Extrinsic Modulation of Human T-Lymphocyte E Rosette Function Associated with Prolonged Hepatocellular Injury after Viral Hepatitis

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ABSTRACT Defective T-lymphocyte E rosette (ER) function associated with viral hepatitis A and B may be due to mechanisms extrinsic or intrinsic to the target lymphocyte. The extrinsic defect is induced by an immunoregulatory plasma lipoprotein (RIF) and has the capacity to regenerate ER function in vitro. The intrinsic defect is refractory to regeneration and is not associated with RIF. Although both mechanisms occur with high frequency during the acute phase of viral hepatitis they tend to segregate in accordance with progression of hepatocellular injury at later stages of the disease. The extrinsic defect was observed in 7 out of 8 patients with longstanding chronic active hepatitis and in 10 out of 10 patients with unresolved hepatitis 12 wk after the onset of jaundice. In contrast, none of nine patients with resolved hepatitis had extrinsically defective ER function 12 wk after the onset of jaundice whereas eight of them displayed an intrinsic defect of ER function at that time. Among the various viral and liver diseases studied RIF appeared to be specific for hepatitis A and B viral infections. None of 64 sera from a variety of viral infections including Epstein-Barr virus cytomegalovirus mononucleosis with associated hepatitis nor 15 sera from patients with several chronic nonviral liver diseases were positive for RIF. RIF and its associated extrinsic defect in ER function therefore appear to correlate with a particular type of hepatocellular injury initiated by the hepatitis A and B viruses that may have a propensity for persistence and(or) progression to an aggressive form of chronic hepatitis.

INTRODUCTION

Human T-lymphocyte E rosette (ER)[1] function is an active cellular process apparently dependent on the expression of surface membrane receptors (1) and susceptible to modulation in vitro by agents which affect oxidative phosphorylation (2), protein and nucleic acid synthesis (3), microfilament function (4), divalent cation concentration (4), and intracellular levels of cyclic nucleotides (5). Concordant with this exquisite sensitivity to intracellular physiologic events is the observation that the normal expression of ER function is reduced in vivo in a variety of pathologic conditions such as cancer (6), autoimmunity (7), and viral infections (6). The mechanism(s) responsible for this phenomenon and the role it may play in the pathogenesis of these diseases are currently undefined.

Reduced numbers of ER positive peripheral blood lymphocytes have been observed during the acute and chronic stages of hepatocellular injury associated with hepatitis B virus infection (8, 9). The significance of this observation has been unclear since the presence of defective ER function per se does not correlate with either the severity of illness, the presence of circulating hepatitis B surface antigen (HBsAg) or antibody (HBsAb) or the eventual

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[1] *Abbreviations used in this paper*: AVH, acute viral hepatitis; CAH, chronic active hepatitis; ER, E rosette, FCS, fetal calf serum; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; IMR, inhibitor of mitogen responsiveness; RIF, rosette inhibitory factor; SI, stimulation index; SIg, surface membrane immunoglobulin.

resolution of disease. Owing to the recent identification of at least two mechanisms (extrinsic and intrinsic) responsible for in vivo suppression of normal T-lymphocyte ER function during acute viral hepatitis (10) a more critical analysis of the relationship between the mechanism responsible for this lymphocyte defect and a variety of clinical parameters is now possible.

The extrinsic mechanism is characterized by the ability of affected lymphocytes to regenerate normal ER function when cultured in the presence of normal, but not autologous serum. The inhibitory potential of such serum is directly demonstrated by its ability to suppress the ER function of normal T lymphocytes in vitro. The active factor (rosette inhibitory factor, RIF) present in these sera has recently been isolated and characterized as a potent immunoregulatory lipoprotein (11). It is biologically active in concentrations as low as 0.1 pM and is avidly bound by a limited number of specific receptor sites on the lymphocyte surface. A second, intrinsic mechanism is characterized by the absence of serum RIF activity and by the lack of regeneration of ER function by such lymphocytes in vitro. The thymus-derived heritage of lymphocytes rendered defective by both mechanisms has been supported by their ability to bind neuraminidase-treated sheep erythrocytes (10), a reasonably specific T-lymphocyte marker (12).

