Abstract

Autoimmune hepatitis (AIH), formerly known as chronic active hepatitis (CAH), first attracted attention around 1948. It probably existed as “subacute hepatitis” before that time, however. Young women were mainly affected and the outlook was poor. Suspicions of an immunological abnormality in CAH were raised by extreme hypergammaglobulinemia, but the likely primary culprit then seemed to be a persistently active virus in the liver. Various anti-tissue antibodies became recognized during 1955 to 1965, detected first to nuclear antigens by the lupus erythematosus (LE) cell test and immunofluorescence (IFL) for antinuclear autoantibody, then to a smooth muscle antigen with the true reactant later identified as the microfilament F actin, and then to an antigen enriched in endoplasmic reticulum (microsomes) of liver and kidney cells. The availability of recombinant or finely purified autoantigen now allows for automated molecular assays for some of these reactivities and this, with improved IFL technologies, has led to serological confidence in diagnosis with institution of highly effective suppressive therapies. Meanwhile immunologists remain sorely challenged in their attempts to define the pathogenetic steps from initiation, relentless persistence to ultimate hepatocytolytic destruction in this enigmatic liver disorder, AIH. Autoimmune hepatitis has been a controversial subject since being named as such in 1965, with eventual international endorsement in 1993. Hence, relevant historical material is included.

Keywords: actin microfilaments, antinuclear antibody, autoimmune hepatitis, hypergammaglobulinemia, immunofluorescence, liver-kidney microsomal antibody

Autoimmune hepatitis (AIH) was formerly known as chronic active hepatitis (CAH) and became recognized after 1948 as a novel progressive inflammatory liver disease mainly affecting young women. Chronic active hepatitis likely existed before 1948 as judged by a description by Himsworth in 1947 of a disease he called “subacute hepatic necrosis,” which fit features of CAH and progressed to a coarsely nodular post-necrotic necrosis initially described in 1943 and attributed then to non-resolving viral hepatitis. Historical timelines are detailed in reference 1 and are summarized in Table 1.

The first hint of an immunological aberration was hypergammaglobulinemia, sometimes extreme, detectable by electrophoresis and later biochemically. This together with impaired synthesis of albumin by a failing liver constituted the earlier laboratory marker, reversal of the serum albumin-globulin ratio. But in the 1950s the nature of CAH was moot; the main suspect remained chronic infection with a...
hepatitis virus, but this was verifiable (for some cases) only later when markers of hepatitis virus infection became available. Advances in the laboratory in the 1950s did, however, provide better insights into CAH. Percutaneous liver biopsy enabled closer study of liver histology throughout the course of disease, and measurement in serum of aminotransferase enzymes released by damaged hepatocytes gave a day-by-day indication of “activity.” These procedures confirmed that CAH was indeed associated with an ongoing active inflammation in the liver. A more provocative test emerged in the 1950s, prompted by the detection in blood in cases of CAH of lupus erythematosus (LE) cells, seen as providing a link between CAH and systemic lupus erythematosus (SLE) that, by the mid-1950s, had joined the newly recognized group of autoimmune diseases based on the associated anti-nuclear autoantibodies (ANA). This finding of LE cells in CAH led to the designation of a subset of cases as “lupoid hepatitis.”1 The status of this entity generated much debate, particularly when LE cell testing was replaced by the easier indirect immunofluorescence (IFL) test for ANA that showed seropositivity in a substantial proportion of cases of CAH. Next, in 1963, a further serological marker was discovered by IFL, an antibody reactive with smooth muscle antibody (SMA) in the gastric mucosa; like ANA it was specific for CAH among other chronic liver diseases.4-5 Despite cases of “lupoid hepatitis” in the 1950s to 1960s being reported worldwide, opinions were that such cases were essentially indistinguishable from CAH in general, and the presumed immunological basis for the disease was unproven. At an international meeting in New York in 1964, the opportunity was taken to designate cases of CAH with immunoserological reactivities as “AIH,”6 a designation formally adopted by the liver community in the 1990s.7 Then followed recognition of other autoantibodies of diagnostic relevance for CAH, including in 1973 an antibody that reacted with liver and kidney microsomes (LKM), so defining type 2 AIH.

