Prevalence of IgG Autoantibody Against F-Actin in Patients Suspected of Having Autoimmune or Acute Viral Hepatitis

Troy D. Jaskowski,^{1*} Eric Q. Konnick,¹ Edward R. Ashwood,^{1,2} Christine M. Litwin,^{1,2} and Harry R. Hill^{1–4}

¹Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, University of Utah School of Medicine, Salt Lake City, Utah

²Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah

³Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah

⁴Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah

Our objectives in this study were to compare results obtained by an enzyme immunoassay (EIA) for F-actin antibody (FAA) immunoglobulin G (IgG) to those determined by an indirect fluorescent antibody (IFA) assay for smooth muscle antibody (SMA) IgG, and to determine the prevalence of FAA in patient sera having serologic evidence of acute viral hepatitis. Sera from 415 patients suspected of having autoimmune hepatitis (AIH), 208 patients suspected of having acute viral hepatitis A, B, or C, and 100 healthy blood donors (HBD) were included in the study. Only one of 100 HBD showed low levels (20–30 Units) of F-actin IgG. In patients suspected

of having AIH, the prevalence of FAA increased as SMA titers increased and all sera with SMA titers of $\geq 1:160$ were FAA-positive. In contrast, there were many sera with negative ($<1:20$) or low (1:20–1:40) SMA titers that contained moderate to high levels (>30 Units) of FAA; many exceeding 80 Units. Moreover, 51.4% of these sera were also positive for anti-nuclear antibody (ANA), which is also utilized in diagnosing type 1 AIH. FAA was detected in 25% of viral hepatitis antibody-positive sera, with the majority (59.3%) containing low levels, and all were ANA-negative. *J. Clin. Lab. Anal.* 21:249–253, 2007. © 2007 Wiley-Liss, Inc.

Key words: anti-smooth muscle; anti-nuclear antibody; indirect fluorescent antibody; enzyme immunoassay; AIH type 1

INTRODUCTION

Indirect fluorescent antibody (IFA) techniques for detecting autoantibody against smooth muscle (SMA) in patients suspected of having autoimmune hepatitis (AIH) have been in use for several decades to aid in serologic diagnosis. In 1976, studies showed SMA to have specificity against the actin component of the cytoskeleton (1,2); subsequently, anti-actin has been advocated by some as a better serologic marker than SMA for autoimmune liver disease (3–5). The SMA found in patients with viral hepatitis and other infectious diseases are said to react against non-actin intermediate filaments in the cytoplasm (1–3,5–7). Autoantibodies against actin have been reported in up to 85% of patients with type 1 AIH (or chronic active hepatitis), in 22% of patients with primary biliary cirrhosis (PBC) (8,9), and are of low prevalence in the normal healthy population (3,10). Moreover, anti-

actin-positive patients have been found to be less responsive to corticosteroid therapy and are more likely to require liver transplantation (3).

The performance of IFA is dependant on several factors (i.e., quality and type of tissue utilized, conjugate specificity, fluorescent microscope and observer) and have not been adequately standardized. In general, IFAs are subjective, often nonspecific, and less sensitive than enzyme immunoassay (EIA) techniques. An antiactin

*Correspondence to: Troy D. Jaskowski, ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108. E-mail: jaskowtd@aruplab.com

Grant sponsor: ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah.

Received 5 March 2007; Accepted 22 March 2007

DOI 10.1002/jcla.20167

Published online in Wiley InterScience (www.interscience.wiley.com).

EIA could offer an objective, more standardized alternative to SMA IFA, which can be automated.

Our objectives in this study were to compare the results obtained with an EIA detecting F-actin antibody (FAA) immunoglobulin G (IgG) to those determined by an IFA detecting smooth muscle antibody (SMA) IgG in sera from patients suspected of having autoimmune liver disease and to determine the prevalence of FAA in sera from patients having serologic evidence of acute viral hepatitis, all in a clinical laboratory setting.

PATIENTS AND METHODS

Patient Samples

A total of 623 patient sera sent to ARUP Laboratories for autoimmune (smooth-muscle IgG) or acute viral hepatitis testing, and 100 sera from healthy blood donors (HBD) were included in the study. All patient samples included in this study were deidentified according to the University of Utah Institutional Review Board–approved protocol #7275 to meet the Health Information Portability and Accountability Act (HIPAA) patient confidentiality guidelines so that actual patient clinical information could not be obtained for these sera.

