Diagnosis and Therapy of Autoimmune Hepatitis

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Abstract: Autoimmune hepatitis (AIH) is a chronic progressive hepatitis, characterized by interface hepatitis with lymphoplasmacellular infiltrates on liver biopsy, high serum globulin level and circulating autoantibodies. It is classified into two types, according to autoantibody profile: type 1 is characterized by anti-nuclear (ANA) and/or anti-smooth muscle (SMA) antibodies; type 2 by anti-liver kidney microsomal type 1 (anti-LKM-1) antibodies. AIH affects all ages, may be asymptomatic, frequently has an acute onset, and can present as fulminant hepatitis. The diagnosis of AIH is based on a scoring system codified by an international consensus.

Corticosteroids alone or in conjunction with azathioprine is the treatment of choice in patients with AIH and results in remission induction in over 80% of patients. Alternative proposed strategies in patients who have failed to achieve remission on standard therapy or patients with drug toxicity include the use of cyclosporine, tacrolimus, budesonide or mycophenolate mofetil.

Liver transplantation is the treatment of choice in managing decompensated disease, however AIH can recur or develop de novo after liver transplantation.

Key Words: Autoimmune hepatitis, autoantibodies, autoimmune liver disease, immunosuppressive treatment.

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic disorder caused by a loss of immunological tolerance against hepatocytes and inducing chronic inflammatory destruction of liver parenchyma, cirrhosis and eventually liver failure. It is characterized by the presence of interface hepatitis on histologic examination, hypergammaglobulinemia and autoantibodies [1].

The incidence of autoimmune hepatitis among northern Europeans ranges from 0.85 to 1.9 cases per 100.000 persons per year, with a prevalence ranging from 10.7 to 16.9 cases per 100.000 persons per year [2,3].

AIH preferentially affects women (gender ratio is 3.5:1) and all ages, from infants to elderly, are susceptible. Originally described in white northern Europeans and North Americans, AIH is now recognized to be worldwide occurring in all ethnic groups, although with greatly different prevalences [4-8].

Classically AIH is subdivided in two types according to serum autoantibody profile: type 1 AIH (AIH-1) is characterized by anti nuclear (ANA) and anti smooth muscle (SMA) antibodies [9]; type 2 AIH (AIH-2) is marked by antibodies to liver and kidney microsomes type 1 (LKM1) and liver cytosol antibody (LC1) [1,10]; differently from AIH-1 that affects all ages, AIH-2 is characteristic of infant-juvenile age.

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AIH-1 is strictly associated with HLA alleles that influence the occurrence, clinical expression, and treatment outcome of the disease. In North America and Europe, HLA-A1-B8, HLA-DRB1*0301 and HLA-DRB1*0401 (DR3 & DR4) have been associated with a susceptibility to AIH [11,12]. DRB1*0301 is the principal susceptibility allele, and DRB1*0401 is a secondary, but independent risk factor.

Different ethnic groups have different susceptibility alleles: HLA DRB1*0404 is the principal risk factor in China and Mexico, HLA DRB1*0405 confers susceptibility in Japan, China, Argentina, while HLA DRB1*07 has been associated wih the development of anti-LKM1 positive AIH in German, Italian and South American patients. DRB1*1301 is associated with AIH in India, Brazil, Argentina, Venezuela, and in DR3/DR4-negative northern American patients [6, 12-16].

It has been shown that alleles also correlate with clinical phenotype and outcome. HLADRB1*03 is associated with early-age onset, SMA with anti-actin specificity, diminished response to corticosteroids, and frequent requirement for liver transplantation.

HLADRB1*04 is associated with a older-age onset, higher frequency in women, presence of ANA, a greater occurrence of other immune diseases (especially autoimmune thyroiditis), more favourable response to immunosuppressive treatment [1, 17].

The pathogenic mechanisms of AIH are still unknown. Various mechanisms have been proposed to explain the onset of an autoimmune hepatocytes response. The "molecular mimicry" hypothesis has been demonstrated to be a primary mechanism in the pathogenesis of autoimmune disease both

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in experimental models and in the human setting at the level of both T and B cells [1].

According to this theory, an immune response to external pathogens may become directed towards structurally similar self components in genetically susceptible individuals. Several agents have been reported as potential triggers of the disease, including certain viruses (hepatitis A, hepatitis C, Epstein-Barr, cytomegalovirus, Herpes virus type 6, Herpes simplex) and drugs (minocycline, atorvastatin, diclo-fenac, isoniazid, α-methyldopa, nitrofurantoin, and propyl-thiouracil). It has been proposed that triggers might share epitopes that resemble self-antigens and they may break self-tolerance by overcoming antigenic ignorance, mimicking sequestered epitopes, or generating neoepitopes [18-20].

Whether drugs and viral infections can induce AIH, unmask a latent disease, or simply cause a form of hepatitis with accompanying autoimmune features is unclear. Moreover, most cases have no identifiable trigger.

Patients with AIH have been reported to have a defect in a subpopulation of T cells, named CD4+CD25+ regulatory T cells (T-regs), controlling the immune response to self antigens

Recent studies suggest that an immunoregulatory dysfunction, characterized by decreased number and function of regulatory T cells, leading to escape from normal suppression of autoreactive T cells, occurs at diagnosis or during relapse of AIH [21].

The percentage of T-regs correlates inversely with anti-SLA and anti-LKM-1 autoantibody titres, suggesting that a reduction in T-regs activity favours the serological manifestations of AIH. These observations suggest that treatment strategies concentrated on restoring the ability of T-regs to expand, with consequent increase in their number and function could be effective. This is at least partially achieved by standard immunosuppression, as numbers of T-regs increase during remission [21-23].

Recurrence of AIH after liver transplantation has been described in both adult and paediatric patients. It is recognized in 22% of patients especially in individuals receiving inadequate immune suppression [24].

Although the rate of this complication increases with the post-transplant time, it may appear as early as 1 month post surgery. The recurrence is usually well controlled by adjustments in the immunosuppressive regimen, but it can potentially lead to cirrhosis and graft failure [25].

