INTRAHEPATIC EXPRESSION OF SERUM HEPATITIS VIRUS-ASSOCIATED ANTIGENS*

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It has been suggested that the same transmissible viral agent may be responsible, not only for parenterally transmitted long-incubation hepatitis, but also for sporadic infectious hepatitis (1, 2). An antigen, variably described as Australia antigen (3–5), serum hepatitis (SH)[1] antigen (6, 7), hepatitis antigen (2), or hepatitis-associated antigen (8), appears to be a structural component of a virus-like particle (9–11) which fulfills the requirements for the serum hepatitis virus. This antigen, or group of antigens (12–14), provides a convenient marker for SH virus particles in blood (9–11). Agents such as the SH virus, because of their persistence and limited cytopathic effects in some individuals, possess unique pathogenetic potential beyond that of direct viral-induced tissue injury. As a persistent endogenous antigen (12), the SH virus may serve as an antigenic focus for immunologically mediated tissue injury. Alternatively, it may participate in the genesis of immune complexes and the initiation of immune complex disease (15, 16) in a fashion similar to that of certain persistent viral infections in experimental animals (17, 18).

This study was initiated to demonstrate cytologic sites and patterns of SH virus-associated antigen (SH antigen) expression in the liver. Sites of SH viral synthesis in vivo have not been established; however, the presence of SH antigen in the nuclei of hepatocytes has been suggested by two groups of investigators (18–21) using heterologous (13, 19, 20) or homologous (21) antisera to SH antigens. Nowoslawski, et al. (21) have demonstrated small virus-like particles in the nuclei of hepatocytes from one individual and have suggested that these might represent SH virus particles. Because of demonstrated differences be-

[1] *Abbreviations used in this paper:* F–anti-GP γ-G, fluorescein-conjugated rabbit anti-guinea pig IgG; F–anti-SH, fluorescein-conjugated antibody to SH antigens; GP–anti-SH, guinea pig anti-SH antigen; LCM, lymphocytic choriomeningitis; PBS, phosphate-buffered saline; SH, serum hepatitis; SH antigen, SH virus-associated antigen.

tween antisera to SH antigens (14) and the well-recognized contribution by the host of cell membranes (22) and virus-induced membrane neoantigens (23–25) to the viral coat, we have used both human and guinea pig antibody to demonstrate SH antigens and presumably the viral particles (9) in tissue and peripheral blood. These studies demonstrated distinct patterns of finely particulate SH antigen in the cytoplasm of hepatocytes and other cells of the liver and in the peripheral blood. Neutralization studies suggest that there is more than one SH antigen determinant and that the response of the guinea pig is relatively deficient in antibody to one of these determinants. Two general patterns of SH antigen distribution were recognizable. Whereas hepatocyte localization was prominent in most, other cells were also observed to contain this antigen in some individuals. Differences in cellular localization may represent differences in host responses to infection with the SH virus, and thus reflect pathogenetic events during SH viral infection.

Materials and Methods

Liver Biopsies.—Liver specimens, obtained by needle biopsy from 78 hospitalized patients,[2] were included in the study. Biopsies were performed on the basis of clinical indications and included a wide variety of primary and secondary hepatic diseases with major emphasis on patients with acute, recurrent, or chronic hepatitis. Serum specimens and peripheral blood smears were obtained at the time of biopsy or collection of other blood specimens.

Preparation of Tissue.—A 5–8 mm segment of each needle biopsy core of liver was embedded in Tissue-Tek (Ames Laboratories, Elkhart, Ind.) on a small cork and snap-frozen in liquid nitrogen. Cryostat sections were cut at 4 μ and permitted to adhere to microscope slides in a humid chamber at 4°C for 16–24 hr and frozen at −80°C for longer storage. The presence of SH viral particles in blood was evaluated on peripheral blood smears dried at room temperature and stored at 4°C. The remaining portion of each liver biopsy was fixed in Zenker's fluid, or 10% formalin, and processed for routine histopathologic examination by the pathology department of each respective institution.

