

Target proteins in human autoimmunity: Cytochromes P450 and UDP-glucuronosyltransferases

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P Obermayer-Straub, CP Strassburg, MP Manns. Target proteins in human autoimmunity: Cytochromes P450 and UDP-glucuronosyltransferases. Can J Gastroenterol 1999;14(5): 429-439. Cytochromes P450 (CYPs) and UDP-glucuronosyltransferases (UGTs) are targets of autoantibodies in several hepatic and extrahepatic autoimmune diseases. Autoantibodies directed against hepatic CYPs and UGTs were first detected by indirect immunofluorescence as antiliver and/or kidney microsomal antibodies. In autoimmune hepatitis (AIH) type 2, liver and/or kidney microsomal (LKM) type 1 autoantibodies are detected and are directed against CYP2D6. About 10% of AIH-2 sera further contain LKM-3 autoantibodies directed against family 1 UGTs. Chronic infections by hepatitis C virus and hepatitis delta virus may induce several autoimmune phenomena, and multiple autoantibodies are detected. Anti-CYP2D6 autoantibodies are detected in up to 4% of patients with chronic hepatitis C, and anti-CYP2A6 autoantibodies are detected in about 2% of these patients. In contrast, 14% of patients with chronic hepatitis delta virus infections generate anti-UGT autoantibodies. In a small minority of patients, certain drugs are known to induce immune-mediated, idiosyncratic drug reactions, also known as 'drug-induced hepatitis'. Drug-induced hepatitis is often associated with autoantibodies directed against hepatic CYPs or other hepatic proteins. Typical examples are tienilic acid-induced hepatitis with anti-CYP2C9, dihydralazine hepatitis with anti-CYP1A2, halothane hepatitis with anti-CYP2E1 and anticonvulsant hepatitis with anti-CYP3A. Recent data suggest that alcoholic liver disease may be induced by mechanisms similar to those that are active in drug-induced hepatitis. Autoantibodies directed against several CYPs are further detected in sera from patients with the autoim-

mune polyglandular syndrome type 1. Patients with autoimmune polyglandular syndrome type 1 with hepatitis often develop anti-CYP1A2; patients with adrenal failure develop anti-CYP21, anti-CYP11A1 or CYP17; and patients with gonadal failure develop anti-CYP11A1 or CYP17. In idiopathic Addison disease, CYP21 is the major autoantigen.

Key Words: *Adrenal failure; Autoimmune hepatitis; Autoimmune polyglandular syndrome; Cytochrome P450; Drug-induced hepatitis; UDP-glucuronosyltransferase*

Les protéines cibles dans l'auto-immunité humaine : les cytochromes P450 et les UDP-glucuronosyltransférases

RÉSUMÉ : Les cytochromes P450 (CYP) et les UDP-glucuronosyltransférases (UGT) sont les cibles des anticorps dans de nombreuses affections auto-immunes hépatiques et extra-hépatiques. Les auto-anticorps dirigés contre les CYP et les UGT hépatiques ont d'abord été détectés par immunofluorescence indirecte comme des anticorps antihépatiques et (ou) antirénaux. Dans l'hépatite auto-immune (HAI) de type 2, les autoanticorps hépatiques et (ou) rénaux microsomiques de type 1 sont décelés et dirigés contre le CYP2D6. Environ 10% des séra d'HAI de type 2 renferment aussi des autoanticorps microsomiques de type 3 dirigés contre la famille des UGT 1. Les infections chroniques causées par le hépatite C ou le virus de l'hépatite delta peuvent provoquer plusieurs phénomènes auto-immuns et de multiples anticorps sont détectés. Les autoanticorps anti-CYP2D6 sont détectés chez jusqu'à 4% des patients qui souffrent d'hépatite C chronique et les anti-CYP2A6 sont détectés chez près de 2% de ces patients. En

voir page suivante

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revanche, 14 % des patients qui présentent une hépatite delta chronique génèrent des auto-anticorps anti-UGT. Chez une faible minorité de patients, certains médicaments provoquent des réactions idiosyncrasiques à médiation immunitaire, aussi connues sous le nom d'hépatite d'origine médicamenteuse. L'hépatite d'origine médicamenteuse est souvent associée à des autoanticorps dirigés contre les CYP et d'autres protéines hépatiques. L'hépatite liée à l'acide tiénilique avec anti-CYP2C9, l'hépatite liée à la dihydralazine avec anti-CYP1A2, l'hépatite liée à l'halothane avec anti-CYP2E1 et l'hépatite liée aux anticonvulsivants avec anti-CYP3A en sont des exemples typiques. De récentes données suggèrent que la maladie

hépatique d'origine éthylique pourrait être déclenchée par des mécanismes semblables à ceux qui sont en cause dans l'hépatite d'origine médicamenteuse. Les autoanticorps dirigés contre plusieurs CYP sont de plus détectés dans les séra provenant de patients qui souffrent du syndrome polyglandulaire auto-immun de type de 1. Les patients atteints de ce syndrome qui souffrent d'hépatite développent souvent des anti-CYP1A2; ceux qui souffrent d'une insuffisance surrénalienne, des anti-CYP21 et des anti-CYP11A1 ou CYP17; et ceux qui souffrent d'insuffisance gonadique, des anti-CYP11A1 ou CYP17. Dans la maladie d'Addison idiopathique, le CYP21 est le principal auto-antigène.

Cytochromes P450 (CYPs) and UDP-glucuronosyltransferases (UGTs) are targets of autoantibodies in several hepatic and extrahepatic autoimmune diseases (1-3). Autoantibodies directed against hepatic CYPs and UGTs were first detected by the staining patterns of liver microsomal (LM) autoantibodies and liver and/or kidney microsomal (LKM) autoantibodies in indirect immunofluorescence (4-6). Later anti-CYP antibodies directed against steroid-producing cells were described (7-9). At least 10 different CYPs and UGTs of family 1 are known targets of autoimmune processes in patients with autoimmune hepatitis (AIH) type 2, in some patients with chronic hepatitis virus, in patients with drug-induced hepatitis, in patients with hepatitis as a disease component of the autoimmune polyglandular syndrome type 1 (APS1) and in patients with Addison disease (Figure 1) (2).

