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ANTIMICROSOMAL ANTIBODIES: WHAT ARE THEY TELLING US?

Bourdi M, Larrey D, Nataf J, Bernuau J, Pessayre D, Iwasaki M, Guengerich FP, et al. Anti-liver endoplasmic reticulum autoantibodies are directed against human cytochrome P-450IA2: a specific marker for dihydralazine-induced hepatitis. J Clin Invest 1990;85: 1967-1973.

EDITOR'S ABSTRACT

Patients with dihydralazine hepatotoxicity have been found to have circulating autoantibodies that react with liver microsomes (anti-liver microsome antibodies) and that are clearly distinct from anti-liver and kidney microsomal antibodies observed in patients with tienilic acid–induced hepatitis and in some patients with autoimmune hepatitis. The authors show that anti-liver microsome antibodies present in the sera of five patients with dihydralazine-induced hepatitis specifically react on immunoblots with a 53 kD protein. They further conclude that this target antigen is the phase I drug metabolizing enzyme termed P-450IA2 based on the following observations: (a) immunoblots of a battery of human liver microsomes produced the identical pattern of relative staining whether the blots were developed with anti-rat P-450IA2 IgG, anti-human P-450IA2 IgG or each of the five patients' sera; (b) P-450IA2 catalytic activity was selectively inhibited when human liver microsomes were preincubated with anti-liver microsome–positive sera; (c) anti-liver microsome–positive sera identified purified human P-450IA2 on immunoblots. Anti-liver microsome antibodies appeared to be specific for dihydralazine hepatitis because they were not present in sera obtained from 28 other patients including patients receiving dihydralazine without a toxic response and patients with other significant liver diseases. Finally, the authors demonstrated that dihydralazine could competitively inhibit catalytic activity characteristic of P-450IA2 in human liver microsomes, suggesting that P-450IA2 may be involved in the metabolism of dihydralazine.

COMMENTS

When we order serum antimitochondrial antibody, antinuclear antibody or antiparietal cell antibody tests on our patients, the test is usually performed by applying serial dilutions of the patient's serum to a commercially prepared microscope slide containing sections of rodent liver, kidney and stomach. To the trained eye, the immunofluorescent patterns produced by each type of autoantibody are diagnostic. Rarely, the serum from a patient will react with liver and kidney endoplasmic reticulum; these organelles can be isolated from whole tissue as tiny membrane vesicles termed microsomes. The antibodies that react with endoplasmic reticulum are therefore called liver/kidney microsomal, or LKM, antibodies. Although all microsomal antibodies produce similar immunofluorescent patterns in the liver tissue, it has been possible to distinguish two types of anti-LKM antibodies: those reacting most intensely with renal cells in the distal third of the proximal tubules (called LKM1) and those reacting most intensely with the cells in the initial third of the tubule (called LKM2). Anti-LKM1 antibodies have been observed in some patients with halothane hepatitis and appear to also identify a subgroup of patients with severe autoimmune hepatitis. Anti-LKM2 antibodies have been observed in patients with tienilic acid–induced hepatitis and appear to be relatively specific markers of this condition. The paper by Bourdi et al. deals with a third type of antimicrosomal antibodies that do not react with kidney tissue at all and are therefore called anti-liver microsomal (anti-LM) antibodies. To date, this immunofluorescent pattern has only been observed using sera from patients who have dihydralazine-induced hepatitis and, recently, from one child with autoimmune hepatitis (1).

The investigators convincingly demonstrate that the major antigen recognized by the anti-LM antibodies in sera from five patients is a cytochrome P-450 (P-450IA2). They also show that P-450IA2 binds and presumably metabolizes dihydralazine. This is the second type of drug-induced liver disease to be associated with autoantibodies to specific cytochromes P-450 (P-450s). Patients with tienilic acid–induced hepatitis usually have serum antibodies (anti-LKM2) that recognize another P-450 (P-450IIC8/9) (2). In this case as well, the antibody appears to recognize a P-450 involved in the metabolism of tienilic acid (2). The simplest explanation for these autoantibodies (2) is that the metabolites produced by P-450s from dihydralazine and tienilic acid are so unstable that they react with, and antigenically alter, the P-450 proteins that produced the metabolites. This is a reasonable hypothesis because P-450s have been shown to convert many substrates to reactive metabolites capable of covalently binding cellular proteins (3). Moreover, the P-450s should be convenient targets for the metabolites because they are likely to be the proteins nearest to the metabolites as they are produced.

