Semin Immunopathol (2009) 31:421–435
DOI 10.1007/s00281-009-0170-7

REVIEW

Autoimmune hepatitis

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Received: 31 May 2009 / Accepted: 1 June 2009 / Published online: 17 June 2009

Abstract Autoimmune hepatitis (AIH) is an inflammatory liver disease affecting mainly females and characterised histologically by interface hepatitis, biochemically by elevated transaminase levels and serologically by the presence of autoantibodies and increased levels of immunoglobulin G. AIH responds to immunosuppressive treatment, which should be instituted as soon as diagnosis is made. Seropositivity for smooth muscle and/or anti-nuclear antibody defines type 1 AIH, while positivity for liver kidney microsomal type 1 antibody defines type 2 AIH. The aetiology of AIH is unknown, though both genetic and environmental factors are involved in its expression. Immune reactions against host liver antigens are believed to be the major mechanism of liver damage.

Keywords Autoimmune hepatitis · Liver · Autoantibody · Humoral immunity · Cellular immunity · Immune-regulation

Abbreviations

AIH	autoimmune hepatitis
IAIHG	International Autoimmune Hepatitis Group
IgG	immunoglobulin G
ANA	anti-nuclear antibodies
SMA	anti-smooth muscle antibodies
LKM	liver kidney microsomal
anti-SLA/LP	anti-soluble liver antigen/liver pancreas antibody
LC1	liver cytosol type 1
LM	liver membrane
p-ANCA	perinuclear anti-neutrophil cytoplasmic antibodies
c-ANCA	cytoplasmic anti-neutrophil cytoplasmic antibodies
p-ANNA	perinuclear anti-nuclear neutrophil antibodies
AMA	anti-mitochondrial antibody
V	vessels
G	glomeruli
T	tubules
LSP	liver-specific protein
ASGPR	asialoglycoprotein receptor
ADH	alcohol dehydrogenase
APECED	autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
tRNP(Ser)Sec	UGA tRNA suppressor associated antigenic protein
MHC	major histocompatibility complex
HLA	human leukocyte antigen
TCR	T-cell receptor
CTLA-4	cytotoxic T-lymphocyte associated antigen 4
NK	natural killer
APC	Antigen-presenting cell
TNF-α	Tumour necrosis factor alpha
T_H	T-helper
Tr	T-regulatory
T-regs	CD4+ CD25+ regulatory T-cells
CTL	cytotoxic T lymphocyte

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CYP2D6	cytochrome P450IID6
CTL	cytotoxic T-cells
IL	interleukin
IL2-R	IL-2 receptor
GITR	glucocorticoid inducible tumour necrosis factor receptor
FOXP3	forkhead winged helix transcription factor box P3
IFN-γ	interferon gamma

Definition

Autoimmune hepatitis (AIH) is an inflammatory liver disease characterised histologically by interface hepatitis, biochemically by elevated transaminase levels and serologically by the presence of autoantibodies and increased levels of immunoglobulin G (IgG). Clinical manifestations are variable. Its onset is often ill-defined, frequently mimicking acute hepatitis and its course may be fluctuating. Hence the old term of 'chronic active hepatitis' has been abandoned. AIH usually responds to immunosuppressive treatment, which should be instituted as soon as diagnosis is made. If left untreated, AIH usually progresses to liver failure requiring transplantation. There are two types of AIH: seropositivity for smooth muscle (SMA) and/or antinuclear antibody (ANA) defines type 1 AIH (AIH type 1), while positivity for liver kidney microsomal type 1 antibody (anti-LKM-1) defines type 2 AIH (AIH type 2) [1, 2]. There is a female predominance in both types.

Epidemiology

The prevalence of AIH is unknown. It occurs worldwide in children and adults [2]. Most of the available information is on AIH type 1 and was collected before the introduction of the International Autoimmune Hepatitis Group Scoring System [3, 4], and therefore no standardised way of evaluating patients was used. Moreover, early studies did not exclude hepatitis C. Reported prevalences range from 1.9 cases of per 100,000 in Norway [5] and one per 200,000 in the US general population [6], to 20 per 100,000 in females over 14 years of age in Spain [7]. It is likely that all these figures are underestimates.

The prevalence of AIH type 2, which affects mainly children and young adults, is unknown, also owing to the fact that the diagnosis is often overlooked. At the King's College Hospital tertiary paediatric hepatology referral centre there has been a sevenfold increase in incidence of both types of AIH over the last decade [2]. AIH represents approximately 10% of some 400 new referrals per year, two third of the cases being AIH type 1 and one third AIH type 2 [2].

Diagnosis and clinical features

The diagnosis of AIH is based on the presence of positive autoantibodies, elevated transaminase and IgG levels and interface hepatitis on liver biopsy. The latter is required to confirm the diagnosis and to evaluate the severity of liver damage [2]. The levels of transaminases and of IgG do not reflect the extent of the histological inflammatory activity, nor indicate presence or absence of cirrhosis. Hepatic disorders that may share some of the above features need to be excluded by appropriate investigations in the work up to the diagnosis. These include viral hepatitis B and C, Wilson disease, non-alcoholic steatohepatitis and drug-induced liver disease. Seventy-five percent of the patients are female. A positive family history of autoimmune disorders is present in about 40% of the patients. Associated autoimmune disorders can be present at diagnosis or develop during follow-up in approximately one fifth of the patients [8], and include thyroiditis, ulcerative colitis, insulin-dependent diabetes, vitiligo, nephrotic syndrome, hypoparathyroidism and Addison disease, the latter two being observed in particular in anti-LKM-1 positive patients or in patients with autoimmune polyendocrinopathy−candidiasis−ectodermal dystrophy (APECED) [9, 10], a monogenic disorder with a variable phenotype that includes AIH in about 20% of the cases.

AIH responds satisfactorily to immunosuppressive treatment, which should be instituted as soon as diagnosis is made. There are three main patterns of disease presentation: an acute onset, characterised by non-specific symptoms of malaise, nausea/vomiting, anorexia and abdominal pain, followed by jaundice, dark urine and pale stools; an insidious onset, with an illness characterised by progressive fatigue, relapsing jaundice, headache, anorexia, amenorrhoea and weight loss and finally a presentation with complications of portal hypertension, such as haematemesis from esophageal varices, bleeding diathesis, chronic diarrhoea, weight loss and vomiting [8]. The mode of presentation of AIH is therefore variable, and the disease should be suspected and excluded in all patients complaining of symptoms and signs of prolonged or severe liver disease. Some patients, however, are completely asymptomatic and are diagnosed after incidental discovery of abnormal liver function tests.