In all instances, autoantibodies directed against specific CYPs or UGTs of family 1 are specific diagnostic markers. Anti-CYP2D6 autoantibodies in the absence of hepatic viral infection are a diagnostic marker for AIH-2 (10-12). AIH may be diagnosed not only as an idiopathic autoimmune disease but also as a severe disease component in APS1 (13-15). In contrast with idiopathic AIH, anti-CYP1A2 autoantibodies are detected in APS1 (16). In sera from patients with APS1, further autoantibodies directed against CYPs activated in steroid biosynthesis are present, such as anti-CYP11A1, anti-CYP17 and anti-CYP21 (7-9,17,18).

The second set of disease complexes characterized by autoimmunity and directed against CYPs and UGTs are autoimmune processes induced by chronic, viral infections (2). Autoantibodies are preferentially found in patients affected by chronic infections with RNA viruses. Fourteen per cent of patients with chronic hepatitis delta virus (HDV) develop anti-UGT autoantibodies (5,19), and up to 4% of patients develop anti-CYP2D6 (20-23). Recently with anti-CYP2A6 a second CYP autoantibody was detected in patients with chronic hepatitis C (24).

A small percentage of patients treated with certain drugs develop severe hepatitis that occurs only after prolonged treatment periods and that is characterized by lymphocytic liver infiltrations and autoantibodies directed against hepatic proteins. It is believed that drug-metabolizing enzymes, mainly CYPs, create reactive metabolites that in turn modify either the metabolizing CYP enzyme and/or other hepatic

proteins (25,26). In susceptible patients these modified proteins induce an immune response resulting in severe drug-induced hepatitis (27). Modified proteins preferentially include CYPs that are often targets for autoantibodies. Tienilic acid-induced hepatitis (28), dihydralazine hepatitis (29), halothane hepatitis (30) and anticonvulsant-induced hepatitis (31) are discussed as typical examples. Recently, alcoholic liver disease was suspected to be caused by an autoimmune reaction against hepatic proteins, directed against both acetaldehyde- and hydroxyethyl-modified hepatic proteins (32,33). Metabolization of ethanol by CYP2E1 has been suggested to generate hydroxyethylradicals that are targets of autoimmune processes in alcoholic liver disease (33).

AIH-2

AIH is a rare autoimmune disease characterized by a marked female predominance, hypergammaglobulinemia, circulating autoantibodies, benefit from immunosuppression, a high prevalence of extrahepatic autoimmunity of 30% and an over-representation of patients with an immunogenetic background of human leukocyte antigens (HLAs) HLADR3 or HLADR4 (34). According to the autoantibody pattern, patients with AIH are further subdivided (35). AIH-1 is characterized by antinuclear and antismooth muscle autoan-

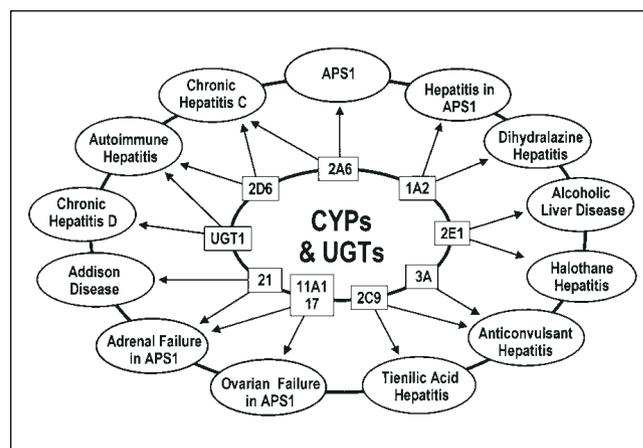


Figure 1) Ten different cytochromes P450 (CYPs) and UDP-glucuronosyltransferases (UGTs) are targets of human autoimmunity (2)

tibodies, AIH-2 is characterized by LKM-1 autoantibodies and AIH-3 is characterized by autoantibodies against soluble liver proteins (2). AIH-1 and AIH-3 are clinically similar and show some overlap in autoantibodies (23,36). In AIH-2, however, LKM-1 autoantibodies almost never overlap with antinuclear autoantibodies, smooth muscle autoantibodies (SMA) or antibodies to soluble liver antigens. AIH-2 is a more severe form of autoimmune hepatitis, with disease onset at younger age, 50% fulminant hepatitis at disease onset and a stronger inflammatory activity (36-38). Standard treatment of autoimmune hepatitis is immunosuppression by a combination of azathioprine and prednisolone.

LKM-1 autoantibodies were first described by indirect immunofluorescence on rodent liver and kidney sections (4). Western blots with hepatic microsomes revealed a protein band of 50 kD. At lower frequencies, 55 kD and 64 kD protein bands were detected (19,39). The 50 kD protein was identified as CYP2D6 (10-12). The immune reaction is highly specific, and no crossreactivity with closely related CYPs is detectable. LKM-1 autoantibodies inhibit the hydroxylation of CYP2D6 substrates in isolated liver microsomes (12). In contrast, the *in vivo* activity of CYP2D6 is not affected by the presence of LKM-1 autoantibodies (40). CYP2D6 is subject to polymorphisms in the Caucasian population. Five to 10% of Caucasians are void of functional CYP2D6 protein, resulting in a low metabolizer phenotype for several drugs, such as debrisoquine (41). So far all patients tested for CYP2D6 activity were of the extensive metabolizer phenotype with functional CYP2D6, indicating that functionally intact CYP2D6 may be a prerequisite for the production of LKM-1 autoantibodies (40,42).