The association between the temporal occurrence of these functional defects in lymphocyte function and the resolution of virus-induced hepatocellular injury has recently been evaluated. Both mechanisms were identified with relatively equal frequency during the first 4 wk after clinical onset of acute viral hepatitis type B. At 12 wk however only the intrinsic defect was identified among those patients with biochemical and histologic evidence of resolved or resolving hepatitis whereas both patients with persistent biochemical evidence of hepatocellular injury and histologic abnormalities indistinguishable from chronic aggressive hepatitis had defective ER function of the extrinsic type (10).

The present study was undertaken to clarify the association between the extrinsic defect and chronic virus-initiated hepatocellular injury and to investigate its occurrence in other viral infections as well as a variety of chronic nonviral liver diseases.

METHODS

Patients. The study group consisted of young adult male outpatients attending the hepatitis clinic and inpatients hospitalized on the acute hepatitis ward of the Naval Regional Medical Center, San Diego, Calif. Controls were age and sex matched healthy naval recruits. All individuals volunteered for the study and informed consent was obtained.

A diagnosis of acute viral hepatitis (AVH) was made as previously described (10). 30 patients were studied within 4 wk of the onset of jaundice. 16 of these (AVH-B) were seropositive for HBsAg by solid phase radioimmunoassay (Ausria III, Abbott Laboratories, Diagnostics Div., South Pasadena, Calif.); and 14 were seronegative for HBsAg. Six of these latter patients (AVH-A) were studied during a documented epidemic of type A virus hepatitis, and their feces contained hepatitis A virus particles by immunoelectron microscopy (13). The remaining eight HBsAg positive patients (AVH-X) represented sporadic cases of unknown point source and specific viral etiology but with clinical and laboratory evidence of typical AVH.

The second major group consisted of 19 separate patients who were studied during the convalescent phase of viral hepatitis A (6 patients) and B (13 patients) between 12–16 wk after the onset of jaundice. Nine of these patients, selected because they had normal SGOT, serum glutamic-pyruvic transaminase, and bilirubin levels and either histologically normal liver biopsies or minimal portal lymphocytic infiltration, were categorized as convalescent phase-resolved (CP-R). The remaining 10 patients were selected because of persistently elevated serum transaminase levels and moderate to severe portal lymphocytic infiltration with minimal piecemeal necrosis of the limiting hepatocellular plate and they were categorized as convalescent phase-unresolved (CP-U).

The third major group consisted of eight patients with biochemical and histopathologic evidence of chronic active hepatitis (CAH) for a minimum of 3 yr. Five of these patients had a well-documented episode of AVH-B at the outset of their illness. The remaining three patients were unaware of an initial icteric episode and were serologically negative for HBsAg and HBsAb at the time of study. The diagnosis of CAH was based on: (*a*) biochemical evidence of hepatocellular injury; (*b*) histopathologic changes compatible with CAH according to the criteria of DeGroote et al. (14); (*c*) onset of AVH (when applicable) no less than 3 yr before diagnosis of CAH; (*d*) absence of clinical and morphological evidence of alcohol abuse of ingestion of known hepatotoxic medication.

Sera. 64 additional sera were evaluated for the presence of RIF a reliable marker of the extrinsic defect in ER function (see below). These sera represented acute and convalescent specimens and occasionally serial samples from patients with the following infections: Influenza A-2; Adenovirus-6; *Mycoplasma pneumoniae*-4; Mumps-3; Rubeola-1; Herpes simplex-1; Rubella-1; Varicella-Zoster-2; Epstein Barr virus mononucleosis-3; Cytomegalovirus mononucleosis-4. The latter 7 sera were generously provided by Dr. Charles A. Horowitz, Minneapolis, Minn. In all cases diagnosis was confirmed by at least fourfold increase in serologic reactivity with the appropriate viral antigen as previously described (15).

15 sera from patients with chronic nonviral liver disease were also studied. Diagnosis was obtained utilizing conventional historical and biochemical criteria and physical examination and was confirmed by liver biopsy. Diagnoses were as follows: Laënnec's cirrhosis-9; hemochromatosis-2; primary biliary cirrhosis-1; extrahepatic bile duct obstruction-1; metastatic liver disease-1; cirrhosis of unknown etiology-1.

All sera were heat inactivated (56°C for 45 min) and absorbed with sheep erythrocytes before use (see below).

Lymphocyte Isolation. A portion of whole blood obtained by venipuncture was allowed to clot at room temperature. A portion of the serum was immediately used for lymphocyte cultivation and a portion frozen at −20°C before subsequent use.