AIH can also develop “de novo” in 3% to 5% of children and adults transplanted for non-autoimmune conditions, regardless of the immunosuppressive regimen [26,27].

It should be considered in the differential diagnosis of all forms of late graft dysfunction after liver transplantation.

Finally, development of AIH has been also reported after allogenic bone marrow transplantation in both adult and paediatric patients [28].

We have recently described a case of autoimmune hepatitis developed after peripheral-blood stem-cell transplantation in an adult man with non-Hodgkin's lymphoma, with appearance of liver/kidney microsomal type 1 (LKM1) autoantibodies [29].

Thus, although rare, AIH should be also considered when liver dysfunction appears after bone marrow transplantation as early recognition and treatment could be lifesaving or prevent the need of liver transplantation.

DIAGNOSIS

AIH does not have pathognomonic features and its clinical, biochemical, serological and histological manifestations can be found in liver diseases caused by different aetiologies [30].

Given the lack of a gold standard for the diagnosis of AIH, the accurate exclusion of any possible liver disease cause is the first step in the diagnostic process. An panel of experts (International Autoimmune Hepatitis Group – IAIHG), by combining different parameters (clinical, biochemical, serological, histological and immunogenetic), established a cumulative score, first issued in 1993 and revised in 1999, in order to simplify AIH identification (Table **1**) [30,31].

The “definite” diagnosis requires compatible histological picture, “hepatitic” biochemical pattern, circulating autoantibodies and abnormalities of serum globulins.

There are no AIH-specific histological changes however, interface hepatitis, plasma cell infiltrates, lobular hepatitis and centrolobular necrosis are typical [1,31].

Recently, a simplified score taking into account four parameters namely autoantibodies, gamma glubulins, histological changes and absence of viral hepatitis, has been proposed as a highly sensitive and specific diagnostic system for routine clinical practice (Table **2**) [32].

Results from a recent study comparing the diagnostic performances have showed that the two proposed scoring systems are not interchangeable, and each may be useful in certain clinical situations. The original scoring system has greater value in diagnosing patients with few or atypical features of AIH, especially in patients with cryptogenic or autoantibody-negative chronic hepatitis, while the simplified scoring system is more useful to exclude the diagnosis in patients with etiologically distinctive disease who have concurrent immune manifestations [33].

A key component of the criteria developed by IAIHG is the detection of autoantibodies by indirect immunofluorescence (IIF) which support the diagnosis and also allow differentiation of AIH into type 1 and type 2. Autoantibodies titres can fluctuate up to disappearance during the course of the disease and the immunosoppressive therapy.

Autoantibody testing is the first diagnostic step in the evaluation of acute and chronic hepatitis of undetermined cause and hepatic dysfunction following liver and bone marrow transplantation. Interpretation of the IIF patterns is not always straightforward and it is largely dependent on the observer’s experience. The operator dependency of the technique and the rarity of AIH can explain the occurrence of

Table 1. Revised Score System for Diagnosis of Autoimmune Hepatitis (1999)

Parameter	Score	Parameter	Score
Female sex:	+2	Liver histology:	
ALP/ALT ratio*:		Interface hepatitis	+3
<1.5	+2	Predominantly plasma cell infiltrate	+1
1.5–3.0	0	Rosetting of liver cells	+1
>3.0	−2	None of above	−5
Serum globulins or IgG above normal:		Biliary changes	−3
>2.0	+3	Other changes***	−3
1.5–2.0	+2		
1.0–1.5	+1	Other autoimmune disease(s)	+2
<1.0	0		
ANA, SMA, or LKM1**:		Optional additional parameters:	
>1:80	+3	Seropositivity for other autoantibodies****	+2
1:80	+2	HLA DR3 or DR4	+1
1:40	+1	Response to therapy:	
<1:40	0	Complete	+2
AMA positive:	−4	Relapse	+3
Hepatitis viral markers:			
Positive	−3	Interpretation of aggregate score	
Negative	+3	Pre-treatment:	
Drug history:		Definite AIH	>15
Positive	−4	Probable AIH	10–15
Negative	+1		
Average alcohol intake:		Post-treatment:	
<25 g/day	+2	Definite AIH	>17
>60 g/day	−2	Probable AIH	12–17

ALP: serum alkaline phosfatase; ALT: serum alanine aminotransferase; ANA: antinuclear antibodies; SMA: smooth muscle antibodies; LKM1: anti liver kidney microsomes type 1 antibodies; AMA: antimitochondrial antibodies; HLA: human leukocyte antigen.

* ratio between the degree of elevation above upper normal limit of these enzymes.

** titres determined by indirect immunofluorescence on rat tissue sections or HEp-2 cells.

*** features suggestive of a different aetiology such as steatosis, iron overload due to genetic hemochromatosis etc.

**** other defined autoantibodies are: anti liver cytosol type 1 antibodies (LC1), anti soluble liver antigen/liver pancreas antibodies (SLA).

Modified from Alvarez F, *et al*., *J. Hepatol.,* **1999** [31].

errors in reporting and discrepancies between different laboratories in autoantibody frequency.

In order to achieve a standardization, the IAIHG established an internationally representative committee to define guidelines and standard procedures for more reliable testing [34].

According to IAIHG recommendation a clinically significant level of positivity would start at the dilution of 1:40 as healthy adults may show reactivity at serum dilution of 1:10. In contrast, in healthy children, autoantibody reactivity is infrequent, so that titres of 1:20 for ANA and SMA and 1:10 for anti-LKM1 are considered clinically relevant.

SMA are non organ specific autoantibodies (NOSA) and they are directed against actin and non actin cytoskeleton components of smooth muscle and other cells. The detection of SMA is classically based on IIF tecnique using conventional substrates such as rodent stomach, liver and kidney. SMA reacts with the wall of small arteries present in all three

Table 2. Simplified Diagnostic Criteria for Autoimmune Hepatitis (2008)

Parameter	Cutoff	Score
ANA or SMA*	1:40	1
ANA or SMA*	1:80	
or LKM *	1:40	
or SLA	positive	2**
IgG:	>Upper normal limit	1
	>1.10 times upper normal limit	2
Liver histology ***:	compatible with AIH	1
	typical AIH	2
Absence of viral hepatitis:	yes	2
Interpretation of aggregate score:	= 6: probable AIH	
	≥ 7: definite AIH	

ANA: antinuclear antibodies; SMA: smooth muscle antibodies; LKM1: anti liver kidney microsomes type 1 antibodies; SLA: anti soluble liver antigen/liver pancreas antibodies;

* titres determined by indirect immunofluorescence on rat tissue sections or HEp-2 cells.

