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Autoimmune Hepatitis: Aetiopathogenesis and Immunotherapies

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Abstract

Autoimmune Hepatitis (AIH) is a rare disorder in which body's own immune system damages the hepatocytes, resulting in a chronic liver inflammation. The main characteristics include histological lymphoplasmacytic infiltration in liver, increased transaminase and immunoglobulin-G levels, and autoantibodies seropositivity. AIH is subclassified into two –type 1 (AIH-1) and type 2 (AIH-2) based on different autoantibody profiles. Despite unknown aetiology, many studies propose the combined roles of genetic and environmental factors. Genetic studies have suggested the strong associations of Human Leukocyte Antigen (HLA) gene with AIH susceptibility, as well as some non-HLA genes that show weaker associations. Viruses may also be potential triggers via a few suggested mechanisms such as molecular mimicry. The pathogenesis of AIH has not been fully clarified, but the loss of self-tolerance and the impaired immunoregulatory system are thought to play major roles, involving a diversity of immune cell populations with T-cells as the main mediators. AIH patients generally have good response towards conventional immunosuppressive treatment (i.e. prednisolone) but relapse rates are high and long-term immunosuppression disrupt patients' immune surveillance against infections and cancer. Therefore, specific immunotherapies such as B-cell-depleting antibodies, anti-TNF-α antibodies, and regulatory T-cells (TREG) expansion via infusion or low-dose IL-2 injection, have been developed; clinical studies involving a limited number of patients have also demonstrated promising results. Future investigations on AIH pathogenesis may uncover more pathways and key molecules/cells that will be good therapeutic targets aimed to restore self-tolerance of AIH, as well as to minimise the use and side effects of immunosuppressive drugs.

Abbreviations

AIH : Autoimmune Hepatitis; AST: Aspartate Transaminase; ANA : Anti-Nuclear Antibody; SMA : and/or Anti-Smooth Muscle Antibody; LKM1 : Anti-Liver Kidney Microsomal Type 1 Antibody; anti-LC1 : Anti-Liver Cytosol Type 1 Antibody; GWAS : Genome-Wide Association Studies; TCR : The T-Cell Receptor; CTLA-4 : Cytotoxic T-Lymphocytes-Associated Antigen 4

Introduction

Autoimmune Hepatitis (AIH) is a type of Autoimmune Liver Diseases (AILD) that primarily affects the liver. The word "autoimmune" refers to autoreactive immunity, whereas "hepatitis" means liver inflammation. AIH is characterised by raising transaminases and circulating immunoglobulin G (IgG) levels, seropositivity for autoantibodies, and presence of interface hepatitis histologically (Liberal, et al. 2016) [1]. Two main forms of AIH have been recognised according to the different serological autoantibody profiles: AIH type 1 (AIH-1), seropositive for Anti-Nuclear Antibody (ANA) and/or Anti-Smooth Muscle Antibody (SMA); and AIH type 2 (AIH-2), seropositive for anti-liver kidney microsomal type 1 antibody [anti-LKM-1; directed against Cytochrome P450 2D6 (CYP2D6)] or anti-liver cytosol type 1 antibody (anti-LC-1) [2].

AIH occurs globally in both children and adults of all ages [3]. AIH also affects both sexes but like many other autoimmune diseases, it is female preponderant with female : male ratios of 4:1 in AIH-1 and 10:1 in AIH-2 [3]. AIH-1 is also more frequent than AIH-2 as it constitutes approximately 80% of all cases [4].

Although it is a chronic disease by definition, up to 40% of AIH patients present an acute onset, alongside one or more non-specific symptoms such as jaundice, malaise, pain in the abdomen and joint [5]. Interestingly, 34-45% of patients are asymptomatic and only diagnosed with AIH following a regular blood test [2]. Indeed, the clinical presentations of AIH are extremely diverse and variable, bringing numerous challenges in the clinical and immunological settings despite the successful sub-classifications. The true problem lies with an unidentified trigger, although current data point towards a combination of genetic and environmental factors that consequently disrupt patients' self-tolerance against their own liver. The exact mechanism is also yet to be fully elucidated, but studies support the synergistic effort between the effector T-cells' autoreactivity and the impairment of regulatory T-cells (TREG) in driving hepatic inflammation and subsequent injury. Early application of immunosuppressive treatments such as corticosteroids are crucial to stop disease progression into end-stage liver failure such as cirrhosis or Hepatocellular Carcinoma (HCC) which will leave patients in need of a Liver Transplant (LT) [1]. While these standard treatments are successful in most patients, discontinuation is hard to achieve with high relapse rates; patients who respond poorly often suffer from adverse side effects and interestingly, this occurs more frequently in juvenile than adult AIH [6]. Thus, alternative treatments have been developed with the aim of restoring self-tolerance to hepatic antigens via targeting specific immune cell subsets or cytokines implicated in AIH perpetuation. In this review, the autoimmune aetiologies and mechanisms of AIH pathogenesis have been summarised, as well as the recent advancement in the development of novel immunotherapies.

