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The Pathogenesis of Autoimmune Liver disease

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Abstract

BACKGROUND: Autoimmune liver disease (AILD) encompasses three main distinct clinical diseases: autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis. These conditions are an important, yet under-appreciated cause of patient morbidity and mortality with ongoing unmet needs for further research and clinical advances.

KEY MESSAGES: There is observational evidence for genetic predisposition, with all three conditions being more common in first degree relatives. AILD is associated with the presence of auto-antibodies and higher risks of other non-hepatic auto-immune conditions. Genetic risk association studies have identified HLA and non-HLA risk loci for the development of disease, with some HLA loci providing prognostic information. This re-enforces the concept that genetic predisposition to autoimmunity is important in the context of environment exposure. Such environmental triggers are unclear but relevant risks include smoking, drug and xenobiotic exposure as well as the complexities of the microbiome. There is evidence for a loss of immune tolerance to self-antigens playing a part in the development of these conditions. In particular the IL-2 and IL-12 regulatory pathways have been implicated in pre-disposing to an unopposed inflammatory response within the liver. Main immunological themes revolve around loss of immune tolerance leading to T-cell mediated injury, imbalance in the regulation of immune cells and defective immune response to foreign antigens. For PBC and PSC there is then the added complexity of the consequences of cholestasis on hepato-biliary injury, immune regulation and liver fibrosis.

CONCLUSIONS: Whilst specific disease causes and triggers are still lacking, autoimmune liver disease arises on the background of collective genetic and

environmental risk, leading to chronic and abnormal hepato-biliary immune responses. Effective and more rational therapy will ultimately be developed when the multiple pathways to liver injury are better understood.

Key words: Autoimmune, Cholangitis, Cirrhosis, Hepatitis, Pathophysiology.

Introduction

The umbrella term of “autoimmune liver disease” (AILD) compromises three disease processes; autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). They are an important, yet underappreciated cause of liver disease, with significant associated morbidity and mortality. These conditions affect people of all ages and persist as life-long chronic diseases. **Autoimmune hepatitis** is a chronic immune-mediated liver disease characterised by hepatitis, the presence of autoantibodies and elevated IgG[1]. Histological features include portal inflammation and interface hepatitis along with lymphocytic infiltrates of both B and T-lymphocytes[2]. AIH, unlike PBC and PSC, is classically very sensitive to immunosuppression. Of the two main cholestatic autoimmune liver disorders, **PBC** is classically seen in women over the age of 40 and almost all are anti-mitochondrial antibody positive. The characteristic morphologic pattern of injury is destruction of small intrahepatic bile ducts [[3]]. Ursodeoxycholic Acid (UDCA) is the mainstay of management for PBC patients and benefit from treatment can be predicted by the biochemical response to UDCA [[4]]. **PSC** is a chronic, inflammatory cholangiopathy, closely associated with inflammatory bowel disease (IBD), and is very distinct from PBC. Histological features of PSC include lymphocytic infiltration and peri-ductal (onion-skin) fibrosis that progresses to stricturing and destruction of the medium-sized intra/extra-hepatic bile ducts [[5]]. PSC classically affects men, and 80% will also be diagnosed with IBD over their lifetime [[6]]. PSC results in clinically significant cholangitis and progresses to cirrhosis and liver failure. There is a 5-10% lifetime risk of cholangiocarcinoma and added risks of colorectal carcinoma if IBD is present [[7]]. Currently no effective medical treatment changes disease natural history.

Overview of Pathogenesis

There is likely a complex sequence of events involving genetic predisposition to auto-immunity (Table 1) in combination with exposure to environmental triggers (Table 2) that lead to the development/immunologic presentation of autoantigens, with a resulting aberrant (and uncontrolled) immune response leading to hepatic/biliary injury (Figure 1). The immune response to injury, the response of the hepatocyte and biliary epithelium to that injury, as well as the effects of cholestasis are key in determining the overall outcome, and rate of fibrosis development. The risk of cancer development seems related to the degree of fibrosis in AIH and PBC, but in PSC cholangiocarcinoma can develop without late-stage disease suggesting additional pathways to malignancy [8].