Immunohistochemical Reagents.—Fluorescein-conjugated antibody to SH antigens (F-anti-SH) was prepared from the 50% saturated ammonium sulfate gamma globulin fraction of a potent human anti-SH by the method of Clark and Shepard (26) using 0.12 mg fluorescein isothiocyanate/mg of gamma globulin. Nonspecific affinity for tissue was abolished by absorption twice with acetone-precipitated guinea pig liver powder at 25 mg/ml. The appropriate dilution necessary to insure maximum reactivity of F-anti-SH with SH antigen in tissues was determined by titration on peripheral blood smears and liver biopsies, from cases of well-established and serologically positive SH virus hepatitis. Negative peripheral blood smears and liver biopsies were also included as controls. Immunologic specificity of reactions for SH antigens was confirmed by neutralization of F-anti-SH with an equal volume of standard SH antigen-positive serum, complement-fixation titer = 512. Absorption of F-anti-SH with SH antigen-negative serum served as a control. Selected reactions were further confirmed by absorption of F-anti-SH with SH antigen: (a) concentrated from serum by ultracentrifugation for 18 hr at 105,000 g; (b) then subjected to sucrose density gradient ultracentrifuga-

tion; (c) rebanding twice by isopycnic ultracentrifugation on cesium chloride gradients; and (d) final reisolation by ultracentrifugation in a sucrose density gradient.[3] Absorption was expressed as the number of complement-fixing units of SH antigen per microliter of anti-SH at standard assay concentration. The serologic specificity of F–anti-SH was also confirmed in micro-Ouchterlony analysis against a panel of SH antigen-positive sera and by demonstrating reactions of identity with a panel of human anti-SH antisera.

The presence and immunocytochemical localization of SH antigen in liver biopsies, peripheral blood smears, and other tissues was also evaluated by indirect immunofluorescence using National Institutes of Health pool No. V801-501-058 of guinea pig anti-SH antigen (GP–anti-SH) and fluorescein-conjugated rabbit anti-guinea pig IgG (F–anti-GP γ-G).

Fluorescein-conjugated purified antibody to human IgG, IgA, and IgM were prepared as previously described (27). Monospecific antisera to human complement components C1q, C4, C3, and C5[4] and to fibrinogen were prepared in goats by repeated immunization with highly purified antigens. Rabbit anti-guinea pig IgG was prepared by repeated immunization with purified guinea pig IgG in complete Freund's adjuvant.[5] Fluorescein-conjugated antibodies were prepared as previously described (28). Specificity of immunofluorescent reagents was confirmed by monospecific reactions in gel double diffusion and by neutralization with specific antigen.

Immunohistochemical Reactions.—The direct immunofluorescent reactions were performed on unfixed peripheral blood smears and cryostat sections of liver. The appropriate immunofluorescent reagent was incubated on each slide for 40 min, the slides were washed three times in phosphate-buffered saline (PBS), and mounted in 90% glycerol, 1% tris (hydroxymethyl) aminomethane-HCl, pH 9.5. Replicate slides were reacted with positive and negative control F–anti-SH. Selected specimens were reacted with F–anti-SH absorbed with purified or semipurified SH antigen to further confirm specificity. Other specificity controls included preincubation of slides with unlabeled guinea pig or human anti-SH antisera to inhibit reaction with F–anti-SH.

Indirect immunofluorescence for SH antigen was performed on unfixed smears or sections by incubation with GP–anti-SH for 40 min, the slides were washed three times with PBS, and reacted with F–anti-GP γ-G for 40 min. After three washes with PBS the slides were mounted in buffered glycerol as for the direct reaction. Controls, similar to those for the direct reaction, were also incorporated in these studies.

Examination of Immunofluorescent Slides.—Preparations were examined using a Leitz Ortholux microscope fitted with a Zeiss NA 1.2–1.4 dark-field condenser, a Leitz HBO 200 illuminator, a 2 mm UG-1 exciter filter, and a K430 barrier filter. Slides were read on two occasions and usually with two separate anti-SH without knowledge of the clinical diagnosis or the presence of SH virus antigen in the serum. Results were photographically recorded on Anscochrome 500 color film or on Polaroid black and white film.

Serologic Tests for SH Antigens.—Sera from patients were tested for the presence of SH antigens by double diffusion in agarose gel by a micro-Ouchterlony method including control positive and negative reactions in each assay (29). Selected sera were also assayed by electroimmunodiffusion (30) or complement fixation (31). Our standard human anti-SH exhibited identity with anti-SH antisera from the New York Blood Center[6] and the NIH pool of guinea

pig anti-SH. Our standard SH antigen-positive serum reacted with all antisera and exhibited identity with other SH antigen-positive sera by gel double diffusion and had a complement-fixation titer of 512.

