Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2008 August ; 47(2): 199–202. doi:10.1097/MPG.0b013e3181709fee.

Trifluoroacetylated IgG4 Antibodies in a Child With Idiosyncratic Acute Liver Failure After First Exposure to Halothane

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Halothane hepatitis, a form of idiosyncratic acute liver failure (Id-ALF), affects 1 in 10,000 to 35,000 adults but only 1 in 80,000 to 200,000 children (1,2). Reports of halothane-induced Id-ALF have decreased the use of halothane for general anesthesia in adults, but it is still being used in children. Certain risk factors have been associated with the development of Id-ALF in adults after exposure to halothane or any of the modern halogenated volatile anesthetics such as enflurane, isoflurane, and desflurane. These factors include age older than 40 years, female sex, obesity, history of autoimmune disease, previous exposure to halogenated anesthetics, and exposure to hepatotoxic drugs such as isoniazid (INH), acetaminophen, or rifampin (3). By contrast, only multiple halothane exposures have been associated with the development of halothane Id-ALF in children, demonstrating a scarcity of known risk factors associated with anesthetic Id-ALF in children (4). Additionally, INH and rifampin, commonly used in children for tuberculosis chemoprophylaxis, have rarely been associated with hepatotoxicity (5). We report a case of a 4-year-old child with a remote history of treatment with INH and rifampin for latent tuberculosis exposure, in whom Id-ALF developed after a first exposure to general anesthesia with halothane.

CASE REPORT

A 4-year-old obese Hispanic girl, weighing 36 kg, needed a tonsillectomy and adenoidectomy. After premedication with midazolam, general anesthesia was induced and maintained with halothane and oxygen, and lasted approximately 39 minutes. There was no intraoperative or postoperative hypoxia or hypotension. She was observed in the recovery room for 45 minutes and discharged home to receive acetaminophen with codeine (120 mg acetaminophen + 12 mg codeine/5 mL) every 4 to 6 hours for pain. Her history was significant for obstructive sleep apnea and mild asthma, well controlled with daily inhaled beclomethasone and intermittent albuterol. Four years before the tonsillectomy and adenoidectomy, she had been treated with INH for 3 months, followed by 4 months of rifampin, because of a positive reaction to the

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The authors report no conflicts of interest.

purified protein derivative test. She had no known drug allergies or previous surgical history. Her immunizations were up to date.

On postoperative day (POD) 4, she was brought to the emergency department (ED) because of a generalized erythematous maculopapular rash on her arms, abdomen, and legs. She was receiving no other medications except acetaminophen with codeine. She received a diagnosis of urticaria and was discharged home. On POD 10 she was brought back to the ED with an elevated temperature of 104°F, vomiting, and malaise. Her serum electrolytes, blood urea nitrogen, creatinine, glucose, total bilirubin, and urinalysis results were within normal limits; however, her aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated (Table 1). She received a diagnosis of hepatitis and was discharged home. The next day she was seen by her pediatrician for persistent symptoms. Her AST and ALT were further elevated (Table 1); consequently, on POD 12, she was sent to the ED a third time.

Examination in the ED showed her to be lethargic and obese. Her temperature was 102.9°F; heart rate, 112 beats/min; respiratory rate, 24 breaths/min; and blood pressure, 119/59mmHg. She had an erythematous rash on her face, knees, elbows, and axillae and a total liver span by percussion of 5 to 6 cm. Her white blood cell count was $14,400 \times 10^9/L$, with 30% neutrophils, 30% bands, and 7% eosinophils. Her hemoglobin, platelets, partial thromboplastin time (PTT), glucose, and amylase were normal; however, the following test results were abnormal: blood urea nitrogen, 65 mg/dL (normal <25); creatinine, 3.1 mg/dL (normal <0.8); ammonia, 65 $\mu\text{mol/L}$ (normal <30); alkaline phosphatase, 356 U/L (normal <350); and fibrinogen, 81 mg/dL (normal 210–439). She had worsening cholestasis, transaminitis, and coagulopathy (Table 1). She did not have toxic salicylate and acetaminophen levels: <5 mg/L and 2.3 $\mu\text{g/mL}$, respectively. The results of serology for infectious hepatitis, cytomegalovirus Ig, hepatitis A IgM, hepatic B surface antigen, core IgM, and hepatitis C IgG were negative. Serology for Epstein-Barr virus indicated previous infection. She received a diagnosis of fulminant liver and renal failure, was admitted to the pediatric intensive care unit, and was treated with vitamin K, fresh frozen plasma, and lactulose.

