmune hepatitis, and the presence of lipopolysaccharide in the systemic circulation suggests altered permeability of the intestinal mucosal barrier (Table 1)\(^{192}\). Gut-derived reactive T lymphocytes in the pancreas and lymph nodes of patients and animal models with type 1 diabetes implicate a trans-intestinal pathway by which immune cells originating in the intestine can influence the systemic immune response\(^{178,196,197}\).

Naïve T lymphocytes may also be sensitised to the gut-derived microbial antigens in the systemic circulation. “Foreign” antigens from the intestine may have epitopes homologous to normal self-proteins, and they may generate a promiscuous adaptive immune response that breaks tolerance to self-antigens by molecular mimicry.
3.6.2 | Intestinal microbiome and gender bias for autoimmunity

The intestinal microbiome may also contribute to a gender bias in the development of autoimmune disease. Male and female non-obese diabetic (NOD) mice have different intestinal microbiota, and this difference is lost after male castration. Female NOD mice develop type 1 diabetes more frequently than male NOD mice, and this difference is lost in germ-free animals. Colonisation of immature female NOD mice with the intestinal microflora from mature male NOD mice protects them from diabetes, and blockade of the androgen receptor in female NOD mice diminishes this effect. These findings suggest that the female propensity for immune-mediated disease in a genetically susceptible host may be influenced by the intestinal microflora. These microflora may in turn be shaped by sex hormones that they can also produce.

3.6.3 | Intestinal microbiome and investigational challenges

Investigations of the intestinal microbiome are challenged by the size, diversity and variability of the microbiota within an individual and a community. The human intestinal tract contains 10-11 trillion bacteria comprising 500-1500 different species distributed in the lumen and the mucosa. Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes are the four major phyla, and 66 operational taxonomic units define a phylogenetic core present in most individuals. Detection methods based on sequencing of the 16S ribosomal RNA (rRNA) gene and DNA hybridisation provide high throughput platforms for detection, but identification relies on databases of known species. Microbial sequences that are uncatalogued are undetected.

Antibiotics, medications, probiotic supplements, personal hygiene, medical history, advancing age and diet influence the composition of the microbiota in an individual, and vaccination programmes, dietary habits and community sanitation influence the intestinal composition within communities. The intestinal microbiota differs in individuals living in rural and urban areas, and the microbiome varies with socioeconomic status. These challenges are formidable, but their recognition is essential in developing experimental models, strengthening study designs, and improving biotechnology.

Cataloguing of the bacterial species within the intestine is a dynamic evolving process and a critical requirement for conducting research in this area. The Human Microbiome Project has defined the composition of the intestinal microbiota, and it has described factors that alter microbial composition and associate with health and disease. Publicly available comprehensive databases and open-source software packages have strengthened the ability to analyse results with updated catalogues, and microarrays have been designed to include explorative probes for unknown microbial sequences.

Awareness of the multiplicity of factors affecting composition of the intestinal microbiota allows studies to be designed that reduce the impact of confounders. The intestinal microbiome tends to be stable from late childhood through adulthood, whereas it becomes less diverse and more variable over short intervals with ageing. This foreknowledge can strengthen the design of clinical studies by directing the selection of cohorts matched for the predetermined confounders. The resources are available to conduct meaningful research of the intestinal microbiome in autoimmune hepatitis, and the investigational precedents in other immune-mediated diseases compel its consideration.

3.6.4 | Transformative management strategies for autoimmune hepatitis

Antibiotics have reduced disease activity in a meta-analysis of 10 randomised clinical trials of patients with rheumatoid arthritis and in a small randomised pilot study of PSC (Table 2). Probiotic supplements have expanded regulatory T cells in cell culture and prevented type 1 diabetes in NOD mice. Gelatin tannate, a protector of the intestinal mucosal barrier, has reduced the activity of experimental acute colitis and altered the composition of the intestinal microbiome in mice. Intestinal re-colonisation in experimental colitis and molecular blockade of TLRs in a murine model of SLE have decreased inflammatory activity. The multiplicity of feasible interventions that may favourably alter the intestinal microbiome and improve immune-mediated disease is a testimony to the investigational interest and preliminary nature of these strategies.