The distinction in type 1 and type 2 AIH is particularly relevant in paediatrics, since anti-LKM-1 positive disease is rare, though not absent, in adults. In paediatrics, anti-LKM-1 positive AIH represents one third of all cases and has a clinical course similar to ANA/SMA-positive AIH, though

anti-LKM-1 positive children present at a younger age, more often with an acute onset, including fulminant hepatitis, and have associated IgA deficiency [8]. The criteria for the diagnosis of AIH have been defined and revised by the IAIHG [3, 4]. This diagnostic system, which includes positive and negative scores, was devised mainly for comparative and research purposes (Table 1) since in most instances clinical, laboratory and histological features allow the diagnosis of AIH to be made without the need of the scoring system. In the IAIHG scoring system, differences between a definite and probable diagnosis of AIH relate mainly to the degree of serum gamma-globulin or IgG elevation, levels of ANA, SMA or anti-LKM-1 and exposure to alcohol, medications or infections that can cause liver injury. Cholestatic laboratory and histological changes carry a negative score. In rare cases, the presence of non-standard autoantibodies, such as those to asialoglycoprotein receptor (anti-ASGPR), liver-specific cytosol antigen type 1 (anti-LC1), soluble liver antigen/liver pancreas (anti-SLA/LP) and atypical perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA; see below for all) supports a probable diagnosis in the absence of

Table 1 International Autoimmune Hepatitis Group diagnostic scoring system

Parameter	Feature	Score
Principal parameters		
Sex	Female	+2
ALP:AST (or ALT) ratio	>3	−2
	1.5–3	0
	<1.5	+2
Serum globulins or IgG (times above normal)	>2.0	+3
	1.5–2.0	+2
	1.0–1.5	+1
	<1.0	0
ANA, SMA, or anti-LKM-1 titers	>1:80	+3
	1:80	+2
	1:40	+1
	<1:40	0
AMA	Positive	−4
Viral markers of active infection	Positive	−3
	Negative	+3
Hepatotoxic drug history	Yes	−4
	No	+1
Average alcohol	<25 g/day	+2
	>60 g/day	−2
Histological features	Interface hepatitis	+3
	Plasma cells	+1
	Rosettes	+1
	None of above	−5
	Biliary changes[a]	−3
	Atypical changes[b]	−3
Optional additional parameters		
Seropositivity for other defined autoantibodies	Anti-SLA/LP, actin, LC1, ASGPR, p-ANCA	+2
HLA	DR3 or DR4	+1
Response to therapy	Remission	+2
	Relapse	+3
Interpretation of aggregate scores		
Pre-treatment		
Definite AIH		>15
Probable AIH		10–15
Post-treatment		
Definite AIH		>17
Probable AIH		12–17

Adapted from reference 4

ALP alkaline phosphatase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *IgG* immunoglobulin G, *ANA* anti-nuclear antibody, *SMA* smooth muscle antibody, *LKM-1* liver kidney microsomal antibody type 1, *AMA* anti-mitochondrial antibody, *SLA/LP* soluble liver antigen/liver pancreas, *LC1* liver cytosol type 1, *ASGPR* asialoglycoprotein receptor, *pANCA* perinuclear anti-neutrophil cytoplasmic antibody, *HLA* human leukocyte antigen, *AIH* autoimmune hepatitis

[a] Including granulomatous cholangitis, concentric periductal fibrosis, ductopaenia, marginal bile duct proliferation with cholangiolitis

[b] Any other prominent feature suggesting a different aetiology

Table 2 Simplified criteria for the diagnosis of autoimmune hepatitis

Variable	Cut-off	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	2[a]
or LKM	≥1:40	
or SLA	Positive	
IgG	Greater than upper limit of normal	1
	>1.10 times upper limit of normal	2
Liver histology	Compatible with AIH	1
	Typical of AIH	2
Absence of viral hepatitis	Yes	2
		≥6 probable AIH
		≥7 definite AIH

Adapted from reference 12

[a] Summation of autoantibody scores cannot exceed a maximum of 2

conventional autoantibodies. Response to steroids weights strongly towards the diagnosis of AIH and has been incorporated into the scoring system, because this condition typically enters remission during corticosteroid therapy and frequently relapses after drug withdrawal. A definite diagnosis before steroid treatment requires a score greater than 15, while a definite diagnosis after steroid treatment requires a score greater than 17 (Table 1). The diagnostic criteria for children are slightly different from those of adults. In view of the fact that autoantibodies are very rarely positive in healthy children, titres as low as 1:20 for ANA and SMA and 1:10 for anti-LKM-1 are compatible with the diagnoses of type 1 and type 2 AIH, respectively [11]. Also in adults, at times autoantibodies have a low titre or are even absent at presentation, the titre rising or becoming detectable during follow-up. Seronegative individuals, therefore, classified at presentation as having cryptogenic chronic hepatitis, may later be firmly diagnosed when conventional markers appear or when autoantibodies that are not generally available are tested.

Recently, a simplified IAIHG set of diagnostic criteria has been proposed for routine clinical practise. A reliable diagnosis of AIH in adults can be made using a very simple diagnostic score based on autoantibodies, IgG, histology and exclusion of viral hepatitis (Table 2) [12].

Histology

Interface hepatitis (hepatitis at the portal–parenchymal interface) is typical, but not exclusive, to AIH [13]. It is characterised by a lymphoplasmacytic infiltrate which crosses the limiting plate, i.e. the hepatocyte boundary surrounding the portal area, invading the liver parenchyma (Fig. 1). Other lesions typically present in AIH are hepatocyte swelling and/or pycnotic necrosis. Lymphocytes, plasma cells and histiocytes surround individual dying hepatocytes at the portal/parenchymal interface and in the lobule. Though plasma cells are usually abundant at the interface and throughout the lobule, their presence in low number does not exclude the diagnosis of AIH. In AIH presenting acutely or relapsing, panlobular hepatitis is often present, associated to bridging necrosis and, in the case of a fulminant presentation, to massive necrosis and multilobular collapse. Fibrosis is present in all but the mildest forms of AIH. Inflammatory changes surrounding the bile ducts, which may be present in a small proportion of patients with AIH, suggest an overlap with sclerosing cholangitis, as reported more frequently in the paediatric setting [14].

Though sampling variation may occur in needle biopsy specimens, especially in the presence of cirrhosis, the severity of the histological appearance is usually of prognostic value. However, it is important to remember that even patients with cirrhosis at presentation respond well to immunosuppressive treatment.

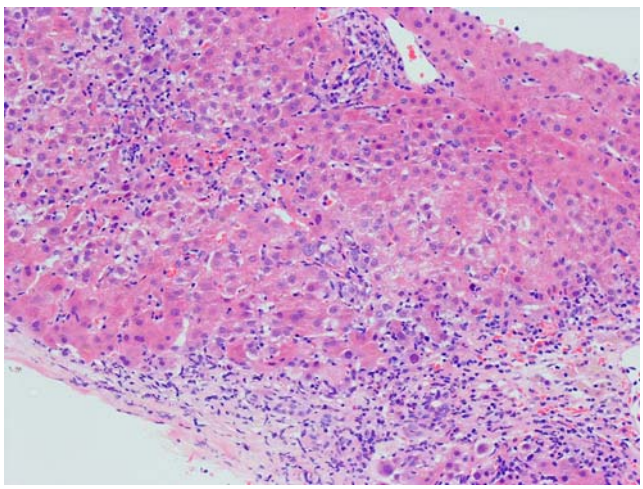

Fig. 1 Portal and periportal cellular inflammation in autoimmune hepatitis: lymphocytes, monocytes/macrophages and plasma cells infiltrate the portal tracts and invade the surrounding parenchyma, resulting in the characteristic picture of interface hepatitis. Haematoxylin and eosin staining (picture kindly provided by Dr. Alberto Quaglia)