On POD 15 the patient experienced stage 2 hepatic encephalopathy and acute tubular necrosis and was transferred to Lucille Packard Children's Hospital. Repeat serological analyses confirmed hepatic failure (Table 1), uremia, renal failure, eosinophilia, and coagulopathy (Table 1) documented in the previous hospital. Factor VII activity was 37%, and ammonia level was 49 $\mu\text{mol/L}$. Urinalysis showed 11 to 20 red blood cells, rare renal cells, and high urine sodium (118 mEq/L). Additional testing showed normal ceruloplasmin and α -1 antitrypsin levels with M2S phenotype, but α -fetoprotein levels were elevated (97.8 ng/mL, normal <10). Test results for autoimmune and infectious liver diseases were negative for anti-smooth muscle antibody, anti-liver-kidney microsomal-1 antibody, anti-nuclear antibody, and also Epstein-Barr virus and cytomegalovirus by polymerase chain reaction. Abdominal Doppler ultrasound showed homogenous liver with hepatopedal flow, gall bladder wall edema without cholelithiasis or biliary tract dilation, and pelvic ascites. Computed tomography of her head was normal, but her chest radiograph showed a large right pleural effusion and pulmonary edema.

The patient was treated with intravenous hydration, fresh frozen plasma, cryoprecipitate, lactulose, vitamin K, and histamine-2 blockade. She also received empiric antibiotics and also *N*-acetylcysteine for its antioxidant and anti-inflammatory properties. She did not require mechanical ventilation or blood pressure support. On POD 21, she was discharged home after improvement of encephalopathy, cholestasis, hepatitis, renal function, and coagulopathy. One month later, her liver, coagulation, and renal function were normal (Table 1). Seven months later, after a viral infection with asthma exacerbation, her AST and ALT increased but subsequently returned to normal values (Table 1). Additional significant history included

normal liver function test results before and after INH therapy (Table 1); no family history of chronic liver disease or hepatitis from exposure to halogenated anesthetics; a half sister who had hepatitis A, which resolved; and a father who experienced elevated transaminases after receiving INH, which resolved after INH was discontinued.

The patient's serum was analyzed in enzyme-linked immunosorbent assays (ELISA) to detect 58 kDa endoplasmic reticulum protein (ERp58), cytochrome P450 2E1 (CYP2E1), and trifluoroacetyl chloride (TFA)-specific IgG and IgG4 antibodies as previously described (6). The serum was tested for ERp58, CYP2E1, or TFA-specific IgG antibodies initially as a screen, because if these were negative we should not find IgG4 subclass antibodies, which we have recently found to be more prevalent in patients with anesthetic hepatitis (7). Because of her history of receiving INH and rifampin and the possibility of induction of serum CYP2E1 autoantibodies at baseline, control sera were obtained from people exposed to halogenated anesthetics without liver disease. Our patient's sera or controls (1:100 dilutions) were incubated with human ERp58, human CYP2E1, trifluoroacetylated ovalbumin, or ovalbumin without covalent modification (0.5 µg/100 µL) for 2 hours followed by 2-hour incubations with 1:1000 dilutions of mouse anti-human IgG or IgG4. The samples were read at 30 or 90 minutes for IgG and IgG4, respectively. The ELISA results were based on the average of 2 samples done in triplicate.