SMA IgG by IFA

Antibody against associated antigens within smooth muscle tissue was detected using substrate (mouse kidney) slides and reagents purchased from INOVA Diagnostics (San Diego, CA). This assay was performed as recommended by the manufacturer. Tissue sections were observed at 400 $\times$ and fluorescence was calibrated using an SMA-positive control demonstrating 3+fluorescence. Sera demonstrating discrete staining of the inner muscular layers of blood vessels of the kidney having fluorescence of 1+(the lowest fluorescence that can be clearly differentiated from the background fluorescence) or greater at a dilution of 1:20 were considered positive for SMA and titrated to endpoint.

Anti-Nuclear Antibody (ANA) IgG by IFA

ANA substrate slides and reagents were purchased from INOVA Diagnostics, Inc. This assay detects IgG antibody against associated nuclear antigens of HEP-2 cells. This assay was performed as recommended by the manufacturer. HEP-2 cells were viewed at 400 $\times$ and fluorescence was calibrated using an ANA-positive control demonstrating a homogenous pattern of 3+fluorescence. Sera that demonstrated nuclear fluorescence of 1+or greater at a dilution of 1:40 were considered positive for ANA IgG antibody and titrated to endpoint. ANA IFA patterns were recorded for positive sera.

FAA IgG by EIA

EIA detecting FAA were donated by INOVA Diagnostics, Inc. This semiquantitative assay detects IgG antibodies in human serum that are directed against the actin (polymerized) component of smooth muscle. Procedures for this assay were followed as stated in the manufacturer's product insert. The reference values (reported as arbitrary units) established by the manufacturer were utilized and are as follows: <20 Units = negative; 20–30 Units = weak positive; and >30 Units = moderate to strong positive.

Serologic Testing for Viral Hepatitis

Acute viral hepatitis infection was evaluated using serologic assays to detect the presence of IgM anti-hepatitis A virus (IgM anti-HAV), IgM anti-hepatitis B virus core antigen (IgM anti-HBc), HBV surface antigen (HBsAg), and anti-hepatitis C virus (anti-HCV). IgM anti-HAV was determined using the DiaSorin ETI-HA-IGMK Plus kit (DiaSorin, Stillwater, MN) using an ETI-MAX 3000 (DiaSorin) instrument. The assay was performed as stated in the manufacturer's product insert, with the exception that a slightly higher assay cutoff, +0.200 *A* instead of the recommended +0.100 *A*, was used to reduce the number of false positives seen during the study interval. IgM anti-HBc, HBsAg, HBsAg confirmation by neutralization, and anti-HCV were determined using the Vitros ECi platform (Ortho Clinical Diagnostics, Rochester, NY) and testing was performed as stated in the manufacturer's product insert. All samples with initial HBsAg signal-to-cutoff values of >500 were diluted 1:151 (5 μ L sample + 750 μ L diluent) prior to HBsAg confirmation testing to guard against prozoning. Acute viral hepatitis samples were selected based on the outcome of serologic testing. Positive samples were selected if they displayed antibody test results consistent with infection. Samples were considered acute HAV-positive when only IgM anti-HAV was reactive, acute HBV was identified by positive IgM anti-HBc with or without HBsAg, while HCV infection was determined by the presence of anti-HCV only.

RESULTS

Analysis of the 415 sera from patients suspected of having autoimmune liver disease showed an increasing prevalence of FAA as SMA titers increased and all sera having SMA titers of $\geq$ 1:160 were FAA-positive (Table 1). Of the 212 SMA-positive sera ($\geq$ 1:20), 74 (34.9%) were positive for FAA, 36 (48.6%) of which were also ANA-positive ($\geq$ 1:40) (homogeneous, speckled, mixed, and centromere patterns were observed). There were a number of sera (*n* = 35) that

TABLE 1. Anti-smooth muscle (SMA) IFA, anti-F-actin EIA and anti-nuclear antibody (ANA) IFA results in 415 sera from patients referred for SMA testing with possible AIH and 100 sera from healthy blood donors