GENETICS

Twin studies, family studies, population studies and an inter-relationship with other autoimmune phenomena suggest a genetic component to risk for each disease. Until recently, understanding of this genetic risk has been limited to HLA haplotypes. Associations with risk-conferring and protective HLA haplotypes are present in all three diseases. Genetic observational studies have suggested that the presence of certain HLA alleles are associated with cholestatic liver disease, in particular HLA-DR8 in PBC and HLA-B*08 with PSC [9]. In addition, in AIH the presence of the HLA haploptype DRB1*03-DRB1*04 is associated with a more aggressive disease phenotype [10]. How HLA associates with disease is unknown but presumed to reflect the strength with which relevant antigens are presented and handled by the immune system. Genome-wide association studies (GWAS), and related genetic association studies, have further increased understanding of the genetic risk

signature of these three diseases and autoimmunity in general. Potential functional implications remain to be confirmed but key findings have pointed towards the importance of a variety of immune and non-immune pathways e.g. *IL-12/STAT4* in PBC, *CD28-IL-2* in PSC and *SH2B3* in AIH [[9]]. Additional pathways are of common relevance to the pathophysiology of autoimmunity generally (pleiotropy), and it is clear that a complex genetic architecture underpins some of the individual patient risk of developing disease.

ENVIRONMENT

Direct evidence for environmental triggers arises from drug induced AIH that has been described related to certain drugs, such as nitrofurantoin, and minocycline [[11],[12]]. This drug-induced form of AIH gives an immunological phenotype indistinguishable from that of traditional AIH, including ANA/SMA antibodies and/or raised IgG [[13]]. Most cases resolve with discontinuation of the offending agent but this can take months or even years and, like traditional AIH, some cases can progress to acute liver failure or chronically to cirrhosis[[11]]. It is proposed that the mechanism of hepatotoxicity is due to drug metabolites eliciting an immune-mediated response [[14]].

Significant environmental risk factors are also associated with the development of PBC, include smoking, use of HRT and a history of recurrent urinary infections [[15],[16]]. Some bacteria such as E.coli have been proposed to cross-react with mitochondrial proteins, and this may explain why recurrent urinary infections are more commonly seen in PBC patients than healthy controls[[17]]. Furthermore the role of xenobiotics in PBC is thought important: in PBC the lipoyl domain of the immunodominant E2 component of pyruvate dehydrogenase (PDC-E2) can be replaced by a chemical

xenobiotic mimic, which experimentally can be shown to be sufficient to break self-tolerance[18]. Potential xenobiotics are speculated to be found in substances patients are exposed to e.g. nail varnish, hair dye, cleaning chemicals [[16]]. Environmental exposures are proposed also to be relevant to the development of PSC. Smoking appears to be protective [[19]] and given the close link between IBD and PSC, it is proposed that changes in the microbiome could act as an environmental trigger. Alternative pathways to liver damage such as ischaemia, infection and toxin related damage can give rise to biliary injury that mirrors PSC with each specific secondary insult highlighting different potential common pathways to injury [[20]].

IMMUNOLOGY

The main immunological themes in disease pathophysiology revolve around loss of immune tolerance leading to T-lymphocyte mediated cell destruction, imbalance in the regulation of immune cells and defective immune response to foreign antigens [[3]].Histologic studies across all three diseases with phenotypic analysis of infiltrating lymphocytes support this [[5]].

T-cells & immune tolerance: T-lymphocytes have been shown to predominate over other cell types in areas of interface hepatitis seen in AIH [[21]]. T-lymphocytes develop in the thymus where they interact with thymic epithelial cells (TECs) which express self-antigens. Thymic cortical TECs (cTECs) utilise self-antigens presented by MHC molecules to produce, via positive selection, T-cells which are self-tolerant. In comparison, thymic medullary TECs (mTECs) express different self-antigens to eliminate auto-reactive T cells (central tolerance) and regulate the production of

regulatory T-cells (Tregs). These Tregs work peripherally to dampen the immune response (peripheral tolerance). [22]

Evidence for the loss of central immune tolerance in AIH comes from murine studies [23,24,25] and inducing non-specific T-cell activation in mice has been shown to result in T-cell mediated liver injury [26]. Evidence for the importance of mTECs in impaired central tolerance leading to AIH has also been shown experimentally [27], and is further corroborated by the description of a few families with AIRE deficiency: this gene plays a part in controlling antigen presentation in mTECs [28], and affected individuals suffer with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a syndrome that includes a 20% prevalence of AIH [29].