The structures recognized by LKM autoantibodies were further characterized by Manns et al (43) who tested 26 LKM-positive sera by using Western blots of partial sequences of recombinant CYP2D6. Eleven of these sera recognized a short minimal epitope of eight amino acids. The amino acid sequence of this minimal epitope is DPAQPPRD. Twelve other clones recognized a larger epitope containing this eight amino acid core sequence. Searching the European Molecular Biology Laboratory database with the minimal epitope revealed a striking match of the minimal epitope with the primary structure of the immediate early (IE) protein IE 175 of herpes simplex virus (HSV) type 1 (Figure 2). Sequence identity is seen for the sequence PAQPPR (43). Therefore, LKM-1 autoantibodies were affinity purified against CYP2D6 and used in Western blots with lysates of baby hamster kidney cells infected with HSV. Interestingly, the autoantibody specifically detected a band at 175 kD, demonstrating crossreactivity with an HSV-specific protein of 175 kD (43). The hypothesis that molecular mimicry may contribute to autoantibody formation in some cases of AIH was suggested on the basis of a case study (44). In a pair of identical, female twins, one sister suffered from AIH-2 and the other twin was healthy. Interestingly, only the sister suffering from AIH was HSV positive and her serum recognized the viral 175 kD protein in lysates of HSV-infected cells (44). Molecular mimicry might con-

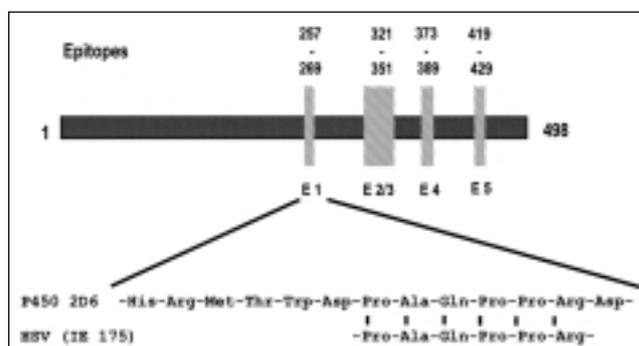


Figure 2) Linear epitopes on cytochrome P450 (CYP) 2D6 and sequence homology to the immediate early protein 175 of herpes simplex virus (2). HSV Herpes simplex virus. Reprinted with permission from reference 2

tribute to the development of AIH-2 by weakening self-tolerance to certain protein targets.

Further work on epitope mapping was performed by Yamamoto et al (45), resulting in the identification of three minor epitopes on CYP2D6 (Figure 2). Yamamoto et al (45) confirmed that most patients with AIH-2 recognize the epitope of amino acid sequence 257 to 269, including the core sequence of DPAQPPRD. With lower frequencies, another epitope of amino acid sequence 373 to 389 was detected and two infrequent epitopes consisting of amino acid sequence 373 to 389 or 410 to 429. Because linear peptides were unable to absorb the inhibitory activity of LKM-1 autoantibodies of CYP2D6 activity, Duclos-Vallee et al (46) suggested the presence of conformational autoantibodies in LKM-1 sera. Recently, another major epitope, located at amino acid sequence 321 to 373, was detected. This epitope is three dimensional and is destroyed if cut into overlapping pieces (unpublished data).

About 10% of patients with AIH have a protein band of 55 kD that is detected by LKM-3 autoantibodies (19). Due to the low abundance of this autoantibody, only five sera with LKM-3 autoantibodies were tested. In the sera of four patients, LKM-3 was associated with LKM-1 autoantibodies. One patient, however, was positive for only LKM-3. Epitope mapping revealed a minimal epitope from amino acid sequence 264 to 373 on UDP-glucuronosyltransferases of family 1. Most of this epitope is located in the constant C-terminus of this enzyme. The autoantibody recognizes human UGT 1.1 and 1.6, as well as rabbit UGT 1.4 and rabbit UGT 1.6 (19). The large size of the epitope cannot be reduced further indicates that this epitope is conformationally dependent.

VIRUS-ASSOCIATED AUTOIMMUNITY

Chronic hepatitis C: Chronic infection with hepatitis C virus (HCV) is known to induce autoimmune reactions. HCV is associated with an array of extrahepatic manifestations, including mixed cryoglobulinemia, membranoproliferative glomerulonephritis, polyarthritis, porphyria cutanea tarda, Sjögren's syndrome and autoimmune thyroid disease (47-50). Not surprisingly, numerous autoantibodies have been

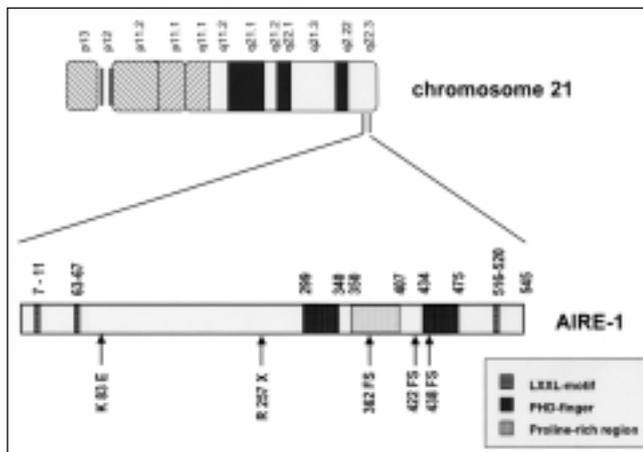


Figure 3) Autoimmune polyglandular syndrome type 1 is caused by a gene defect in a single gene product located on the long arm of chromosome 21 (64,65). AIRE Autoimmune regulator; PHD Plant homeo domain

found to be associated with chronic HCV. Similar to AIH antinuclear, SMA, LKM and antithyroid autoantibodies are found with a high prevalence. A hepatitis C-specific autoantibody was found with anti-GOR that is present in at least 80% of sera from patients with HCV hepatitis (51).