The patient had elevated levels of IgG autoantibodies to ERp58 (1.663 ± 0.001 OD) and CYP2E1 (4.000 ± 0.001 OD) and also significantly elevated levels of TFA antibodies (4.000 ± 0.001 OD) in her sera in comparison with 2 standard deviations above mean control values (0.863 ± 0.628 , 2.228 ± 1.445 , and 2.557 ± 0.514 OD, respectively). She also had elevated serum levels of IgG4 subclass antibodies to TFA (1.018 ± 0.291 OD) in comparison with 2 standard deviations above control values ($0.277 \pm .006$). The ELISA results supported the diagnosis of halothane Id-ALF.

DISCUSSION

Halothane Id-ALF has rarely been reported in children. Multiple exposure to halothane is the commonly accepted risk factor that may promote the development of halothane Id-ALF in children (4). We demonstrate for the first time, as far as we are aware, TFA IgG4 subclass antibodies in a child with remote exposures to INH and rifampin in whom halothane Id-ALF developed after the first general anesthetic with halothane.

Anesthetic Id-ALF after exposure to halothane or any of the modern halogenated volatile anesthetics is believed to be caused by immune-mediated reactions generated by the TFA hapten formed from the oxidative metabolism of the anesthetic by CYP2E1 (8). The TFA hapten covalently alters native liver proteins and promotes the development of TFA antibodies, autoantibodies to native proteins such as CYP2E1 (6,9,10) and ERp58 (6,11) in the sera of susceptible patients, and also hepatitis. A recently described murine model demonstrating antibodies, autoantibodies, and hepatitis after immunization with TFA-altered mouse liver proteins has helped to confirm that TFA-altered liver proteins are immunogenic (12).

Surprisingly, we found TFA IgG4 subclass antibodies for the first time in a child with halothane Id-ALF without detecting CYP2E1 or ERp58 IgG4 autoantibodies. IgG4 subclass antibodies have been previously documented in patients with inhalant allergies, asthma (13), and autoimmune thyroiditis (14). We have recently found CYP2E1 IgG4 subclass autoantibodies in the majority of adults with anesthetic Id-ALF, which suggests that CYP2E1 IgG4 subclass antibodies have a role in the development of hypersensitivity and autoimmunity to anesthetics (7). Perhaps the absence of IgG4 subclass antibodies to the more common native proteins seen in adult patients suggests that in some children, autoantibodies develop to other native proteins

and not CYP2E1 or ERp58. Additionally, finding TFA IgG4 subclass antibodies could directly connect this hapten to the development of hypersensitivity and autoimmunity to anesthetics after first exposure, as has been previously hypothesized by earlier studies (8,15,16).

It is noteworthy that in our patient halothane Id-ALF developed after the first halothane exposure. This is an unusual phenomenon, especially in children; however, certain aspects of her medical history may help us understand why she may have been at increased risk for halothane Id-ALF. A key finding is the history of treatment with INH. Equally interesting is the finding that her treatment with INH occurred almost 4 years before the development of halothane Id-ALF. Nevertheless, previous studies have shown that INH can induce CYP2E1 in the liver (17). CYP2E1 is the major enzyme that metabolizes halogenated anesthetics, and previous studies have shown that halothane undergoes 35% to 40% metabolism. Thus, the history of INH administration may be a factor that may have placed this patient at increased risk for the development of liver injury from increased halothane metabolism and the generation of TFA-altered proteins. A second key finding is that current epidemiological studies suggest that childhood tuberculosis may be more common in some children, such as foreign-born children from Mexico who are younger than 5 years of age (18). In this respect, our patient may have been at increased risk for tuberculosis exposure and treatment with potentially hepatotoxic drugs that may have placed her at higher risk for hepatic injury after halothane exposure.