Aetiopathogenesis

Genetic Factors

The cause of AIH is unknown but with the suggestion that AIH develops in genetically susceptible individuals after being exposed to one or more environmental triggers such as pathogens. In terms of inheritance, although a Dutch AIH study has reported no twin concordance in AIH [7] and family studies of AIH are still scarce, about 40% of AIH individuals have family members with past occurrences of autoimmune disorders [1], suggesting a possible critical role of genetic factors in AIH.

HLA: Like virtually all autoimmune diseases, the susceptibility of AIH is strongly linked to the most polymorphic human gene region -Human Leukocyte Antigen (HLA), which is located on chromosome 6 and contains many adaptive immunity-related genes [1]. Several candidate gene-based studies in different populations have confirmed the association of HLA in AIH-1 (summarised in Table 1). Although different findings have been observed in different populations, these studies reveal an overall strong association of a HLA class II gene called DRB1 in AIH-1 susceptibility, particularly DRB1*0301 and DRB1*0401 alleles which are prevalent in North America and Northern Europe (Table 1). GWAS, genome-wide association study.

Regions	Countries	Year of Publish	Associated Risk	Remarks	Reference
America	North America	1997	DRB1*0301, DRB1*0401		[8]
	United States	1997	DRB1*0301, DRB1*0401		[9]
	Mexico	1998	DRB1*0404		[10]
	Brazil	1999	DRB1*13, DRB1*03		[11]
	Argentina (children)	1999	DRB1*0301, DRB1*1301		[12]
	Venezuela (Mestizo population)	2007	DRB1*0301, DRB1*1301		[13]
	North America	2008	DRB1*13		[14]
	Latin America	2009	DQB1*02, DQB1*0603, DRB1*0405, DRB1*1301	meta-analysis	[15]
	Brazil (children)	2015	DRB1*03, DRB1*13		[16]

Europe	Italy	2005	B*0801-DRB1*03-DQB1*02		[17]
	Germany	2006	B*0801-DRB1*03-DQB1*02		[18]
	UK	2006	A*01-DRB1*03/DRB1*04		[19]
	Netherlands	2014	DRB1*0301, DRB1*0401	GWAS	[20]
	Germany	2014	DRB1*0301, DRB1*0401	GWAS	
	Finland (children)	2017	B*0801-DRB1*03/DRB1*13		[21]
Asia	Western India	2005	DRB1*1501, DRB1*14, DRB1*0301, DRB1*1301		[22]
	Thailand	2006	DRB1*0301, DQA1*0101		[23]
	Korea	2008	DRB1*0405, DQB1*0401		[24]
	North India	2014	DRB1*04, DRB1*08		[25]
	Japan	2014	DRB1*0405, DQB1*0401		[26]
	Iran	2016	DRB1*03, DRB1*04, DRB1*08, DRB1*13		[27]
	Japan	2017	DRB1*0401, DRB1*0405, DQB1*0401		[28]

Table 1: HLA associations of AIH-1 in different countries.

The first AIH Genome-Wide Association Studies (GWAS) conducted within Dutch and German adults have also verified these two allelic variants as the respective first and second key loci that predispose AIH-1 [20]. Studies for AIH-2, on the other hand, are limited due to its low prevalence; nevertheless, it has also been linked to DRB1*0301, as well as DRB1*0701 and DQB1*0201 [29,30]. There are also very little studies comparing the genetic predisposition of juvenile and adult AIH, though early investigations have reported association of DRB1*1301 and DRB1*0301, but not DRB1*0401 in juvenile AIH-1 [12,31].

Importantly, the DRB1 alleles also seem to affect the clinical phenotypes, serological profile, treatment response, and disease prognosis. For example, DRB1*0301 carriers usually have hyper-IgG, seropositivity for antibodies to soluble liver antigen/liver pancreas (anti-SLA/LP), lower chance for remission after immunosuppressive treatment, frequent LT requirement, and are male-predominant [32,33]. In contrast, DRB1*0401 carriers are usually females and display higher chance of complete remission and lesser occurrence of cirrhosis [34,35]. These data suggest that HLA typing may emerge as a helpful tool in clinical practice to predict AIH prognosis.