The highly variable expressions of CAH long proved challenging to clinicians, especially on the introduction in the 1980s of diagnostic virology revealing that cases of CAH could be accounted for by chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). Numerous committees were convened in the 1960s and later to derive diagnostic criteria for CAH,1 but it was not until 1993 that success (global consensus) was achieved at a meeting of hepatologists convened in Brighton, U.K., when the International Autoimmune Hepatitis Group (IAIHG) was constituted to “modernize” diagnostic criteria for CAH/AIH. This “Brighton Report” developed multiple criteria with allocated “scores” for the presence (or absence) of clinical, histological, and serological indices by comparison with a “gold standard” set of cases diagnosed at 1 experienced Institute in London: the final “score” reflected either a definite or probable category of AIH.7 This system proved robust and versatile and was widely adopted in epidemiological studies and for definition of cases nominated as AIH in published articles. But, there were some drawbacks: 1) the scoring system was overly cumbersome for routine clinical practice; 2) allocation of scores for absence of features of other liver diseases, like primary biliary cirrhosis (PBC) or chronic hepatitis C (CHC), tended to disallow consideration of liver disease overlap syndromes; 3) “cut-offs” that specified levels of diagnostic confidence as either definite and probable left the latter with relatively low specificity. The system was revised and updated by the IAIHG in 1999.8

### Table 1: Timeline for Discoveries/Recognition of Autoimmune Hepatitis (AIH)

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1947</td>
<td>Disease described as “subacute hepatitis” resembled latter-day AIH</td>
</tr>
<tr>
<td>1948-51</td>
<td>Chronic active hepatitis (CAH) applied to a novel inflammatory liver disease with hyperglobulinemia in young women as forerunner of AIH</td>
</tr>
<tr>
<td>1956</td>
<td>CAH with positive LE cell test called lupoid hepatitis: autoimmunity suspected</td>
</tr>
<tr>
<td>1957</td>
<td>Serum transaminase levels in CAH promptly reduced by corticosteroid therapy</td>
</tr>
<tr>
<td>1963-65</td>
<td>Immunofluorescence (IFL) using CAH serum reveal ANA and SMA positivity</td>
</tr>
<tr>
<td>1965</td>
<td>CAH is nominated as an autoimmune disease of the liver</td>
</tr>
<tr>
<td>1968</td>
<td>Efficacy of long-term (2-year) immunosuppressive treatment in CAH</td>
</tr>
<tr>
<td>1973 and 1981</td>
<td>IFL reveals anti-LKM autoantibody that distinguishes 2 types of AIH</td>
</tr>
<tr>
<td>1973 and 1976</td>
<td>Filamentous (F) actin revealed as the main serological reactant for SMA</td>
</tr>
<tr>
<td>1989</td>
<td>Cytochrome P450 2D6 isoenzyme revealed as the molecular reactant for anti-LKM</td>
</tr>
<tr>
<td>1993</td>
<td>IAIHG promulgates first universally accepted set of diagnostic criteria for AIH</td>
</tr>
</tbody>
</table>

### Autoimmune Hepatitis in 2010

The presentations of AIH (ie, clinical, biochemical, histological, and serological) are each highly diverse, explaining why criteria committees have experienced such difficulty in defining the disease. These presentations, except serological, are described relatively briefly here since they are covered in several recent authoritative reviews (Table 2).

### Clinical Presentations

Autoimmune hepatitis can present as an acute or even an alarmingly fulminant hepatitis or, conversely, be asymptomatic and recognized only incidentally by routine biochemical tests of liver function. The physical signs of disease are likewise wide-ranging, from those interpretable merely as transaminitis (ie, virtually nil) to indices indicative of advanced liver cell dysfunction or those seen in classical cirrhosis. Among the physical findings, emphasis can be placed on the likelihood of an accompanying disease, particularly one with a likely autoimmune basis (eg, thyroiditis, polyarthritis, serositis, hemocytolytic disease, ulcerative colitis) directing thought toward an immunopathologically based disease.

### Histological Expressions

Liver biopsy was widely used hitherto to assess progression and/or response to therapy, thus giving pathologists an extensive histological experience. Yet no definitive histological...
indicator of AIH has emerged since features resemble those seen in most other types of hepatitis, particularly viral, namely lobular necrosis, bridging necrosis and hepatocellular apoptosis (acidophilic or Councilman bodies). This sharing of features is no surprise since the pathogenesis of both conditions is similar, namely a T-cell attack on either a virus-infected or a normal but autoantigen-bearing liver cell. The features directing the histopathologist to AIH shown in Image 1 include the intensity of perilobular “piece-meal” necrosis, now called “interface hepatitis,” penetration by lymphocytes into the cell body of hepatocytes - emperipolesis - and prominence among the lymphoid infiltrates of plasma cells. The latter has been repeatedly observed in AIH, even justifying an earlier descriptive name of “plasma cell hepatitis,” but the pathogenetic significance (if any) has never been established. Is it that these plasma cells signify production of a specific autoantibody within the liver itself? If so, this could further contribute to the ongoing destruction of hepatocytes, presently attributed to the harmful effects of activated CD4+ve T lymphocytes such as the release of proinflammatory lymphokines by activated CD4+ve T cells (Th1, Th17), and cytolitic effects of activated CD8+ve T lymphocytes.