RESULTS

Immunochemical Features of SH Antigenic Expressions.—SH antigens were recognized by immunofluorescence in selected unfixed peripheral blood smears and liver biopsies using both human and guinea pig antisera to SH antigens. These antigens were seen in peripheral blood as numerous minute fluorescent green granules which usually appeared to be within the plasma (Fig. 1). Intra-hepatic SH antigens were expressed as fine granules in the cytoplasm of hepato-cytes and other intrahepatic cells (Fig. 2), but not within nuclei. Reactivity or integrity of SH antigens, present in peripheral blood, was abolished by exposure to acetone or ethanol and markedly attenuated by ether:ethanol (Table I). The integrity of these antigens in liver was significantly attenuated in a parallel fashion although not abolished by these solvents, thus subsequent studies were performed on unfixed tissues.

Parallel titration of human anti-SH and GP–anti-SH on peripheral blood smears from serological SH-positive and negative individuals indicated concordance of these two anti-SH antisera. The blood from all serologically negative individuals was negative by immunofluorescence; and 12 of 14 serologically positive individuals had positive SH antigen reactions by immunofluorescence (Table II). Parallel titration of both human anti-SH and GP–anti-SH on selected SH antigen-positive peripheral blood smears and liver sections demonstrated reduced relative sensitivity for intrahepatic SH antigen as compared with peripheral blood. A four- to eightfold greater concentration of anti-SH antiserum was required for maximum reactivity of intrahepatic SH antigen than for demonstration of peripheral blood particles, suggesting either steric masking of intracellular SH antigens by other proteins or quantitative and qualitative antigenic deficiency of intracellular SH particles.

Immunochemical specificity of the immunofluorescent reactions for SH antigens was demonstrated by competitive inhibition with anti-SH antisera and by neutralization with SH antigen. Human anti-SH was apparently more poly-valent than the GP–anti-SH. Nonfluoresceinated human anti-SH, applied to the SH antigen substrate in a fourfold excess relative to the fluorescein-con-jugated antibody, completely blocked subsequent immunofluorescent reactions of both human anti-SH and GP–anti-SH (Table III). By contrast, GP–anti-SH in similar excess, inhibited the GP–anti-SH reaction but only partially blocked human anti-SH. This suggested the presence in human anti-SH of antibodies to antigenic determinants on SH particles that are immunochemically independent of, and sterically distant from, determinants recognized in common by the two antisera.

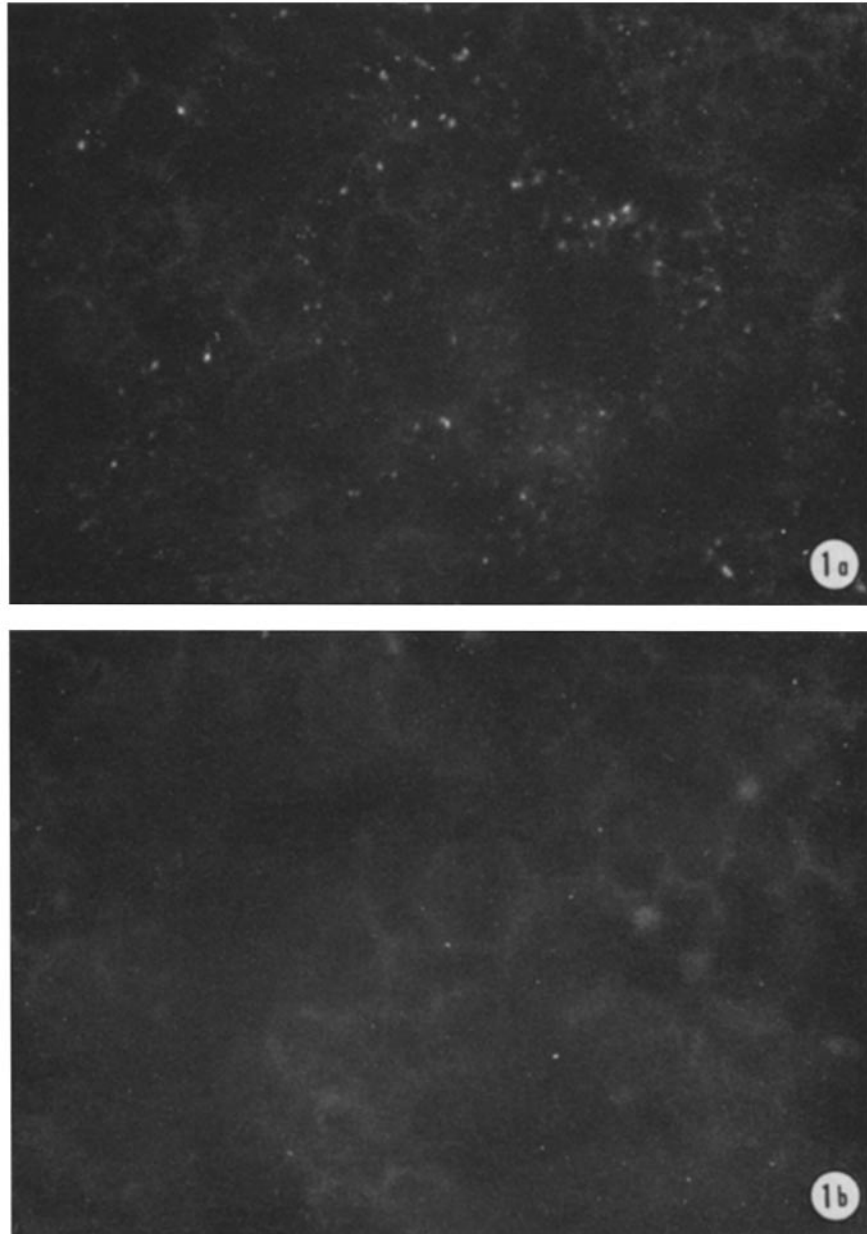Fig. 1. The immunofluorescent reactions of human fluorescein-conjugated anti-SH (F–anti-SH) with SH antigen particles present in peripheral blood (1 a). The particles are randomly scattered in smears, appear to be extracellular, and can be transferred to negative blood with serum. The immunofluorescent reaction is abolished by neutralizing the F–anti-SH with SH antigen-positive serum (1 b). × 1020.