** addition of points achieved for all autoantibodies (maximum, 2 points).

*** evidence of hepatitis is a necessary condition.

The simplified score was found to have 88% sensitivity and 97% specificity (cutoff 6) and 81% sensitivity and 99% specificity (cutoff 7) for the diagnosis of autoimmune hepatitis.

Modified form Hennes E, *et al*., *Hepatology*, **2008** [32].

tissues, the muscular layer of the stomach and the vascular axis of the lamina propria of the gastric mucosa [35].

The examination of the kidney reactivity is of relevance, since it allows to recognize three immunomorphological patterns: a) SMA-V (vessels): isolated positivity of small/ medium-size vessel walls; b) SMA-G (glomeruli): positivity of glomerular mesangial cells in addition to vessels; c) SMA-T (tubuli): positivity of peritubular structures in addition to vessels and glomeruli. High titres of SMA-G and, especially, SMA-T were found to be associated with anti-filamentous actin (F-actin) reactivity [36].

In our experience anti F-actin reactivity, revealed by SMA-T/G patterns or anti-microfilaments antibodies (anti-MF), is highly predictive of AIH-1, with a sensitivity of approximately 80% and a specificity of about 90% [35].

Other suitable substrates for SMA research, especially for anti-actin reactivity are represented by cultured human fibroblasts, vascular smooth muscle cell lines, HEp-2 cells.

Recently, a commercially available ELISA assay, where purified filamentous actin (F-actin) in its native form is used as antigen, has been set-up. We have compared the diagnostic performance of anti F-actin antibodies by ELISA with that of SMA in 78 consecutive patients with AIH-1 and 160 controls. SMA *tout court* were detected in 78% of our AIH-1 patients and in 20% of the controls. Anti F-actin antibodies were present in 70.5% of AIH-1 patients and in 6% of controls. However, the SMA-T/G patterns, were never detected in the controls thus showing the highest specificity for AIH-1 with a sensitivity of 60%. Positivity for anti F-actin antibodies did not identify a clinically distinct subgroup of AIH patients [37].

ANA occurring in AIH-1 are directed against different and heterogeneous nuclear components, such as double stranded DNA, histones, ribonucleoproteins, lamins and others not yet identified [38].

ANA are routinely searched by IIF on rat tissue sections or HEp-2 cell slides. Most commonly, “speckled” and “homogeneous” or “diffuse” ANA patterns are detectable. ANA positivity and the IIF pattern associated does not correlate with treatment response or disease outcome [39].

The target of LKM1 is the cytochrome P450 2D6 (CYP2D6), a drug-metabolizing enzyme situated in the endoplasmic reticulum of the hepatocytes while the antigen recognized by LC1 has been identified as a liver-specific 58-kd metabolic enzyme named formiminotransferase cyclodeaminase [40,41].

The identification of the molecular targets of anti-LKM-1 and LC1 has led to the development of accurate immunoassays based on the use of the recombinant or purified antigens.

Other autoantibodies, such as atypical perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), antibodies to double stranded DNA (anti-dsDNA) and antibodies to a soluble liver antigen (SLA), have been reported in patients with AIH-1 and can support the diagnostic suspect in the absence of conventional autoantibodies [42-44].

The target of anti-SLA has been identified as a ~50 kDa UGA serine tRNA-associated protein complex (tRNP(Ser) Sec). Detection of anti-SLA, renamed anti-SLA/liver-pancreas (LP) when these antibodies were discovered to be identical to LP antibodies, was initially considered to identify a

Table 3. Autoantibodies Frequencies at Presentation in 163 Italian Patients with AIH

	All patients	Type 1 AIH (n=125)	Type 2 AIH (n=38)	*p*
ANA-D	55 (34%)	54 (43%)	1 (3%)	<0.0001
ANA non-D	25 (15%)	22 (18%)	3 (8%)	ns
SMA-AA	67 (41%)	66 (53%)	1 (3%)	<0.0001
SMA non-AA	24 (15%)	17 (14%)	7 (18%)	ns
Anti-LKM1	25 (15%)	0/125	25 (66%)	<0.0001
Anti-LC1	20 (12%)	0/125	20 (53%)	<0.0001
Anti-SLA	12/104 (11%)	12/71 (17%)	0/33	0.008
pANCA	41/106 (39%)	41/72 (57%)	0/34	<0.0001
Anti-dsDNA	31/151 (20%)	31/117 (26%)	0/34	<0.0001

ANA-D: "diffuse" ANA pattern

SMA-AA: SMA anti-actin pattern

Modified from Muratori P, *et al*., *J. Hepatol*., **2009** in press [46].

third type of AIH, seronegative for the conventional ANA, SMA, anti-LKM1 autoantibodies. Subsequent studies showed that really anti-SLA/LP positive patients have clinical, serological and laboratory features indistinguishable from patients with AIH-1 [42]. Thus, anti-SLA/LP antibodies, in virtue of their high specificity, may be considered as additional marker, useful in reclassifying as AIH cases initially considered as cryptogenic chronic hepatitis [42, 45].

According to literature data about thirteen percent of adults with chronic hepatitis, without viral markers, satisfy diagnostic criteria for AIH but are autoantibody negative. Although these patients are often designated as having cryptogenic chronic hepatitis, they are very similar to patients with classical AIH in age, female sex prevalence, serum liver tests, histological findings, HLA repertoire. In addition, autoantibody-negative patients with cryptogenic chronic hepatitis respond as well to corticosteroid treatment as do patients with classical AIH [45].

