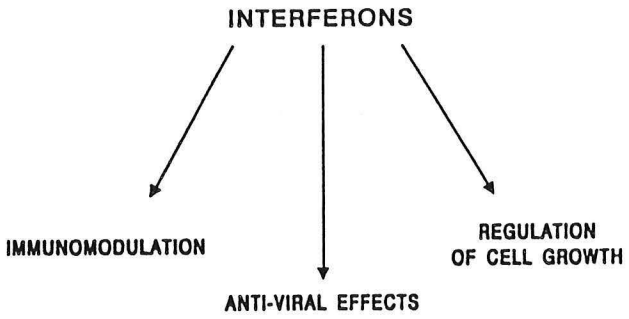

MANAGEMENT OF CHRONIC HEPATITIS B IN THE 1990's:

A NEW ERA?

Internal Medicine Grand Rounds

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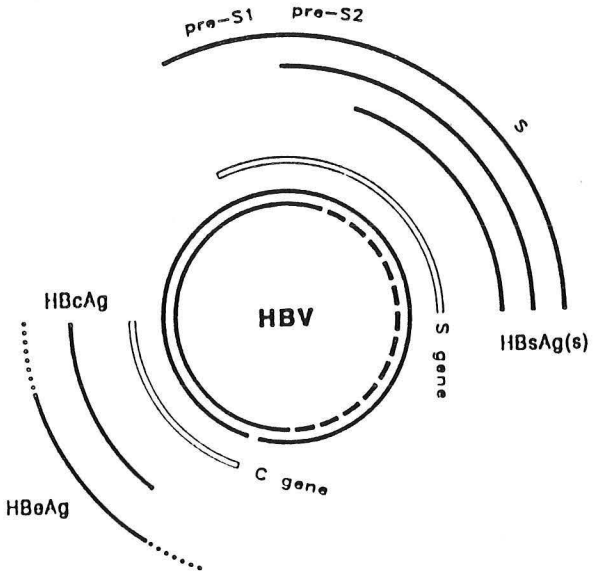
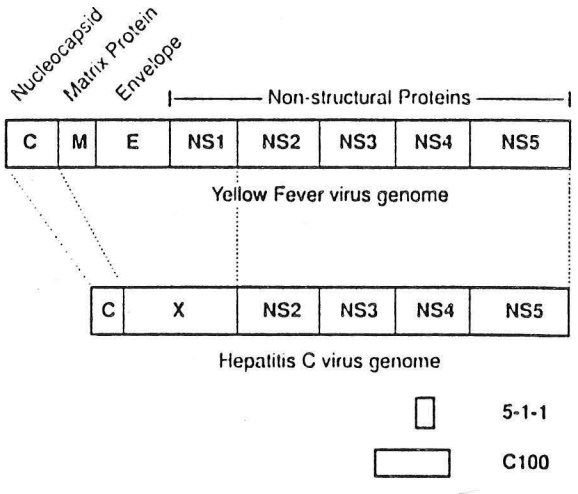




Chronic hepatitis is a term used to describe a necroinflammatory disease of the liver that continues beyond the period for recovery expected after an acute episode of viral or toxin induced hepatitis. While the histologic features used to define this clinicopathologic syndrome can be seen in Wilson's disease, alpha-1-antitrypsin deficiency, primary biliary cirrhosis, or even alcoholic liver disease, this term is most commonly used by clinicians to describe patients in which such etiologies have been excluded. In this context the term chronic hepatitis describes a family of diseases resulting from chronic viral infections, drug induced liver injury or putative autoimmune or other unknown mechanisms. Despite these diverse etiologies, the histologic and biochemical similarities of these diseases in conjunction with longstanding deficiencies of serodiagnostic testing has led to often confusing and frustrating attempts to formulate a unified approach to their management. While three prospective randomized trials of corticosteroid therapy of "chronic active liver disease" performed in the late 1960's and early 1970's established the efficacy of at least one form of therapy for chronic active hepatitis (1-3), no significant advances in accepted therapy for this syndrome entered the clinical arena over the next two decades. However, two recent developments, one in serodiagnostics and one in therapeutics have led to major changes in the manner in which such patients are likely to be managed over the next decade.