Biochemical Expressions

Routine biochemical tests of liver function (in non-treated subjects) show a hepatitis profile, but with variable features according to the type and stage of disease at presentation (Table 3). There are moderate to highly elevated levels in the serum of hepatocellular amino transferases, >2 to 10-fold

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**Table 2: Reviews on AIH: Author(s), Year, Reference, and Major Theme(s)**

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Reference</th>
<th>Major Theme(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obermayer-Straub P et al</td>
<td>2000</td>
<td>32</td>
<td>General overview and disease expressions</td>
</tr>
<tr>
<td>Zachou K, et al</td>
<td>2004</td>
<td>56</td>
<td>Autoantibody specificities</td>
</tr>
<tr>
<td>Storch W</td>
<td>2004</td>
<td>57</td>
<td>Autoantibodies and pathogenesis</td>
</tr>
<tr>
<td>Krawitt EL</td>
<td>2006</td>
<td>58</td>
<td>Clinical features and therapy</td>
</tr>
<tr>
<td>Nimmo MC</td>
<td>2007</td>
<td>59</td>
<td>Histology and laboratory diagnosis</td>
</tr>
<tr>
<td>Mackay IR</td>
<td>2008</td>
<td>1</td>
<td>Historical reflections</td>
</tr>
<tr>
<td>Bogdanos DP, et al</td>
<td>2008</td>
<td>12</td>
<td>Laboratory diagnosis</td>
</tr>
<tr>
<td>Mieli-Vergani G, et al</td>
<td>2009</td>
<td>52</td>
<td>Pediatric expressions</td>
</tr>
<tr>
<td>Longhi MS, et al</td>
<td>2010</td>
<td>55</td>
<td>Immunopathogenesis</td>
</tr>
</tbody>
</table>

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**Table 3: Biochemical Abnormalities in 5 Clinical Presentations of Autoimmune Hepatitis**

<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Bilirubin</th>
<th>Albumin</th>
<th>Globulins</th>
<th>Transaminases</th>
<th>Alk. phos</th>
<th>Gamma GT</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild, asymptomatic</td>
<td>N*</td>
<td>N</td>
<td>N</td>
<td>↑**</td>
<td>N</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>Protracted, indolent</td>
<td>N→↑</td>
<td>N</td>
<td>↑</td>
<td>↑→↑↑↑</td>
<td>N→↑</td>
<td>N→↑</td>
<td>↑→↑↑</td>
</tr>
<tr>
<td>Advanced, cirrhotic</td>
<td>↑→↑↑</td>
<td>↓↓↓**</td>
<td>↑↑</td>
<td>↑→↑↑</td>
<td>↑→↑↑</td>
<td>↑</td>
<td>↑→↑↑</td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>↑→↑↑</td>
<td>N</td>
<td>N</td>
<td>↑→↑↑↑</td>
<td>↑</td>
<td>↑</td>
<td>↑→↑↑</td>
</tr>
<tr>
<td>Fulminant</td>
<td>↑↑↑</td>
<td>↓</td>
<td>↑</td>
<td>↑↑↑↑</td>
<td>↑</td>
<td>↑</td>
<td>N→↑</td>
</tr>
</tbody>
</table>

*N, normal value.

**↑—↓↓↓ degree of increase or decrease.**

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**Image 1:** Photomicrographs of liver in autoimmune hepatitis. (A) Low magnification showing lymphoid infiltration into a portal tract and perilobular interface hepatitis (H&E, ×160). (B) High magnification showing swollen damaged liver cells invaded by contiguous lymphocytes (emperipolesis) and prominence of plasma cells (H&E, ×640)
above the upper normal limit (UNL), alkaline phosphatase and gamma glutamyl peptidase are moderately but variably increased, and albumin decreases as liver function begins to fail, as in inadequately treated patients. An underappreciated test is the level of immunoglobulin G (IgG), typically highly raised, from 1.1 to 5-fold UNL. Serum levels of transferases, IgG, and albumin at 1 to 3 month intervals are critical for monitoring the biochemical response to immunosuppressive regimens of treatment, and responses per se are of some diagnostic value in cases wherein other features are equivocal.