As some patients can develop conventional autoantibodies later in the course of disease, seronegative individuals may be classified at presentation as having cryptogenic chronic hepatitis until conventional markers appear [45].

In Table **3** is summarized our experience about autoantibody repertoire in a consecutive series of 163 Italian AIH patients [46]. Fig. (**1**) illustrates the main IIF patterns of autoantibodies detectable in AIH.

a: Smooth muscle antibodies (SMA) with "peritubular" pattern. Positivity of vessels, glomerular mesangial and peritubular structures on rodent kidney section (magnification 40 X). **b:** Antinuclear antibodies (ANA) on rodent liver section (magnification 40 X).

c: Antinuclear antibodies (ANA) with "homogeneous" or "diffuse" pattern on HEp-2 cells with extremely positive mitotic cell nuclei (magnification 40 X). **d:** Anti Microfilaments pattern: typical positivity of microfilaments/"stress fibres" on vascular smooth cell line derived from the thoracic aorta of rat embryo (VSM47, Euroimmun AG, Lübeck, Germany) (magnification 40 X).

e: Perinuclear anti-neutrophil cytoplasmic antibodies (pANCA): pattern of positivity on alcohol-fixed human neutrophils (magnification 40 X). ***f***: Antibodies to double stranded DNA (ds-DNA): pattern of positivity on *Crithidia Luciliae* with staining of the kinetoplast (magnification 40x). **g:** Liver-kidney microsome type 1 (LKM1) antibodies: staining of the third portion of the proximal tubules on rodent kideny section (magnification 20x).

h: Liver-kidney microsome type 1 (LKM1) antibodies: homogenous staining of the hepatocyte cytoplasm on rodent liver section (magnification 20x).

i: Liver cytosolic antigen type 1 (LC-1) antibodies: uneven staining of the liver lobule, with sparing of the hepatocytes around the central vein on rodent liver section (magnification 20x).

The clinical presentation of AIH is heterogeneous, varying from a symptomatic disease (where fatigue, arthralgia, myalgia and anorexia, are the most common symptoms) to an asymptomatic onset [47]. In these latter patients the diagnosis is generally suspected and performed during routine general medical examinations that include the screening of liver tests. Nearly a quarter of patients present an acute icteric *poussèe* which requires a differential diagnosis with acute viral hepatitis. In this case the main differential parameter is represented by the gamma globulin serum level which is highly suggestive of AIH when it is higher than 19 g/L [29]. Finally, a fulminant presentation has been also reported, particularly in cases of type 2 AIH [48-50].

Concurrent immune diseases are frequent. The most frequent associated autoimmune diseases include autoimmune thyroiditis (particularly in elderly patients), Graves' disease, ulcerative colitis, alopecia, vitiligo, insulin-dependent diabe-

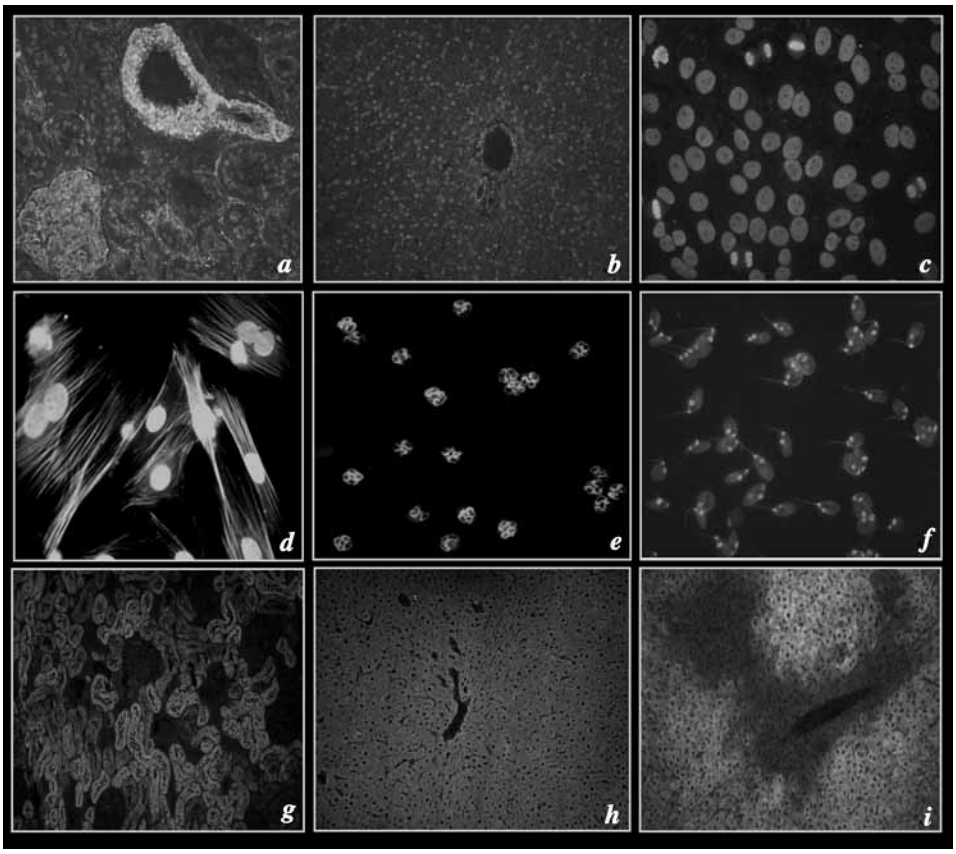

Fig. (1). Indirect Immnufluorescence autoantibody patterns with diagnostic relevance in autoimmune hepatitis (AIH). Modified from Muratori P *et al.*, *J. Hepatol.*, **2009** in press [46].

tes mellitus, rheumatoid arthritis, autoimmune haemolytic anaemia, Sjögren's syndrome [17,51].

Moreover, 3%-6% of patients with AIH are affected by asymptomatic celiac disease. In these patients usually autoimmune liver damage is unaffected by the gluten free diet. Nevertheless, there are obvious benefits from the early detection and treatment of celiac disease in terms of normal absorption of medication and calcium with maintenance of skeletal integrity in corticosteroid-treated patients [52].