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Neutralization of both anti-SH antisera by SH antigen more firmly established immunochemical specificity of these reactions (Table IV). Immunofluorescent anti-SH reactions were consistently abolished by neutralization of

TABLE I

Effects of Organic Solvent Fixation on the Reactivity of SH Antigens

Fixation	Liver		Peripheral blood	
	Human anti-SH*	Guinea pig anti-SH‡	Human anti-SH*	Guinea pig anti-SH‡
None	3+	3+	4+	4+
Ether:ethanol (1:1)	3+	3+	1+	1+
Ethanol (95%)	1+	1+	0	0
Acetone	2+	2+	0	0

* Direct immunofluorescence with F–anti-SH.
‡ Indirect immunofluorescence with GP–anti-SH followed by F–anti-GP γ-G.

TABLE II

Comparison of Serologic and Immunofluorescent Methods for the Demonstration of SH Antigen in Blood

Gel double diffusion (serum)	Number	Immunofluorescence*	
		Positive	Negative
Positive	14	12	2
Negative	50	0	50

* Direct immunofluorescence on peripheral blood smears with human F–anti-SH.

TABLE III

Competitive Inhibition of Immunofluorescent Anti-SH Reactions by Anti-SH Antisera

Blocking antibody	Reactivity of SH antigens by immunofluorescence			
	Liver		Peripheral blood	
	Human anti-SH*	Guinea pig anti-SH‡	Human anti-SH*	Guinea pig anti-SH‡
None	3+	3+	4+	4+
Human anti-SH	0	0	0	0
Guinea pig anti-SH	1-2+	0	2+	0

* Direct immunofluorescence with F–anti-SH.
‡ Indirect immunofluorescence with GP–anti-SH followed by F–anti-GP γ-G.

the antisera with SH-positive serum, crude, semipurified, and purified SH antigen. Although purified SH antigen appeared slightly less efficient in neutralization of human F–anti-SH than in complement fixation, complete neutralization was readily achieved. The observed difference between tissue substrates

parallels the serologic difference in reactivity of these two substrates; because of the four- to eightfold greater reactivity of peripheral blood SH antigen, more complete absorption of anti-SH is necessary to neutralize this reaction.