Lymphocytes were isolated from peripheral blood collected into preservative-free heparin and isolated by the colloidal iron method as previously described (5). Yields are

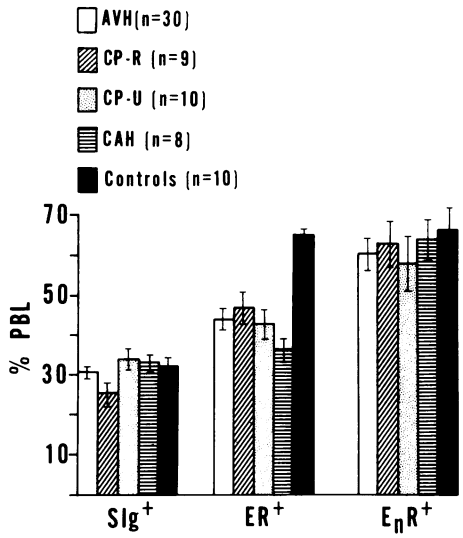

FIGURE 1. Lymphocyte distribution according to surface Ig, ER, and EnR markers during various phases of viral and postviral hepatitis. AVH, acute viral hepatitis; CP-R, convalescent phase-resolved; CP-U, convalescent phase-unresolved; CAH, chronic active hepatitis; PBL, peripheral blood lymphocytes.

>95% lymphocytes, <1% monocytes, and <5% polymorphonuclear leukocytes. The latter cells were excluded from subsequent analyses based on cytologic criteria.

Lymphocyte surface marker studies. Lymphocytes were assayed for the presence of surface membrane immunoglobulin (SIg) as previously described (10) utilizing fluorescein conjugated polyvalent anti-immunoglobulin reagents. This assay detects both SIg stable as well as SIg labile cells as described by Lobo et al. (16).

ER function was evaluated according to the method of Jondal et al. (17). Lymphocytes were also assayed for the ability to bind neuraminidase treated sheep erythrocytes (E_n) as previously described (10).

In vitro regeneration of ER function. Lymphocytes were analyzed for ER function after 24-h cultivation in the absence of autologous serum (10). Regeneration of ER function was considered positive if the mean % ER^+ lymphocytes present after cultivation exceeded the mean % ER^+ freshly isolated lymphocytes by 2 SD.

Serum mediated inhibition of ER function (RIF Assay). Sera were assayed for the ability to inhibit the ER function of normal donor lymphocytes (10, 11). Briefly, lymphocytes were isolated from the peripheral blood of healthy donors according to the method of Boyum (18) and cultures were established as previously reported (10, 11). After a 24-h incubation, with either autologous donor serum, fetal calf serum, or test serum (all at 20% vol/vol concentrations), the cells were washed and assayed for ER function. Experiments were performed in duplicate. The result was expressed as relative percent inhibition of ER function induced by test serum and was calculated: % inhibition = $(C - X/C) \times 100$ where C equals mean % ER^+ cells cultured in test serum. Significant inhibition was considered to be present if C differed from X by at least 2 SD.

Serum mediated inhibition of mitogen responsiveness. Sera were assayed for the capacity to modulate the activation of normal donor lymphocytes by PHA. Lymphocytes (5×10^5/ml in RPMI-1640 containing 100 U/ml penicillin, 500 μg/ml streptomycin, and 2 mM l-glutamine) were cultured in sterile microculture plates. Control cultures were supplemented with 20% (vol/vol) heat inactivated fetal calf serum while test cultures contained 20% (vol/vol) serum from patients and healthy controls. 1 μl (0.6 μg) PHA-P (Difco Laboratories, Detroit, Mich.), determined to represent an optimal concentration from more than 20 serial titration experiments on normal donor lymphocytes, was added at initiation of the cultures. After a 48-h cultivation in a humid atmosphere containing 5% CO_2 in air, 4 μCi of [^{3}H]methyl thymidine (50 mCi/mmol) was added and incubation was continued for an additional 16 h. Cultures were harvested onto glass fiber filters utilizing a multiple automated sample harvester (MASH II, Microbiological Associates, Bethesda, Md.) according to the method of Hartzman et al. (19). The dried filters were placed into vials containing 3 ml of scintillation cocktail composed of Omnifluor (New England Nuclear, Boston, Mass.) in toluene and counted. Experiments were performed in triplicate. Results were expressed as mean±SD cpm/10^6 lymphocytes and converted to stimulation index (SI) as follows:

$$SI = \frac{\text{cpm}/10^6 \text{ lymphocytes stimulated}}{\text{cpm}/10^6 \text{ lymphocytes unstimulated}}$$

Sera were considered inhibitory if: (*a*) they suppressed the SI by at least 2 SD below the control SI of 164.6±32.1 (SD); and (*b*) the cpm in the unstimulated test cultures was within 2 SD of the mean cpm of the unstimulated control cultures (75.2±12.7 SD).