Reactive metabolites can cause hepatotoxicity by binding to, or reacting with, proteins whose functions are vital to the hepatocyte (3). The reaction of metabolites with P-450IA2 and P-450IIC8/9 should not directly cause toxicity because these enzymes are unlikely to be of critical importance to the hepatocytes. However, it seems reasonable to assume that the P-450s would not be the only protein targets for reactive metabolites, some of which might be more crucial for hepatocyte survival. If metabolites are directly causing the drug toxicity observed, the production of antibodies to the P-450s might be occurring only after hepatocytes have lysed; this would explain why anti-LM antibodies were ob-

served only in patients with dihydralazine hepatitis and not in all patients treated with the drug. In other words, the presence of anti-P-450 antibodies could be incidental and merely imply that toxicity is the direct result of reactive metabolite(s) produced by the P-450 recognized by the antibody. The fact that only a small fraction of patients receiving the drug develop toxicity may reflect the recognized interpatient differences in the catalytic activities of the P-450s or detoxifying enzymes (3).

A more exciting possibility is that antimicrosomal antibodies may directly contribute to the pathogenesis of some forms of liver disease. Data supporting this view are indirect and largely obtained from studies of halothane hepatitis (4). Anti-LKM1 antibodies are often present in sera of patients with halothane hepatitis. These antibodies will react with hepatocytes isolated from halothane-pretreated rabbits and will also activate the destruction of the hepatocytes by lymphocytes *in vitro*. It appears that the major hepatocyte proteins recognized by these antibodies have become antigenic by covalently binding the trifluoroacetyl metabolite of halothane produced by P-450(s). One trifluoroacetylated protein has the molecular weight of a P-450 and is postulated to be the P-450 that produces trifluoroacetyl chloride. A second protein identified is a carboxyl esterase, which is a major protein present in the endoplasmic reticulum (5). This enzyme is unlikely to be involved in metabolism of halothane and may therefore become trifluoroacetylated as an "innocent bystander." Trifluoroacetylated proteins are not confined to the endoplasmic reticulum; they also appear to be present on the surface membrane of halothane-treated hepatocytes. P-450s and other microsomal proteins may be present on the hepatocyte membrane (6) but need not be the antigens stimulating an immune attack. This is because the majority of proteins present on the surface of the hepatocyte are synthesized in ribosomes bound to the hepatocyte's endoplasmic reticulum. Thus proteins could be trifluoracetylated immediately after synthesis and before they are sorted to the plasma membrane. This idea is supported by studies of isaxonine, which is converted to a reactive metabolite in the endoplasmic reticulum. When ^{14}C isaxonine was administered to rats *in vivo,* radiolabeled protein was detected first in endoplasmic reticulum and subsequently in the plasma membrane (7). *In vitro* metabolism of ^{14}C isaxonine in a mixture of liver microsomes and plasma membranes (which were attached to beads) resulted in radiolabeling of microsomal proteins, but there was little labeling of plasma membrane proteins (7). These studies suggest that a metabolite of isaxonine capable of covalent binding does not diffuse far from its site of production within the endoplasmic reticulum, presumably because it is extremely reactive. Proteins bound to the metabolite presumably appear in the surface membrane of the hepatocyte after the binding has occurred within the endoplasmic reticulum (7).

By analogy to the current thoughts about halothane hepatitis, antimicrosomal antibodies may indicate that reactive metabolites capable of creating neoantigens are being produced in the endoplasmic reticulum; some of these neoantigens could reasonably end up on the surface membrane and stimulate an immune attack in an "allergic" individual. An antibody directed to a specific P-450 probably identifies the enzyme responsible for generating the reactive metabolite but need not imply that this antibody is directly involved in an immune attack on the liver.

It may be argued that observations made in halothane hepatitis, which has clinical features suggesting hypersensitivity, are not relevant to other drugs, such as tienilic acid and dihydralazine, which generally produce hepatitis in the absence of the classic clinical hallmarks of hypersensitivity (skin rash, fever and eosinophilia). However, hepatotoxicities caused by many drugs have at least some features consistent with an immunological basis. For example, although tienilic acid hepatitis generally occurs months after patients have started therapy, it often recurs within 24 hr after patients who have recovered from the toxicity are rechallenged with the drug (8). Hepatitis also develops sooner on rechallenge in patients who have recovered from dihydralazine hepatitis (9). Finally, it should also be remembered that patients with autoimmune hepatitis do not generally exhibit skin rashes, fever or eosinophilia, although an immunological basis for this disease is accepted. The absence of the classic clinical signs of hypersensitivity therefore do not in itself exclude the possibility that autoimmunity is playing a role in the pathogenesis of drug-induced liver disease.