Table 3 Methods, associations and reactants for autoantibodies in liver diseases

Autoantibody	Conventional method of detection	Molecularly based assays	Disease association	Molecular target(s)
ANA	IIF	N/A	AIH-1; overlap syndromes	Multiple targets, particularly chromatin
SMA	IIF	N/A	AIH-1; overlap syndromes	Microfilaments (actin?), intermediate filaments (vimentin etc.)
Anti-LKM-1	IIF[a]	ELISA, IB, RIA	AIH-2; HCV infection (5%)	Cytochrome P450 2D6
Anti-LC1	IIF, DID, CIE	ELISA, RIA	AIH-2	Formimino-transferase cyclodeaminase
SLA/LP	Inhibition ELISA	ELISA, IB, RIA	AIH-1; AIH-2 and AIH negative for other reactivities	tRNP(Ser)Sec (see text)
Atypical p-ANCA (p-ANNA)	IIF	N/A	AIH-1; sclerosing cholangitis	Antigen(s) at the nuclear periphery, possibly tubulin V
AMA	IIF[a]	ELISA, IB, RIA	Primary biliary cirrhosis	E2 subunits of 2-oxo-acid dehydrogenase complexes, particularly PDC-E2

ANA anti-nuclear antibody, *SMA* anti-smooth muscle antibody, *LKM-1* anti-liver/kidney microsomal antibody type 1, *LC1* anti-liver cytosol type 1 antibody, *SLA/LP* anti-soluble liver antigen/liver pancreas antibody, *p-ANCA* perinuclear anti-neutrophil cytoplasmic antibody, *p-ANNA* perinuclear anti-neutrophil antibody, *AMA* anti-mitochondrial antibody, *IIF* indirect immunofluorescence (recommended cut-off titre for positivity is 1/40 except in children—see text), *DID* double-dimension immunodiffusion, *CIE* counter-immuno-electrophoresis, *ELISA* enzyme-linked immunosorbent assay, *IB* immunoblot, *RIA* radio-immunoprecipitation assay

[a] Anti-LKM-1 and AMA both stain renal tubules and are frequently confused (see text)

Autoantibodies

A key component of the criteria developed by the IAIHG [3, 4, 15] is detection by indirect immunofluorescence of autoantibodies to constituents of the nuclei (ANA), smooth muscle (SMA) and liver kidney microsome type 1 (anti-LKM-1) (Table 3, Fig. 2). Autoantibody detection not only assists in the diagnosis but also allows differentiation of AIH in type 1 and type 2. ANA and SMA that characterise AIH type 1 and anti-LKM-1 that defines AIH type 2 are practically mutually exclusive [15]; in those rare instances when they are present simultaneously, the clinical course is similar to that of AIH type 2. Recognition and interpretation of the immunofluorescence patterns is not always

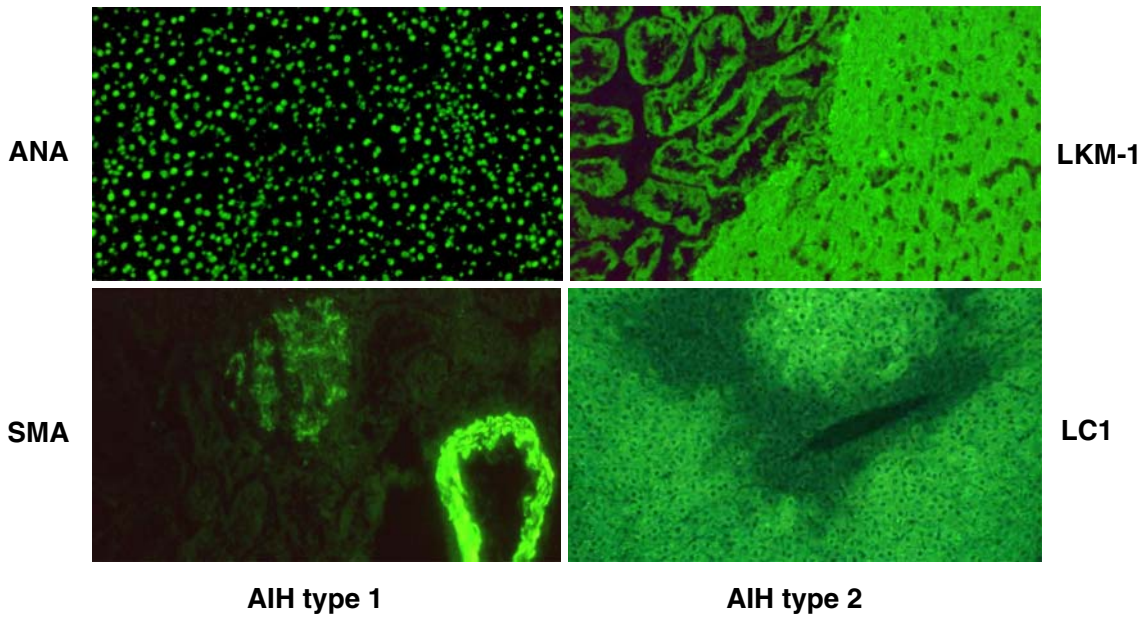

Fig. 2 Indirect immunofluorescence appearance on rodent tissue substrates of the autoantibodies diagnostic of autoimmune hepatitis (AIH). *Left* AIH type 1. *Top* anti-nuclear antibody (*ANA*), *bottom* anti-smooth muscle antibody (*SMA*) staining a vessel (V pattern) and a glomerulus (G pattern). *Right* AIH type 2. *Top* anti-liver kidney microsomal type 1 (LKM-1) antibody, *bottom* anti-liver cytosol type 1 (LC1) antibody

straightforward. The operator dependency of the technique and the relative rarity of AIH explain the non-infrequent occurrence of errors in reporting, particularly of less frequent specificities such as anti-LKM-1. Problems do exist between laboratory reporting and clinical interpretation of the results that are partly dependent on insufficient standardisation of the tests, but also partly dependent on a degree of unfamiliarity of some clinicians with the disease spectrum of AIH [16]. In regard to standardisation, a lead has been taken by the IAIHG, which has established an internationally representative committee to define guidelines and develop procedures and reference standards for more reliable testing [15].

The basic technique for the routine testing of autoantibodies relevant to AIH is indirect immunofluorescence on a freshly prepared rodent substrate that should include kidney, liver and stomach to allow the detection of ANA, SMA, anti-LKM-1 as well as anti-LC1, but also of anti-mitochondrial antibody (AMA), the serological hallmark of primary biliary cirrhosis. Positive sera should be titrated to extinction, while the pattern of nuclear staining for those ANA positive may be further characterised by the use of HEp2 cells (Fig. 3). Of particular importance is the plan of section and orientation of the kidney because both anti-LKM-1 and AMA stain renal tubules, but with different patterns distinguishable only in the presence of both proximal and distal tubules. Thus AMA stains preferentially the distal tubules, which are smaller in size, whereas anti-LKM-1 stains characteristically the third portion of the proximal tubules. The sections of liver, kidney and stomach should be dried in air and used without further fixation. Commercially available sections are of variable quality because, to lengthen shelf-life, they are treated with fixatives, which readily result in enhanced background staining that may hinder the recognition of diagnostic autoantibodies, especially when these are present at low titre.

Since healthy adults may show reactivity at the conventional starting serum dilution of 1/10, the arbitrary dilution of 1/40 has been considered clinically significant by the IAIHG. In contrast, in healthy children autoantibody reactivity is infrequent, so that titres of 1/20 for ANA and SMA and 1/10 for anti-LKM-1 are clinically relevant [14, 15]. Hence, the laboratory should report any level of positivity from 1/10, and the attending physician should interpret the result within the clinical context and the age of the patient.