Character of the SH Antigen Expressions.—Typical intrahepatic expressions of SH antigen were observed as minute granular confluences within the cytoplasm, but not the nucleus, of hepatocytes (Fig. 2). This was usually sparse and focal (0.5–5% of cells), although in four biopsies it was diffuse. Patterns of cytoplasmic localization varied between individual livers but was consistent for that individual when replicate biopsies were compared. Typical patterns of hepatocellular localization included: (*a*) diffuse cytoplasmic, (*b*) local cyto-

TABLE IV

The Capacity of Progressively Purified SH Antigen to Neutralize Anti-SH Antisera

SH antigen	SH antigen concentration required for neutralization*			
	Human anti-SH‡		Guinea pig anti-SH§	
	Liver	Peripheral blood	Liver	Peripheral blood
Serum	8	80	8	80
Ultracentrifugal concentrate	8	80	8	80
First density gradient	8	80	8	80
Third density gradient	8	160	8	80
Fourth density gradient	20	160	8	160

* Expressed as complement-fixing units of SH antigen per microliter of antibody.

‡ Direct immunofluorescence with human F–anti-SH.

§ Indirect immunofluorescence with guinea pig anti-SH followed by F–anti-GP γ-G.

plasmic including occasional pericanalicular concentration, and (*c*) hepatocyte perimeter and surface (Fig. 2).

The second generic pattern of SH antigen expression was nonhepatocellular (Fig. 2). These antigens were observed as fine to medium-sized granules scattered through the cytoplasm of Kupffer cells and sinusoidal lining cells. Granular expressions of SH antigen were also observed in cellular infiltrates at the periphery of lobules and within portal triads. These cells were mononuclear cells that could not be specifically identified but may have included histiocytes, mast cells, monocytes, or even large lymphocytes.

When intrahepatic SH antigen was correlated with clinical disease and the presence of SH antigen in the serum, moderate agreement between serum and liver SH antigen was found (Table V). Among 37 individuals with acute hepatitis by history and histopathology, 46% were positive for serum SH antigen, but only seven, or 41%, of these exhibited intrahepatic SH antigen. A similar proportion of serum SH antigen-negative individuals exhibited SH antigens within the liver; however, all of these latter individuals had histories of paren-
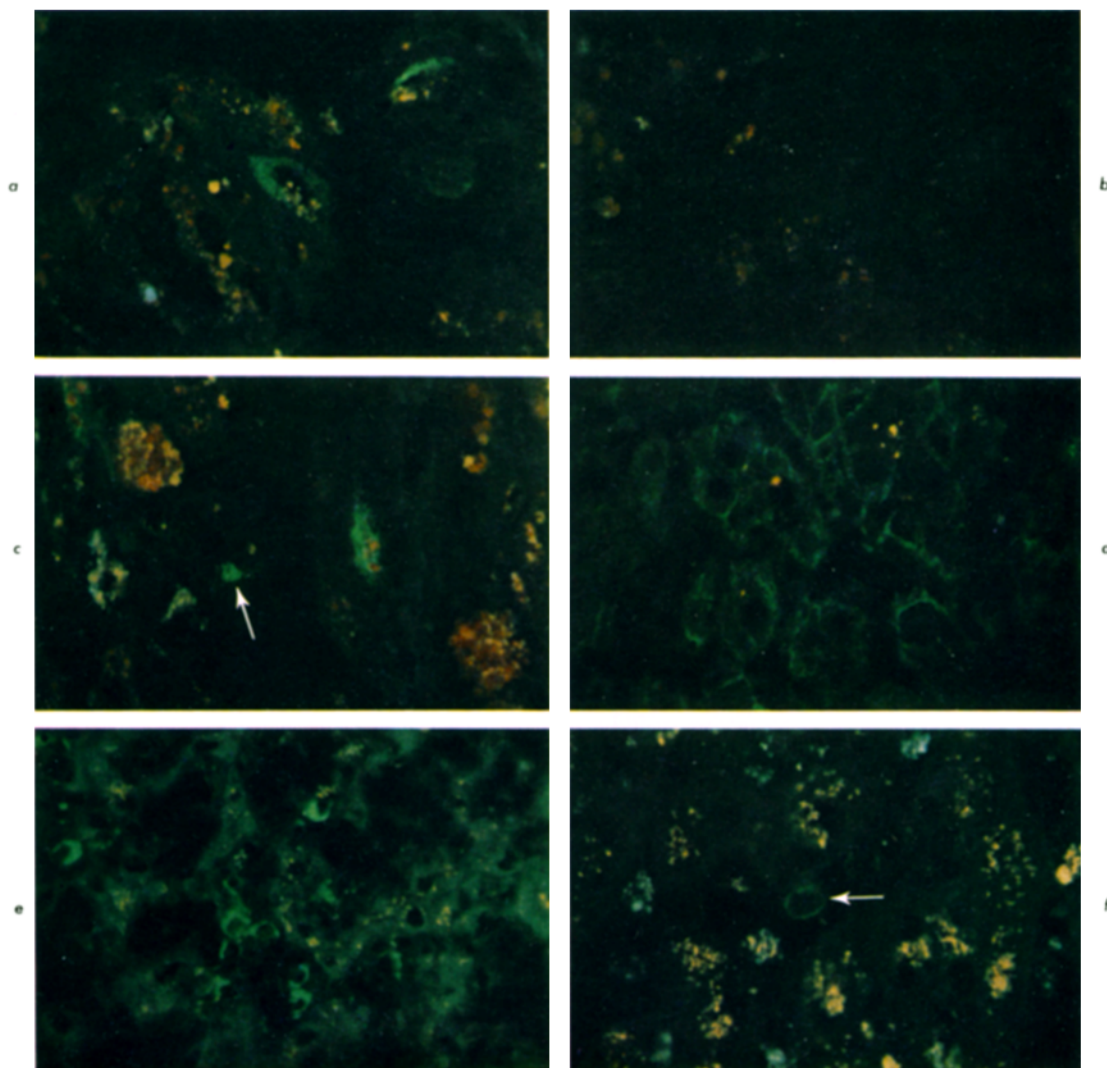
FIG. 2. Intrahepatic localization of SH virus-associated antigens with fluorescein-conjugated human anti-SH. (a) Focal localization of SH antigens in the cytoplasm of hepatocytes in chronic hepatitis is seen as discrete green granules. Other autofluorescent material is seen as orange granules. × 335. (b) The specific reaction seen in A is abolished by neutralization of F–anti-SH with purified SH antigen. × 335. (c) In the center a hepatocyte exhibits very local accumulation of SH antigen in the cytoplasm (arrow); and in a second hepatocyte to the right, pericanalicular concentration of SH antigen is observed. × 335. (d) Diffuse hepatocellular distribution of SH antigen is seen in a case of acute hepatitis. The antigen is finely particulate and almost entirely limited to the perimeter or surface of cells. × 335. (e) Nonhepatocellular SH antigen is seen as medium-sized granules in the cytoplasm of numerous mononuclear cells infiltrating a hepatocellular plate. ×155. (f) SH antigen is observed in the cytoplasm of a cell along the sinusoid of a hepatic lobule (arrow). Peripheral blood leukocytes from this patient were negative and the positive intrahepatic cells may be hypertrophied sinusoidal lining cell. × 335.