Depending on geographical origin, a variable proportion of patients with LKM-1 antibody-associated liver disease is infected with HCV. The prevalence of HCV infection among LKM-1 positive patients is about 90% in Italy (21), about 50% in France and Germany (51,52), and less than 10% in Great Britain (21). HCV-negative patients show a pathogenesis typical of AIH. They show a low age of onset, a high prevalence of female patients (80%), a high inflammatory activity and a good response to immunosuppression. In contrast the LKM-1 positive patients with hepatitis C have a very different pathogenesis. The age of onset usually is above 40 years, inflammatory activity is low, response to corticosteroids is not convincing and the majority of these patients do not require treatment because disease activity is low.

Further characterization of LKM autoantibodies revealed that although anti-CYP2D6 titres were similar to titres in AIH-2, differences existed in epitopes recognized by LKM autoantibodies (53-56). In patients with AIH-2, the epitope of amino acid sequence 257 to 269 is recognized with a significantly higher prevalence than in chronic hepatitis C (55). Further, the immune reaction seems to be more heterogeneous than in AIH. Additional protein targets are detected at 59 kD and 70 kD (57).

LKM autoantibodies in chronic hepatitis C seem to indicate an increased risk of exacerbation of the disease (58-60). Dalekos et al (60) studied antibody titres and performed epitope mapping of LKM-1 positive sera from patients with chronic hepatitis C. Interestingly, a patient with high LKM-1 titre and autoantibodies directed against an epitope of amino acid sequence 257 to 269, which is preferentially recognized in patients with AIH-2, showed exacerbation of the disease under interferon treatment. In contrast with other patients with HCV infection, a rarely detected epitope

on the third C terminal of the protein was recognized in this patient. These results suggest that epitope mapping may be helpful to detect patients at risk of exacerbation of disease (60).

Recently, another autoantibody was detected in patients with HCV and hepatitis G virus (HGV). In general, about 2% of HCV-positive sera and 7.5% of LKM-1 positive HCV sera recognize CYP2A6. Interestingly, anti-CYP2A6 autoantibodies are not detected in patients with AIH-2, who are characterized with high levels of LKM-1 autoantibodies. The clinical relevance of this finding remains to be determined (24).

Chronic HDV: HDV is caused by a small viroid of 1698 base pairs that is dependent on coinfection with hepatitis B virus (HBV) to produce its envelope. HDV either coinfects with HBV or superinfects patients with pre-existing HBV. Interestingly, autoantibody production in chronic HDV is much more pronounced than in HBV (61). In their original report, Crivelli et al (5) observed serum antibodies in 14% of patients with chronic HDV that were directed against hepatic microsomes and microsomes of the proximal renal tubules. In contrast to LKM-1 and LKM-2 autoantibodies, which only react with liver and kidney tissues, additional fluorescence signals may be detected in the pancreas, adrenal gland, thyroid and stomach. Western blots revealed several, molecular targets around 55 kD (19,62). This novel autoantibody was called LKM-3 (5). The molecular targets of LKM-3 autoantibodies were identified as UGTs of family 1 (19). CYPs are active in phase one of drug metabolism with UGTs, and for the first time autoantibodies directed against enzymes of phase two were detected. LKM-3 autoantibodies are exclusively detected in patients with HDV or AIH-2. They are not detected in sera from patients with chronic HBV, chronic hepatitis C, primary biliary cirrhosis, primary sclerosing cholangitis or lupus erythematosus (19). Autoantibodies are specific for a conformationally dependent epitope of amino acid sequence 264 to 373 (63). In addition to the major epitope on family 1 UGTs, a minor epitope was found on UGT 2B13 (19). This epitope was recognized by two of eight LKM-3 positive patient sera with chronic HDV infection, and the signal was much lower than signals detected with UGT family 1 (19). Autoantibody titres in patients with chronic HDV usually are lower than in patients with AIH-2 (62).

ANTI-CYP AUTOANTIBODIES IN APS1

APS1 – A complex autoimmune syndrome results from defects in a single gene: APS1 is caused by mutations in a single gene called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) or autoimmune regulator (AIRE) (64,65). AIRE is one of the first gene loci involved in the regulation of autoimmunity that is known outside the HLA locus. The AIRE gene product was implicated as a transcription factor based on the presence of two plant homeo domain (PHD)-finger motifs and an LXXXL motif (64,65). Because AIRE is expressed in the thymus, studies of AIRE function may provide new insights into

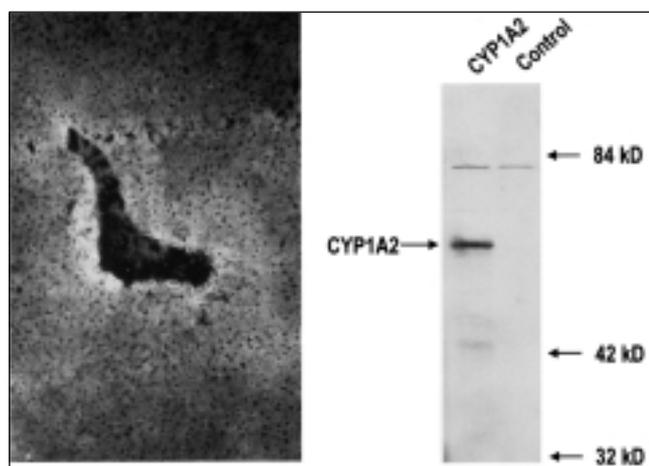


Figure 4) Indirect immune fluorescence of liver microsomal autoantibodies detected in hepatitis as a disease component of autoimmune polyglandular syndrome type 1 (70). CYP Cytochrome P450. Reprinted with permission from reference 16

mechanisms that establish and maintain self-tolerance. APS1 is found in patients homozygous for defects of the AIRE gene that eliminate one or both PHD-finger motifs of the AIRE-1 protein and result in a lack of functional AIRE-1 gene products (Figure 3). APS1 is characterized by a variable combination of disease components such as mucocutaneous candidosis, autoimmune tissue destruction and ectodermal dystrophy (14,66,67) (Table 1). In contrast with other autoimmune diseases, APS1 shows 100% penetrance, lack of female preponderance, and little or no association with HLA-DR. Typically, the onset of APS1 occurs in childhood and multiple autoimmune manifestations evolve throughout the patient's lifetime (Table 1) (14). Organ-specific autoantibodies are associated with several disease components such as hypoparathyroidism, adrenal and gonadal failures, insulin-dependent diabetes melitus, hepatitis and vitiligo.