Serological Expressions

Given the general acceptance of AIH as a diagnostic entity, one could assume autoantibodies would be a major feature of diagnosis and characterization. However, assays remain insufficiently well standardized and performance varies with the location and style of laboratory practice; use of assays is influenced by operator-dependent indirect IFL being more labor intensive than automated molecular assays. Such assays are referred to herein as “molecular” since these depend on the use of highly purified or recombinant autoantigenic substrates, although “molecular” is often reserved for assays that detect DNA or RNA.

Details of Serological Assessment in Autoimmune Hepatitis

The several informative reactants for liver disease diagnosis include cell nuclei, tissues enriched for F-actin (classically gastric smooth muscle), and cytoplasmic antigens including soluble liver antigen and liver pancreas (SLA/LP), LKM antigen, and liver cytosol antigen type 1 (LC-1), as discussed below. An adequate panel of serological assays should be performed at initial assessment before beginning treatment and in most cases, assays need to be done only once. It is generally believed that results are not sufficiently useful for subsequent monitoring of disease activity, although levels of reactivity do fall concurrently with remission. An important point is that in the infrequent cases of acute or fulminant onset of AIH, initial serological tests can be only weakly positive and may need to be repeated to establish the diagnosis serologically.

Principles of Technology for IFL

Immunofluorescence is generally preferable to automated molecular assays using purified or recombinant autoantigens, and for some reactants it provides information not otherwise obtainable: detailed procedures are specified in recent reports.11,12 The IFL methodology requires unfixed (or lightly fixed), air-dried tissue sections as a multi-tissue block (liver, kidney, stomach) that is incubated with the test serum potentially containing an autoantibody (first antibody). Unbound serum is removed by washing, and then a fluorochrome-labeled second antibody, raised in an animal and specific for human immunoglobulin (lg), is applied to detect binding of the first antibody. In testing for anti-F actin, heating sera to 60 degrees eliminates a thermolabile interfering factor.13 The multi-tissue block enables simultaneous detection of most relevant autoantibodies except anti-SLA/LP that requires a molecular assay. The first serum dilution recommended for autoantibody detection by IFL (before titration) is for adults 1:40, and for children 1:20 for ANA and SMA and 1:10 for anti-LKM.11 HEP-2 cells and IFL should be used for assessing a positive test for ANA at a starting serum dilution of 1:160 for optimal assessment of patterns of nuclear staining. Automated molecular assays satisfactorily screen out negative reactors, but IFL is required for refined analysis of positive reactions and for checking discrepancies if any with the clinical opinion. Titration of sera is recommended since the higher the titer the more confident becomes the serological diagnosis, although this has not been proven.

Specific Serological Reactants Measured in Autoimmune Liver Disease (Image 1 and Image 2)

Anti-Nuclear Autoantibody

An ANA is detectable on each of the tissues of the composite block. While the liver section provides a degree of identification of patterns of ANA reactivity, HEP-2 cells with their large nuclei are superior for recognizing one of the speckled patterns signifying reactivity with ribonucleoproteins, centromeres, nucleoli, or other (see below). The International Union of Immunological Societies (IUIS) Standardization Committee14 has specified cut-offs for positivity for ANA on HEP-2 cells in a multicenter study on normal sera: the percentage of positive frequencies cited according to serum dilution at 1:40, 1:80, 1:160, and 1:320 were, respectively, 32, 13, 5, and 3, illustrating the sensitivity-specificity trade-off. Conventional cut-off titers for ANA positivity by IFL are cited as 1:40 for tissue cells and 1:160 for Hep-2 cells, but the level of reactivity will depend on factors such as tissue substrate, disease activity, and the likelihood that a given serum can have multiple ANA specificities with differing levels of reactivity. In AIH the frequency of a positive ANA test is cited at about 70%,15 and homogeneous staining is the most frequent pattern particularly in active AIH. For this the nucleosome and its main constituents, nuclear chromatin and histones, are the components mainly responsible in AIH as well as in SLE (Image 2A). With remission, the frequency of ANA positivity decreases to 34%, and homogeneous ANA becomes replaced by a speckled pattern (Image 2B) in 38% of cases,16 specifying reactivity with ribonucleoproteins, centromeres, or nucleoli, or other as yet unidentified moieties.