The pathogenic role of DRB1 alleles in AIH have been suggested since they play a part in the autoimmune development of Rheumatoid Arthritis (RA) and multiple sclerosis [36]. Investigators initially hypothesised a common amino acid motif in the antigen-binding grooves of HLA molecules encoded from the multiple AIH-associated risk alleles that might favour presentation of hepatic autoantigen. Although majority have at most partial amino acid sequence similarities, analysis found that both DRB1*0301 and DRB1*0401 alleles encode an identical 6-amino-acid sequence ("shared motif") at positions 67-72 of DRβ polypeptide chain located at pocket 4 of HLA class II antigen-binding groove, with a positively-charged lysine residue at position 71 (DRβ-Lys-71) which seems to be the critical determinant of AIH-1 susceptibility because of its location at a crucial site of antigen-binding groove where antigen interacts with the T-Cell Receptor (TCR) [37]. DRβ-Lys-71 may affect disease severity in a gene dosage-dependent way as homozygous DRB1*0301 patients displayed a more severe disease (i.e. substantial clinical manifestations and poorer treatment responsiveness) compared to the heterozygotes [35]. In addition, this gene dosing concept alongside Linkage Disequilibrium (LD) may explain the worse clinical outcomes seen in DRB1*0301 than DRB1*0401, because DRB1*0301 has strong LD with DRβ-Lys-71-encoding DRB3*0101, resulting in greater gene dosing effect of DRβ-Lys-71 than DRB1*0401 which has strong LD with DRB4*0103 that encodes an arginine instead of lysine at DRβ71 [38].

Essentially, the DRβ-Lys-71 model suggests that arginine -structurally and electrostatically similar to lysine- at DRβ71 (DRβ-Arg-71) may exert the same influences as DRβ-Lys-71 does but in an attenuated way as there may be slight conformational differences in the antigen-binding grooves that affect marginally on the way the triggering epitope is presented. DRβ-Arg-71 can also be encoded by DRB1*0404 and DRB1*0405 alleles which are associated with AIH-1 in Mexican, Korean, and Japanese (Table 1). However, DRB1*1301 allele which is associated with AIH-1 in Brazil especially juvenile patients (Table 1), encodes for a negatively-charged glutamic acid at DRβ71 that forms an electrostatically different pocket 4 and thus argues against the DRβ-Lys-71 model. Nevertheless, the key susceptibility component of AIH-1 risk in Brazil seems to be the amino acid at DRβ86 instead of DRβ71 because studies found that DRB1*1302 which differs from DRB1*1301 by only one amino acid residue at DRβ86 (valine to glycine) confers protection instead of risk to AIH-1 in the same population [12].

Overall, the underlying mechanism of HLA association with AIH is still unclear. The 'shared lysine/valine' hypothesis does not tell the nature of etiologic autoantigen, but it is definitely not universal and given the geographical variations of HLA association, it is possible that the environmental factors which differ from population to population, generate distinctive peptides or epitopes to be each presented on the diverse HLA molecules. On the contrary, the real 'culprit' allele may lie in a distinct locus in HLA haplotype and associate with the disease through LD with the 'mistaken' HLA allele. It is also noteworthy that GWAS have only been conducted in two cohorts so more data from different populations are required to fully clarify the role of HLA.

Non-HLA gene: Thus far, the reported polymorphisms outside HLA locus that predispose AIH are mostly immune-related genes, such as those encoding for immunoregulatory molecules. A meta-analysis examining the association of cytotoxic T-lymphocytes-associated antigen 4 (CTLA-4) polymorphism with AIH-1 has revealed that the CTLA-4 G allele (exon-1 $^{+}$49A>G) could increase the disease risk [39]. CTLA-4 is an immune checkpoint molecule that downregulates immune responses and studies have found that AIH-1 patients carrying G allele have significantly lower number of regulatory T-cells (Treg) and diminished expression of functional CTLA-4 protein [40,41], indicating the impact of CTLA-4 polymorphism in the impairment of T-cell regulation and self-tolerance, thereby contributing to AIH-1 autoimmunity. Besides, Src homology 2 adaptor protein 3 (SH2B3) polymorphism is reported in the AIH-1 GWAS and the risk allele is rs3184504*A allele [20]. Currently, the role of SH2B3 in AIH-1 are still under investigation but SH2B3-deficient mice showed overactive immune responses characterised by increased secretion of multiple cytokines, including IL-18 and IL-21 which have been implicated in AIH pathogenesis in both humans and murine models [42].

Furthermore, genes implicated in enhancement of immune response have also been described. The tumour necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine and significant association between polymorphisms at TNFA gene promoter sequence (-308 A/G) and AIH-1 susceptibility has been reported in a meta-analysis [43]. The TNF-α-308 polymorphism has been associated with increased serum levels of TNF-α in patients of other autoimmune diseases such as RA and Systemic Lupus Erythematosus (SLE) [44], which is also a feature of AIH and may contribute functionally to AIH by promoting hepatic inflammation and injury which increase tendency to autoimmunity [45]. Besides polymorphisms, gene mutations may also lead to AIH development. For example, mutations in the Autoimmune Regulator (AIRE) gene which is important in establishing central tolerance within the thymus, causes a syndrome called autoimmune polyendocrine syndrome type-1 (APS-1). Interestingly, 20% of people suffering from APS-1 develops a disease form resembling AIH-2 [46]. This is supported by AIRE-deficient mouse models where some of them exhibit AIH-like characteristics such as lymphoplasmacytic infiltration in the liver, aminotransferase elevations, and positivity for autoantibodies [47].