Anti-nuclear antibody ANA is readily detectable as a nuclear staining in kidney, stomach and liver. On the latter in particular, the ANA pattern may be detected as homogeneous, or coarsely or finely speckled. In most cases of AIH, but not in all, the pattern is homogeneous. To obtain a much clearer and easier definition of the nuclear pattern, HEp2 cells that have prominent nuclei should be used (Fig. 3). HEp2 cells, however, should not be used for screening purposes, because nuclear reactivity to these cells is frequent at low serum dilution (1/40) in the normal adult population [14, 17]. For ANA, likely molecular targets include nuclear chromatin and histones, akin to lupus, but there are probably several others. The advent of new techniques using recombinant nuclear antigens and immuno-assays will enable a better definition of ANA target antigens, an assessment of their specificity for diagnosis and their possible role in the pathogenesis of AIH type 1 [16].

Smooth muscle antibody SMA is detected on kidney, stomach and liver, where it stains the walls of the arteries. In the stomach it also stains the muscularis mucosa and the lamina propria. On the renal substrate, it is possible to visualise the V, G and T patterns; V refers to vessels, G to glomeruli and T to tubules [18]. The V pattern is present also in non-autoimmune inflammatory liver disease, in autoimmune diseases not affecting the liver and in viral infections, but the VG and VGT patterns are more specific for AIH. The VGT pattern corresponds to the so called ‘F actin’ or microfilament (MF) pattern observed using cultured fibroblasts as substrate. Neither the VGT nor the anti-MF patterns are, however, entirely specific for the

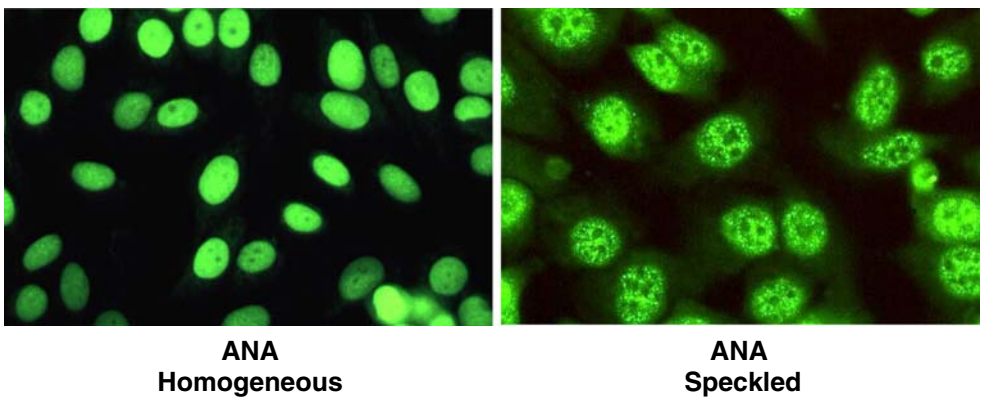

Fig. 3 Indirect immunofluorescence appearance on HEp2 cells of anti-nuclear antibody (ANA). The homogeneous pattern, *left* is the most common in autoimmune hepatitis. The speckled pattern, *right* is rare in autoimmune hepatitis, but frequent in other conditions such as chronic hepatitis C

diagnosis of AIH type 1. Though the VGT-MF pattern has been suggested to be due to a specific antibody uniquely found in AIH type 1, it may just reflect high titre SMA. The molecular target of the microfilament reactivity that is observed in AIH type 1 remains to be identified. Though 'anti-actin' reactivity is strongly associated with AIH type 1, some 20% of SMA-positive AIH type 1 patients do not have the F actin/VGT pattern [19]. The absence, therefore, of anti-actin SMA does not exclude the diagnosis of AIH.

Anti-liver kidney microsomal antibody Anti-LKM-1 stains brightly the liver cell cytoplasm and the P3 portion of the renal tubules, but does not stain gastric parietal cells [20]. Anti-LKM-1 is often confused with AMA, since both autoantibodies stain liver and kidney. Compared to LKM-1, AMA stains the liver more faintly and the renal tubules more diffusely with an accentuation of the small distal ones. In contrast to anti-LKM-1, AMA also stains the gastric parietal cells. In the context of AIH, there can be positivity for AMA in a small subset of patients (3–5%), who respond to classical treatment for AIH [21, 22]. The identification of the molecular targets of anti-LKM-1, i.e. cytochrome P4502D6 (CYP2D6) and of AMA, i.e. enzymes of the 2-oxo-acid dehydrogenase complexes, has led to the establishment of immuno-assays based on the use of the recombinant or purified antigens. Commercially available enzyme-linked immunosorbent assays (ELISAs) are accurate for detection of anti-LKM-1, at least in the context of AIH type 2, and reasonably accurate for the detection of AMA [15]. Therefore, if a doubt remains after examination by immunofluorescence, this can be resolved by the use of molecularly based immuno-assays.

Variant liver microsomal antibodies: anti-LM, anti-LKM-2 and anti-LKM-3 [16] These antibodies are mostly directed against P450 cytochrome isoforms different from 2D6. Anti-LM (liver membrane) antibodies stain only the liver cytoplasm, react with liver-specific cytochrome P4501A2 and occur in dihydralazine-induced hepatitis and in AIH associated with APECED. An anti-LKM-1-like pattern of immunofluorescence is given by autoantibodies to P4502A6 that occur in APECED and occasionally in hepatitis C. The term anti-LKM-2 was originally applied to anti-LKM-1-like microsomal antibodies produced during hepatitis induced by the no longer marketed anti-hypertensive tienilic acid and are directed against cytochrome P4502C9. Anti-LKM-3, which target members of the family 1 UDP glucuronosyltransferases, also give an immunofluorescent pattern similar to anti-LKM-1, but occur mainly in hepatitis D (delta).

Anti-liver cytosol type 1 This antibody was originally described either in association with anti-LKM-1, or in isolation, in both instances defining a clinical entity resembling AIH type 2 [23]. Later, anti-LC1 has been also found occasionally in association with the serological markers of AIH type 1 [24] and in patients with chronic hepatitis C virus infection [25]. Anti-LC1 can be detected by indirect immunofluorescence using the standard tissue panel composed of rodent liver, kidney and stomach. It stains the cytoplasm of liver cells with relative sparing of the centrilobular area, but is usually obscured by concurrent presence of anti-LKM-1 (Fig. 2). In the presence of anti-LKM-1, anti-LC1 can be detected by the use of liver cytosol in double-dimension immunodiffusion, or counter-immunoelectrophoresis, and a positive reference serum [26]. In Western blot, anti-LC1 reacts with a 58–60 kD protein when human liver cytosolic fraction is used as substrate. The molecular target has been identified as formimino-transferase cyclodeaminase [27]. The presence of anti-LC1 in isolation scores positively towards a diagnosis of AIH type 2, allowing prompt initiation of treatment.

Anti-soluble liver antigen/liver–pancreas antigen Anti-SLA [28] and anti-LP [29], earlier described separately in AIH, target the same antigen and are therefore the same autoantibody [30]. Anti-SLA was thought to identify a third type of AIH in which tests for conventional autoantibodies were negative [28]. However, early reports predated the publication of the IAIHG recommendations and used a cut-off point for conventional autoantibody levels higher than that currently used for the diagnosis of AIH. Several patients considered to have AIH type 3 were probably positive for conventional autoantibodies and therefore had type 1 or 2 AIH. Screening of cDNA expression libraries using high-titre anti-SLA serum has allowed to identify the molecular target antigen as UGA tRNA suppressor-associated antigenic protein (tRNP(Ser)Sec) [29, 31, 32]. Molecularly based diagnostic assays have become available, but their full evaluation is still under way. Though anti-SLA/LP is found occasionally in patients with AIH who are negative for ANA, SMA and anti-LKM-1, it is also frequently present in typical cases of AIH type 1 and AIH type 2, and also in AIH/sclerosing cholangitis overlap syndrome. Anti-SLA appears to be highly specific for the diagnosis of AIH. Its detection at the time of diagnosis identifies patients with more severe disease and worse outcome [33].