One such advance, the identification, cloning and development of serodiagnostic tests for hepatitis C (4,5), was reviewed in Medical Grand Rounds by Dr. Cuthbert in September of 1989. Release during the past year of the long awaited first diagnostic test for Hepatitis C virus (HCV) infection has already changed our diagnostic approach to chronic hepatitis. In addition, large quantities of interferons produced by recombinant technology have become available for clinical testing during the past decade. During the past three years the first large prospective randomized trials to demonstrate efficacy of interferon-alpha in treatment of chronic hepatitis B and C have appeared in the literature. Especially if, as seems imminently likely, the U.S. Food and Drug Administration licenses interferon for the treatment of chronic HCV, these latter developments are likely to have an even greater impact on management of patients with chronic hepatitis. While selected aspects of the diagnosis and management of putative autoimmune chronic hepatitis will be covered in this protocol, the major goal of this presentation will be to review our knowledge of the effects of interferon-alpha therapy on chronic viral hepatitis and to discuss the issues which must be addressed by physicians who are contemplating use of this form of therapy.

CORTICOSTEROID THERAPY OF CHRONIC HEPATITIS

Four decades have passed since a chronic active liver disease of young women associated with hypergammaglobulinemia and response to steroid therapy was first described (6,7). This syndrome, subsequently termed "lupoid" hepatitis (8, because of frequent presence of LE cells) or simply chronic active liver disease, is now most commonly known as autoimmune chronic active hepatitis (AICAH). In addition to fatigue, anorexia, malaise, jaundice and other nonspecific symptoms

of liver disease, arthralgias and skin rashes are common symptoms in such patients. In addition, these patients may also suffer from a variety of associated diseases of putative autoimmune origin such as Hashimoto's thyroiditis, ulcerative colitis, Sjogren's syndrome, fibrosing alveolitis and autoantibody positive hemolytic anemia. Such disease associations in addition to linkage to the B8, DR3 haplotype in some families has been offered as evidence of an autoimmune etiology of this disease (9).

While half or more of such patients have anti-nuclear antibodies (ANA) in the presence or absence of autoantibodies to smooth muscle, no widely accepted laboratory or histologic criteria for definitive diagnosis of this syndrome exists. Recently a series of other autoantibody tests including anti-liver kidney microsomal (anti-LKM_1), anti-soluble liver antigen (anti-SLA) and anti-asialoglycoprotein receptor (anti-ASGPR) have been proposed as alternative tests for diagnosis of ANA negative cases of AICAH (10,11). However, with the exception of anti-LKM_1 autoantibodies which appear to characterize only a small subset of such patients, the remaining such recently described AICAH-specific autoantibody tests have yet to be validated in more than one or two major centers and are not available in a standardized form in this country. Thus, while the presence of autoantibodies and associated "autoimmune" diseases may serve as evidence in support of this diagnosis, definition of this syndrome has also relied heavily on exclusion of other known causes of liver diseases.

In part for these reasons, the only widely accepted therapy for this disease has been validated in trials in which entry criteria was based on relatively non-specific histologic and biochemical criteria. Three such prospective, randomized trials of corticosteroid therapy in chronic active liver disease (CAH +/- cirrhosis) have been performed. The treatment protocols employed and the survival data achieved in such trials are summarized in Table 1. In the first of these trials begun in 1963 (1), patients were selected on the basis of otherwise unspecified "characteristic" clinical and biochemical features of CAH. In the next two trials, clearer definitions of disease chronicity, histologic criteria, and degree of hypertransaminasemia were detailed in the published reports. However, the only potential "autoimmune" or immunologic criteria for entry into these latter two trials was the use of hypergammaglobulinemia as an alternative entry criteria (2,3). Indeed, a number of HBsAg positive patients were enrolled in these trials and retrospective evaluation of such patients and three subsequent prospective trials of corticosteroid therapy of chronic hepatitis B virus (HBV) infections indicated a lack of benefit from corticosteroid therapy in this subgroup of chronic hepatitis patients (12-15). However, among HBsAg negative patients in either the Mayo Clinic or the King's College series, no difference in steroid responsiveness or outcome of ANA negative or ANA positive patients could be discerned (16.17).