Hepatic anti-CYP autoantibodies in APS1: Chronic hepatitis is a serious disease component present in 10% to 18% of patients with APS1 (13,14,67,68), and occasional deaths related to hepatitis occur in patients with APS1 without warning signs (14,69). Recently, a hepatic autoantigen in AIH, related to APS1, was identified as CYP1A2 (70). Anti-CYP1A2 autoantibodies may be detected by a predominant staining of the perivenous rat hepatocytes (16,71) (Figure 4). In Western blots of human hepatic microsomes, a band of 54 kD was detected (16,72). Retrospectively, this finding identified an unusual case of autoimmune hepatitis that was reported initially as a patient with APS1 (71,72). This patient suffered from hepatitis, vitiligo, alopecia, nail dystrophy and had a brother who died from Addison disease (71,72). In the serum, anti-CYP1A2 autoantibodies were detected that were able to inhibit the enzymatic activity of CYP1A2 (72). In Finnish patients with APS1, seven of 68 patients were affected by hepatitis. Four of 68 patients with APS1 were anti-CYP1A2 positive. All anti-CYP1A2 positive patients suffered from hepatitis (73). Similarly, one of six Sardinian patients with APS1 and three of eight Swedish

TABLE 1
Disease components in autoimmune polyglandular syndrome type 1

Disease component	Prevalence (%)
Endocrine components	
Hypoparathyroidism	79
Adrenal failure	12
Insulin-dependent diabetes mellitus	12
Parietal cell atrophy	13
Hypothyroidism	4
Ovarian failure in females (13 years and older)	60
Testicular failure in males (16 years and older)	14
Nonendocrine components	
Candidiasis	100
Alopecia	29
Vitiligo	13
Keratopathy	35
Hepatitis	12
Intestinal malabsorption	18
Enamel hypoplasia	77
Tympanic membrane calcification	33
Nail dystrophy	52

Data from reference 14. IDDM Insulin dependent diabetes mellitus

patients were anti-CYP1A2 positive. All anti-CYP1A2 positive patients were affected by hepatitis (16,74). These results indicate that, although not all hepatitis patients with APS1 are CYP1A2 positive, the presence of this autoantibody seems to be a marker for hepatitis in APS1. Further investigations with recombinant CYPs revealed that autoantibodies directed against CYP2A6 are detected in APS1 patients (75). However, investigations in the Finnish patient population revealed that autoantibodies directed against CYP1A2, but not against CYP2A6, correlate with hepatitis as a disease component in APS1 (73). It is interesting to note that in the sera from 300 patients with idiopathic AIH types 1 to 3, different autoimmune liver diseases and chronic viral hepatitis anti-CYP1A2 could not be detected (unpublished data). Anti-CYP2A6 autoantibodies, however, were detected in about 2% of sera from patients with chronic hepatitis C and HGV (24). Interestingly, both viruses belong to the flavivirus group.

EXTRAHEPATIC ANTI-CYP AUTOANTIBODIES IN APS1

Adrenal failure: Another frequent endocrine disease component of APS1 is adrenal failure, and it affects more than 70% of patients with APS1 (13-15,67,68). Adrenal failure in APS1 is diagnosed between ages four and 41 years, and is associated with the presence of steroidal cell autoantibodies (14,76). Several autoantigens such as CYP21, CYP11A1 and CYP17 were identified (7-9,17,18,77-79). All three enzymes are involved in steroid biosynthesis (Figure 5) (80); however, they differ in tissue specificity. CYP21 is expressed in the adrenal cortex only, CYP17 is expressed in the adrenal

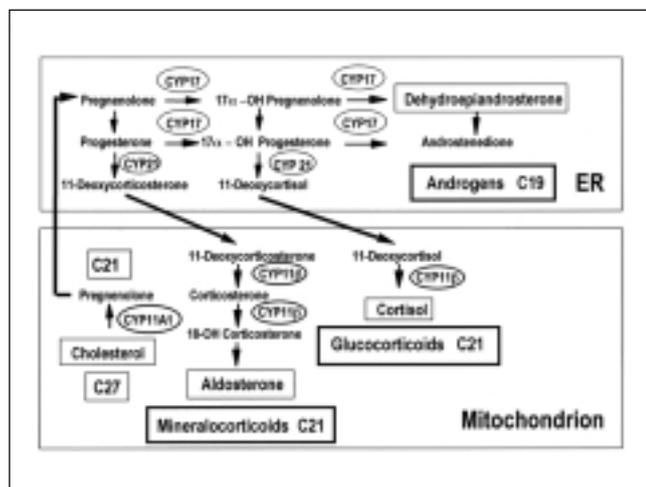


Figure 5 Cytochromes P450 (CYP) active in steroid biosynthesis are autoantigens in autoimmune polyglandular syndrome type 1 (80). ER Endoplasmic reticulum. Adapted with permission from reference 80

cortex and gonads, and CYP11A1 is expressed in the adrenals, gonads and placenta (18). Several other steroidogenic enzymes were tested for autoantibodies in 46 patients with APS1, CYP11, aromatase, 3β-hydroxysteroid dehydrogenase and adrenodoxin. However, no autoantibodies were detectable in 46 Finnish patients with APS1 (81).