In the development of PBC specific loss of immune tolerance to a mitochondrial antigen, pyruvate dehydrogenase E2 subunit (PDC-E2,) is archetypal. Such high titre antimitochondrial autoantibodies are directed against a highly specific epitope within the lipoic acid binding domain of the PDC-E2. The uniqueness of AMA epitope specificity alongside the conformational changes of the PDC-E2 lipoyl domain during physiological acyl transfer is central to disease, and as above, chemical xenobiotic modification of the lipoyl domain of PDC-E2 can break self-tolerance. [28]

In addition, murine models of autoimmune cholangitis have indicated a role for Tregs and auto-reactive CD8 positive T cells in the disease process as well as a lack of regulatory B cells[30]. In PSC there is a clear T cell predominant infiltrate [5] but little knowledge to date of relevant auto-antigenic triggers. Fewer functioning Tregs can be demonstrated in PBC, PSC and AIH cohorts, supporting the concept of defective loss of immune tolerance [27,31].

Cytokines & leukocyte recruitment: The recruitment of T helper 1 & 17 and cytotoxic CD8 T-cells into the liver via the inflamed endothelium of hepatic sinusoids is mechanistically important. Interactions between these cells and the hepatocyte lead to apoptosis of the hepatocyte. Th-17 cells produce IL-17 and IL-22 which promote hepatocyte regeneration. Regulatory T-cells at the site of damage act to control Th-1, Th-17 and cytotoxic T-cells, to limit hepatic injury. [3]

All three main AILDs have been linked to abnormalities in the Th17 pathway [32,33,34].A higher frequency of circulating and intrahepatic Th-17 cells and their resulting cytokines (IL-17, IL-23) have been found in the livers of patients with AIH [33]. In particular, the IL-23/Th17 signalling pathway appears to be important in the pathogenesis of PBC but the exact mechanisms are not clear. Th17 cells secrete pro-inflammatory cytokines (such as IL-17 and TNFα) and promote epithelial repair via IL-22, when activated [3]. IL-12, IL-17 and IL-23 cytokines play an important part in regulating cell-mediated immunity and are implicated by genome-wide studies in the pathogenesis of PBC [35,36]. Patients with PBC have a preponderance of Th17 lymphocytes and IL-12 cells infiltrating their livers [37,38]. IL-17 induces IL-6 which further stimulates Th-17 cells [3]. IL-12 stimulates the function of T lymphocytes and NK cells via activating signalling factors such as STAT4 to produce cytokines critical for the function of Th1 cells [39]. IL-12 is implicated in the development of other T cell mediated auto-immune disease, such as auto-immune myocarditis [40] and SLE [41].IL-12 and IL-23 are mainly produced by antigen-presenting cells and are key

parts of the Th-17 immune pathway. STAT 4 and Tyrosine Kinase 2 proteins are important for receptor signalling for the Th-17 pathway [39].

The question remains whether modifying the IL-12 pathway has an effect on auto-immune liver disease. In mouse models, deletion of the IL-12p40 subunit appears to lead to worsening biliary inflammation and, suggesting that IL-12 may also have negative immunoregulatory functions [42].

Histological studies of patients with PSC have similarly shown a preponderance of T cell infiltration in the liver [43]. These T cells express IL-2 receptors, highlighting this pathway in disease. . IL-2 is essential for developing regulatory T cells and IL-2RA deficient mice develop fatal auto-immunity with peri-portal infiltrates [44]. GWAS studies also implicated the IL-2RA receptor as a risk locus for patients with PSC [9].

Cholangiocytes, Biliary injury & Fibrosis

In the biliary diseases PBC and PSC, the cholangiocytes are not merely passive victims of the immune system but are key to disease progression. Additionally it is clear that cholestasis itself is potentially damaging, and that the effects of bile acid signalling are complex, and of relevance to immunity, metabolism and liver regeneration. Powerful pathways for bile acid signalling, such as the FXR axis, have thus arisen as potential targets for new therapies for patients [45].

During the normal cellular life cycle, as well as in response to injury, cells, including cholangiocytes, are programmed to undergo apoptosis. Cholangiocytes that fail to complete apoptosis correctly can preserve the self-antigen on apoptotic bodies

(apotopes) which in turn can be recognised by auto-antibodies to create an immune response, a phenomenon that has been described in PBC [46]. In both PBC and PSC, cholangiocytes also show increased cellular senescence, wherein damaged cells permanently withdraw from the cell cycle but do not apoptose [47,48]. These cells accumulate in biliary tissue and secrete chemokines (such as CX3CL1 and CXCL8) and growth factors that promote immune cell recruitment and tissue repair [47]. These cells however cannot proliferate so damaged cells cannot be replaced, which may contribute to the ductopenia seen in PBC. Cholangiocytes also express Toll-like receptors that recognise pathogens and initiate a pro-inflammatory cascade to aid in tissue healing [49]. This cascade includes release of pro-inflammatory cytokines which recruit and activate T cells, macrophages, neutrophils and NK cells [3]. The presence of inflammation has been shown to cause disruptions in the tight-junctions between cholangiocytes, thus inappropriately exposing these cells to potentially toxic substances, such as the constituents of bile [50]. This has been demonstrated in animal models of PSC [51] and can also be demonstrated in colonic epithelial cells [52]. Persistent exposure to antigens via the enterohepatic circulation could pre-dispose cholangiocytes to being pro-inflammatory, in a genetically pre-disposed individual. These antigens may serve as “molecular mimics”, where foreign antigens share similar structure to self-antigens and this promotes the inflammatory response [3]. Lymphocytes activated in the gut can also be recruited towards the liver by expression of a variety of adhesion molecules, including VAP-1, which is expressed by the liver and recruits lymphocytes to areas of inflammation as well as catabolizing enteric-derived pathogens [53].