Anti-dsDNA

Anti-double-stranded (ds) DNA by radioimmunooassay is the hallmark reactivity in SLE and is seldom lacking in active disease; but the frequency in AIH is controversial. Studies in the 1970s (on cases designated then as CAH) gave divergent results probably attributable to technical issues such as contaminant single-stranded (ss) DNA in the antigen preparation. In later, more specific immunoassays in AIH for anti-dsDNA based on radioimmunoprecipitation of immune complexes,17 or IFL on Crithidia luciliae,18 positivity rates were lower at 10% to 16% respectively and, in unpublished personal experience with cases of AIH, an immunoprecipitation assay gave an even lower frequency. Thus, since the frequency of a positive anti-dsDNA in AIH is relatively low (<10%), routine testing will add little.

ANCA-Like Reactants

In AIH (then CAH) in the 1960s a granulocyte-specific ANA was recognized at titers greatly in excess of those for coexisting conventional ANA;19 this reactivity likely represented the later-recognized anti-neutrophil cytoplasmic ANCA (pANCA).

There are 2 species of ANCA: cANCA seen in Wegener’s disease and some vasculitides and perinuclear ANCA (pANCA)
seen in systemic necrotizing vasculitis. The respective identified autoantigens are proteinase-3 and myeloperoxidase. In autoimmune hepatic and colitic diseases there is recognized an atypical pANCA for which, in type 1 AIH, the frequency was 65% compared with 13% in CHC infection with autoimmune features but, surprisingly, 0% in type 2 AIH. The reactant for this atypical pANCA is unidentified. In fact, it is claimed to be anti-neutrophil nuclear rather than anti-neutrophil cytoplasmic, thus leading to the designation peripheral anti-neutrophil nuclear antibody (pANNA)11,12 (Image 1C). While pANNA has high sensitivity for diagnosis of AIH, the specificity is low by reason of its occurrence in other gastrointestinal diseases. However, in cases wherein diagnosis is equivocal, positivity can be useful in pointing to type 1 AIH.

Smooth Muscle (Cellular Filament) Autoantigens

Autoimmune hepatitis is characterized by autoantibodies to cytoskeletal proteins that support cellular structure, contractility, and locomotion: microfilaments, including actin, 6 nm; intermediate filaments, vimentin, desmin, etc., 15 nm; and microtubules, tubulin, 30 nm. These were first detected by IFL on smooth muscle in rodent gastric mucosa, and this remains the convenient substrate to detect SMA diagnostically (Image 3A). The frequency in CAH/AIH by IFL is about 70% to 80%, decreasing with remission. A positive test for SMA at lower titers (1:40 to 1:160) is given in liver diseases other than AIH, including PBC, viral hepatitis, and miscellaneous non-hepatitic virus infections, and here testing for the more specific antibody to F actin becomes important (see below). The non-specificity of low-titer SMA has led to the idea that this reactivity is attributable merely to a response to liver cell damage of any provenance; this, however, is
inapplicable because SMA is not expressed in anti-LKM positive type 2 AIH despite substantial degrees of cellular breakdown. Also important is the fact that positivity for SMA distinguishes lupoid AIH from classic SLE, indicating that a type of liver cell destruction occurs in AIH that does not occur in SLE. In diagnostic practice, a negative test for SMA by IFL is in itself sufficient. But a positive test, especially if weak, should be followed up either by IFL on other tissues to ascertain the filament subspecificity, as described below, or a molecular assay using purified F-actin.

Reverting to observations in the 1970s by IFL on different tissues, SMA+ve sera gave differing reactivities, either limited to smooth muscle in blood vessel walls (called SMAv), or more broadly with renal glomerular mesangium (SMAg) and the periphery of renal tubular cells (SMAt) (Image 3B).23 The idea arose that SMAv aligned with non-AIH-specific reactivity, whereas SMAgt pointed to autoimmune-type hepatitis, and some laboratory serologists retain the designations SMAv and SMAgt in their diagnostic reporting. The reactant for SMAgt+ve sera was identified in the 1970s by use of F actin for absorption of reactivity of SMA+ve serum,24 and then by use of an SMA-positive serum specifically to immunoprecipitate purified F-actin.25 Hepatocytes give a polygonal pattern of staining with anti-F-actin sera (Image 3C) due to an abundance of submembranous actomyosin enabling hepatocytes to propel bile flow along biliary ductules.26 For diagnostic purposes, a cultured rat intestinal cell line enriched in F-actin is a convenient IFL substrate (Image 3D). Commercially available enzyme-linked immunosorbent assays (ELISAs) based on purified F-actin also enable these distinctions to be made but with lower sensitivity.27 In AIH, anti-F actin correlates with younger age, progressive disease, and the genetic risk alleles HLA B8 and DR3.28 The reactant for weakly positive SMA tests from non-AIH cases may be intermediate filaments rather than F actin.29