Virus as potential trigger

The genetic technologies have indeed uncovered dozens of risk alleles or mutations for complex liver diseases like AIH and are increasingly being used for clinical purposes. However, it is also true that the genetic susceptibility for AIH is heterogenous as evidenced by the geographical variations of HLA and non-HLA associations, and not everyone carrying the risk factors develop AIH. This indicates that genetics do not account fully for the overall disease liability and non-genetic factors such as pathogens may be at play on the background of genetic predisposition as key drivers of AIH.

A broad variety of pathogens, especially viruses, have been associated with the initiation of many autoimmune diseases with molecular mimicry as the most described mechanism in which immune reactions towards microbial antigens turn against structurally equivalent self-antigens [46]. Molecular mimicry is well-exemplified in AIH-2 because the autoantigen CYP2D6 and its autoantibody anti-LKM-1 have been defined. Interestingly, anti-LKM-1 autoantibodies have been detected in sera of patients with chronic Hepatitis C Virus (HCV) infection [48], whereas a large proportion of AIH-2 patients are positive for antibodies against HCV [49,50], suggesting an association between AIH-2 and HCV infection. A few epidemiological studies have confirmed this link [51,52] and the involvement of molecular mimicry has been suggested when HCV polypeptide was found to share homologous sequence with the immunodominant region of CYP2D6 protein [53]. Studies later confirmed that HCV proteins-specific antibodies could cross-react with a CYP2D6 conformational epitope

consisting of the immunodominant region [54], and a case study of a child who developed AIH-2 10 years after HCV infection has also been reported [55], providing further evidences regarding the role of molecular mimicry and etiologic agent HCV in AIH-2 pathogenesis. Besides, Manns, et al. also found a homologous sequence between CYP2D6 immunodominant epitope with a protein of herpes simplex virus type 1 (HSV-1) called ICP4, suggesting that HSV-1 infection may potentially be an etiologic factor of AIH-2 through molecular mimicry; but unlike HCV, no firm epidemiological data reporting an association between HSV-1 and AIH-2 has been published. Nevertheless, in an old case study of identical twin sisters, one was normal while the other one who was diagnosed with AIH-2, was also tested positive for HSV and reactivity between her serum and ICP4 was detected in HSV-infected cell lysates [56].

There are several other suggested mechanisms of how pathogens could potentially trigger AIH. For example, pathogens may directly damage hepatocytes, thereby causing the release of autoantigens and/or inducing an intense inflammatory response that ultimately results in autoimmunity. It is noteworthy that such initiating pathogen is very difficult to find out as the pathogen may have already been cleared at diagnosis [57]. In addition, it is also difficult to detect a potential structural homology between the initiating pathogen and autoantigen because the reactivity to the initial epitopes from the pathogen might be lost after some time, leaving behind the immunodominant epitopes that share no similarities with the pathogen structure, resulting in no apparent proof for an occurrence of infection with a pathogen [57].

Several case studies have reported the development of adult AIH-1 [58,59] or juvenile AIH-2 [60] following infection with Epstein-Barr virus (EBV). However, no other comprehensive research has been done in finding out the association between EBV infection and AIH, neither the role of EBV in AIH pathogenesis. EBV has been implicated in many other autoimmune diseases and a recent study by Harley, et al. has described a novel mechanism by which EBV could trigger autoimmunity -it produces a protein called EBNA2 that recruits host transcription factors to bind and activate some of the host genes associated with the susceptibility to SLE and six other autoimmune disorders [61]. Although this has not yet been evaluated in AIH, this research has provided novel mechanistical insights into how environmental factors could interact with risk-associated genes to induce the initiation of autoimmunity.

Autoimmune Mechanisms

Regardless of the aetiologies, the pathogenesis of AIH operates in a complex scenario, involving both innate and adaptive immune responses. The latter is particularly important due to the presence of autoantibodies and T-cells, as well as their antigen-specific functional roles. The putative immunopathogenic mechanism of AIH has been summarised (Figure 1). Although the detailed mechanism is still poorly understood due to unknown aetiologies, investigations on AIH clinical features and functions of AIH-associated immune cells have provided valuable insights into the underlying mechanism driving the autoimmune attack against hepatocytes, including the breakdown of immune tolerance which has been found to play an important part in the perpetuation of AIH pathogenesis. Since the discovery of CYP2D6 as the main hepatic autoantigen target of anti-LKM-1 in AIH-2 [62], thorough investigations have been done over the years, hence it is without surprise that most knowledge on AIH pathogenesis derives from AIH-2 studies. Recently, a novel humanised murine model of AIH has been developed by using CYP2D6 immunisation of autoimmune-prone Non-Obese Diabetic (NOD) mouse that contains the human DRB1*03 gene [63]. This model is more representative of AIH than other previously described models because it resembles AIH closely in terms of disease manifestations and incorporates the influence of HLA-risk gene, providing a good tool for future elucidation of AIH pathogenesis.