Anti-neutrophil cytoplasmic antibodies Most ANCA, described originally in liver unrelated diseases, are directed at cytoplasmic components of neutrophils and give either a perinuclear (p-ANCA) or a cytoplasmic (c-ANCA) pattern. c-ANCA mainly reacts with proteinase 3 and is found in Wegener's granulomatosis; p-ANCA reacts with myelo-

peroxidase and is frequently detected in microscopic polyangiitis. In AIH type1, akin to primary sclerosing cholangitis and inflammatory bowel disease, p-ANCA are frequently detected, but they are atypical, since they reportedly react with peripheral nuclear membrane components (perinuclear anti-nuclear neutrophil antibodies, p-ANNA) [32]. In contrast to AIH type 1, p-ANNA are virtually absent in AIH type 2. Detection of atypical p-ANCA can act as an additional pointer towards the diagnosis of AIH, particularly in the absence of other autoantibodies [15].

Treatment

The recognition in the early 1970s that AIH responds to immunosuppressive drugs has revolutionised its prognosis [34]. Immunosuppression should be started as soon as possible, without waiting for 6 months as suggested in early studies, particularly in patients with severe symptomatic disease. Most patients, including those with cirrhosis [35], achieve remission on 30 mg prednisolone daily for 1 month, after which azathioprine can be introduced at 1 mg/kg/day and the dose of prednisolone reduced to 5–15 mg/day to maintain the aminotransferase activity within the normal range. Once achieved, remission can be maintained by azathioprine alone [36]. It is prudent not to attempt withdrawal of immunosuppression within 2 years of diagnosis.

When the standard treatment fails, other drugs that have been tried include cyclosporin, ursodeoxycholic acid, budesonide and mycophenolate mofetil. Though encouraging results are described, these reports require further validation.

Liver transplantation is the ultimate treatment for most patients who present with fulminant hepatic failure and those who reach end-stage chronic liver disease, but AIH may recur after transplant [37].

Genetics

AIH is a ‘complex trait’ disease, i.e. a condition not inherited in a Mendelian autosomal dominant, autosomal recessive or sex-linked fashion. The mode of inheritance of a complex trait disorder is unknown and involves one or more genes, operating alone or in concert, to increase or reduce the risk of the trait and interacting with environmental factors.

Susceptibility to AIH is conferred by genes within the human leukocyte antigen (HLA) region on the short arm of chromosome 6, especially those encoding DRB1 alleles [38, 39]. Since the role of class II major histocompatibility complex (MHC) molecules (HLA in man) is to present peptide antigens to CD4 T-cells, this suggests the involvement of HLA class II antigen presentation and T-cell activation in the pathogenesis of AIH.

In Europe and North America, susceptibility to AIH type 1 is conferred by the possession of HLA DR3 (*DRB1*0301*) and DR4 (*DRB1*0401*), both heterodimers containing a lysine residue at position 71 of the DRB1 polypeptide and the hexameric amino acid sequence LLEQKR at positions 67–72 [38, 39]. In Japan, Argentina and Mexico, susceptibility is linked to *DRB1*0405* and *DRB1*0404*, alleles encoding arginine rather than lysine at position 71, but sharing the motif LLEQ-R with *DRB1*0401* and *DRB1*0301* [40]. Thus, K or R at position 71 in the context of LLEQ-R may be critical for susceptibility to AIH, favouring the binding of autoantigenic peptides, complementary to this hexameric sequence. However, an alternative model based on valine/glycine dimorphism at position 86 of the DR-beta polypeptide has been proposed, better representing the key HLA associations in patients from Argentina and Brazil [38, 39]. In a study from Japan, patients with AIH type 1 were found to have DRB1 alleles which encode histidine at position 13 [38, 39]. These HLA associations may be the molecular footprints of the prevailing environmental triggers that precipitate AIH type 1 in different environments. In this context, it is of interest that in South America possession of the HLA *DRB1*1301* allele, which predisposes to paediatric AIH type 1 in that population, is also associated with persistent infection with the endemic hepatitis A virus [41].

Susceptibility to AIH type 2 is conferred by the possession of HLA DR7 (*DRB1*0701*) and DR3 (*DRB1*0301*), patients positive for *DRB1*0701* having a more aggressive disease and severe prognosis [42].

Genetic studies looking outside MHC genes have identified the cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), an adhesion molecule that down-regulates peripheral T-cell immune responses, as a possible non-MHC gene predisposing to autoimmune disease. CTLA-4 gene polymorphisms involving a transition from adenine (A) to guanine (G) in exon 1 at position 49 confer susceptibility to a number of autoimmune conditions, including AIH type 1 where the genotype distribution is significantly different in patients in comparison to normal controls, with 32% of patients bearing the *CTLA-4*A,A* genotype, 54% the *CTLA-4*A,G* genotype and 14% the *CTLA-4*G,G* genotype, compared to 50%, 30% and 13% in the normal controls [43]. This finding, however, was not confirmed in Brazilian patients with AIH type 1 and 2 [44]. Polymorphism of the tumour necrosis factor alpha (TNF-α gene) may also confer susceptibility to AIH, Czaja et al. having reported that polymorphism at position 308 in its promoter region is associated with severity of AIH type 1 in

Europe and North America in synergy with DRB1*03 [45], a finding, however, not confirmed in Japanese patients [46].

As mentioned above, a form of AIH resembling AIH type 2 affects some 20% of patients with APECED, which is a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene and is characterised by a variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis [9, 10]. The *AIRE1* gene sequence consists of 14 exons containing 45 different mutations, with a 13-bp deletion at nucleotide 964 in exon 8 accounting for more than 70% of APECED alleles in the UK [9]. The protein encoded by *AIRE1* is a transcription factor. *AIRE1* is highly expressed in medullary epithelial cells and other stromal cells in the thymus involved in clonal deletion of self-reactive T-cells. Studies in a murine model indicate that the gene inhibits organ-specific autoimmunity by inducing thymic expression of peripheral antigens in the medulla leading to central deletion of autoreactive T-cells. Interestingly, APECED has a high level of variability in symptoms, especially between populations. Since various gene mutations have the same effect on thymic transcription of ectopic genes in animal models, it is likely that the clinical variability across human populations relates to environmental or genetic modifiers. Of the various genetic modifiers, perhaps the most likely to synergize with *AIRE* mutations are polymorphisms in the HLA region. HLA molecules are not only highly variable and strongly associated with multiple autoimmune diseases, but are also able to affect thymic repertoire selection of autoreactive T-cell clones. Carriers of a single *AIRE* mutation do not develop APECED. However, although the inheritance pattern of APECED indicates a strictly recessive disorder, there are anecdotal data of mutations in a single copy of *AIRE* being associated with human autoimmunity of a less severe form than classically defined APECED [9, 10]. The role of *AIRE1* heterozygote state in the development of AIH remains to be established. *AIRE1* mutations have been reported in three children with severe AIH type 2 and extrahepatic autoimmune manifestations [47] and in four children with AIH type 1 and a family history of autoimmune disease [48].