Illustrations for Image 2 and Image 3 provided by Bob Wilson, Division of Immunology, Pathology Queensland (2A-D and 3B) and Ban-Hock Toh, Faculty of Medicine, Monash University, Melbourne (3D).
Actin is an interesting molecule. Cellular monomeric globular (G) actin, 46 kDa, exists in all cells in a dynamic state, undergoing polymerization to filamentous (F) actin.30 The antibody autoepitope on F actin microfilaments is likely conformational, since reactivity is lost under the denaturing conditions of Western blotting, but the binding site(s) of autoantibodies and its possible correspondence with attachment sites for the numerous other actin-binding proteins of the cell are unknown. There is evidence in vitro for functional effects of anti-F actin on cell motility,31 but in AIH anti-F actin has not been shown to be relevant to liver cell damage in vivo. Moreover, how anti-F actin is provoked in the first place is undecided. Perhaps if liver cell destruction from any cause resulted in spillage of liver-abundant F-actin, this could act as a provoking and perpetuating autoantigen in individuals at genetic risk for autoimmune (see below), in which case the distribution of F actin in apoptosis fragments after cell death becomes important. Finally, in the effector phase, further study is needed to determine if impaired permeability of the liver cell membrane in the course of hepatocyte injury leads to exposure of submembranous actin in hepatocytes to autoimmune attack.

The SLA/LP Molecule

A liver-pancreas antigen (LP) was recognized in 1981 by complement fixation and was co-identified with a reactant described in 1987 by radioimmunoassay called soluble liver antigen (SLA), hence referred to as SLA/LP. Anti-SLA/LP has a frequency in AIH of only 25% to 30%, so it is not highly sensitive for diagnosis; however, since anti-SLA/LP positivity is limited to AIH and CHC with autoimmune features, the specificity is high.32 Anti-SLA/LP usually coexists with other autoantibodies detectable in AIH but may exist as the sole serological marker. As a result its use in diagnostic test panels is for cases of suspected AIH in which assays are negative. The antigenic reactant, which is liver/pancreas-specific, was erroneously attributed to various liver cell proteins, but it is now characterized as a tRNA-associated protein (tRAP).33,34 for general usage it remains known as SLA/LP. Structural considerations place SLA/LP within the liver specificity,35 but reactivity to SLA/LP in vivo has not been linked to pathogenesis.

Liver-Kidney Microsomal Autoantigens

In CAH in 1973 a cytoplasmic reactivity was described by IFL on liver and kidney sections and characterized as microsomal36 (Image 2D). Microsomes are the in vitro equivalent of particles of the endoplasmic reticulum wherein the LKM autoantigens reside. A study in 1985 showed a polar segregation of cases of AIH, either positive for ANA/SM-A or for anti-LKM, with the latter defining an alternative serological type, called type 2.37 Connotations of this for pathogenesis of AIH are discussed below. Later, other LKM reactants were identified with different disease associations, particularly intrahepatic adverse drug reactions, so the original antigen was called LKM1 and successors became LKM2 and LKM3.32

Type 2 AIH is an infrequently occurring variant and has a rather restricted demographic and geographic impact, being more frequent among cases of AIH affecting young children and certain European populations vs populations of the United States, Australia, and Japan. Interestingly, there has been clear molecular identification of the specific autoimmune reagents in this less frequent type 2 AIH variant vs the still uncharacterized serological reactant(s) in classic type 1. The identity of the LKM antigen was revealed first by immunoblotting32 and more precisely by using reactive sera to screen gene expression libraries as an isoform (2D6) within the large cytochrome oxidase P450 enzyme family.38 Various linear epitope sequences have been identified, the most reactive being the amino acid sequence DPAQPQPRD within residues 257-269,38 but the suspicion is that these are segments of an incompletely characterized conformational epitope structure. The LKM reactant for serum recognized in cases of hepatitis occasionally induced by exposure to medicinal drugs such as tienilic acid (withdrawn in 1980) and called LKM2 was identified as isoform 2C9, and that detected in a proportion of cases of chronic hepatitis D-infected patients, and seen also (rarely) in spontaneous AIH, was called LKM3. This differed in staining human exocrine pancreas and thyroid as well as liver-kidney and reacted with uridine diphosphate glucuronosyl transferases (UGT).32 A microsomal antibody occurring in hepatitis induced by dihydroalazene stained only centrilobular hepatocytes but not kidney (anti-LM) and reacted with CYP450 1A2. There is yet a fourth category of anti-LKM reactive with CYP450-1A2 and 2A6; these anti-LKM are detected in some 20% to 25% of patients with a type 2 AIH that occurs in the syndrome of autoimmune polyendocrinopathy - candidiasis-ectodermal-dystrophy (APECED),32 also known as autoimmune polyendocrine syndrome type 1 (APS-1). The IFL patterns of these alternate anti-LKMs are mostly indistinguishable from that of anti-LKM1.