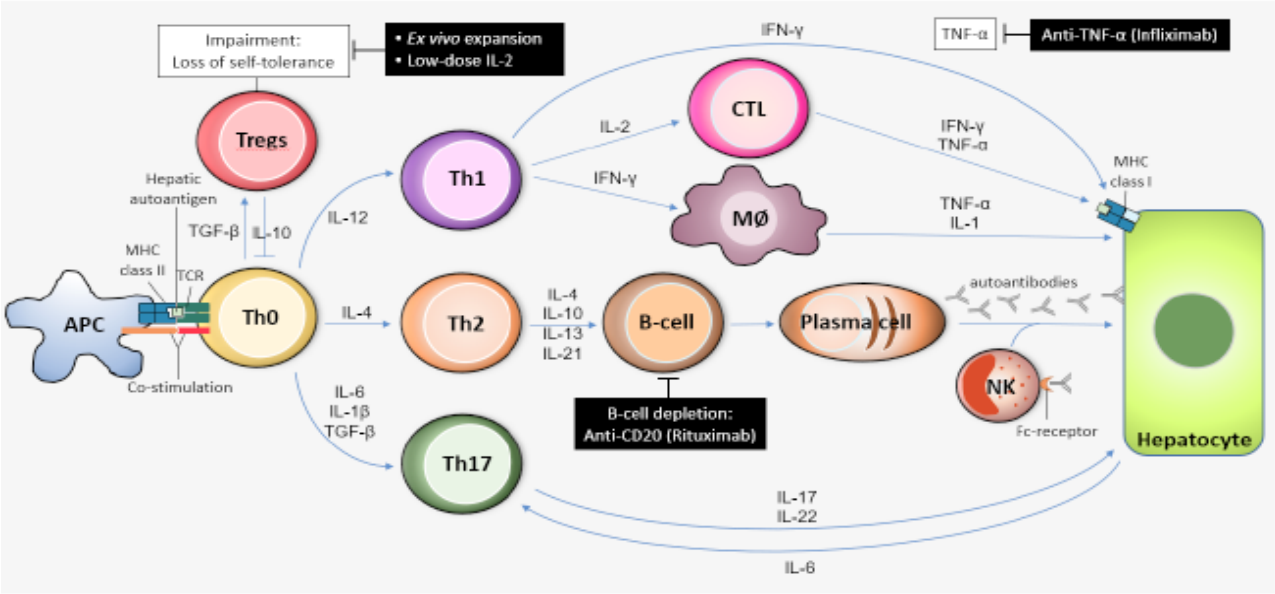

Figure 1. Putative immunopathogenic pathways and targeted immunotherapies of AIH.

Hepatic damage is believed to be initiated by the presentation of hepatic autoantigen on MHC class II to naïve $CD4^+$ Th cell (Th0). In the presence of co-stimulation, Th0 is activated and differentiated into Th1, Th2, Th17, or Tregs according to the exposure of different cytokines IL-12, IL-4, IL-6/IL-1β/TGF-β, or TGF-β alone respectively. Th1 is primarily IFN-γ secreting and it drives activation of CTLs and MØ -both of which contribute to the increased TNF-α levels (TNF-α inhibition by rituximab). IFN-γ upregulates MHC class I expression on hepatocytes, promoting autoantigen presentation for the recognition and cytotoxic killing by CTLs. Th2 secretes IL-4, IL-10, IL-13, and IL-21 that drive maturation of B-cell (depletion therapy via infliximab) into autoantibody-producing plasma cells; autoantibodies may involve in ADCC, mediated by Fc-receptor bearing NK cells. Th17 enhances inflammation by releasing pro-inflammatory cytokines IL-17 and IL-22; it also induces IL-6 secretion by hepatocytes which in turn promotes Th17 generation. Anti-inflammatory IL-10-producing Tregs are defective in AIH (Ex vivo or in vivo expansion strategy), thereby perpetuate the disease. APC, antigen-presenting cell; CTL, cytotoxic T-lymphocytes ($CD8^+$); Fc, crystallizable fragment; IL, interleukin; IFN, interferon; MØ, monocyte/macrophage; MHC, major histocompatibility complex; NK, natural killer cell; TNF, tumour necrosis factor; Th, T-helper cell; Treg, regulatory T-cells. (Figure adapted from Liberal, et al. 2016).

Effector Immune Responses

Humoral Immunity: In the context of humoral immunity, several findings have suggested the involvement of autoantibodies in driving hepatocytic damage. For instance, an early study by Vergani, et al. detected cytotoxic activity against hepatocytes within the non-T-lymphocytes compartment in chronic active hepatitis patients negative for Hepatitis B Surface Antigen (HBsAg); the cytotoxicity could be inhibited by aggregated IgG, indicating the possible participation of Antibody-Dependent-Cell-Mediated Cytotoxicity (ADCC), potentially facilitated by autoantibodies [64]. Subsequent investigation found immunoglobulins bound on the surface of hepatocytes extracted from AIH patients, and these hepatocytes were sensitive to cytotoxic killing upon incubation with autologous Fc receptor-bearing Peripheral Blood Mononuclear Cells (PBMCs) [65]. Later, study on AIH-2 patients found CYP2D6 on hepatocyte surfaces, allowing their detection by anti-LKM-1 autoantibodies (Muratori, et al. 2000). In addition, studies also found that the autoantibody titires are associated with parameters of disease severity [33], though this might also be the bystander effect of the aggravated hepatic damage.