In idiopathic Addison disease, the major autoantigen associated with adrenal failure is CYP21 (8,9,18,82-84). Betterle et al (83) performed a prospective study involving adult patients with adrenal cell autoantibodies (ACA). Only 50% of patients with ACA were found to progress to Addison disease in a 10-year period, suggesting that ACA in adults are a marker for low progression to Addison disease (83). Autoantibodies directed to CYP11 or CYP17 in APS1 are rare findings. In adrenal failure in APS1, however, the situation is different. Anti-CYP11 and anti-CYP17 autoantibodies are frequently associated with anti-CYP21 autoantibodies (9,18,81). The second difference is the fact that, in contrast with ACA in adults, in children the occurrence of ACA resulted in progression to adrenal failure in nine of 10 patients with a mean latency period of 2.7 years. These results demonstrate that autoantibodies directed against steroidogenic enzymes of the adrenal cortex in patients with APS1 are markers of high progression to clinical Addison disease (85). This result is in accordance with an earlier report (76), which described ACA as risk factors for the development of adrenal failure in APS1.

Gonadal failure: Gonadal failure in patients with APS1 is found in 60% of females above 13 years of age and in 14% of males (14). The targets of autoantibodies that stain steroid-producing Leydig cells were identified. In most cases CYP11A1 and/or CYP17 antibodies are detected (17,18,81,85,86). In accordance with a high frequency of gonadal failure in patients with APS1, gonadal CYPs, especially CYP11A1, are detected as autoantigens in APS1 with high prevalence (8,17,18,81,85,86). It is interesting to note that testicular failure in men is found with a much lower

prevalence than ovarian failure in women (14). This difference may indicate that the blood-testis barrier provides an immunologically privileged zone.

DRUG-INDUCED HEPATITIS

Because of their chemical nature, many drugs are characterized by direct toxic effects on hepatocytes. During detoxification, hydrophobic drugs are first hydroxylated by CYPs. The hydroxylated reaction product is then conjugated to water soluble components, resulting in hydrophilic conjugates, which may be excreted via bile or urine (Figure 6, top left). However, sometimes these 'detoxification reactions' result in reactive metabolites, which may interact with cellular components such as proteins, DNA and membranes. Covalent modifications inflicted by drugs or their metabolites irreversibly disturb cellular processes and may result in cell death by apoptosis or necrosis (Figure 6, top right). Characteristics of direct toxicity are a clear dose-response curve, manifestation in the majority of people, reproducibility in animal models and manifestation shortly after drug usage. In contrast, in immune-mediated, drug-induced hypersensitivity, covalent modifications induced by the drug result in a light, often unmeasurable toxicity. However, in a minority of people, covalent binding of drugs or their metabolites to cellular components results in an immune response. This response may be directed against the covalently bound metabolite, a haptene protein domain or the native protein (Figure 6 top right). The following characteristics may help to detect immune-mediated, drug-induced hypersensitivity (1,26,87).

Disease is not observed immediately following drug usage. It always occurs with a significant lag period that may range from weeks to months.

There is no obvious dose dependence between drug dosage and toxicity.

Symptoms disappear on discontinuation of treatment and recur upon re-exposure.

Often disease is accompanied by symptoms of an immune reaction (fever, eosinophily, rash).

Usually autoantibodies against hepatic proteins are detected.

Over-representation of females.

TIENILIC ACID-INDUCED HEPATITIS

Tienilic acid is an antihypertensive drug that was withdrawn from the market because of clinical reports of rare but severe hepatotoxicity (88). In 0.1% to 0.7% of patients, severe clinical hepatitis developed in a dose-independent manner. Reactions occurred 14 to 240 days following drug treatment. Cellular infiltrates of neutrophils, eosinophils and lymphocytes were characteristic of the liver. After discontinuation of drug treatment, liver damage resolved. Rechallenge with the drug resulted in a recurrence of symptoms after a shorter