Even so, inasmuch as the exact mechanism for the development of hepatitis after exposure to halothane or any of the other halogenated volatile anesthetics has not been completely elucidated, induction of CYP2E1 by INH is only one possible mechanism that may have increased this child's susceptibility to the development of hepatitis after halothane exposure. Possibly her obesity may have increased her risk for the development of hepatitis. Obesity is a well-known risk factor for the development of halothane hepatitis (3) and has been recently linked to nonalcoholic fatty liver diseases, cryptogenic cirrhosis (19), and Id-ALF from other drugs. Unfortunately, a liver biopsy was not performed in this patient, so we do not know whether nonalcoholic fatty liver disease, although commonly associated with obesity in adult patients, may have also been present. Moreover, normal transaminases approximately 1 year before the event do not guarantee the absence of nonalcoholic fatty liver disease.

There is no widely used test for anesthetic Id-ALF, but most clinicians agree that finding eosinophilia, hepatitis, CYP2E1 or ERp58 autoantibodies, or TFA IgG antibodies after the exclusion of infectious and other autoimmune forms of hepatitis increases the index of suspicion for anesthetic Id-ALF. In light of recent studies, finding TFA IgG4 antibodies further increases the likelihood of anesthetic Id-ALF. The demonstration of eosinophilia and TFA IgG4 antibodies also suggests that the TFA hapten can induce allergy-mediated hepatic injury in susceptible patients even after 1 exposure to halothane. Clearly, INH, rifampin, or obesity may also have played a significant role in this process. In summary, we highlight a child who may have been at risk for Id-ALF because of previous exposure to tuberculosis chemoprophylaxis drugs, and we demonstrate for the first time, to the best of our knowledge, sera TFA IgG4 antibodies during active anesthetic Id-ALF. These findings underscore the importance of pediatricians, emergency room physicians, anesthesiologists, and gastroenterologists having a high index of suspicion for Id-ALF in potentially higher risk children after the first exposure to halothane and possibly other halogenated volatile anesthetics such as isoflurane and desflurane.

Acknowledgments

D.B.N. is supported by NIHR21DK075828. This work is supported in part by a grant from the American Autoimmune Related Diseases Association, Mr and Mrs Joseph Scoby, and the Gail I. Zuckerman Foundation.