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teral exposure. SH antigen may be detectable only when present at relatively high density and a negative immunofluorescence assay does not exclude the presence of SH antigen within the liver, only the quantitative level of its expression.

The incidence of intrahepatic SH antigens in chronic or recurrent hepatitis was similar to the acute hepatitis group, although a better correlation with serum SH antigen was found (Table V). Intrahepatic SH antigen of hepatocyte

TABLE V

The Incidence and Character of SH Virus-Associated Antigens in Liver Biopsies

Disease group	Number	Parenteral exposure	SH Antigen in liver*			
			Hepato-cyte	Non-hepato-cyte	Both	Total
Acute hepatitis						
Serum SH antigen positive	17	11	3	2	2	7
Serum SH antigen negative	20	11	5	1	1	7
Total	37	22	8	3	3	14
Recurrent or chronic hepatitis						
Serum SH antigen positive	8	8	1	0	5	6
Serum SH antigen negative	16	14	0	2	1	3
Total	24	22	1	2	6	9
Alcoholic liver disease						
Serum SH antigen positive	0					
Serum SH antigen negative	9	1	1	0	0	1
Miscellaneous						
Serum SH antigen positive	0					
Serum SH antigen negative	8	8	0	0	1	1

* Direct immunofluorescence with human F–anti-SH.

pattern was clearly demonstrated in one patient at the time of acute hepatitis as well as 3 months later when his biopsy showed chronic active hepatitis. Liver biopsies from two individuals without recognizable hepatitis exhibited intrahepatitic SH antigen. The reactions were quite definite, could be neutralized by SH antigen, and presumably represented SH viral infection in the presence of other primary hepatic disease.