Statistical analyses. All individual experimental data is reported as arithmetic mean and standard deviation of replicate assays. The patient groups were compared by reference to mean and standard error of the sample means within the group. Comparison of groups was performed by two-tailed *t* test in accord with Goldstein (20).

RESULTS

Expression of lymphocyte surface markers during various phases of viral hepatitis. The percent of SIg^+ lymphocytes was normal in all patient groups (Fig. 1). In contrast, there was a significant ($P < 0.001$) reduction in the incidence of ER^+ lymphocytes during every phase of disease: acute phase 44.0±2.7%; convalescent phase, resolved 46.9±3.9%; convalescent phase, unresolved 42.9±3.8%; chronic phase 36.5±2.0% in comparison with matched controls 64.0±1.6% (mean±SEM). This resulted in the appearance of a population of SIg^-, ER^- lymphocytes representing approximately 23–31% of total peripheral blood lymphocytes in the patient groups compared with control levels of 4.21±1.7%. This population of defective lymphocytes was capable of forming rosettes with neuraminidase treated SRBC indicated by normal levels of E_nR^+ lymphocytes in all patient groups (Fig. 1). The total lymphocyte count was similar in patient and control groups.

The frequency and degree of defective ER function and the result of all subsequent assays were independent of the type of virus implicated as the etiologic agent, thus permitting analysis of disease groups on the basis of temporal phase of hepatic injury.

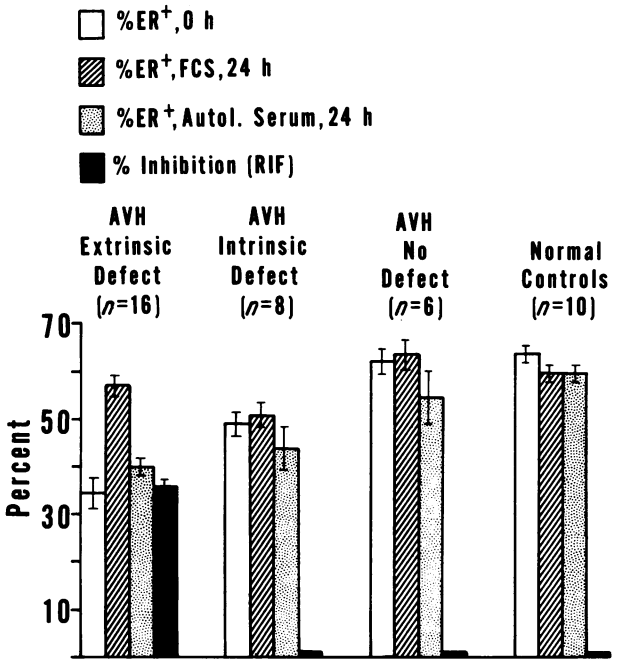

FIGURE 2. Regeneration of ER function and coexistent serum RIF during the acute phase of viral hepatitis. %ER+, 0 h, assay on freshly isolated lymphocytes; %ER+, FCS/autologous serum, 24 h, assay performed after 24 h incubation of washed lymphocytes in fetal calf or autologous serum; % inhibition (RIF), relative percent inhibition of normal lymphocyte ER function by patient sera.

Modulation of ER function during the acute phase of viral hepatitis. During the first 4 wk of AVH 24 out of 30 patients had decreased levels of ER+ lymphocytes (Fig. 2). The ER function of lymphocytes from 16 of these cases increased from 34.5±3.0% to 57.1±2.2% upon cultivation in fetal calf serum (FCS) ($P < 0.01$) but not after cultivation in autologous serum (42.3±1.6%). Each of their sera inhibited the expression of ER function of normal lymphocytes by 35.3 ±2.3% (mean±SEM) and these patients were classified as having an extrinsic defect in ER function.

Eight patients with AVH had decreased levels of ER+ lymphocytes (48.9±2.4%; $P < 0.001$ relative to control) which did not regenerate after cultivation in either FCS (51.4±2.7%) or autologous serum (44.3 ±4.7%) and their sera were devoid of RIF activity (Fig. 2). These patients were classified as having an intrinsic defect in ER function. This group had slightly higher initial levels of ER+ lymphocytes (48.9±2.4%) than the AVH patients with an extrinsic defect (34.5 ±3.0%) ($P < 0.05$).