The intestinal microbiome has already been implicated in the pathogenesis of autoimmune hepatitis, and this association must be strengthened by investigations in animal models and in patients with the disease. Sequencing of the 16S rRNA gene can determine signature sequences that reconstruct the intestinal microbiome and advances in microarray technology and open-source software packages can provide high-throughput platforms that recognise known and previously unknown microbial species. The antigenic triggers of autoimmune hepatitis are uncertain, and the intestinal microbiome constitutes a discovery field that could lead to transformative concepts of cause and management.

3.7 | Anticipated advances in management

Current immunosuppressive regimens have undirected and unsustainable actions, and they may fail to suppress inflammatory activity or prevent cirrhosis. Seven per cent of treated patients worsen...
during therapy (treatment failure), and 14% improve but fail to normalise liver tests and liver tissue (partial response). Treatment withdrawal is followed by relapse in 50-87%,240-243 and 60-81% of patients become treatment-dependent.241,242 Autoimmune hepatitis progresses to cirrhosis in as many as 40%,244 and 1%-6% of patients with cirrhosis develop hepatocellular carcinoma.245,246 Deficiencies in current management strategies can be improved, supplemented or replaced, and progress can be anticipated in each area.

3.7.1 | Key prospects for improved current management

The basic deficiency in managing autoimmune hepatitis is the lack of a biomarker that reflects suppression or elimination of the principal pathogenic mechanisms that sustain the disease. Autoimmune hepatitis can progress or exacerbate despite treatment to normal liver tests or liver tissue, and an anticipated improvement in management will be characterisation of the treatment response by biomarkers that reflect immune reactivity, inflammatory response, fibrotic activity or apoptotic cell death. Serum levels of organ-specific miRNAs (miR-22 and miR-122) have already been proposed as biomarkers of inflammatory activity in autoimmune hepatitis, and serum determinations of nitric oxide metabolites may emerge as biomarkers of hepatic inflammation and fibrosis. Other candidate biomarkers are the serum chemokines and cytokines.

Management strategies guided by biomarkers reflective of pathogenic pathways and serial non-invasive tests of hepatic fibrosis (transient elastography by ultrasonography and magnetic resonance elastography) will facilitate the individualised adjustments of medication, the addition of supplemental therapies, and the optimal timing of drug withdrawal. These advances will improve the results of current management regimens, but they will not improve the targeting of specific pathogenic mechanisms. Molecular and cellular interventions developed for this purpose may supplement or supersede current blanket immunosuppressive agents. Site-directed interventions are likely to constitute the next transformative advance in management.

3.7.2 | Key prospects for next-generation therapy based on pathogenic mechanisms

The key prospects for transforming the management of autoimmune hepatitis in the next generation are likely to involve gene products of risk-laden genetic loci outside the MHC, manipulations of the environment and the intestinal microbiome, epigenetic factors that enhance pro-inflammatory gene activity or signalling pathways that promote inflammatory, immune and fibrotic responses. Future management strategies will be founded on insights derived from current preliminary efforts to manipulate mediators of cell trafficking, apoptosis, oxidative-nitrosative stress, immunosuppression and fibrosis.

Tumour necrosis factor-alpha-induced protein-8 like-2 (TIPE2) inhibits signalling pathways that promote inflammatory, immune activity,256,257 and its expression in the peripheral blood mononuclear cells of a murine model of autoimmune hepatitis is decreased (Table 2).258 Furthermore, laboratory and histological features of inflammatory activity and the production of pro-inflammatory cytokines are increased when the murine model is based on TIPE2-deficient animals.258 Agonists of TIPE2 activity are feasible molecular interventions that warrant consideration in the future management of autoimmune hepatitis.

Chemokines that modulate the trafficking of inflammatory and immune cells to sites of liver injury can affect injurious and reparative processes. Neutrophils, antigen-presenting cells, and various lymphocytes are increased when the murine model is based on TIPE2-deficient animals.258 Agonists of TIPE2 activity are feasible molecular interventions that warrant consideration in the future management of autoimmune hepatitis.

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Hepatic fibrosis is stimulated by activating the cannabinoid CB1 receptors on HSCs, and it is inhibited by activating the cannabinoid CB2 receptors. Rimonabant blocks the CB1 receptor, but this drug has been withdrawn because of severe neuropsychiatric side effects. Next-generation antagonists of the CB1 receptor or agonists of the CB2 receptor are required to advance this anti-fibrotic therapy.