A differential intrahepatic expression of SH antigen between hepatocytes and nonhepatocytes and its possible significance was briefly explored. In acute hepatitis more than half exhibited intrahepatic SH antigen that was limited to hepatocytes, whereas in recurrent or chronic hepatitis in only one case was the antigen limited to hepatocytes. (Table V). Most of the chronic or recurrent

hepatitis group exhibited both hepatocellular and nonhepatocellular SH antigen. This differential localization is consistent with hypothetical secondary localization of SH antigen in nonhepatocytes.

Intrahepatic Deposition of Immunoglobulins and Complement.—Within 21 of 27 liver biopsies, increased deposition of immunoglobulin and/or complement was demonstrable in a very limited fashion. The deposits were usually granular in character, frequently found along sinusoidal walls, but also observed in rare solitary hepatocytes and other scattered cells in the perilobular and portal regions. The most consistent observation was that of nucleolar deposition of C1q. These reactions were quite distinct and neutralized by absorption of F-anti-C1q with purified C1q. 16 of the 21 biopsies exhibiting nucleolar deposition of C1q were positive for SH antigen in the serum and/or in the liver suggesting a relationship between SH antigen associated hepatitis and nucleolar binding of C1q (Table VI).

TABLE VI

Correlation between Immunofluorescent Demonstration of Nucleolar C1q and Hepatitis

SH antigen in serum or liver	Number	Nucleolar localization of C1q	
		Positive	Negative
Positive	16	12	4
Negative	19	2	17

DISCUSSION

Fluorescein-conjugated human antibody to SH antigens binds in a highly specific fashion not only to putative SH virus particles in blood of individuals with serum hepatitis, but also to antigenically identical particulate material in the cytoplasm of hepatocytes and other cells of the liver. Similar, immunologically specific, reactions were observed with guinea pig anti-SH antigen. It is presumed that the SH antigens are viral-coat antigens induced in host cell membranes by the viral genome and incorporated during intracellular viral synthesis (23–26) and/or during evagination from the host cell (22). Thus, while SH antigen in the blood might be restricted to virus particles, certain viral-coded neoantigens may be induced in cells in sufficient quantity to be detected not only on the virus particle but also in the unincorporated state. The observation of SH antigens in cells thus implies the presence of the SH genome but not, of necessity, complete SH virus particles. The local intracellular distribution and particulate character of the SH antigen, observed in this study, is similar to that observed with many viruses.

The use of homologous antisera for the detection of SH antigens has the advantage that antibodies to species-specific or organ-specific antigenic con-

stituents of the host cells should not be encountered, whereas heterologous antisera to this antigen could include antibody to host-cell antigens not coded for by the SH genome. In spite of this hypothetical objection to heterologous antisera, identical reactions were observed with guinea pig anti-SH antigen. Levene and Blumberg (14) have observed serologic differences between individual rabbit antisera to SH antigens and have demonstrated the presence of at least two antigenic determinants. Our results also suggest more than one SH antigen and indicate a more polyvalent antibody response to these antigens by our human antisera than by guinea pig anti-SH. The disparity between our results and those of Millman et al. (19) and Coyne et al. (20), who observed nuclear staining with fluorescein-conjugated rabbit anti-SH, may be attributable to technical differences and/or unrecognized differences in specificity of the antisera to SH antigens. Technical details are significant, for acetone fixation as employed by others (19–21) attenuates or destroys the reactivity of SH antigen. Our contention that SH antigen, and by inference the virus, is present in the cytoplasm is supported by the observation of SH virus-like particles in the mitochondria of lymphoid cells infected in vitro with this agent (32). The specificity of the cytoplasmic localization of SH antigen was established by neutralization studies with SH antigen-positive sera and purified SH antigen, as well as by competitive inhibition. The demonstrated correlation between SH antigen particles in blood of serologically positive individuals by immuno-fluorescence further corroborates identity between SH antigen particles in blood and similar particles in the cytoplasm of liver cells. Demonstration of SH antigen in blood by immunofluorescence is indicative of aggregation rather than distribution as solitary 160–250-A particles (11).