Bile itself is clearly not inert, containing bile acids, bilirubin, cholesterol, complex carbohydrates, phospholipids and proteins which can prove potentially toxic to cholangiocytes and hepatocytes [52]. Exposure to bile acids can result in cell apoptosis and necrosis [54] and cholangiocytes have mechanisms in place to protect them from such bile-related injury. Defects in these mechanisms can lead to injury and fibrosis. Once such mechanism is the binding of bile acids to cholesterol to prevent toxicity [55] and defects in transporter proteins that maintain this process (such as MDR3) have been shown to lead to biliary damage [56]. Another protective mechanism is the "biliary HCO_{3-} umbrella" whereby bicarbonate is used to further protect cholangiocytes from bile acid damage [57] . Defects in this mechanism have been described in biliary disease and represent a potential target for therapy [58].

Conclusion

In the absence of clear aetiologic agents for autoimmune liver diseases it is clear that appropriate therapy will be hampered for patients. Nevertheless over time it has become more apparent that these diseases are the result of complex and dynamic interactions between host genetic risk and environmental exposure. Subsequent immunologic and biliary driven injury results in chronic hepato-biliary inflammation and fibrosis. Approaches based on these understandings will in time hopefully lead to better and more rational therapy for patients.

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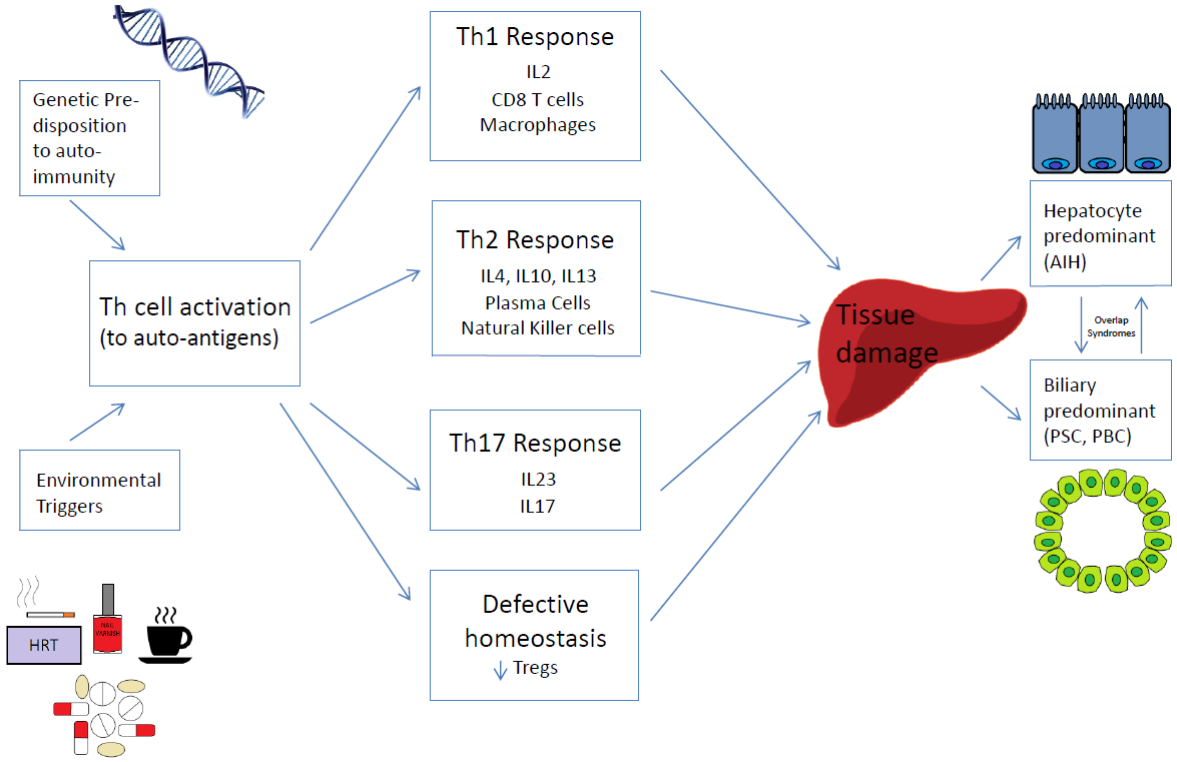
TABLE 1: Genetic Risk Factors for Autoimmune Liver Disease (adapted from Webb G, Hirschfield GM. Using GWAS to identify genetic predisposition in hepatic autoimmunity. *J Autoimmun.*2015 Sep 4.pii: S0896-8411(15)30034-2.)