20% of 30 patients examined during the first 4-wk of AVH had normal levels of ER+ lymphocytes (62.8 ±2.8%) which remained unchanged after cultivation in FCS (63.8±2.4%) or autologous serum (52.9±5.5%) and their sera were devoid of RIF activity (Fig. 2) rendering them indistinguishable from the healthy controls.

Despite the heterogeneity within the AVH group with regard to ER function, all three subgroups had normal levels of SIg+ lymphocytes. The excellent correlation between the presence of serum RIF activity and regeneration of ER function permitted its subsequent use as a criterion for determination of the mechanism responsible for defective ER function.

Modulation of ER function during the convalescent phase of acute viral hepatitis. Nine patients with biochemical and histologic evidence of resolved ($n = 7$) or resolving ($n = 2$) hepatitis (convalescent phase-resolved [CP-R]) were examined 12–16 wk after the onset of jaundice. ER function was depressed in eight of these nine patients (46.9±3.9%) none of whom had serum RIF activity (Fig. 3) indicative of the presence of an intrinsic defect at that time.

In contrast, RIF activity was present in the serum of all 10 patients with biochemical and histologic evidence of persistent hepatocellular injury 12–16 wk after the onset of jaundice (convalescent phase-unresolved [CP-U]). Such sera inhibited the ER function of normal lymphocytes by 27.7±4.0% (Fig. 3). The co-

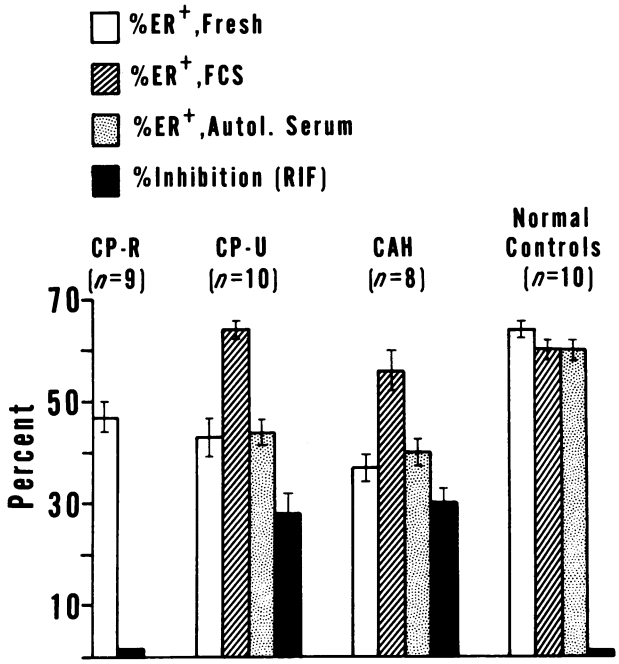

FIGURE 3. Regeneration of ER function and coexistent serum RIF during the convalescent and chronic phases of viral and postviral hepatitis. CP-R, convalescent phase-resolved; CP-U, convalescent phase-unresolved; CAH, chronic active hepatitis. %ER+, 0 h, assay on freshly isolated lymphocytes; %ER+, FCS/autologous serum, 24 h, assay performed after 24 h incubation of washed lymphocytes in fetal calf or autologous serum; % inhibition (RIF), relative percent inhibition of normal lymphocyte ER function by patient sera.

TABLE I

Mechanisms Responsible for Defective ER Function during Various Phases of Viral and Postviral Hepatitis

Phase of hepatitis	Type of lymphocyte defect			
	Extrinsic	Intrinsic	None	Total
	No. of patients			
Acute	16	8	6	30
Convalescent (resolved)	0	8	1	9
Convalescent (unresolved)	10	0	0	10
Chronic	7	1	0	8

existence of serum RIF activity with depressed levels of ER^+ lymphocytes (42.9±3.8%) in all patients is indicative of the presence of an extrinsic defect in this subgroup. Regeneration experiments corroborated the validity of these observations in this group as they did in patients examined during the acute phase of hepatitis.