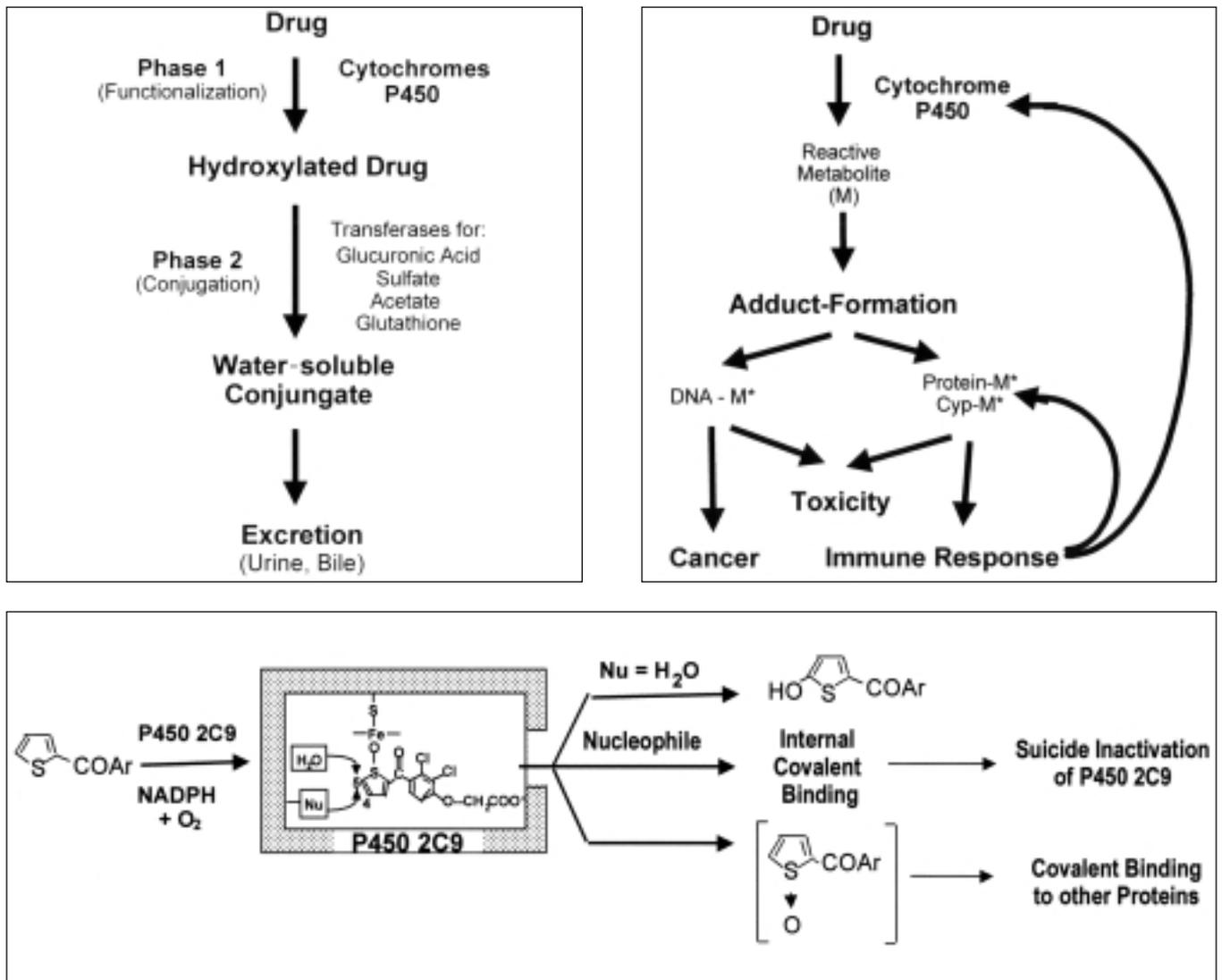


Figure 6) Top left Phase 1 and phase 2 of drug metabolism. Adapted with permission from reference 3. Top right Bioactivation of drugs by cytochromes P450 and hypothesis for induction of autoimmunity in drug-induced hepatitis. Adapted with permission from reference 3. Bottom Metabolism of tienilic acid by CYP2C9. Adapted with permission from reference 1

time period than before (88). In 60% of patients suffering from hepatitis after administration of tienilic acid, a specific antibody directed against unmodified LKM proteins, the LKM-2 autoantibody, was detected (6,89). The molecular target of this autoantibody was CYP2C9, the major tienilic acid-metabolizing enzyme (90). Based on the available data, a mechanism for LKM-2 induction in patients with tienilic acid-induced hepatitis was proposed (1,27,28) (Figure 6, bottom). According to this hypothesis, tienilic acid is activated in the active centre of CYP2C9 to form a reactive sulphoxide. The sulphoxide covalently binds to the metabolizing enzyme, CYP2C9, and causes enzyme inactivation. After suicide inactivation, CYP2C9 may be presented to the immune system, where autoreactive B cells and T cells specific for the alkylated peptide may be present at low frequency. These components of the immune system may activate the immune system against CYP2C9 and cause the formation of anti-CYP2C9 or LKM-2 autoantibodies (1,27).

DIHYDRALAZINE HEPATITIS

Long term treatment with dihydralazine, which has vasodilatory effects, resulted in severe hepatitis in numerous patients. At the Pathological Institute in Berlin, Friedrichshain, from 1981 to 1985, 70 cases of acute, drug-induced hepatitis were registered (87). Seventy-five per cent of patients were female. In addition, most patients were 'slow acetylators' because they were deficient in the acetylation pathway for drug metabolism (87,91). Hepatitis did not manifest immediately after onset of drug treatment but was recorded after an average duration of 14 weeks of drug treatment, with variations in drug exposure from two weeks to 11 months (87). Dihydralazine-induced hepatitis did not show any obvious dose dependence, daily intake varied from 20 to 200 mg and cumulative dosage until development of hepatitis ranged from 350 mg to 36 g. Usually dihydralazine-induced hepatitis resolved after discontinuation of treatment. After re-exposure, hepatitis usually recurred after a

shorter lag period (92). In sera of patients with dihydralazine hepatitis, autoantibodies against LMs, but not renal microsomes, were detected (29). The molecular target of LM autoantibodies, which are highly specific, is CYP1A2. CYP1A2 and CYP1A1 share more than 80% sequence homology, but CYP1A1 is not detected by LM autoantibodies (29,93,94). A mechanism similar to the induction of LKM-2 autoantibodies was proposed in dihydralazine hepatitis. Dihydralazine is metabolized by CYP1A2 and a reactive metabolite that binds to the active centre of CYP1A2 is generated by this process (27,28,94). The covalently modified CYP1A2 is presented to the immune system as a neoantigen and induces an immune reaction, which results in the production of LM autoantibodies (1,94). Interestingly, dihydralazine is also metabolized by acetylation. *N*-acetyltransferase is the enzyme mediating this second pathway of dihydralazine metabolism. About 50% of the Caucasian population is void of *N*-acetyltransferase activity resulting in the phenotype of a 'slow acetylator' (95,96). Slow acetylators are dependent exclusively on the metabolism of dihydralazine by CYPs and are more likely to form drug adducts than extensive acetylators. In accordance with the hypothesis of drug activation by dihydralazine as the initial event in the induction of dihydralazine hepatitis, slow acetylators are over-represented in the patient population affected by dihydralazine-induced hepatitis (91,97). This effect is due most likely to a shift away from acetylation, toward an increased oxidation by CYP1A2. Shifts toward dihydralazine metabolism by CYP1A2 may also occur due to induction of CYP1A2 by long term treatment with dihydralazine or by smoking.