Immunopathogenesis

Impairment of T-regulatory cells

The mechanisms underlying the breakdown of self-tolerance in autoimmune disease have not been fully elucidated, though there is mounting evidence that a defect in homeostatic processes, normally keeping the response to self-antigens under control, is involved (Fig. 4). Early studies have shown that patients with AIH have impaired 'suppressor' cell function, which could be corrected following in vitro exposure to therapeutical doses of steroids [49]. Later on a defect specifically involving a subpopulation of T-lymphocytes controlling immune responses directed against a liver-specific membrane autoantigen was reported [50]. In line with these observations, evidence provided over the last 5 years has indicated that an impairment in regulatory T-cells is key to the loss of immune tolerance in AIH and to the emergence of uncontrolled effector autoimmune responses [51–53]. Among recently described regulatory T-cell subpopulations, namely natural killer T (NKT) cells, T-helper 3 (T_H3), T-regulatory 1 (Tr1), CD8+ CD28− and γδ T-cells, the CD4 T-lymphocytes constitutively expressing the IL-2 receptor (IL-2R) α chain (CD25) have emerged as a major subset, being central to immune-tolerance maintenance [54]. CD4+ CD25+ regulatory T-cells (T-regs), which in health constitute 5–10% of the total population of peripheral CD4 T-lymphocytes control innate and adaptive immune responses by preventing proliferation and effector function of autoreactive T-cells. In addition to CD25, also present on activated T-cells, T-regs express a number of defining markers, including the glucocorticoid inducible tumour necrosis factor receptor (GITR), CTLA-4 and the forkhead winged helix transcription factor box P3 (FOXP3), the best marker for murine T-regs and still the most specific human T-reg marker, being associated with the ability of these cells to suppress [55].

In AIH, T-regs are defective in number, this defect being more marked at disease presentation than during drug-induced remission, when a partial T-reg restoration is observed [52, 53]. T-reg frequency inversely correlates with markers of disease severity, such as levels of anti-SLA and anti-LKM-1 autoantibody titres, suggesting that T-reg reduction favours the serological manifestations of autoimmune liver disease [52].

Functional studies have demonstrated that, at variance with healthy subjects, T-regs isolated from patients with AIH are unable to regulate proliferation and interferon gamma (IFN-γ) production by CD4 and CD8 T-cells, both involved in the autoimmune liver attack [52, 53]. Akin to cell number, T-reg suppressor function improves during drug-induced remission, though never reaches levels seen in health. Autologous cross-culture experiments suggest that loss of control over T-cell effector function in AIH is due to a primary T-reg defect and not to target unresponsiveness [53].

T-regs from AIH patients are defective at promoting regulatory cytokine secretion by their targets, which in health create a regulatory environment supporting and enhancing the function of T-regs themselves [51]. In addition to impaired regulation of effector T-cell function, T-regs in AIH are

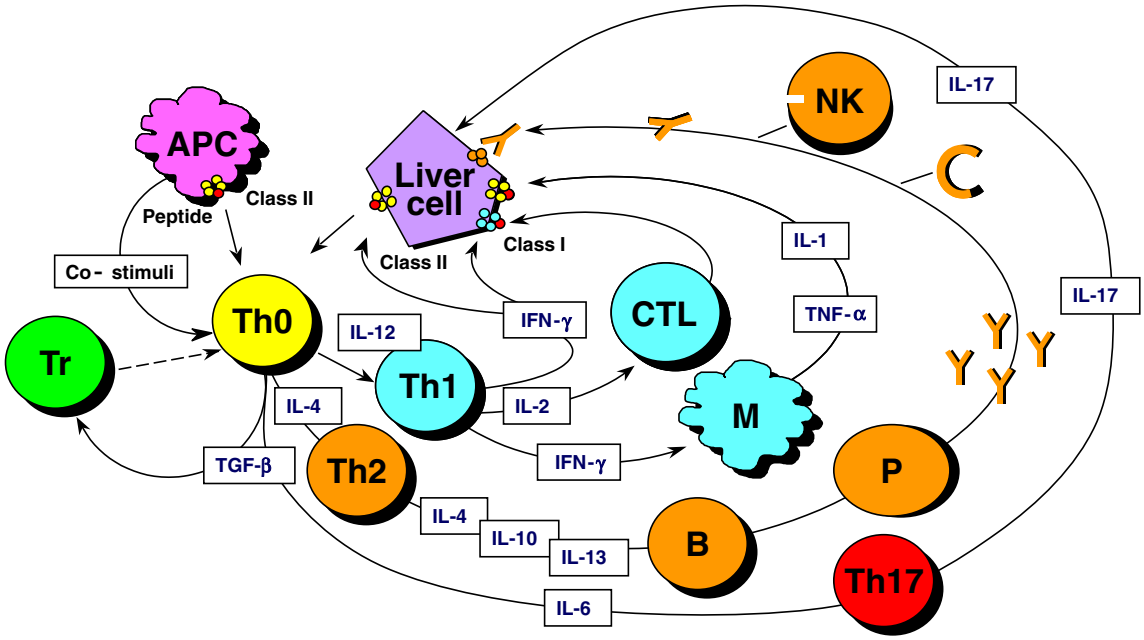

Fig. 4 Autoimmune attack to the liver cell. A specific autoantigenic peptide is presented to an uncommitted T-helper (T_H0) lymphocyte within the HLA class II molecule of an antigen-presenting cell (*APC*). T_H0 cells become activated and, according to the presence in the microenvironment of interleukin (IL)-12 or IL-4 and the nature of the antigen, differentiate into T_H1 or T_H2 and initiate a series of immune reactions determined by the cytokines they produce: T_H1 secrete IL-2 and interferon gamma (*IFN-γ*), which stimulate cytotoxic T-lymphocytes (*CTL*), enhance expression of class I and induce expression of class II HLA molecules on hepatocytes and activate macrophages; activated macrophages release IL-1 and TNF-α. T_H2 secrete mainly IL-4, IL-10 and IL-13, and direct autoantibody production by B-lymphocytes. If regulatory T-cells (*Tr*) do not oppose, a variety of effector mechanisms are triggered: liver cell destruction could derive from the action of CTL; cytokines released by T_H1 and recruited macrophages; complement activation or engagement of Fc receptor-bearing cells such as natural killer (*NK*) lymphocytes by the autoantibody bound to the hepatocyte surface. The role of the recently described Th17 cells, which arise in the presence of transforming growth factor beta and IL-6, is under investigation

unable to restrain, and even enhance, the activation of monocytes, cells of the innate immune system abundantly present in the portal–periportal inflammatory infiltrate [56].

Because of their ability to control autoaggression, T-regs represent an attractive candidate for immune intervention aiming at reconstituting self-tolerance in autoimmune conditions. The immunotherapeutic use of CD4+ CD25+ T-regs, however, has been so far hindered by their limited ability to proliferate and by their propensity to apoptosis [57], making difficult their expansion to numbers adequate for treatment. The observation of a partial T-reg restoration observed in patients during remission [52, 53] indicates that T-regs in AIH, though impaired, have the potential to expand and regain their function. Following in vitro exposure to a polyclonal T-cell stimulation, T-regs can be expanded not only in healthy subjects, but also in patients with AIH [58]. While maintaining the phenotypic features of original CD4+ CD25+ T-cells, expanded T-regs increase their FOXP3 expression alongside their suppressor function. Using the same approach, in AIH T-regs were also generated de novo and expanded from the pool of CD4+ CD25− T-cells [58], a heterogeneous population mainly composed of effector cells but also including a subset of lymphocytes with regulatory potential.

