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ASSOCIATION OF ANTI-58 kDa ENDOPLASMIC RETICULUM ANTIBODIES WITH HALOTHANE HEPATITIS

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Abstract—We recently showed that when rats were administered the inhalation anesthetic halothane, a 58 kDa liver endoplasmic reticulum protein became covalently trifluoroacetylated by the trifluoroacetyl chloride metabolite of halothane. Although the 58 kDa protein showed 99% identity to that of the deduced amino acid sequence of a cDNA reported to correspond to phosphatidylinositol-specific phospholipase C-α, it did not have phosphatidylinositol-specific phospholipase C activity. It was concluded that the reported cDNA of phosphatidylinositol-specific phospholipase C-α actually encoded for the 58 kDa endoplasmic reticulum protein of unknown function. Other researchers have come to the same conclusion and have shown that the 58 kDa protein has protein disulfide-isomerase and protease activities. We now report that patients with halothane hepatitis have serum antibodies that react with both purified trifluoroacetylated and native rat liver 58 kDa proteins. These results suggest that when patients are exposed to halothane a human liver orthologue of the rat liver trifluoroacetylated-58 kDa protein is formed. In certain patients, this protein may become immunogenic and lead to the formation of specific antibodies and or specific T-cells, which may react with both trifluoroacetylated and native 58 kDa proteins, and ultimately be responsible, at least in part, for the hepatitis caused by halothane.

Several recent reports by investigators from independent laboratories indicate that the putative cDNA of phosphatidylinositol-specific phospholipase-C-α (PI-PLC-α‖) was incorrectly assigned and actually encodes for an endoplasmic reticulum protein mass of approximately 55–60 kDa [1–3]. Moreover, it now appears that PI-PLC-α may actually be derived from PI-PLC-δ [4]. The 55–60 kDa endoplasmic reticulum protein has protein disulfide-isomerase (PDI) activity [2], can catalyze the proteolysis of other endoplasmic reticulum proteins, such as calreticulin and PDI [3, 5], and appears to alter complex formation between nuclear proteins and regulatory regions of interferon-inducible genes [6]. In addition, it has been shown to be a target of the reactive trifluoroacetyl (TFA) chloride (CF_3COCl) metabolite of the inhalation anesthetic halothane ($CF_3CHClBr$) [1]. We now report that patients diagnosed with halothane hepatitis have serum antibodies that react with both TFA-labeled and native forms of a 58 kDa protein purified from rat liver microsomes, suggesting that human orthologues may have an immunopathological role in the etiology of this disease.

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‖ Abbreviations: PI-PLC-α, phosphatidylinositol-specific phospholipase-C-α; PDI, protein disulfide-isomerase; TFA, trifluoroacetyl; and ELISA, enzyme-linked immunosorbent assay.

MATERIALS AND METHODS

Purification of TFA-58 kDa and native 58 kDa proteins. TFA-58 kDa and native 58 kDa proteins were purified from liver microsomes of halothane-treated and control rats, respectively, as previously reported [1].

Human antisera. Human sera were obtained after informed consent and the study protocol was approved by the Joint Committee on Clinical Investigations. Sera were obtained from patients with a clinical diagnosis of halothane hepatitis (N = 40) by J. G. Kenna, as described in detail elsewhere [7, 8]. The sera of patients with unexplained hepatitis following halothane anesthesia were negative for serological markers of hepatitis A and B infection, cytomegalovirus, and Epstein–Barr virus, and none had received any potentially hepatotoxic drugs or had a history of excess alcohol use. The interval between halothane exposure and the onset of hepatic injury ranged from 3 to 28 days (median 8 days). The interval between exposure and sera collection was 4–67 days (median 20 days) and time to death (31%) was 6–74 days (median 28 days). Sera were also collected from the following control patients by J. G. Kenna from Kings College Hospital, London, U.K., and by J. L. Martin from The Johns Hopkins Medical Institutions, Baltimore, MD. Control patients included those with multiple halothane exposures without developing evidence of liver dysfunction (N = 5); normal control subjects, who had not had prior halothane exposure, hepatitis or liver dysfunction (N = 5); subjects exposed to subclinical doses of halothane such as anes-

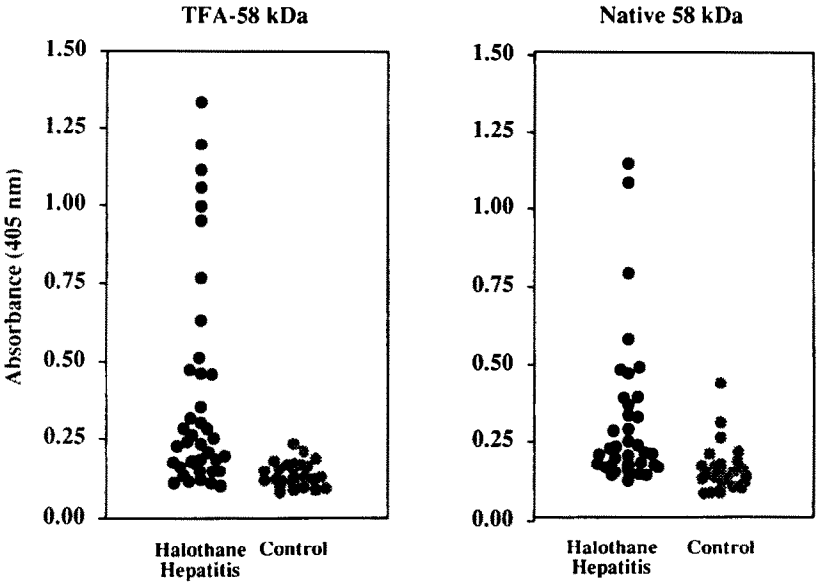

Fig. 1. ELISA screening of halothane hepatitis patients' sera for antibodies that react with TFA-58 kDa and native 58 kDa proteins. All sera from 40 halothane hepatitis and 32 control patients were assayed at a 1/100 dilution.