HALOTHANE HEPATITIS

Halothane is one of the most frequently used anesthetics. About 20% of patients show a slight increase in transaminase levels after halothane exposure, which seems to be due to the direct toxic effects of the drug (98). One in 10,000 patients, however, develop severe hepatitis characterized by highly increased transaminase values, centrilobular necrosis and a high rate of mortality (99,100). Female sex is a risk factor for the development of halothane hepatitis, resulting in a female to male ratio among patients with halothane hepatitis of two to one. A second known risk factor is obesity. Some cases of halothane hepatitis are reported after a single exposure to halothane (101). However, the risk of developing halothane hepatitis strongly increases with multiple exposures to halothane (102). Under physiological conditions, oxidative metabolism of halothane is mainly due to CYP2E1 (103-105). A reactive trifluoroacetylchloride (TFA) is formed and a small proportion may bind to the active centre of CYP2E1. However, most of the TFA molecules will leave the active centre of CYP2E1 and modify the epsilon-amino group of lysine residues of multiple hepatic proteins (106). TFA products may act as neoantigens and induce an immune response in patients with a genetic predisposition. Some identified autoantigens in halothane hepatitis are GRP94, BiP/GRP78, ERp73, calreticulin, carboxylesterase, polysulphidesterase epoxidhydrolase and CYP2E1 (25,26).

To reduce the risk of idiosyncratic drug reactions of halothane, other polyhalogenated, volatile anesthetics were developed that are metabolized less efficiently, namely enflurane, isoflurane and desflurane. Rates of metabolism are 29% for halothane, 2.4% for enflurane, 0.2% for isoflurane and 0.01% for desflurane (26). Registered cases of immune-induced toxicity parallel metabolism rates of these substances. Nine hundred cases of halothane hepatitis are known, but only 15 to 24 cases were enflurane-induced hepatitis, five cases were isoflurane-induced hepatitis and one case was desflurane-induced hepatitis (26). Interestingly, anesthesia used in patients with desflurane-induced hepatitis was preceded by two exposures with halothane and most likely due to 'preimmunization' with identical adducts generated before by halothane (26,107).

HEPATITIS INDUCED BY ANTICONVULSANTS

Life-threatening systemic reactions were recorded in one of 10,000 patients treated with aromatic anticonvulsants such as phenobarbital, phenytoin and carbamazepine (108). First symptoms are recorded one to 12 weeks after start of therapy. Reactions are characterized by fever, rash, lymphadenopathy and sometimes by hepatitis or nephritis (108). In the serum of nine of 24 patients with anticonvulsant-induced hepatitis, antimicrobial autoantibodies that recognize a hepatic protein of 53 kD were detected (31). These autoantibodies were not found in the sera of healthy controls or of patients after therapy with anticonvulsants without these adverse side effects (31). Western blot experiments using a series of purified rat CYPs showed that the sera of all eight patients tested recognized rat CYP3A1, but the sera of six of the eight patients recognized rat CYP2C11 (31). When human LMs were tested, only marginal signals were detectable. To investigate further the nature of the sequence recognized by the sera, a genebank with fusion proteins of rat CYP3A1 sequences was screened with patient serum that recognized rat CYP3A1. Positive clones contained a consensus sequence of amino acid sequence 355 to 367, which was recognized by all patients with anticonvulsant hepatitis (109). The exact nature of the epitope recognized in humans by these autoantibodies remains to be established.

ALCOHOL-INDUCED LIVER DISEASE

Chronic intake of large quantities of alcoholic beverages is a major cause of liver disease and cirrhosis. Only 10% to 20% of patients who abuse alcohol are affected by alcohol-induced liver disease (ALD), indicating that host factors may be involved in the pathogenesis. Several investigations were performed in animal models and in humans, suggesting that adduct formation and an immune response to covalently modified neoantigens may be involved in the pathogenesis of ALD (110-112). Israel et al (111) showed that both humans and mice chronically exposed to alcohol developed persistent, circulating antibodies that recognized acetaldehyde protein adducts. The reactivity to the acetaldehyde protein adducts was independent of the protein carrier used. A second population of autoantibodies was found by Clot et

al (110) in alcoholic liver disease. These autoantibodies were directed against hydroxyethyl domains of proteins and did not crossreact with autoantibodies directed against acetaldehyde modified proteins. The generation of hydroxyethyl-modified proteins was found to be dependent on CYP2E1 (32,33).

DISCUSSION

Significant progress has been made in recent years concerning the molecular identification of hepatocellular autoantigens. For autoimmune diseases associated with the repeated formation of anti-LKM autoantibodies, drug-metabolizing enzymes were determined to be targets of autoimmunity. In drug-induced hepatitis, formation of reactive metabolites is the first event in the disease process. This step, in a small minority of patients with a genetic predisposition, will induce an immune response, which is followed by drug-induced hepatitis. The nature of this genetic predisposition is not known. In contrast, in APS1, the genetic predisposition, a defect in a transcription factor, may be identified. Because this transcription factor is expressed strongly in the thymus, it may be involved in the establishment and maintenance of tolerance. The AIRE-1 gene product, therefore, may help to

elucidate intracellular signal transduction pathways, which may lead to a better understanding of idiopathic autoimmune diseases, where neither genetic predisposition nor factors that make certain proteins preferred targets of autoimmunity are known. Potentially, modification of CYP2D6 by foodborne substrates may help to induce idiopathic AIH-2; however, infections by certain viruses such as herpes viruses may facilitate the loss of self-tolerance. In chronic, viral hepatitis, constant triggering of the immune system and shift of cytokine patterns by chronic infections with RNA viruses may facilitate the development of autoimmune reactions. Further viruses tend to avoid immune reactions by mimicking epitopes on cellular proteins and obtaining protection of tolerance. Cryptic epitopes generated by viral proteins, however, may help to break tolerance to self proteins and provide a risk factor for the development of autoimmunity.

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