Modulation of ER function in chronic active hepatitis. ER function was depressed (36.5±2.9%) in 8 out of 8 patients with CAH (Fig. 3) and RIF activity was present in the serum of 7 out of 8 patients (29.8 ±4.2% relative inhibition) indicative of the presence of an extrinsic defect. Regeneration of ER function upon cultivation in FCS but not autologous serum confirmed these observations. A single patient with CAH had a decreased number of ER^+ lymphocytes (46.0 ±3.5%) which did not regenerate upon cultivation in FCS and his serum was devoid of RIF activity, indicative of an intrinsic defect.

The initial level of ER^+ lymphocytes in this group was comparable ($P > 0.5$) to the other groups which shared an extrinsic defect (AVH-extrinsic: 34.5±3.0%; convalescent, unresolved: 42.9±3.8%) and was significantly lower than those groups characterized by an intrinsic defect (AVH-intrinsic: 48.9±2.4%, $P < 0.01$; convalescent, resolved: 46.9±3.9%; $P < 0.05$). Furthermore, the mean level of serum RIF activity in the CAH group (29.8±4.2%) was comparable ($P > 0.5$) to both the AVH, extrinsic subgroup (35.3±2.3%), and the convalescent, unresolved group (27.7±4.0%).

Comparative frequency of mechanisms responsible for defective ER function during each phase of hepatitis. Extrinsic defectiveness in ER function was observed in 53.3% of patients during the acute phase of viral hepatitis, 26.7% of these patients displayed an intrinsic defect and 20.0% were normal in this regard (Table I). In contrast to the heterogeneity observed during the acute phase, separate groups of patients examined at a later stage of disease were considerably more homogeneous with regard to this function and appeared to segregate into two separate and distinct categories. Although ER function remained depressed in 8 out of 9 patients recovered from hepatitis none of them displayed an extrinsic defect whereas an extrinsic mechanism was responsible for the depression of ER function seen in all 10 patients with unresolved hepatitis and 7 out of 8 patients with chronic active hepatitis (Table I).

TABLE II

Serum Mediated Inhibition of ER Function (RIF) and Mitogen Responsiveness (IMR) during the AVH versus the unresolved and chronic (CP-U) and CAH phases of viral and postviral hepatitis

		RIF^+	RIF^-
		% of patients	
AVH	IMR^+	23.8	14.3
$n = 21$	IMR^-	38.1	23.8
CP-U + CAH	IMR^+	26.7	6.6
$n = 15$	IMR^-	66.7	0
Total	IMR^+	25.0	14.3
$n = 36$	IMR^-	47.2	23.8

Comparison of serum-mediated inhibition of ER function (RIF) and mitogen responsiveness (IMR). To determine whether the serum activity responsible for inhibition of ER function (RIF) might be related to previously described serum inhibitors of mitogen responsiveness (IMR), 36 sera (27 RIF^+, 9 RIF^-) were assayed for the capacity to modulate the response of normal lymphocytes to stimulation by phytohemagglutination. Culture conditions were similar and criteria for designation of a particular serum as inhibitory in both assays were identical (suppression of each function by more than 2 SD below control levels). As indicated in Table II, no consistent relationship between RIF and IMR activity was apparent. Derivative calculations from Table II indicate that RIF activity was present in 69.2 and 73.9% of IMR^+ and IMR^- sera, respectively; and IMR was present in 26.1% and 40.0% of RIF^+ and IMR^+, respectively, there is no significant association between the two serum activities.

Incidence of RIF in related diseases. To ascertain whether the extrinsic defect in ER function is a specific accompaniment of hepatitis A and B virus induced hepatocellular injury, the presence of RIF was sought in sera from patients with a variety of viral diseases and from other patients with chronic nonviral hepatopathies (Table III). In striking contrast to the observations in hepatitis A and B virus infection none of 64 sera from 27 patients with 10 other viral and mycoplasma infections and none of 15 sera from as many patients with chronic nonviral liver disease were positive for RIF.

DISCUSSION

The present study extends our observations of decreased ER positive lymphocytes in association with viral hepatitis B and confirms our previous suggestion (8, 10) that at least two mechanisms (extrinsic and intrinsic) are responsible for the suppression of T-lymphocyte ER function in these patients. Both mechanisms appear to affect the same general class of lymphocytes. The thymus-derived heritage of lymphocytes rendered defective by the extrinsic mechanism is revealed by their ability to regenerate ER function when cultivated in the absence of autologous serum. Although the intrinsic defect is not readily reversible, the T-cell lineage of these cells is apparent from their ability to form rosettes with neuraminidase treated sheep erythrocytes. It is notable that both mechanisms are also observed in hepatitis A virus infection and that a persistent extrinsic defect in T-lymphocyte function is characteristically associated with progression of disease and the development of virus initiated chronic active hepatitis.