No.	SMA titer	F-actin +(%)	ANA +(%)	F-actin & ANA +(%)
1	1:5120	1 (100.0)	0 (0.0)	0 (0.0)
3	1:640	3 (100.0)	1 (33.3)	1 (33.3)
6	1:320	6 (100.0)	3 (50.0)	3 (50.0)
1	1:160	1 (100.0)	1 (100.0)	1 (100.0)
13	1:80	10 (76.9)	5 (38.5)	4 (30.8)
21	1:40	7 (33.3)	7 (33.3)	6 (28.6)
167	1:20	46 (27.5)	25 (15.0)	21 (12.6)
203	<1:20	23 (11.3)	22 (10.8)	5 (2.5)
100	Healthy donors ^a	1 (1.0) ^b	4 (4.0)	1 (1.0)

^aFour of 100 sera from healthy blood donors were SMA IgG positive at 1:20; all four sera were F-actin IgG negative.

^bNegative (<1:20) by SMA IgG. F-actin IgG value was 25 Units (reference = <20 Units). This serum was ANA IgG positive by IFA at 1:40 (mixed pattern).

TABLE 2. Thirty-five patient sera with negative (<1:20) or low (1:20–1:40) IFA titers for smooth muscle antibody (SMA) that had moderate to high levels of antibody against F-actin, with anti-nuclear antibody (ANA) IFA results

No.	SMA titer	Range of F-actin EIA Units ^a	ANA+ (%)
7	1:40	31–117	6 (85.7)
21	1:20	32–132	11 (52.4)
7	<1:20	31–105	1 (14.3)

^a>30 Units = moderate to strong positive.

had negative (<1:20) or low (1:20–1:40) SMA titers that possessed moderate to high (>30 Units) levels of IgG antibody against F-actin (Table 2). A total of 18 of these 35 (51.4%) sera were also positive for ANA by IFA, a pattern of results suggestive of a diagnosis of type 1 AIH in these patients. Fluorescence of the mitochondria (anti-mitochondria antibody; AMA), which is associated with PBC, was also noted in the ANA IFA in three of these 35 (8.6%) sera.

One of 100 sera from HBD possessed low levels (25 Units) of IgG antibody against F-actin (Table 1). This sample was negative for SMA by IFA, but produced an ANA IFA mixed pattern with a titer of 1:40 (Table 1). Four of the 100 sera from HBD were positive for SMA at 1:20, all of which were negative (<20 Units) for F-actin IgG.

When assessing the 108 (HAV, *n* = 36; HBV, *n* = 36; and HCV, *n* = 36) sera with serologic test results consistent with acute viral hepatitis, the prevalence of

TABLE 3. F-actin and anti-nuclear antibody (ANA) IFA results for 208 acute viral hepatitis patient sera

No.	Acute viral hepatitis	F-actin EIA+ (%)	Range of F-actin EIA Units ^a	F-actin and ANA+ (%)
36	Type A	9 (25.0)	20–42	0 (0.0)
36	Type B	9 (25.0)	21–70	0 (0.0)
36	Type C	9 (25.0)	24–50	0 (0.0)
100	Negative	3 (3.0)	20–40	1 (1.0)

^a<20 Units = negative; 20–30 Units = weak positive; >30 Units = moderate to strong positive.

F-actin IgG was 25.0% for acute HAV, 25.0% for acute HBV, and 25.0% for HCV (Table 3). Over one-half (16 of 27; 59.3%) of these positive sera contained low levels (21–30 Units) of F-actin IgG and none were found to be ANA-positive by IFA (Table 3). AMA reactivity for two HAV IgM-positive sera was noted in the ANA IFA. One of these, HAV IgM+/AMA+ sera, was also F-actin-positive (41 Units). A total of 100 sera from patients suspected of viral hepatitis, but having negative tests for HAV, HBV, and HCV were also tested for F-actin IgG. Only three of these 100 sera were F-actin-positive (20, 22, and 40 Units). One of these three F-actin-positive (22 Units) sera produced a homogenous ANA pattern at 1:40.