Variant forms of AIH with concomitant biochemical and/or histological features typical for the other autoimmune liver diseases such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), have been extensively reported. The term of "overlap" syndrome is used when the characteristics of PBC or PSC coexist with the features of AIH [1].

The diagnosis of overlap syndrome is at present difficult, since the IAHG cumulative score used to identify AIH has not been validated in this particular setting; PBC and PSC are identified on the basis of antimitochondrial antibody positivity and the demonstration of biliary beadind respectively. The prevalence of AIH/PBC and AIH/PSC is around 10–15% [53].

The occurrence of hepatocellular carcinoma in patients with AIH is low even in those with histological cirrhosis. Nevertheless, malignancy is a possible consequence of the disease, and continued surveillance of these patients is justified . Male gender, history of blood transfusion, features of portal hypertension, treatment failure, immunosuppressive treatment for ≥3 yr, and cirrhosis of ≥10 yr duration seem to identify AIH-1 patients with a higher risk for hepatocellular carcinoma [54].

STANDARD TREATMENT OF ADULTS WITH AUTOIMMUNE HEPATITIS

Prednisone alone or in combination with azathioprine is the standard treatment for all forms of AIH [55].

The first controlled clinical trials, published in the early 1970s, demonstrated that prednisone alone or prednisone/ prednisolone with azathioprine, but not azathioprine monotherapy, improve clinical, biochemical and histological parameters and are effective in reducing mortality. Eighty percent of treated patients reach remission within 3 years [56-58].

The life expectancy of succesfully treated patients exceeds 80% after 20 years of follow-up and is similar to that of age- and sex- matched normal subjects. Moreover, the presence of cirrhosis at presentation does not preclude the success of corticosteroid therapy or survival expectation [59].

According to the published guidelines, absolute indications for treatment are: i) serum aminotransferase levels

Table 4. Treatment Schedules According to Guidelines of AASLD

	Monotherapy	Combination Therapy	
	Prednisone only (mg/day)	Prednisone (mg/day)	Azathioprine (mg/day)
Week 1	60	30	50
Week 2	40	20	50
Week 3	30	15	50
Week 4	30	15	50
Maintenance until end point	20	10	50
Conditions that favor each regimen	Cytopenia Absent thiopurine methyltransferase activity Pregnancy Malignancy Short trial (≤6 months)	postmenopausal state Osteoporosis Brittle diabetes Obesity Acne Emotional lability Hypertension	

Modified from Czaja AJ and Freese DK, *Hepatology*, **2002** [60].

greater than 10-fold the upper normal limit or, ii) serum aminotransferase levels ≥ 5-fold with gamma globulin elevation ≥ 2-fold the upper normal limit or, iii) bridging necrosis and/or multiacinar necrosis on liver biopsy irrespective of aminotransferase/gamma globulin serum levels [60,61].

In young patients the appropriate starting therapeutic regimen is prednisone monotherapy at the dose of 0.5-1 mg/kg/d; in patients who have preexistent comorbid diseased (e.g. osteoporosis, diabetes, hypertension, obesity), or in middle-aged patients, the combination theraphy of prednisone (0.5 mg/kg/d) with azathioprine (50-100 mg/d) is preferred.

In patients having symptoms (fatigue, jaundice, myalgia, arthralgia) and/or serum aminotransferase/gamma globulin elevation, but not satisfying the abosolute criteria of treatment, the therapeutic regimen should be individualized [1].

In Table **4** is illustrated treatment schedules proposed by American Association for the Study of Liver Diseases (AASLD) in 2002 [60].

Both compliance and treatment outcome can be monitorated by aminotransferase serum levels. Immunosuppressive treatment should be slowly tapered to avoid a relapse of disease which frequently occurs when the tapering is inappropriately fast.

In this connection, azathioprine could be used as a corticosteroid-sparing agent especially when high doses of prednisone have been required to control the disease activity.

Remission is defined by absence of symptoms, normal serum bilirubin and gamma globulin levels, serum aminotransferase levels normal or less than twice normal, inactive or normal liver histology [60-61].

There is no firm guidelines for decision regarding duration of treatment after remission; the physician should decide whether to completely stop the immunosuppressive therapy, with the risk of relapse, or to prescribe the minimum dose possible to maintain the remission.

Maintenance of the biochemical remission, even with low-dose steroids, is the most important goal in treating AIH, and prevents evolution of the disease [46].

It has been recently reported by Czaja that a mild form of AIH-1, characterized by patients who did not satisfy preestablished criteria for severe disease, is not rare and that untreated asymptomatic patients with mild disease have a lower 10-year survival expectation than treated patients who satisfied similar criteria for mild disease [62].

Patients with a fulminant presentation who fail to improve biochemical parameters after 2 weeks of corticosteroid therapy are candidates for liver transplantation. Liver transplantation should be also considered in patients with decompensated cirrhosis or those developing hepatocellular carcinoma that meets transplantation criteria. The actuarial 10-year survival after transplantation is 75% [48].

Typically, recurrence of AIH after liver transplantation can be easily managed by introduction or increased dose of corticosteroids. Treatment of "de novo" AIH is similar to that commonly used in classical AIH and usually resulting in excellent graft- and patient survival [63].

MECHANISMS OF DRUG ACTION

Mechanisms of glucocorticoid action involve the glucocorticoid receptors, the glucocorticoid-responsive genes, and the release of anti-inflammatory molecules [64]. Prednisone (Fig. **2**) is converted to prednisolone within the liver and the unbound prednisolone is the biologically active metabolite which produces therapeutic, but also well known side effetcs [65].

Prednisolone can diffuse across cell membranes into the cytosol to bind the glucocorticoid receptor (GR) that is com-

plexed with two molecules of a 90 kDa heat shock protein (HSP-90) (Fig. **3**).

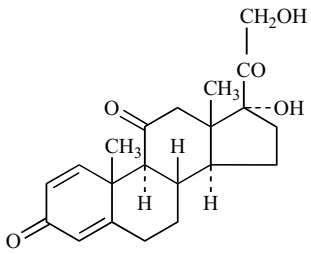

Fig. (2). Structural formula of prednisone.