Liver Cytosol-1 Autoantigen - FTCD

In 1988 autoantibodies were recognized,39 and later further characterized as soluble liver cytosolic antigen 1 (LC-1). Notably, anti-LC-1 reactivity is organ- (liver-) specific and also relatively disease-specific for type 2 AIH. Unlike anti-LKM1, anti-LC-1 is seldom demonstrable in CHC and only very occasionally (in children) in type 1 AIH.40 The LC-1 molecule has been eluted from liver cytosol as a protein of 240-290 kDa and, since the signal by immunoblot under reducing conditions is at 62kDa, it likely exists as a tetramer.40 Anti-LC1 shows by IFL a characteristic decreasingly homogeneous staining of hepatocytes toward the central vein, usually obscured by accompanying anti-LKM. Reactivity is demonstrable also by immunodiffusion, immunoblot, or counterimmunoelectrophoresis. Anti-LC-1 has been identified as formiminotransferase cyclodeaminase (FTCD)31 that comprises 2 domains; the globular FT domain joined by a short linker to the CA domain. Epitopes for autoantibodies have been mapped, and there was defined a conformational epitope spanning much of the molecule and 2 linear epitopes have been mapped, and there was defined a conformational epitope spanning much of the molecule and 2 linear epitopes located in the C-terminal domain, aa 428-434 and 440-447.41 Molecular assays using recombinant antigen are available, but mostly anti-LC-1 associates with anti-LKM1, so its independent detection has limited applications.

A Liver-Specific and Disease-Specific Autoantigen?

This has been long sought since AIH resembles an organ-specific autoimmune disease. Assessment of the autoantigenic potential of a liver-specific lipoprotein (LSP)42 proved indecisive
but, stemming from this work, came the recognition of a liver-specific membrane antigen, the asialoglycoprotein receptor (ASGPR). However, earlier hopes that this could serve as an informative liver-specific reactant have not been fully realized. Conformationally intact receptor (H1 subunit) as a recombinant protein should facilitate further evaluation of anti-ASGPR, but so far data are limited.

Liver cell membrane preparations of variable purity have been studied by Western immunoblotting using AIH sera for a molecular signal corresponding to a disease-specific autoantigenic moiety. Numerous studies have consistently demonstrated multiple reactive components, mw 20 to >100kDa, but with none standing up as a credible disease-specific autoantigen; perhaps some components were cytoplasmic contaminants of incompletely purified membrane preparations. Findings that there is a marked decrease in intensity of signal by immunoblot with increasing serum dilution beyond 1:1000, and after therapeutically induced remissions and that normal sera show equivalent (albeit much weaker) reactivities, suggest the multiple bands are associated with and even consequential to hepatocellular damage. In the latest and most detailed of the studies, several of the reactive components were molecularly identified as potential constituents of the liver membrane (liver arginase, cytokeratins, heat shock proteins, and valosin-containing protein). As in previous studies, these membrane components also reacted weakly with normal sera (ie, as natural antibodies) and became amplified during hepatocellular destruction. Of course, destruction-dependent amplification of a naturally-occurring reactivity could well be an integral part of pathogenesis in autoimmunity.

Autoimmune Hepatitis—The Next Decade


In 1993 it was the right time for a definitive distinction of the autoimmune from other (known or unknown) causes of CAH. This was successfully met by the widely endorsed IAIHG criteria, but these were too cumbersome for everyday use. Recently simplified criteria were developed and have been extensively trialed with satisfactory results. As a derivative from these, critical and readily measurable indices could be nominated as reflecting the essence of AIH: 1) clinical, no evidence of current viral infection but possibly an accompanying autoimmune disorder; 2) biochemical, transferases >2x UNL and IgG >1.1x UNL; 3) histological, interface hepatitis with plasma cell prominence; 4) serological, positivity to an acceptable titer for SMA with anti-F actin specificity, ANA, anti-SLA/LP, or alternatively, anti-LKM. The inclusion of a clearly evident therapeutic response to prednisolone is sometimes used as a criterion, but specification of AIH must be established before, not after, therapy is initiated.