The first 4 wk of acute viral hepatitis are characterized by a remarkable degree of heterogeneity with regard to the expression of this lymphocyte surface membrane marker. Although every patient displays defective ER function on at least one occasion (10), approximately 20% of patients will be normal in this respect at any single sampling interval during that period. Approximately two-thirds of the remaining patients have an extrinsic defect in ER function while the residual one-third manifest a defect in ER function which appears to be mediated by mechanisms intrinsic to the lymphocyte itself. Although the intrinsic defect tends to be quantitatively less severe neither the degree of the defect nor the responsible mechanism has predictive value at this early stage of the disease (10).

Such heterogeneity is consistent with the dynamic and complex interactions which occur between pathogen and host at the outset of an acute viral infection. If either the qualitative or quantitative aspects of defective ER function are indeed related to these interactions one would predict evolution towards homogeneity in this regard as the eventual clinical outcome of the host-virus encounter becomes apparent. Such is the case in viral hepatitis. As previously (10) and currently reported, recovery from acute viral hepatitis is uniformly associated with the disappearance of serum RIF activity despite the fact that nearly 90% of such patients continue to display defective ER function.

In marked contrast, RIF was found to be present in all 10 patients who displayed biochemical and histologic evidence of continuing hepatocellular injury when examined 12–16 wk after the onset of acute viral hepatitis. The ability of these lymphocytes to regenerate ER function when cultured in vitro in the absence, but not the presence, of autologous serum further attests to the fact that mechanisms extrinsic to the lymphocyte were operative in these patients. Serum RIF activity and extrinsic suppression of ER function were also identified in 7 out of 8 patients with long-standing CAH. The single CAH patient with an intrinsic defect was clinically indistinguishable from the remaining eight. The significance of this deviation from the norm is unknown but may be resolved by continued clinical observation.

TABLE III
Occurrence of RIF in Hepatitis Virus Infection and Other Viral and Liver Diseases

Group	Cases RIF⁺/tested	Sera RIF⁺/tested
1. Hepatitis viruses	65/119	82/133
Type A	25/44	
Type B	37/70	
"Non-A, Non-B"	3/5	
2. Mononucleosis with hepatitis EBV,* CMV	0/7	0/20
3. Other viruses	0/16	0/32
Influenza A, adenoviruses, mumps, rubeola, rubella, Herpes Simplex, Varcella-Zoster		
4. Mycoplasma	0/5	0/12
5. Chronic nonviral liver diseases	0/15	0/15
Laënnec's cirrhosis, hemochromatosis, primary biliary cirrhosis, extrahepatic bile duct obstruction, metastatic liver disease, cirrhosis of unknown etiology		

* CMV, cytomegalovirus; EBV, Epstein-Barr virus.

The current data therefore indicate a very strong association between the mechanism responsible for defective ER function and the clinical outcome of disease when the assays are performed 3 or more mo after the onset of AVH. The presence at that time of an intrinsic mechanism correlates with recovery whereas an extrinsic mechanism indicates persistence of hepatocellular injury and is strongly associated with the subsequent development of CAH. Although the extrinsic defect tends to be slightly more severe on a quantitative basis the differences are too small and too variable to assume any prognostic value.

Defective ER function is not limited to viral hepatitis but is observed in a variety of viral infections (21) and in chronic nonviral liver diseases (22) as well. The uniform absence of RIF from a large number of sera from patients with such illnesses suggests that mechanisms intrinsic to the lymphocyte may be responsible for defective ER function in these diseases and that

the extrinsic defect is relatively specific for hepatitis A and B virus initiated hepatocellular injury.

It is noteworthy that hepatitis is a common clinical accompaniment of cytomegalovirus and Epstein-Barr virus-associated mononucleosis. Indeed moderately abnormal liver function tests were documented in 1 out of 4 and 3 out of 3 of our patients with Epstein-Barr virus and cytomegalovirus mononucleosis, respectively. The absence of RIF in such patients suggests that mechanisms different from those responsible for hepatocellular injury in hepatitis A and B virus infection may be operative in these diseases. Furthermore, the absence of RIF in a significant percentage of patients during the acute phase of viral hepatitis types A and B suggests that RIF is not merely a nonspecific consequence of hepatocellular injury but rather it appears to be associated with a particular type of injury that has the potential to become progressive or chronic. Assuming the validity of this hypothesis the majority of patients with acute viral hepatitis A and B must be able to terminate such events since RIF is common during the acute phase of hepatocellular injury whereas chronic (RIF^+) hepatitis is a relatively rare sequela.