It is unknown how other autoantibodies like SMA recognise intracellular autoantigen such as F-actin; one suggested mechanism would be the release of antigens during hepatocytic damage. Besides, nearly all the autoantigens addressed by autoantibodies in AIH are not expressed only in the liver, like CYP2D6 which is also found in the central nervous system. Investigators also face challenges in designing a study that would provide a solid evidence for a pathogenic role of AIH autoantibodies in humans as it might have to involve invasive procedures such as depletion of antibody-secreting B-cells, or disease transmission via blood transfusion. Nevertheless, attenuated inflammatory response has

been observed in AIH patients treated with anti-CD20 antibody (rituximab) therapy, suggesting an involvement of autoantibodies [66].

Cellular Immunity: In the early 1990s, Wen, et al. did a clonal assessment and detected cytotoxicity against hepatic antigens within the T-lymphocytes subpopulations [67]. Interestingly, investigators also detected a great number of HLA-DR-expressing $CD4^+$ T-cell clones and subsequent proliferative inhibition via anti-HLA-DR and anti-CD4 deduced that these clones recognise antigens as complexes with HLA class II molecules -the classic rule of immunorecognition. Investigators also found predominance of hepatic autoantigen-specific $CD4^+$ T-cells in the blood and $CD8^+$ T-cells in the liver, indicating that $CD8^+$ Cytotoxic T Cells (CTLs) are indeed present in AIH liver and help from $CD4^+$ T-cells may be crucial for the stimulation of CTLs and production of IgG autoantibodies.

Epitopes on antibodies and T-cells were mapped and presence of CYP2D6-specific $CD4^+$ T-cells and CTLs, as well as their cytotoxic activity (mainly by CTLs), were detected within the liver-infiltrating immune cells in anti-LKM-1-positive AIH-2 patients [68]. Furthermore, Ma, et al. discovered that different peptides of CYP2D6 stimulate $CD4^+$ T-cells to produce different cytokines such as IFN-γ or IL-4/IL-10, reflecting the distinctive activation profile of type 1 T-helper (Th1) cells (IFN-γ-secreting) and Th2 cells (IL-4-secreting) [29]. Moreover, the frequency of epitopes recognised and the amount of cytokine secreted by $CD4^+$ T-cells, as well as CYP2D6-specific CTLs reactivity, are in parallel with the indices of disease activity, suggesting their important contribution in AIH pathogenesis [29,69]. The CTLs could also produce IFN-γ and elicit cytotoxic activity upon recognising CYP2D6 in context of HLA class I. Besides, an overlapping sequence have been found in CYP2D6 epitopes from B-cells, $CD4^+$ and $CD8^+$ T-cells [53,69], suggesting a functional interconnection between these immune cells to this specific region, in which B-cells can present antigens to $CD4^+$ T-cells which subsequently give help for CTLs activation. In this context, $CD4^+$ Th1 might be more important than Th2 due to its production of IFN-γ which could stimulate CTLs and upregulate expression of HLA class I on hepatocytes for CTLs targeting. In fact, a recent study has reported increased expression of Th1 transcription factors (T-bet) and IFN-γ proteins in PBMCs of AIH patients while no differences were observed in Th2 transcription factor (GATA-3) and IL-4 expressions, indicating the dominant role of Th1 response in AIH [70].

$CD4^+$ Th17 cells have also been found in AIH patients, supporting the findings of increased circulating pro-inflammatory IL-17 and IL-23 levels, and upregulated IL-17, IL-23, IL-6, and IL-1β expressions in hepatocytes of AIH patients [71]. Recently, a study found that Th17 cells in juvenile AIH have heightened inflammatory and diminished immunomodulatory properties as observed from their significantly downregulated expression of CD39 (an ectonucleotidase with important immunoregulatory function), suggesting a Th17 effector characteristic that may promote AIH perpetuation [72].

Indeed, adaptive immune cells, especially T-cells, have been studied extensively and demonstrated prominent roles in AIH pathogenesis. However, there are emerging roles of innate cells such as monocytes/macrophages and Natural Killer (NK) cells. The pathogenic role of monocytes in AIH was first investigated by Longhi, et al. who reported aberrant activation of monocytes during active disease of juvenile AIH, characterised by increased migration from blood to liver, exhibition of pro-inflammatory phenotypes such as increased TNF-α production and upregulated Toll-like-receptor 4 (TLR-4) expression [73]. Recent study in adult AIH also found upregulated expression of an inflammatory signal adapter called RIP3 in monocytes which could be responsible for their aberrant activation and subsequent hepatic inflammation in AIH because RIP3 signal is associated with monocytes activity and disease severity measured by biochemical indices [74]. The pathogenic role of NK cells, on the other hand, is still elusive. However, recent study found that cytotoxic NK cells recruited from the blood into liver may account for AIH progression rather than those reside in the liver which showed marginal expansion [75].