REFERENCES

1. Carney FM, Van Dyke RA. Halothane hepatitis: a critical review. *Anesth Analg* 1972;51:135–160. [PubMed: 4399967]
2. Warner LO, Beach TP, Garvin JP, et al. Halothane and children: the first quarter century. *Anesth Analg* 1984;63:838–840. [PubMed: 6465580]
3. Njoku D, Laster MJ, Gong DH, et al. Biotransformation of halothane, enflurane, isoflurane, and desflurane to trifluoroacetylated liver proteins: association between protein acylation and hepatic injury. *Anesth Analg* 1997;84:173–178. [PubMed: 8989020]
4. Kenna JG, Neuberger J, Mieli-Vergani G, et al. Halothane hepatitis in children. *Br Med J (Clin Res Ed)* 1987;294:1209–1211.
5. Ormerod LP. Rifampicin and isoniazid prophylactic chemotherapy for tuberculosis. *Arch Dis Child* 1998;78:169–171. [PubMed: 9579163]
6. Njoku DB, Greenberg RS, Bourdi M, et al. Autoantibodies associated with volatile anesthetic hepatitis found in the sera of a large cohort of pediatric anesthesiologists. *Anesth Analg* 2002;94:243–249. [PubMed: 11812677]
7. Njoku DB, Mellerson JL, Talor MV, et al. Role of CYP2E1 immunoglobulin G4 subclass antibodies and complement in pathogenesis of idiosyncratic drug-induced hepatitis. *Clin Vaccine Immunol* 2006;13:258–265. [PubMed: 16467335]
8. Neuberger J, Mieli-Vergani G, Tredger JM, et al. Oxidative metabolism of halothane in the production of altered hepatocyte membrane antigens in acute halothane-induced hepatic necrosis. *Gut* 1981;22:669–672. [PubMed: 7286784]
9. Eliasson E, Kenna JG. Cytochrome P450 2E1 is a cell surface autoantigen in halothane hepatitis. *Mol Pharmacol* 1996;50:573–582. [PubMed: 8794896]
10. Bourdi M, Chen W, Peter RM, et al. Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis. *Chem Res Toxicol* 1996;9:1159–1166. [PubMed: 8902272]
11. Martin JL, Reed GF, Pohl LR. Association of anti-58 kDa endoplasmic reticulum antibodies with halothane hepatitis. *Biochem Pharmacol* 1993;46:1247–1250. [PubMed: 8216376]
12. Njoku DB, Talor MV, Fairweather D, et al. A novel model of drug hapten-induced hepatitis with increased mast cells in the BALB/c mouse. *Exp Mol Pathol* 2005;78:87–100. [PubMed: 15713433]
13. Vance GH, Thornton CA, Bryant TN, et al. Ovalbumin-specific immunoglobulin G and subclass responses through the first 5 years of life in relation to duration of egg sensitization and the development of asthma. *Clin Exp Allergy* 2004;34:1542–1549. [PubMed: 15479268]
14. Silva LM, Chavez J, Canalli MH, et al. Determination of IgG subclasses and avidity of antithyroid peroxidase antibodies in patients with subclinical hypothyroidism: a comparison with patients with overt hypothyroidism. *Horm Res* 2003;59:118–124. [PubMed: 12637791]
15. Pohl LR, Kenna JG, Satoh H, et al. Neoantigens associated with halothane hepatitis. *Drug Metab Rev* 1989;20:203–217. [PubMed: 2680380]
16. Pohl LR, Thomassen D, Pumford NR, et al. Hapten carrier conjugates associated with halothane hepatitis. *Adv Exp Med Biol* 1991;283:111–120. [PubMed: 2068977]
17. Kenna JG, Martin JL, Satoh H, et al. Factors affecting the expression of trifluoroacetylated liver microsomal protein neoantigens in rats treated with halothane. *Drug Metab Dispos* 1990;18:788–793. [PubMed: 1981738]
18. Nelson LJ, Schneider E, Wells CD, et al. Epidemiology of childhood tuberculosis in the United States, 1993–2001: the need for continued vigilance. *Pediatrics* 2004;114:333–341. [PubMed: 15286213]
19. Maheshwari A, Thuluvath PJ. Cryptogenic cirrhosis and NAFLD: are they related? *Am J Gastroenterol* 2006;101:664–668. [PubMed: 16464222]

TABLE 1

Results of liver function tests and coagulation studies

Time	Total/direct bilirubin, mg/dL	AST, U/L	ALT, U/L	GGT, U/L	Albumin, g/dL	PT, s	INR
Before INH (July 2000)	0.1	32	29				
After INH and rifampin (December 2004)		26	23				
POD 10 (2005)	0.3	1073	734				
POD 12	2.2	4434	8531	192	3.5		
POD 13	3.5/2.4	7876	6090	211	3.1	20	1.7
POD 16	6.0/3.8	274	1748	282	2.1	24.3	2.1
Discharge	2.3/0.8	67	455	132	3.2	13.1	1.1
Follow-up visit (POD 43)	0.7/0.1	42	44	103			
7-mo follow-up	0.4/0.1	313	129	35	4.7		
10-mo follow-up	<0.1/<0.1	29	22	15	4	11.2	1

GGT = γ -glutamyltransferase; PT = prothrombin time; international normalized ratio.

Normal values: total bilirubin, <1.5 mg/mL; direct bilirubin, 0.4 mg/mL; AST, <60 U/L; ALT, <30 U/L; GGT, <60 U/L; albumin, 3.5–5 g/dL; PT, 10–13 s; INR, 0.9–1.1.