The mechanisms responsible for hepatocellular injury in viral hepatitis are not established, although they have been the subject of extensive hypotheses based on indirect or circumstantial evidence (23–26). The course of disease associated with hepatitis virus infections is quite variable (27, 28) and it appears that host factors probably play a significant role in determining the biological outcome of the encounter between virus and host. Available circumstantial evidence suggests that such host factors may be immunological in nature and a variety of conjectural hypotheses have been formulated which implicate both cellular and humoral immune responses as pathogenic determinants of virus associated hepatic disease (23–26). If the validity of such hypotheses is confirmed, coexistent immunoregulatory events would be of importance as modulators of the implicated immunopathogenetic systems.

In the current study we have identified two types of functionally defective T lymphocytes and have evaluated the occurrence of these two mechanisms involved in the regulation of T-lymphocyte ER function during infection with the hepatitis viruses as well as during the postviral phase of persistent hepatocellular injury. The significance of these mechanisms is suggested by the observations that: (*1*) ER function is modulated by intracellular levels of cyclic nucleotides (5) and by thymosin (29) which also regulate other aspects of the immune response (30); (2) the nonimmune binding of sheep erythrocytes in ER formation appears to trigger the release of immunosuppressive lymphokines by T lymphocytes (31); (3) ER function appears to be dependent on the expression of specific membrane receptors (1) and as such it represents a recognitive event at the T-lymphocyte surface. Hence modulation of ER function may be associated with alteration of specific pathways of differentiation and function of T lymphocytes that in turn influence other immunologic functions more directly involved in hepatocellular injury.

Extrinsic suppression of ER function is mediated by an unusual lipoprotein, RIF, which is not present in normal serum. RIF is a discrete low density lipoprotein with a density of 1.050 ± 0.004 g/cm^3 and beta electrophoretic mobility (11). It has recently been isolated and purified and appears to represent a structural association complex of apolipoproteins A_{II}, B, and C_{III}. RIF is remarkably potent with activity measurable in concentrations as low as 0.1 pM. It progressively and actively inhibits ER function after an initial 4 h lag period subsequent to binding by a limited number of specific lymphocyte membrane receptor sites. In these respects extrinsic modulation of ER function resembles the active regulation of cholesterol synthesis by fibroblasts due to the binding of exogenous lipoprotein by specific cell surface receptor sites as recently described by Goldstein and Brown (32). Although the source of RIF is not known, the liver is a major locus of lipoprotein biosynthesis. Furthermore, it has been suggested that human liver contains as yet undefined factors which inhibit other lymphocyte functions (33). It appears that hepatitis A and B virus infection may induce hepatic synthesis and(or) release of RIF. Because of its immunoregulatory properties, this molecule may serve as a feedback regulator of the immune system and influence the pathogenic mechanisms underlying the observed hepatocellular injury.

The present study suggests that RIF and previously described hepatitis-associated serum factors (IMR) which inhibit the DNA synthetic response of lymphocytes to PHA (34) are distinct and separate entities. IMR and RIF exhibit independent segregation in our panel of sera, and only RIF had a statistically significant association with progressive liver disease.

Wands et al. (35) have recently described the existence of a serum factor in patients with acute and chronic hepatitis which inhibits spontaneous and induced lymphocyte cytotoxicity for Chang liver cells. This factor, like RIF, apparently disappears with clinical recovery. The relationship between this interesting phenomenon and RIF is unclear at present and warrants further investigation.

A second immunoregulatory low density lipoprotein, distinct from RIF in its apoprotein moieties and biological properties, has recently been isolated from normal human serum (36). This lipoprotein is a potent regulator of the inductive phase of the triggering of human lymphocytes by mitogens and allogeneic cells.

It remains to be established whether the observed capacity of serum from some hepatitis patients to inhibit lymphocyte mitogen responsiveness may be due to a quantitative elevation in this species of immunoregulatory low density lipoprotein.

The significant association of an abnormal immunoregulatory lipoprotein (RIF) with progression of postviral hepatocellular injury suggests the potential pathogenetic role of the immune system in this disease. It is hoped that further studies may elucidate pivotal events involved in the immunobiology and clinical course of hepatitis virus infections.

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