thesiologists, critical care technicians, and recovery room nurses (N = 5); patients diagnosed with primary biliary cirrhosis (N = 5), acute fulminant liver disease (N = 5), and chronic active liver disease (N = 5); and patients diagnosed with viral hepatitis, who had been exposed to halothane (N = 2).

Enzyme-linked immunosorbent assay (ELISA) screening of patients' sera. The ELISA was conducted by a previously reported method [8], except that the antigens employed were either TFA-58 kDa or native 58 kDa proteins and the incubations of the patients' sera with the antigens were for 3 hr instead of 2 hr. Two statistical hypotheses were tested. The first was whether control and halothane hepatitis patients differed in response to the TFA-58 kDa or native 58 kDa antigens. The Wilcoxon Rank Sum test was applied and two-sided P values less than or equal to 0.05 were deemed to be statistically significant. The second hypothesis, whether the response to TFA-58 kDa and native 58 kDa proteins differed within control and halothane hepatitis patient groups, was done using the binomial test on the proportion of patients with a TFA response greater than or equal to a response with native protein. Any two-sided P value less than or equal to 0.05 was considered statistically significant.

RESULTS

It was found in an ELISA that serum antibodies of the group of halothane hepatitis patients reacted to a significantly greater extent with either the purified TFA-58 kDa protein ($P < 0.001$) or native 58 kDa protein ($P < 0.001$) than did serum antibodies from the group of control patients (Fig. 1). Of the individual halothane hepatitis patients studied, 12 of 40 and 7 of 40 responded noticeably stronger with the purified TFA-58 kDa and native 58 kDa proteins, respectively, than did any of the control patients. Although as a group there was no significant difference in the response of the halothane hepatitis patients with the two antigens (P = 0.64), there were 5 of 40 of the halothane hepatitis patients, but none of the control patients, who had serum antibodies that reacted appreciably greater with the TFA-58 kDa protein than with the native 58 kDa protein (absorbances of 1.111 vs 0.489, 1.054 vs 0.365, 0.955 vs 0.579, 0.766 vs 0.487, and 0.458 vs 0.291).

DISCUSSION

Immunoblotting studies of liver tissue from halothane-treated rats [9], rabbits [10], and humans [11] have revealed that serum antibodies of halothane hepatitis patients react with one or more liver microsomal protein fractions of 100 kDa, 80 kDa (formerly designated 76 kDa), 59 kDa, 57 kDa, and 54 kDa that are covalently trifluoroacetylated by the TFA chloride metabolite of halothane [9]. This and other findings have suggested that halothane hepatitis may result from an immunological reaction mediated by specific antibodies or specific T-cells that are directed against human orthologues of these TFA-proteins [12, 13].

To understand better how halothane might cause hepatitis by an immune mechanism, we have begun recently to purify and characterize TFA-proteins from rat liver microsomes that are recognized by the halothane hepatitis patients' serum antibodies. The TFA-59 kDa protein was identified as a carboxylesterase (EC 3.1.1.1) [14] and reacted in an ELISA with antibodies in the sera of 2 of 10

patients with halothane hepatitis [15]. During the purifications, two new TFA-liver microsomal proteins were identified. One was a 63 kDa protein, which corresponds to the calcium binding protein calreticulin [16]. Serum antibodies of only 1 of 40 patients with halothane hepatitis reacted in an ELISA with this protein. The other protein was a 58 kDa protein [1], which we have now shown to be another antigen associated with halothane hepatitis (Fig. 1).

Except for 5 patients, serum antibodies of the halothane hepatitis patients reacted with the TFA-58 kDa and native 58 kDa proteins to nearly the same extent. This was a surprising finding because previous immunoblotting studies indicated that the halothane hepatitis serum antibodies only react with TFA-microsomal proteins and not with native proteins of similar molecular masses [9–11]. One possible explanation for these results is that the halothane hepatitis patients' antibodies may react with sequential epitopes on the TFA-58 kD protein and conformation epitopes on the native 58 kDa protein [17]. If this were the case, then it is possible that the conformationally dependent epitopes of the native 58 kDa protein were destroyed by the denaturing conditions of immunoblotting, but were not appreciably altered by the relatively mild conditions of the ELISA [18–20]. It may also be possible that the patients' antibodies recognized novel TFA-58 kDa epitopes with high affinity and the same population of antibodies recognized the unmodified 58 kDa carrier with lower affinity. These lower affinity antibody responses may only be detected by the intrinsically more sensitive ELISA technique. Although these possibilities as well as others cannot be confirmed until epitope mapping studies of the TFA-58 kDa and native 58 kDa have been done, the finding of patients' antibodies reacting with both proteins does suggest that immune reactions against either or both of these proteins may have a role in the development of halothane hepatitis.

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