Defective immune tolerance

Tregs are crucial in the maintenance of immunological tolerance against self-antigens. In fact, healthy individuals also have autoreactive T-cells which escape thymic selection but their reactivity in the periphery is suppressed by functional Tregs whereas those with dysfunctional Tregs develop autoimmune diseases [76]. In AIH, reduced frequency and proliferative ability of $CD4^+CD25^+$Tregs have been reported, as well as diminished functionality characterised by their inability to regulate $CD4^+$ and $CD8^+$ T-cell activation [77,78] and downregulated expression of immunomodulatory molecules like galectin-9 [79]. The galectin-9 ligand -Tim-3, expressed on $CD4^+CD25^+$ cells has also shown downregulation, suggesting that AIH may also be linked to effector cells resistance to Treg control. Furthermore, Tregs expressing the immunomodulatory molecule CD39 has shown several defects in AIH, including a decrease in number, failure to control IL-17 secretion by effector cells, and an inclination to convert into IFN-γ or IL-17-secreting effectors, all of which contribute to the defective immunosuppression and AIH hepatic damage [80].

Despite many reports on numerical and functional impairments of $CD4^+CD25^+$Tregs, Peiseler, et al. has reported normal number and functionality of $CD4^+CD25^+Foxp3^+$Tregs [81]. The cause of these opposing results lies in the difficulties to accurately identify Tregs according to expression markers such as

CD25 and Foxp3 which could also be expressed momentarily by non-regulatory $CD4^+$ T cells [82]. A study has re-investigated the phenotypic and functional characteristics of Tregs and the redefined (or 'bona fide') Tregs characterised by $CD4^+CD25^+CD127^-$ demonstrated significantly lower frequency in circulation of AIH patients compared to controls, and the frequency is inversely correlated with indices of disease activity [83]. Moreover, the same study also found dysfunctionalities of these Tregs in the production of anti-inflammatory IL-10 cytokines and suppression of effector $CD4^+$ cells.

Immunotherapies

Standard treatments

There is currently no cure for AIH, therefore the ultimate goal of treatment is to promote remission, defined as the normalisation of transaminases and IgG levels [1]. Corticosteroids (i.e. prednisolone or prednisone), with or without azathioprine (a purine analogue) have been the first line since 1970s to dramatically improve the survival rates [84]. Prednisolone should be used at diagnosis to effectively stop disease progression by suppressing hepatic inflammation while azathioprine is normally given at later time for the maintenance of remission [85]. This combination could reduce the incidences of prednisolone monotherapy side effects such as cosmetic changes, brittle diabetes, and osteopenia [2]. Budesonide, another corticosteroid, is used to replace prednisolone if those side effects cannot be tolerated by a patient; despite the fewer adverse effects, budesonide has shown lower effectiveness inducing remission in juvenile AIH compared to prednisolone [86]. In general, AIH patients demonstrate excellent response towards these conventional therapies with about 80% of them achieving complete remission after 3 years [1]. However, the remaining 20% who are refractory may progress into end-stage liver disease and require LT. In addition, relapse happens very frequently, in approximately 50-86% patients who previously had complete remission, especially during the first half a year of treatment withdrawal [2]. A more potent immunosuppressive treatment such as cyclosporine A, tacrolimus or Mycophenolate Mofetil (MMF) is considered for patients with poor response to initial conventional therapy or relapsed AIH. Besides, lifelong immunosuppressive treatment is not without risk because repressing immune system renders patients more susceptible to opportunistic infections and cancer development (e.g. HCC) [2]. Therefore, immunotherapies targeting specific immune cells or cytokines (summarised in Figure 1) could induce rapid and full remission more effectively and at the same time minimalize deleterious side effects.

Anti-TNF-α

Using Monoclonal Antibody (mAb) to block the action of certain cytokine is rare for treating AIH patients, probably due to the complexity of the pathogenesis and the difficulty to determine that one single cytokine responsible for the hepatic inflammation. Infliximab, a recombinant humanised chimeric mAb targeting against TNF-α, has been an effective treatment for several other immune-mediated diseases such as RA and inflammatory bowel disease (IBD). In a study of 11 difficult-to-treat AIH patients (i.e. unresponsive to conventional therapies and developed serious side effects) who received infliximab infusions as a rescue therapy, all showed marked reduction of inflammation while 60% achieved long-term remission, suggesting a good efficacy and also an important role of TNF-α in driving AIH hepatic damage [87]. However, 4 of them developed infectious side effects which caused them to be hospitalised and infliximab treatment had to be discontinued. Generally, the difficult-to-treat AIH patients are more vulnerable to infections following strong immunosuppressive therapies in comparison to the good responders to conventional treatment because cirrhosis, frequently seen in these patients, is a considerable risk factor for the development of infections [88]. In addition, there are several reports on the induction of AIH due to infliximab treatment in patients with RA, psoriasis, and IBD [89-91]. Hence, further studies are required to understand better the pathogenic role of TNF-α in AIH, and identifying biomarkers specifically for TNF-α activity could help to select patients that would most likely benefit from anti-TNF-α therapy.