Once the glucocorticoid bind to GR, the two HSP-90 molecules dissociate from the receptor thus allowing the activated prednisolone-GR complex to translocate to the nucleus where it binds to positive or negative GR-responsive elements (GREs) in the promoter regions of target genes [66].

Receptor binding to the regulatory sequences of the GREs increases or decreases their expression. The transcritpion of inflammatory genes including interleukin (IL) 1β, IL-2, IL-4, IL-5, IL-6, IL-12, tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ), is suppressed through binding to negative GREs [67,68].

Conversely, the transcription of immunosuppressive genes such as annexin-1, mitogen-activated protein kinase (MAPK), the IL-1 receptor antagonist (that blocks the binding of IL-1 to its receptors thus counteracting the effect of the pro-inflammatory cytokine IL-1) and IL-10 is induced *via* positive GREs [69].

Glucocorticoids also antagonize the activity of transcription factors required to drive optimal cytokine transcription, including Nuclear Factor Kappa B (NF-kB), activated protein-1 (AP-1) complex, and nuclear factor of activated T cells (NF-AT).

It has been shown that glucocorticoids induce the transcription of the gene encoding the inhibitor of Nuclear Factor Kappa B subtype a (IkBa), which reduces the amount of NF-kB that translocates to the nucleus, thus markedly down-regulating pro-inflammatory cytokines secretion [70].

These "genomic" effects determine inhibition of cytokine expression and secretion which results in a profound inhibition of T-cell effector function [70,71].

Before the recent description of CD4+CD25+ regulatory T cells, a defective function of T "suppressor" cells was described in AIH patients, and *in vitro* improvement of suppressor T-cell activity was reported after preincubation of AIH patients' lymphocytes with low-dose of prednisolone [72,73].

More recently, it has been demonstrated that glucocorticoids increase the number and function of the CD4+CD25+ regulatory T cells and can restore to some extent their suppressive actions on the cell-mediated cytotoxic response [23].

The thiopurine drugs, azathioprine, 6-mercaptopurine (6-MP), and 6-thioguanine (6-TG) are commonly used in a variety of clinical conditions such as hematological malignancies, inflammatory bowel diseases, solid organ transplantation and autoimmune diseases. Azathioprine (Fig. **5**) and 6-MP are purine analogues that act as antagonists to the endogenous purines and exert cytotoxic effects after metabolism to thiopurine nucleotides. [74].

Azathioprine is a pro-drug which is almost entirely (88%) converted to 6-MP and methylnitroimidazole in the liver. Following intracellular uptake, 6-MP is futher converted, by three different enzymes, into 6-thiouric acid (6-TU), 6-methyl-MP (6-MMP), and 6-TG nucleotides (6-TGN) (Fig. **5**) [75].

The 6-TGN, as a result of their structural similarity to the endogenous purine-base guanine, are incorporated into DNA of leukocyte leading to cell-cycle arrest and apoptosis with following immunosuppression [74-75].

Moreover, one of the 6-TGN, the 6-thioguanine triphosphate (6-TGTP) exerts immunosuppressive effects because of inhibition of Rac1 upon CD28 co-stimulation, inducing T-cell apoptosis. Rac1 is a small GTPase that mediates a number of important physiologic functions including inhibition of T-cell apoptosis [76].

6-TGTP binds to Rac1 instead of GTP thereby suppressing the activation of Rac1 target genes such as mitogen-activated protein kinase (MEK), NF-kB, and bcl-x_L, leading to a mitochondrial pathway of apoptosis [76].

The immunosuppressive mechanism of azathioprine usually takes at least 1 to 2 months before obtaining the full clinical effects [74].

ADVERSE EFFECTS

The dramatic success of the immunosuppressive therapy in AIH patients is offset by the development of drug-related side effects and the difficulty in maintaining remission without a long-term therapeutic regimen [60].

Glucocorticoid-related side effects are the main causes leading to premature discontinuation of therapy. The most common side effects are mild and include cosmetic changes such as facial rounding, weight gain, striae rubrae, acne and hirsutism which are reversible decreasing the dosage or withdrawing the drug. Severe adverse effects include diabetes mellitus, osteoporosis, psychiatric disturbance, cataracts, hypertension, and opportunistic infections, but these events usually occur after prolonged therapy with high doses [78].

Hypoalbuminemia and protracted hyperbilirubinemia have been associated with increased levels of free prednisolone in the blood and, therefore, with an enhanced risk of drug toxicity.

It has been shown that the frequency of glucocorticoid-related adverse effects is significantly higher in patients with cirrhosis than in those without cirrhosis [79].

The most common azathioprine-related adverse events include nausea, vomiting, fever, arthralgias, skin rashes, bone marrow suppression with cytopenia, pancreatitis and hepatotoxicity. They typically resolve after dosage reduction of drug discontinuation.