A total of 100 sera negative for SMA (<1:20) were tested for viral hepatitis using IgM anti-HAV, IgM anti-HBc, HBsAg, and anti-HCV. A total of 19 of these sera had serologic test results consistent with viral hepatitis; one sample was positive for IgM anti-HAV only, two were positive for HBsAg only, 14 were positive for anti-HCV only, one was positive for HBsAg/anti-HBc/anti-HCV, and one was positive for anti-HBc/anti-HCV. The HAV IgM-only-positive sample had a signal/cutoff (S/CO) of seven, while the HBsAg-only-positive samples had S/CO of 15 and 2,580. The anti-HBc IgM-positive sample had an S/CO of 1.69 with an HBsAg S/CO of 3,390 and an anti-HCV S/CO of 19. All but two anti-HCV-positive samples had strong positive S/CO results, greater than 8.0 (range 16.8–34.1); two samples had weak positive S/CO results, 1.24 and 4.41. Only three of these 19 (15.8%) sera had positive results for F-actin IgG (24 and 33 Units for two anti-HCV+ sera; 26 Units for one anti-HBc+/anti-HCV+ sample), none of which were ANA-positive by IFA. The distribution of F-actin IgG for all sera included in this study is shown in Fig. 1.

DISCUSSION

Early diagnosis of AIH and immunosuppressive treatment are essential to help prevent severe liver damage. Unfortunately, lack of certainty in establishing

Fig. 1. Distribution of F-actin IgG in normal blood donors and patient sera sent to ARUP Laboratories for SMA or viral hepatitis serology.

a diagnosis can lead to a delay in treatment and the continued progression of the disease. Likewise, mistaking viral hepatitis for AIH can lead to inappropriate treatment.

In our experience, the standard IFA method used for detecting SMA in patients suspected of having AIH often produce results that are of low titer ($\leq 1:40$), which may be of little value to the clinician. The present study shows that high levels of F-actin IgG can be present in patients suspected of having AIH that are SMA-negative ($< 1:20$) or have low ($\leq 1:40$) SMA titers (Table 2). In addition, 52.4% of the sera in this category were also ANA-positive by IFA, which helps support a diagnosis of type 1 AIH (AIH-1) in these patients. This observation (SMA-/F-actin+/ANA+) was first reported in 2004 by Jaskowski et al. (20).

Recently, Granito et al. (11) showed a similar discord between SMA IFA titers and levels of F-actin IgG in patients with AIH-1. A total of 44% (7 of 16) of their SMA negative AIH-1 patients were positive for F-actin IgG when using the manufacturer's recommended cut-off value of 20 Units (11). Moreover, when using the manufacturer's recommended cutoff, F-actin IgG was detected in 55% (17 of 31) of their AIH-1 patients that were negative for the SMA peritubular/glomerular (SMA T/G) IFA pattern (11), which is said to be highly predictive of AIH-1 (12,13). These findings suggest a lower sensitivity of the SMA IFA method in detecting this autoantibody in patients with AIH-1. One explanation for the higher sensitivity observed with F-actin IgG in AIH-1 is that immunodominant epitopes may be more immunoaccessible in purified F-actin than in rodent kidney tissue (11).

Antibodies to F-actin can also be found in patients with viral hepatitis, but usually they are present in lower

levels than those seen in AIH-1 (14,15). The present study is in agreement with these observations, as we found 25% of patient sera having serologies consistent with viral hepatitis were positive for F-actin IgG with the majority (59.3%) of these sera having low levels (Table 3). In addition, ANA was not found in any of the viral hepatitis-positive sera that were F-actin IgG-positive. The prevalence of F-actin IgG in patient sera with suspected viral hepatitis with negative serologies was much less (3%; Table 3). In contrast, 19.0% of patients suspected of having AIH that were SMA IFA-negative had serologic test results consistent with acute viral hepatitis. The high anti-HCV S/CO values observed in these samples suggest that the majority of individuals (14 of 16) with anti-HCV positive results had encountered and mounted an immune response to HCV (16–18). These data suggest that patients suspected of having AIH with negative SMA IFA results may benefit from viral hepatitis marker testing.

Since other studies have suggested anti-actin to be a better marker for AIH (3–5) and that anti-actin-positive patients are less responsive to corticosteroid therapy and more likely to suffer liver failure (3), we recommend that all patients suspected of having autoimmune liver disease be tested for IgG antibody against F-actin. Patients suspected of having AIH-1 should also be tested for ANA at the same time (19). To better serve the patient and the physician, sera sent to our laboratory for SMA testing are assessed for F-actin IgG by EIA. Sera producing F-actin+/SMA–results may provide the physician with useful information, depending on the level of anti-F-actin and results from other related hepatitis testing. Further studies on patients that produce F-actin +/SMA–results are needed to assess the clinical utility of F-actin IgG in autoimmune liver disease in these patients.