The patterns of SH antigen localization in the liver are entirely consistent with the focal character of hepatocellular injury and necrosis characteristic of SH virus hepatitis (33). SH antigens were observed in hydropic as well as relatively normal appearing hepatocytes, while in some cells these virus-associated antigens were observed in association with the pericanalicular region of hepatocytes. The latter feature may be pathogenetically related to the associated cytologic and functional abnormalities of hepatocytes and the presence of intracytoplasmic bile in viral hepatitis (33). The presence of SH antigens in the cytoplasm and in proximity to the hepatocyte surface may be pathogenetically important in directing host immune responses to infected cells and mediating injury. Immunofluorescent studies of immunoglobulins and complement in liver biopsies produced some evidence for the participation of antibody and/or complement in the cytopathic effects associated with the hepatocellular form of infection and in the nonhepatocellular localization of SH antigen. The presence of nonhepatocellular SH antigen was usually associated with granular deposits of C1q in similar or identical cells. The granular deposition of SH antigens, immunoglobulin, and complement in the cytoplasm of sinusoidal endothelium,

Kupffer cells, and cells of the portal-perilobular region is consistent with the hypothesis that these deposits may represent phagocytosed SH antigen–antibody–complement complexes (31).

The frequent presence of C1q in the nucleoli of hepatocytes in serum hepatitis introduces the possibility of an immunologic reaction, although a similar deposition of immunoglobulin is not observed. Alternatively, we believe that these reactions may reflect a hepatitis-associated augmentation of in vitro binding of C1q to nucleic acids (34). The immunofluorescent reactions for C1q are remarkably similar to some of the reactions demonstrated by Millman et al. (19) and Coyne et al. (20) for SH antigen. The reactions demonstrated by Nowoslawski et al. are far less discrete (21).

Intrahepatic SH antigen localization, and by analogy SH virus infection, was frequently rather focal in character and quantitatively limited; this contrasts with peripheral blood where high concentrations of SH virus particles are frequently encountered. This dichotomy suggests that organs other than the liver may function as occult sites of viral synthesis; and the studies of Coyne et al. (20) suggested that SH antigens might be present in tissues other than liver. Alternatively, the liver may be the only site of viral or SH antigen synthesis, but the sensitivity of the immunofluorescent assay may be insufficient to detect all cells possessing the agent and antigens. Such a relationship has been observed between the density of lymphocytic choriomeningitis (LCM) virus infection of cells in vitro and the number of cells exhibiting viral antigens by immunofluorescence (35).

The ability to demonstrate SH antigens in tissues may implement study of the immunopathogenetic events in SH virus hepatitis as well as diagnosis. The occurrence of chronic SH antigenemia is well recognized (12, 36) and intrahepatic persistence has been observed in this study. The presence of a replicating endogenous antigen introduces a pathogenetic hazard similar to that found in chronic LCM virus infection (17) in which potentially phlogogenic virus–antibody complexes (18) may be chronically generated. Detailed immunochemical elucidation of the structural antigens of SH virus particles may resolve the disparities observed with different antisera and may permit discrimination between immune responses to: (a) virus-specific neoantigens and (b) host-cell antigens incorporated in the virus (22–25). Immune responses to the former could mediate heteroimmune reactions to the infected host cells or lead to an exogenous immune complex process (37), whereas responses to the latter would theoretically represent an autologous immune complex process (38). In this context it is significant that Gocke et al. (15) have recently suggested a pathogenetic role for SH antigens in the lesions of periarteritis nodosa, and preliminary evidence from Nowoslawski et al. (16) supports the phlogogenic character of immune complexes containing SH antigen. Alternatively, anti-tissue immune responses may also account for many of the pathogenetic features

of hepatitis. Demonstration of persistent intrahepatic SH antigen in the acute as well as the chronic active phase of one case of hepatitis suggests that this agent may be directly implicated in the chronic pathogenesis. Interestingly, the SH antigen persisted in hepatocytes with prominent surface localization, no antigenemia, and little evidence of nonhepatocyte localization which may implicate cytopathic properties or directed anti-tissue host responses rather than immune complex mechanisms in this form of chronic disease.

SUMMARY

SH virus-associated antigens have been identified in the liver and blood by immunofluorescence. This antigenic expression of the hepatitis agent or its genome is found in the cytoplasm of hepatocytes and other cells of the liver. Acute hepatitis is associated with hepatocellular localization of SH antigen, whereas alternative or additional SH antigen in the cytoplasm of nonhepatocytes is observed in chronic or recurrent hepatitis. Persistent intrahepatic SH antigen has also been observed in conversion from acute to chronic hepatitis, and the persistence of this virus-associated antigen in the liver may serve a significant pathogenetic role in mediating direct anti-tissue responses as well as local immune complex reactions. Competitive inhibition studies of SH antigen suggest at least two antigenically and sterically independent determinants on the SH particle.

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