In summary then, the findings of Bourdi et al. are important because they support the idea that anti-P-450 antibodies (a) indicate that reactive metabolites are being produced in the liver that are capable of altering protein structure and creating new antigens and (b) point to which P-450 is producing these metabolites. The observed injury may be the direct result of the reactive metabolites, it may be the result of immune responses to new antigens formed as a result of the metabolites or both mechanisms could be involved. As a final point, anti-LKM1 antibodies observed in a subset of patients with generally severe autoimmune hepatitis have been shown to selectively identify P-450IID and several other microsomal antigens (10, 11). Recently, a child with autoimmune hepatitis has been shown to have anti-LM antibodies recognizing P-450IA2 (1), the identical antigen identified by anti-LM antibodies in dihydralazine-induced hepatitis. Although drugs do not appear to be involved in autoimmune hepatitis, many substances encountered in our diet appear to be metabolized by P-450s (3). The observations made in hepatitis caused by tienilic acid and dihydralazine support the hypothesis that some forms of autoimmune hepatitis may result when reactive, neoantigen-forming metabolites are produced from dietary substances by P-450s (10). If this is so, it may be possible to identify and remove potential of-

fending compounds from the diet of these individuals. Alternately, the administration of selective inhibitors of the P-450s identified by the autoantibodies might provide a rational long-term treatment for these patients.

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EPIDEMIOLOGICAL STUDIES WITH ANTI-HEPATITIS C VIRUS

Stevens CE, Taylor PE, Pindyck J, Choo Q-L, Bradley DW, Kuo G, Houghton M. Epidemiology of hepatitis C virus: a preliminary study in volunteer blood donors. JAMA 1990;263:49-53.

ABSTRACT

In a survey carried out from 1985 through 1986, volunteer blood donors to The Greater New York Blood Program were tested for two surrogate markers for non-A, non-B hepatitis—elevation of alanine aminotransferase level and presence of antibody to hepatitis B core antigen. Stored serum samples from selected donors were also recently tested for antibody to hepatitis C virus (anti-HCV). Anti-HCV was detected in 0.9% to 1.4% of donors and was higher in black and Hispanic donors than in white donors. Anti-HCV prevalence increased with increasing age through the fourth decade of life, but decreased thereafter, possibly reflecting the disappearance of detectable antibody with time. Anti-HCV correlated with both alanine aminotransferase level and the presence or absence of antibody to hepatitis B core antigen. These associations suggest that donor screening for elevation of alanine aminotransferase level and presence of antibody to hepatitis B core antigen was, as expected, at least partially effective in preventing transfusion-associated non-A, non-B hepatitis. The detection of anti-HCV in donors who have neither an elevation of alanine aminotransferase level nor presence of antibody to hepatitis B core antigen suggests that donor screening for anti-HCV will further reduce the risk of transfusion-associated hepatitis.

COMMENTS

The search for a viral agent in patients with non-A, non-B hepatitis (NANBH) after transfusion has been long and difficult. Hundreds of putative agents and tests have been proposed, evaluated and then discarded when it became clear that each lacked the requisite specificity. In 1985 Bradley did identify and characterize *a small* enveloped virus that was readily transmissible to chimpanzees (1), but it remained for Michael Houghton and his coworkers at the Chiron Corporation to take the next major step forward (2). They used a molecular biological approach to identify a clone that encoded for a polypeptide associated with NANBH. The polypeptide was then synthesized in recombinant yeast and used as the basis of an assay for antibodies to at least one major etiological virus of NANBH.

The availability of a test for anti-hepatitis C virus (anti-HCV) has opened new vistas for hepatologists throughout the world. As this new test is applied to patients with (and without) liver disease, some long-standing problems surely will be resolved. Certainly, at the same time new ones will be uncovered.

From early studies we have learned that a high proportion of patients with documented NANBH after transfusion are anti-HCV positive (3). There is a high prevalence of anti-HCV in intravenous drug abusers, hemophiliacs and hemodialysis patients and a somewhat lower prevalence in homosexual men. This later finding was unexpected, as was the high prevalence in patients with autoimmune CAH and cirrhosis. About 1% of healthy blood donors are also anti-HCV positive.

In the paper under discussion here, Stevens and her coworkers have begun the important task of large-scale epidemiological studies designed to learn about this 1% of the population. They have analyzed a selected sample of blood donors from New York City, which has shed