The endocannabinoid system can also influence the inflammatory and immune responses to liver injury (Table 2). Natural (delta 9-tetrahydrocannabinol) and endogenous (anandamide, 2-arachidonoylglycerol) endocannabinoids can exert anti-inflammatory and immunosuppressive effects by activating the CB1 and CB2 receptors and expanding the regulatory T cell population in an experimental model of autoimmune hepatitis. Inhibitors of the endocannabinoid hydrolysing enzyme, fatty acid amide hydrolase, can achieve similar results by impairing degradation of the endogenous endocannabinoids. Agonists of the endocannabinoid system are now feasible treatments in autoimmune hepatitis.

### Caveats of a changing paradigm

The abundance of pathogenic targets and feasible counter-interventions in autoimmune hepatitis creates problems in decision-making and resource allocation. The critical pathogenic mechanisms or defective immunomodulatory pathways that initiate or sustain the autoreactive response remains unclear, and the therapeutic effort cannot be confidently directed. Promising interventions in one immune-mediated disease may be ineffective in another immune-mediated disease because the pivotal pathogenic pathways differ. For this reason, CTLA-4Ig may be effective in rheumatoid arthritis but not in inflammatory bowel disease. Differences in key pathogenic pathways may also exist in experimental animal models of the human disease as treatments effective in one species may not be effective in another species. Most importantly, therapeutic manipulations of key homeostatic mechanisms may have serious and unexpected consequences by disrupting normal defences against pathogens and malignant transformations or by inducing liver injury. Autoimmune hepatitis is at the point where the unmet clinical need for improved treatment must interface with the trajectory of investigations in biological therapy. Progress along this trajectory would constitute a transformative advance in the management of autoimmune hepatitis.

## OVERVIEW

The next-generation transformative advances in autoimmune hepatitis can be predicted by the progress already made in describing its variable incidence, genetic predisposition, and responsiveness to investigational site-specific molecular and cellular interventions. Findings in other immune-mediated systemic and liver-related diseases provide insights that should also drive progress. The disruption of homeostatic mechanisms that regulate lymphocyte activation, differentiation, proliferation, and migration is a pathogenic concept common to all autoimmune diseases, and discoveries that clarify and modify the pathways of inflammation, apoptosis, and fibrosis are potential stepping-stones for progress in autoimmune hepatitis regardless of their disease origin.

The identification of risk-laden genetic foci outside the MHC and the targeting of implicated gene products has been an area of progress in PBC. Epigenetic analyses promise to unmask environmental cues that influence the onset and outcome of NAFLD and PBC and the intestinal microbiome is emerging as a discovery field for antigens and activated immune cells that affect the inflammatory responses in PSC, NAFLD and autoimmune hepatitis. Population-based epidemiological studies in autoimmune hepatitis and controlled interview-based surveys in PBC have the power to implicate environmental and xenobiotic factors that shape the disease, and clinical studies evaluating molecular and cellular interventions have the prospect of transforming current therapies into highly individualised, site-specific management strategies in multiple autoimmune diseases.

These projections may not be validated in autoimmune hepatitis, but they constitute evidence-based starting points for gaining fresh insights into the mechanisms and management of this disease. All expectations must be shaped by the realities that autoimmune hepatitis is rare, large international collaborations will be necessary to validate findings, diverse clinical phenotypes must be sharply defined, and study designs must be restricted to well-characterised cohorts of similar phenotype. Furthermore, the evaluation of new therapies will require predefined metrics of response that are uniformly applied, awareness of possible adverse consequences, and monitoring mechanisms that assess tolerability, promote early detection of side effects and evaluate compliance.

Transformative advances in autoimmune hepatitis have lagged behind those in other autoimmune diseases mainly because current immunosuppressive regimens have been life-saving, generally available and cost effective. Research funding has strengthened complacency with current treatment regimens by allocating resources preferentially to other rare but currently untreatable or less adequately treated autoimmune liver diseases. The unmet clinical needs of autoimmune hepatitis must be promulgated and the applicability of research in this disease to other similar diseases recognised. Advances already made in other autoimmune diseases should encourage and direct the investigational efforts in autoimmune hepatitis. These efforts in turn should generate a reciprocated legacy of insights.

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### AUTHORSHIP

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REFERENCES


118. Dozmorov MG, Coit P, Maksimowicz-McKinnon K, Sawalha AH. Age-associated DNA methylation changes in naive CD4+ T cells


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