Whether T-regs with autoantigen specificity, shown in animal studies to suppress more efficiently than their non-antigen-specific counterpart, would represent an immunotherapeutic option also in AIH patients, is still under investigation. In this regard, AIH type 2 would be an excellent model for attempting reconstitution of self-tolerance by specific immunological intervention since not only is the key autoantigen, i.e. CYP2D6, known [59] but also the antigenic regions ($CYP2D6_{217–260}$ and $CYP2D6_{305–348}$), target of B, CD4 and CD8 T-cell immune responses well characterised [42, 60, 61]. Generation of T-regs specifically controlling effector immune responses to CYP2D6 would be critical for re-establishing immune tolerance in AIH type 2 and therefore achieving earlier disease remission, while limiting or maybe even preventing life-long immunosuppression.

Mechanisms leading to autoimmune liver damage

Regardless of the factors triggering the autoimmune process, the pathogenic mechanism leading to the liver damage operates in a complex scenario, which involves the intervention of both innate and adaptive immune responses. The histological picture of interface hepatitis (Fig. 1) with its striking infiltrate of lymphocytes, plasma cells and

monocytes/macrophages was the first to suggest an autoaggressive cellular immune attack in the pathogenesis of AIH. Immunohistochemical studies, focused on the phenotype of the inflammatory cells infiltrating the liver parenchyma in AIH, have revealed a predominance of αβT-cells [62]. Amongst these cells, a majority was found to be positive for the CD4 helper/inducer phenotype and a sizeable minority for the CD8 cytotoxic/suppressor phenotype. Lymphocytes of non-T-cell lineage included NK cells, monocytes/macrophages and B-lymphocytes [62].

Liver damage is believed to initiate with the presentation of a self-antigenic peptide to the T-cell receptor of an uncommitted T-helper (T_H0) lymphocyte by professional antigen-presenting cells (APCs), such as macrophages, dendritic cells and B-lymphocytes, in the presence of co-stimulating signals, induced by the interaction of CD28 on T_H0 and CD80 on APC. Once exposed to the antigen embraced by a HLA class II molecule on the APC, the T_H0 lymphocytes become activated and, according to the presence in the milieu of IL-12 or IL-4, they differentiate into T_H1 or T_H2 cells and initiate a series of immune reactions determined by the cytokines they produce. T_H1 cells predominantly secrete IL-2 and IFN-γ the latter being the main orchestrator of tissue damage because of its ability to stimulate cytotoxic T-cells (CTL), to enhance the expression of HLA class I molecules on APCs and of HLA class II molecules on hepatocytes [63, 64] and activate monocytes/macrophages, which in turn release IL-1 and TNF-α. The expression of HLA class II on hepatocytes, that normally do not express it, enables them to present the autoantigen to T_H1 lymphocytes and hence the perpetuation of the autoimmune process. The function of T_H1 cells is counterbalanced by that of T_H2 cells, arising in the presence of IL-4 and mainly producing IL-4, IL-10 and IL-13. These cytokines induce also the maturation of B cells into plasma cells, with consequent production of autoantibodies. As regulatory cells in AIH are numerically and functionally deficient, as described above, effector responses are perpetuated with ensuing persistent liver cell destruction by the direct action of CTL, cytokines released by T_H1 cells and monocytes/macrophages, complement activation or engagement of natural killer cells by the autoantibodies bound to the hepatocyte surface. The role of the recently described T_H17 cells, which arise in the presence of transforming growth factor beta (TGF-β) and IL-6, is under investigation.

Mechanisms leading to and perpetuating autoimmune attack in AIH are illustrated in Fig. 4.

Humoral immunity

The role of autoantibodies in the pathogenesis of autoimmune liver damage has been suggested by the finding that hepatocytes, isolated from patients with AIH, are coated with immunoglobulins and are susceptible to cytotoxicity when exposed to autologous Fc receptor-bearing mononuclear cells [65, 66]. It has been also shown that titres of anti-liver specific protein (LSP) antibody, as well as antibodies to the LSP components asialoglycoprotein receptor and alcohol dehydrogenase (ADH), correlate with biochemical and histological indices of disease severity [67–69]. The finding that CYP2D6 is expressed on the surface of hepatocytes, and therefore susceptible to be recognised by anti-LKM-1 autoantibodies, has led to the hypothesis that these autoantibodies could be directly involved in the pathogenesis of autoimmune liver damage in AIH type 2 [70]. Following the identification of CYP2D6 as the target antigen of LKM-1, the search for B-cell epitopes on CYPD26 has been the focus of intensive investigation. In AIH type 2 anti-LKM-1 antibodies recognise linear regions (autoepitopes) of CYP2D6 in a hierarchical manner. Thus, the principal linear B-cell epitope, $CYP2D6_{193–212}$ is recognised by 93% of patients, $CYP2D6_{257–269}$ by 85%, $CYP2D6_{321–351}$ by 53% and two additional minor epitopes $CYP2D6_{373–389}$ and $CYP2D6_{410–429}$ are recognised by 7% and 13%, respectively [60].

Using two complementary strategies, consisting in the expression of both the full-length and a series of truncated CYP2D6 proteins in a eukaryotic system, and in the characterization of the identified epitope by mutagenesis, Ma and colleagues have provided a conformational epitope mapping of CYP2D6 [71], showing that the antigenicity was confined to the C terminal portion of the molecule where it increased stepwise towards the C terminal. In addition, a short amino acid sequence, $CYP2D6_{316–327}$, further characterised by molecular modelling, was noted to be located on the surface of the molecule, suggesting that it may be directly involved in autoantibody-mediated liver cell damage [71].

Cellular immunity

Early investigations of cellular immune mechanisms involved in the pathogenesis of autoimmune liver damage led to the conclusion that patients suffering from AIH had circulating lymphocytes ‘sensitised’ to liver antigens and able to kill hepatocytes in vitro. Studies performed using separated T- and non-T-cell subsets from the peripheral blood of AIH patients and autologous hepatocytes as targets demonstrated that cytotoxic cells were present in the non-T-cell subpopulation [65]. The authors also observed high cytotoxic activity in all untreated patients with AIH but in only 40% of those in remission induced by immunosuppressive treatment [65]. The findings that the cytotoxic cells were present in the non-T-cell compartment and that aggregated IgG were able to block the cytotoxic activity

led to the suggestion that an antibody-dependent-cell-mediated cytotoxicity is involved in the pathogenesis of the autoimmune liver damage, a hypothesis confirmed later by the observation that hepatocytes from AIH patients carrying IgG on their surface were susceptible to damage by lymphocytes from healthy subjects [66]. Subsequent studies of clonal analysis, performed in the early 1990s, showed that cytotoxicity against liver-specific antigens could also be detected within the T-cell compartment [72]. Studies conducted later to identify liver-specific T-cells in peripheral blood demonstrated that patients with AIH have a tenfold higher frequency of liver antigen-specific precursors in their circulation than normal subjects [73]. Experiments of clonal analysis, performed by the same authors to investigate the function of activated T-lymphocytes in AIH, revealed a high frequency of CD4 T-cell clones expressing the HLA-DR molecule. Inhibition of proliferation with anti-HLA-DR and anti-CD4 monoclonal antibodies led to the conclusion that these clones follow the classical rules of immune recognition, being able to recognise antigens in the context of class II HLA molecules [72]. Subsequent investigations showed that in AIH the largest number of clones generated from the peripheral blood are CD4+ T-cells bearing the αβ T-cell receptor, while the highest proportion of clones obtained from liver biopsies, are CD4− CD8− T-cells bearing the γδ T-cell receptor or CD8+ αβ T-cells. Both types of liver derived clones were able to proliferate in response to liver-specific antigens, such as ASGPR and ADH; clones that proliferated in the presence of ASGPR, were also able to induce the production of ASGPR-specific autoantibodies from B-lymphocytes in vitro [73].