ACKNOWLEDGMENTS

We thank INOVA Diagnostics (San Diego, CA) for donating the F-actin IgG EIAs and Diana Knapp, Rose Bartel, Jill Prigmore, Charlin Masterson, Rene Borja, and Amit Phansalkar for their technical assistance during this study.

REFERENCES

- Andersen P, Small JV, Sobieszek A. Studies on the specificity of smooth-muscle antibodies. *Clin Exp Immunol* 1976;26:57–66.
- Bottazzo GF, Florin-Christensen A, Fairfax A, Swana G, Doniach D, Groeschel-Stewart U. Classification of smooth muscle autoantibodies detected by immunofluorescence. *J Clin Pathol* 1976;29:403–410.
- Czaja AJ, Cassani F, Cataleta M, Valentini P, Bianchi FB. Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. *Hepatology* 1996;24:1068–1073.
- Fusconi M, Cassani F, Zauli D, et al. Anti-actin antibodies: a new test for an old problem. *J Immunol Methods* 1990;130:1–8.
- Toh BH. Smooth muscle autoantibodies and autoantigens. *Clin Exp Immunol* 1979;38:621–628.
- Kurki P, Linder E, Miettinen A, Alfthan O. Smooth muscle antibodies of actin and “non-actin” specificity. *Clin Immunol Immunopathol* 1978;9:443–453.
- Toh BH, Yildiz A, Sotelo J, et al. Viral infections and IgM autoantibodies to cytoplasmic intermediate filaments. *Clin Exp Immunol* 1979;37:76–82.
- Bretherton L, Brown C, Pedersen JS, et al. ELISA assay for IgG autoantibody to G-actin: comparison of chronic active hepatitis and acute viral hepatitis. *Clin Exp Immunol* 1983;51:611–616.
- Dighiero G, Lymberi P, Monot C, Abuaf N. Sera with high levels of anti-smooth muscle and anti-mitochondrial antibodies frequently bind to cytoskeleton proteins. *Clin Exp Immunol* 1990;82:52–56.
- Fagraeus A, Norberg R. Anti-actin antibodies. *Curr Top Microbiol Immunol* 1978;82:1–13.
- Granito A, Muratori L, Muratori P, et al. Antibodies to filamentous actin (F-actin) in type 1 autoimmune hepatitis. *J Clin Pathol* 2006;59:280–284.
- Cassani F, Cataleta M, Valentini P, et al. Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. *Hepatology* 1997;26:561–566.
- Muratori P, Muratori L, Agostinelli D, et al. Smooth muscle antibodies and type 1 autoimmune hepatitis. *Autoimmunity* 2002;35:497–500.
- Chretien-Leprince P, Ballot E, Andre C, et al. Diagnostic value of anti-F-actin antibodies in a French multicenter study. *Ann NY Acad Sci* 2005;1050:266–273.
- Zamanou A, Tsirogianni A, Terzoglou C, Balafas A, Economidou I, Lymberi P. Anti-smooth muscle antibodies (ASMAs) and anti-cytoskeleton antibodies (ACTAs) in liver diseases: a comparison of classical indirect immunofluorescence with ELISA. *J Clin Lab Anal* 2002;16:194–201.
- Dufour DR, Talastas M, Fernandez MD, Harris B. Chemiluminescence assay improves specificity of hepatitis C antibody detection. *Clin Chem* 2003;49(Pt 1):940–944.
- Dufour DR, Talastas M, Fernandez MD, Harris B, Strader DB, Seeff LB. Low-positive anti-hepatitis C virus enzyme immunoassay results: an important predictor of low likelihood of hepatitis C infection. *Clin Chem* 2003;49:479–486.
- Alter MJ, Kuhnert WL, Finelli L. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2003;52(RR-3):1–13, 15; quiz CE11–14.
- Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929–938.
- Jaskowski TD, Litwin CM, Hill HR. Detection of immunoglobulin (Ig)G antibody against F-actin and anti-smooth muscle in normal blood donors and patients suspected of having autoimmune liver disease. Presented at the Association of Medical Laboratory Immunologists 17th Annual Meeting, Baltimore, MD. August 2004.