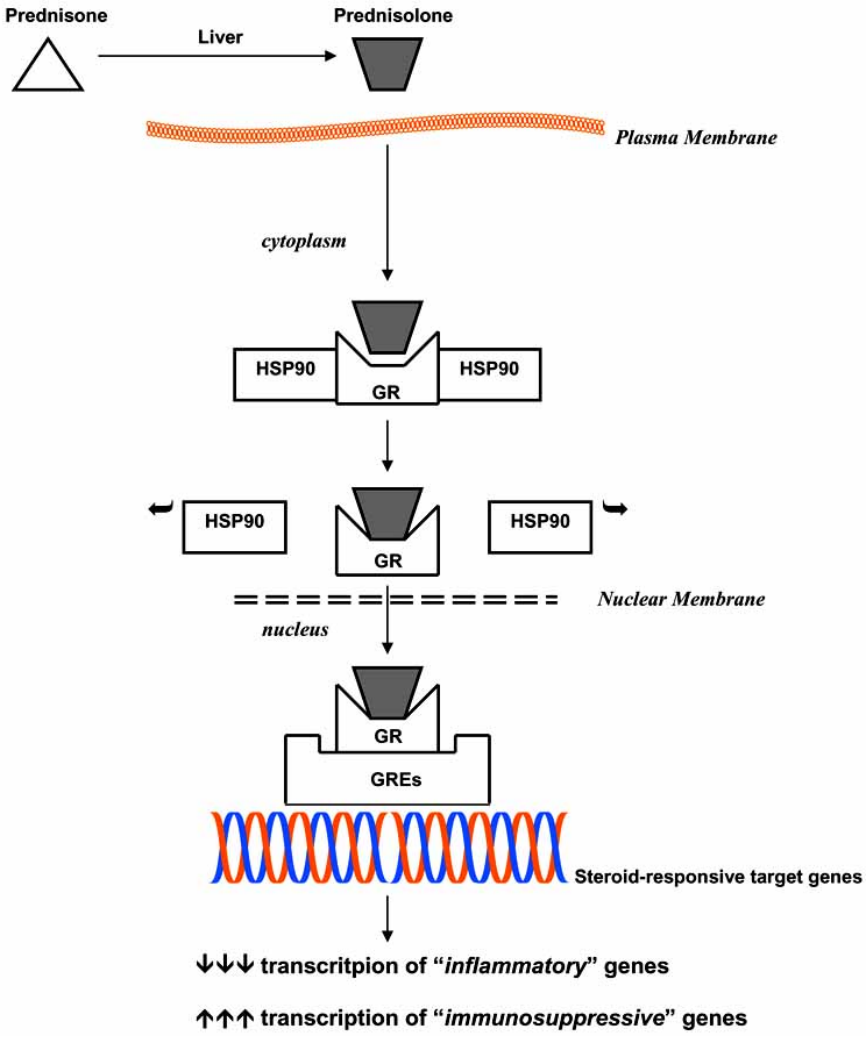

Fig. (3). Mechanism of action of Prednisone. GR: glucocorticoid receptor; HSP90: 90 kDa heat shock protein; GREs: GR-responsive elements.

As above reported, the 6-TGN are the active metabolites responsible for both therapeutic and toxic effects of azathioprine. Drugs that inhibit xanthine oxidase activity (XO), such as allopurinol, or deficiencies / variations in thiopurine methyltransferase (TPMT), reduce the competing enzymatic conversion of 6-MP in inactive metabolites and increase the available pool of 6-MP for conversion to 6-TGN with following enhance of therapeutic action and drug toxicity of azathioprine.

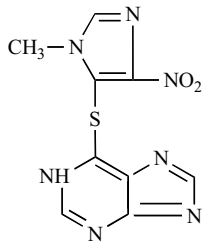

Fig. (4). Structural formula of azathioprine.

Moreover, it should be considered that the genes encoding TPMT are highly polymorphic with 90% individuals having high activity, but 10% have intermediate activity and 0.3% low or not detectable enzyme activity [74].

Although routine screening for TPMT activity has not been established in the treatment of AIH, determination of TPMT activity (phenotype or genotype) could be a useful instrument for individualizing therapeutic regimen [60,78].

Azathioprine therapy has been associated with birth defects in animal models while data on birth outcome among women exposed to AZA during pregnancy remain limited. As AIH is usually well managed with corticosteroids in pregnant women, it is reasonable to avoid azathioprine during pregnancy [77].

NEW THERAPIES

Multiple alternative therapies have been proposed to treat AIH patients with inadequate response or intolerance to standard treatment with glucocorticoids and azathioprine.

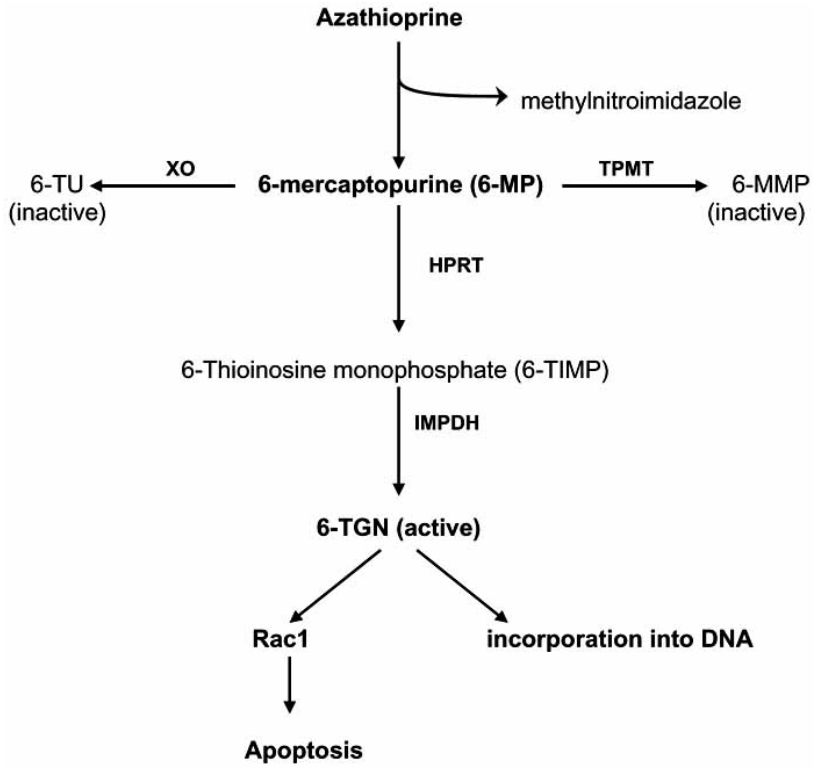

Fig. (5). Mechanism of action of azathioprine. XO: xanthine oxidase; TPMT: thiopurine methyltransferase; 6-TU: 6-thiouric acid; 6-MMP: 6-methyl-MP; HPRT: hypoxanthine phosphoribosyl transferase; IMPDH: inosine monophosphate dehydrogenase; 6-TGN: 6-TG nucleotides.

These include cyclosporine, mycophenolate mofetil, tacrolimus and budesonide.