Autoreactive CD4 T-cells T-cell epitopic targets have been mainly studied in AIH type 2, where the autoantigen is known. The observation that autoantibodies to CYP2D6 belong to the IgG isotype, implicating a CD4-dependent class switch, and that the majority of T-cells infiltrating the liver in AIH are CD4 lymphocytes has led to studies investigating CD4 T-cell immune response against CYP2D6 in LKM-1 positive patients. $CYP2D6_{262–285}$-specific T-cell clones generated from liver tissue and peripheral blood express a T_H1 CD4+ phenotype [74, 75]. At variance with these studies, which focused on a short antigenic sequence of CYP2D6, a systematic approach based on the construction of overlapping peptides covering the whole CYP2D6 molecule was adopted to define the specificity of ex vivo CYP2D6 reactive T-cells in patients with AIH type 2 [42]. This study showed that T-cells from patients positive for the predisposing HLA allele *DRB1*0701* recognise in a proliferation assay seven regions of CYP2D6, four of which are also partially recognised by T-cells of *DRB1*0701* negative patients. While distinct peptides induce production of IFN-γ, IL-4 or IL-10, peptides that induced IFN-γ and proliferative responses overlap. There was also an overlap between sequences inducing T- and B-cell responses. The number of epitopes recognised and the quantity of cytokine produced by T-cells are directly correlated to biochemical and histological markers of disease activity. These results indicate that the T-cell response to CYP2D6 in AIH type 2 is polyclonal, involves multiple effector types targeting different epitopes and is associated with hepatocyte damage [42].

Autoreactive CD8 T-cells In the early 1990s CD8 T-cell clones specific for ASGPR were described in patients with AIH [72]. More recent studies have identified CYP2D6-specific CD8 T-cells capable of secreting IFN-γ and of exerting cytotoxicity after recognition of CYP2D6 epitopic sequences in an HLA class I restricted fashion [61]. The possibility that CYP2D6-specific CD8 T-cell immune responses are directly involved in the liver damage is suggested by the association of their strength with indices of disease activity and by their presence within the portal tract cellular infiltrate [61]. The $CYP2D6_{245–254}$ sequence, which flanks a major B-cell epitope ($CYP2D6_{254–271}$) [76] and overlaps with $CYP2D6_{225–260}$, a T-cell epitope able to induce CD4 T-cell proliferation and IFN-γ production [42], was identified as the HLA-A2 restricted sequence most effective at inducing CD8 T-cell functions [61]. The co-localisation of B, CD4 and CD8 T-cell epitopes suggests functional interrelations between immune responses to this particular region. B-cells can function as antigen-presenting cells to CD4 T-helper lymphocytes that in turn provide help to cytotoxic T-lymphocytes. The fact that B and T-cell immune responses converge on the same antigenic region indicates that this part of the CYP2D6 molecule may play a key role in the initiation and perpetuation of the autoimmune process in AIH type 2, by promoting the activation of different arms of the immune system. This region could therefore be an appropriate candidate for potential antigen-specific immune intervention.

Monocytes In addition to CD4 and CD8 T-lymphocytes, monocytes/macrophages represent a major component of the portal/periportal cellular infiltrate in AIH. A recent study has demonstrated that compared to health, peripheral blood monocytes, isolated from children with AIH, have a more vigorous spontaneous migration, which cannot be further augmented by exposure to migration-inducing stimuli [56]. In addition, monocytes in AIH are skewed towards a pro-inflammatory phenotype, as indicated by the higher TNF-α over IL-10 production and by the elevated level of expression of toll-like-receptor-4, whose engagement is key to the initiation of adaptive immune responses.

The finding of a marked monocyte activation during active disease, a time when also CD4 and CD8 T-cell autoimmune responses are at their highest in terms of proliferation and IFN-γ production, suggests a monocyte participation in the pathogenesis of liver damage in AIH, possibly promoted by autoreactive cells belonging to the adaptive arm of the immune system [56].

Taken together, the data presented above indicate an involvement of both humoral and cellular immune responses in the pathogenesis of AIH, involvement which is likely to be permitted by a failure of immune homeostatic processes.

Animal models

Research on the pathogenesis of AIH has been hampered by the lack of animal models reproducing faithfully the human condition. The ideal model for AIH should have a well-defined initiating event followed by chronic inflammation leading to fibrosis. Recently, researchers have focused on animal models of AIH type 2, since in this condition the autoantigen is well defined. The model produced by the group of Alvarez [77] is based on immunising every 2 weeks for three times C57BL/6 female mice with a plasmid containing the antigenic region of human CYP2D6, the target of anti-LKM-1 and formiminotransferase cyclodeaminase, the target of anti-liver cytosol-1, an additional marker for AIH type 2 [27], together with the murine end terminal region of CTLA-4. The latter was added to facilitate antigen uptake by antigen-presenting cells. In a parallel set of experiments a plasmid containing the DNA encoding IL-12, a T_H1 skewing pro-inflammatory cytokine, was also used. When autoantigens and IL-12 were used to break tolerance, antigen-specific autoantibodies were produced, a relatively modest elevation of transaminase levels at 4 and 7 months was observed, and a portal and periportal inflammatory infiltrate composed of CD4 and CD8 T-cells and, to a lesser extent, B-cells was demonstrated 8–10 months after the third immunisation. When the same immunisation protocol was used in different mouse strains, either a mild hepatitis or no inflammatory changes were observed indicating the importance of a specific genetic background. Another model of AIH type 2 uses CYP2D6 transgenic mice and aims at breaking tolerance with an Adenovirus-CYP2D6 vector [78]. While focal hepatocyte necrosis was seen in both mice treated with the Adenovirus-CYP2D6 vector and control mice treated with Adenovirus alone, only the former developed chronic histological changes, including fibrosis, reminiscent of AIH. The hepatic lesion was associated to a specific immune response to an immunodominant region of CYP2D6 and a cytotoxic T-cell response to Adenovirus-CYP2D6 vector-infected target cells. Though these two experimental approaches provide useful information on the possible pathogenic mechanisms leading to AIH type 2, a model mimicking closely AIH in humans is still missing.

Conclusion

The recognition that AIH is exquisitely responsive to immunosuppressive therapy has not only dramatically improved its outcome, but has also highlighted its autoimmune nature. Over the past five decades, several pathogenic aspects of AIH have been elucidated, including predisposing genetic factors and disease-specific humoral and cellular immune responses. Research aims for the future include a deeper understanding of its pathogenesis, possibly through the development of animal models faithfully reproducing the human disease, and the establishment of novel treatments aimed at arresting specifically liver autoaggression or, ideally, at reinstating tolerance to liver antigens.

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