Autoimmune Overlap Diseases of the Liver

The tendency of autoimmune diseases to cluster in the 1 patient or among relatives is one of their defining features, AIH included. But further, there is the special category of overlap diseases wherein there is coexpression of features of 2 usually distinct autoimmune disease entities within the 1 organ, to such a degree that criteria for both diseases can be fulfilled. Overlapping liver disease with AIH, often reviewed over the past 20 to 30 years, include PBC or primary sclerosing cholangitis (PSC) particularly in children. There is still no answer to the nosological dilemma posed by the overlapping syndromes. Clinical, histological, and genetic features point to PBC and PSC as primary partners in these overlaps, with AIH being a secondary expression, but there is a series of cases of clear-cut AIH described with a persisting AMA yet no other features of PBC. So it remains undecided whether 2 separate coexisting diseases should be specified or a single PBC-AIH (or PSC-AIH) overlap entity.

Pathogenesis of Autoimmune Hepatitis

Mechanisms of Autoimmune Disease

The evidence for autoimmunity as 1 of the major causes of chronic hepatitis and the diagnostic utility of reliable immunoserological tests are now well established. Still lacking, however, are mechanistic immunological explanations particularly for those autoimmune diseases (eg, type AIH, PBC, Sjogren’s syndrome) wherein a relatively organ-specific form of tissue autoimmunity coexists with non-organ-specific serological accompaniments.

First of all, are there fundamental differences between the non-overlapping type 1 and type 2 AIH? Probably yes since, despite similarities in clinical features, histology, and therapy, type 2, in terms of its clinical accompaniments, could be regarded as an organ-specific form of autoimmunity. Moreover this is the type seen in the inherited APECED syndrome (APS-1), which is due to a homozygous loss of function mutation of the thymus-expressed transcription factor called autoimmune regulator (AIRE) that facilitates the intrathymic deletional tolerance operating particularly for organ-specific autoantigens; and likely operative also in the loss of tolerance to the (relatively) tissue-specific autoantigens of the CYP450 multi-enzyme family. In contrast type 1 AIH clinically shows multisystem autoimmune expressions and reactivities with non-organ-specific autoantibodies. Although speculative, the possibility is that the nature of the tolerance failure in types 1 and 2 is different, in that in the former the main tolerance deficit is “peripheral” due to impaired numbers or function of regulatory T cells, and in the latter is “central” meaning impaired deletion (negative selection) of thymic lymphocytes reactive with tissue-specific antigens, including the LKM autoantigens.

In a recent and perhaps oversimplified attempt to codify the nature of autoimmune responses, the sequence of events was compared, using a descriptive cartoon, with the 3 stages of normal eliminative immune responses as follows. Stage 1, initiation, occurs presumably in any peripheral tissue under inflammatory conditions, wherein there is provocative tissue damage associated with endocytosis by resident-activated dendritic cells of a self-antigen mimic or an immunogenic apoptosis fragment, an apoptope, with transfer to the regional lymph node (RLN). Stage 2, establishment, occurs in the lymphoid follicle/germinal center of the RLN, under conditions of deficient natural immune tolerance (impaired thymic deletion of self-reactive T cells/inefficient generation...
of regulatory T cells (Tregs), so allowing for induction of reactive sets of anti-self effector lymphocytes. Stage 3, destruction/elimination, occurs when such effector lymphocytes home back to the site of initial injury and react damagingly with the autologous tissue to which they have become sensitized. The effector elements will include CD4 Th1 and/or Th17 T cells, CD8 cytolytic T cells, NK cells, and local autoantibody-producing B cells.53 Thus, the process can become autonomous (self-perpetuating), reflected in the later stages by the well-recognized development of ectopic lymphoid folicles/germinal centers in the target tissue itself. Insufficiently addressed issues in type 1 AIH, particularly relating to the F actin autoantigen, include ascertaining the site and nature of antibody and T cell epitopes and the role of reactivity to these in pathogenesis. Such knowledge is now available for type 2 AIH since the culprit CYP450 autoantibody has been well identified.53

The stages codified above can be modulated by 3 major determinants. First, environmental processes, acting mostly at stage 1, initiation, but proving difficult to identify. Second, genetic influences, acting in all 3 stages, into which earlier on HLA associations provided much insight and currently genome-wide association studies are becoming highly informative, although not as yet applied to AIH. Third, the poorly considered element of random chance operating from the macro-level of the intact individual to the micro-level of the myriad cellular interactions necessary for immune responses to be completed.52 There is indeed much unfinished business to be dealt with before the full story of AIH unfolds.54