Cyclosporin A (CyA) and tacrolimus, are calcineurin inhibitors which suppress the immune system by preventing interleukin-2 (IL-2) production in T cells. CyA and tacrolimus are structurally different molecules that bind to the intracellular immunophilins cyclophilin and FKBP-12, respectively.

When bound, both molecules inhibit the phosphatase action of calcineurin, which is required for the movement of nuclear factors in activated T cells to the chromosomes where subsequent cytokine synthesis occurs. Decreased secretion of IL-2 prevents proliferation of the inflammatory response *via* B cells and T cells [80].

These treatment could be effective in decreasing T cell–mediated inflammation in the liver of patients with AIH. At present their use in AIH is not still well documented.

Malekzadeh *et al.* reported the results of CyA treatment for 26 weeks in 19 patients, nine of whom had not received previous corticosteroid treatment and 10 of whom had either been unsuccessfully treated or had discontinued treatment due to intolerable side-effects. The mean serum AST and ALT levels decreased significantly, the histological activity index improved, and the medication was well tolerated [81].

CyA was also tested in a pilot, multicenter clinical trial involving thirty-two children with AIH. CyA alone was administered for 6 months, followed by combined low doses of prednisone and azathioprine for 1 month, after which CyA was discontinued. Twenty-five children normalized alanine aminotransferase activity levels by 6 months and all the patients by 1 year of treatment with few and well-tolerated adverse effects [82].

Fewer data are available about use of tacrolimus in AIH. Aqel et observed signifcant improvement of AST and ALT serum level in 11 patients with steroid refractory AIH treated with tacrolimus [83]. In a following study Larsen *et al.* confirmed efficacy of tacrolimus in nine patients with steroid refractory AIH [84].

Mycophenolate mofetil (MMF) is a reversible and non-competitive inhibitor of inosine monophosphate dehydrogenase which inhibits the *de novo* synthesis of guanosine nucleotides in lymphocytes reducing T and B lymphocyte proliferation. The selectivity of MMF for lymphocytes is due to its inhibition of the *de novo* pathway as other cells can make use of the salvage pathway for synthesis of guanosine nucleotides [85].

Recent reports have indicated that it could be effective in problematic AIH patients.

A retrospective study was performed in 15 AIH patients who received MMF either as monotherapy or in combination with prednisone after failure or intolerance of the initial regimen. Administration of MMF, either as monotherapy or

in combination with prednisone, resulted in biochemical and histologic improvement without the development of significant complications [86].

In a retrospective analysis of 29 patients receiving MMF (12 were switched to MMF after intolerance or nonresponse to prednisone +/- AZA, whereas 17 received MMF +/- prednisone as initial therapy) MMF was associated with a high rate of intolerance (34%). In those who could tolerate it, it was associated with a high rate of remission (84%) [87].

In another study, of 36 patients who failed standard therapy and were treated with MMF, 14 patients (39%) experienced remission. A total of 22 patients (61%) did not respond sufficiently to MMF. Of eight patients with prior nonresponse to azathioprine, six (75%) did not respond to MMF and only two (25%) reached biochemical remission. Of 28 patients with azathioprine intolerance, in 16 (57%) patients the response to MMF was insufficient and in 12 patients (43%) remission was reached [88].

Budesonide is a synthetic glucocorticoid with a high degree of first-pass metabolism which reduces its systemic bioavailability, and it has a 15-fold greater affinity for the glucocorticoid receptor than prednisolone. Budesonide has been tried both as frontline and salvage treatment in AIH patients.

In a first pilot study Czaja reported that budesonide therapy with 3 mg thrice daily for 5 +/- 1 months, was associated with a low frequency of remission and high occurrence of treatment failure in ten patients, who were dependent on continuous treatment to prevent exacerbation of their disease [89].

Csepregi *et al.* reported a clinical and biochemical remission in fifteen of the eighteen patients (11 with AIH alone and 7 with an overlapping primary biliary cirrhosis or primary sclerosing cholangitis) who received 3 mg thrice daily. Ten patients received budesonide as first-line therapy and seven of them entered remission [90].

Wiegand *et al.* treated 12 patients for three months and found budesonide monotherapy to be effective in inducing complete remission in seven patients and a partial response in three [91].

In a small series of Canadian patients, budesonide has been successfully used in seven of nine patients with AIH who were either intolerant to prednisone and azathioprine or prednisone-dependent. No adverse effects were reported with budesonide [92].

Ursodeoxycholic acid (UDCA) is a hydrophilic and nontoxic bile acid which is recognized as a main treatment for chronic cholestatic liver diseases. It is presumed to have immunomodulatory properties, to alter HLA class I antigen expression on cellular surfaces, and to suppress immunoglobulin production. UDCA can improve laboratory tests when administered with standard corticosteroid therapy, however does not facilitate reduction in the dose of corticosteroids or reduce histological activity. The setting in which UDCA may be beneficially used is AIH form with cholestatic fetaures and overlap sindromes with PBC or PSC [93].

Overall, these preliminary studies have shown possible benefit as potential second-line treatment strategies in patients refractory or intolerant to standard therapy, but there are not evidences to suggest their use as first line therapy in AIH. Multi-center trials with large patient number are needed to better define efficacy and target population of new therapies.

Other drugs such as methotrexate, cyclophosphamide and deflazacort, have been empirically used for treatment of AIH but in a very limited number of patients therefore no general conclusions can be drawn [94-97].

FUTURE PROSPECTS

In the last decades several pathogenic aspects of AIH, including genetic factors and humoral and cellular immune responses have been elucidated. Identification and characterization of disease-associated autoantibodies has allowed to define age distribution and to improve diagnostic process.

Standard immunosuppressive treatment with corticosteroid and azathioprine is associated with an excellent prognosis in the vast majority of patients. However, treatment failure occurs in a subgroup of patients where the liver disease progresses either despite appropriate treatment, either because of intolerance to standard treatment. These patients represent the main therapeutic challenge requiring new alternative treatment strategies.

The notion that functionally enhanced Tregs can be expanded and generated "de novo" will hopefully pave the way to ex vivo strategies aiming at the reconstitution of the compromised immune system regulation in patients with AIH [98].

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