Anti-CD20

The pathogenic role of autoantibodies in AIH remains controversial but auto aggressive B-cells in AIH could act as professional antigen-presenting cells that present autoantigens to naïve T-cells and thereby contribute to the perpetuation of autoimmune attack against hepatocytes. As mentioned above, the B-cells-depleting anti-CD20 mAb (rituximab) has shown efficacy in treating difficult-to-treat juvenile and adult AIH patients [66-92]. All patients attained full remission and the treatment was well-tolerated without any serious adverse events. However, these studies involve only a small cohort of people so more extensive studies are required to confirm the effectiveness of rituximab. Besides, regulatory B cell (Bregs), a subset of CD20+ B-cells, have demonstrated inhibitory function against pathogenic CD4+ T-cells which ameliorated AIH in experimental murine model, so targeting only the pathogenic B-cells while sparing the Bregs may improve treatment efficacy [93-94].

Tregs-based therapies

The immunoregulatory impairments observed in AIH Tregs and the fact that functional Tregs can inhibit autoreactivity in health have led to the suggestion of Tregs being a potential effective treatment in AIH aimed at re-establishing self-tolerance to own hepatic antigens. In the study by Longhi, et al. *ex vivo* generated CYP2D6-specific Tregs has significantly suppressed the expansion and function of CYP2D6-specific effector cells derived from HLA-DR3- and DR7-positive AIH-2 patients, including cytokine release

and cytotoxic activity [95]. Later, adoptive transfer of ex vivo expanded CXCR3+ Tregs in an experimental AIH-2 murine model has also successfully re-established peripheral tolerance to hepatic autoantigens and induced remission [96]. It is true that Tregs in the AIH setting have unstable lineages and are prone to adopt effector cell properties as mentioned above, thus exploiting autoantigen-specific Tregs instead of non-autoantigen-specific Tregs may be a better approach but this also means that only AIH-2 patients may benefit from this treatment since the autoantigen has been known.

Moreover, Tregs can also be expanded in vivo by injecting patients with low-dose IL-2 (LDIL-2). IL-2 is a T-cell growth factor which favours the proliferation of Tregs due to their high-affinity IL-2 receptor (CD25) expression [97]. Patients of other autoimmune diseases such as SLE and type 1 diabetes have demonstrated clinical improvements associated with increased number of circulating Tregs following LDIL-2 injections in several studies (reviewed in [98]). In a study investigating the clinical and immunological impacts of LDIL-2 in 2 difficult-to-treat AIH patients at King's College Hospital (London), it showed that LDIL-2 effects were confined to Tregs as only Tregs were significantly induced in number while other non-Treg immune cells showed insignificant changes [99]. While one of the patients who was not cirrhotic had normalisation of AST and IgG levels by the end of treatment, the other one who was cirrhotic did not experience significant changes in both levels between the baseline and end of treatment, indicating that the presence of severe hepatic damage (i.e. cirrhosis) may hamper the therapeutic effect of LDIL-2 in Tregs expansion. Although this study was small, it has provided the initial rationale for the investigation of LDIL-2 therapy in AIH. However, it is noteworthy that IL-2 can also induce effector T cells proliferation so further studies on how IL-2 affects the balance between Tregs and effector T cells are essential.

Conclusion

Despite the fact that intensive research on AIH has been conducted for six decades, the exact aetiology and mechanisms of the hepatic destruction in AIH remain poorly understood. However, progresses towards better understanding has been made: (1) genetic studies have discovered a strong link of HLA genes, and weaker link of non-HLA genes for AIH susceptibility, seriousness, and treatment responsiveness although the underlying mechanisms of these associations are still largely unknown; (2) more evidences have indicated that EBV might be a real trigger to initiate AIH development. However, the pathways leading to hepatocytic damage have not been thoroughly uncovered. Nevertheless, it is important to appreciate the tremendous advancement made since the first report about AIH six decades ago [100], especially from the finding of defective immune tolerance associated with numerical and functional diminished Tregs to current immunotherapeutic strategies aiming to re-establish tolerance by promoting Tregs expansion such as the LDIL-2 therapy. Lastly, using the newly developed "humanised" murine model developed by Yuksel, et al. [63] in future studies may potentially uncover novel mechanisms involving key immune molecules or cells that could be targets for treatment.

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