Diagnosis	Genetic Association	
Autoimmune hepatitis	HLA associations	Associated with increased risk:- A1, B8, DQA1*05:01, DQB1*02:01, DQB1*06, DR4*04:04 DRB*04:05, DRB1*01, DRB1*03:01, DRB1*04:01, DRB1*13:01, DRB1*14, DRB3*01:01 Protective:- DRB5*01:01, DRB1*15:01, DQB1*04, DQB1*03:01
	Non-HLA association candidate genes	*SR2B3*
Primary biliary cirrhosis	HLA associations	Associated with increased risk:- DPB1*03:01, DQA1*04:01, DQB1*03:02, DQB1*04:02, DQB1*04:01, DQB1*06:01, DRB1*04:05, DRB1*08:01, DRB1*08:03, DRB1*14, B*39:05 Protective:- DQA1*01:02, DQA1*05:01, DQB1*03:01, DQB1*06:02, DQB1*06:04, DRB1*11:01, DRB1*11:04, DRB1*13:02, DRB1*15:01, B*07:02
	Non-HLA association candidate genes	*SH2B3, MMEL1, DENND18, IL1RL1, IL1RL2, STAT4, STAT1, CCL20, PLCL2, CD80, IL12A, , DGKQ, NAKB1, IL7R, IL12B, IL12RB2, OLIG3, ELMO1, TNFSF15, TNFSF1A,TNFSF11, POU2AF1, CXCR5, DDX6, LTBR, TYK2, SPIB*
Primary Sclerosing cholangitis	HLA associations	Associated with increased risk:- B*08:01, DRB1*03:01, DQA1*05:01, DQB1*02:01, DRB1*13:01, DQA1*01:03, DQB1*06:02, DRB1*01:01, DQA1*01:01 Protective:- DQA1*02:01, DQA1*03, DQA1*05:01, DQB1*03:01, DQB1*03:02, DQB1*03:03, DRB1*04:01, DRB1*07:01, DRB1*11:01, DRB4*01:03, DRB4*01:03, DRB4*02:02
	Non-HLA association candidate genes	*SH2B3, MMEL1, TNPSFR1, BCL211, CD28, GPR35, MST1, IL2, IL21, BACK2, IL2RA, SIK2, HDAC7, FUT2, PSMG1*

TABLE 2: Environmental Risk Factors associated with development of autoimmune liver injury

	Associations with disease
Autoimmune hepatitis	Nitrofurantoin Minocycline Hepatitis A infection
Primary biliary cirrhosis	Smoking HRT Frequent urinary infections Nail varnish Hair dye Cholestasis of pregnancy Industrial waste sites Paracetamol use
Primary Sclerosing cholangitis	Inflammatory bowel disease Smoking (protective) Coffee Hormonal contraception Diet

FIGURE 1: Overview of the pathogenesis of autoimmune liver disease.



There is likely a complex sequence of events that occur in the development of AILD. A genetic predisposition to auto-immunity in combination with exposure to environmental triggers leads to the development of CD4 T helper cells which are activated to recognise autoantigens. This can lead to a variety of immune responses, all of which can lead to tissue damage; the Th1 response involving IL-2 in the differentiation of CD8 cytotoxic T cells and macrophages; the Th17 response involving IL-17 and IL-23; the Th2 response involving IL-4, IL-10 and IL-13 as well as differentiation of B cells into plasma cells resulting in immunoglobulin production as well as activation of monocytes and Natural Killer (NK) cells; Defective cell homeostasis results in increased cell senescence and defective apoptosis. Decreased numbers of circulating Tregs affects all of these pathways. A combination of these immune responses leads to tissue injury, although it is not certain exactly

what factors are involved in the development of biliary or hepatic-predominant disease.