A number of other features of these early trials of treatment of chronic hepatitis are important to reconsider when planning current management of chronic hepatitis. First of all, despite the above described diagnostic ambiguities and the small size of patient groups in each of these trials (only 14-27 patients per therapy group), the results of each trial indicated a significant reduction in disease mortality among steroid treated patients (see Table I). In part this fortuitous outcome was related to the very significant short term mortality in control (or azathioprine treated) patients (48% at 4.4 years, 28% at two years,

and 41% in ≤3.5 years follow-up). As noted by previous reviewers (18), the patients enrolled in these trials were identified well before the modern era of widespread liver enzyme screening of asymptomatic individuals. Thus, virtually all subjects were symptomatic patients with often advanced liver disease at time of presentation and enrollment. Therefore, while there is no basis for questioning the applicability of these results to similar patients with symptomatic "chronic active liver disease", it has become widely recognized that the majority of modern patients referred for evaluation and management of chronic hepatitis have lesser degrees of both symptomatology and biochemical or histologic abnormalities than did patients enrolled in any of these early steroid therapy trials.

TABLE I

RANDOMIZED, PROSPECTIVE TRIALS DEMONSTRATING EFFICACY OF CORTICOSTEROIDS IN THE TREATMENT OF CHRONIC ACTIVE HEPATITIS

Trial	Therapy	# Patients in Group	Follow-up (mean years)	Mortality
Cook et al (1) (Royal Free Hospital, London)	Nil	27	4.4	48%
	Prednisolone[a]	22	4.4	14%*
Murray-Lyon et al (3) (King's College Hospital, London)	Azathioprine[b]	25	2.0	28%
	Prednisone[c]	22	2.0	5%
Soloway et al (2) (Mayo Clinic, Rochester, Minn.)	Placebo	17	≤3.5	41%
	Azathioprine[d]	14	≤3.5	36%
	Prednisone[e]	18	≤3.5	6%**
	Aza + Pred[f]	14	≤3.5	7%**

[a]Prednisolone therapy initiated at a dose of 15 mg po qd and then tapered empirically following remission
[b]Azathioprine 75 mg po qd
[c]Prednisone 5 mg po tid
[d]Azathioprine 100 mg qd
[e]Prednisone 60 mg po qd x 1 weeks, 40 mg qd x 1 week, 30 mg qd x 2 weeks, then 20 mg qd
[f]Azathioprine 50 mg po qd + Prednisone 30 mg qd x 1 weeks, 20 mg qd x 1 week, 15 mg qd x 1 weeks, then 10 mg po qd
*p<0.01 compared to control
**p<0.05 compared to control

A final issue that is likely to be raised repeatedly until such time as improved diagnostic tests for AICAH and HCV are available relates to the possible role of HCV in the pathogenesis of steroid responsive chronic active liver disease. The presence of a non-A, non-B hepatitis virus was not even suspected until 5-10 years after patient selection for these landmark steroid therapy trials and little or no data regarding risk factors for this infection are available for these patients. However, investigators at two of the institutions at which these initial steroid therapy trials were performed have recently reported on the frequency of anti-HCV reactivity among their current population of patients being treated for AICAH (19,20). Fully 65% of Kings College Hospital patients with "active" AICAH were found to be reactive in the first generation Ortho-HCV ELISA test (19). Yet, such reactivity tended to disappear as hypergammaglobulinemia resolved during immunosuppressive therapy (corticosteroids +/- azathioprine) and this report appears to be among the first to document a

high false-positivity rate for the first generation anti-HCV tests among patients with hypergammaglobulinemia of various etiologies. Preliminary results of re-screening of these samples with the RIBA confirmatory test for HCV reactivity (see later sections of protocol for additional details) indicates that approximately 75% of these positive anti-HCV results represent false positives (personal communication, I.G. McFarlane). Czaja et al (20) from the Mayo Clinic have reviewed results of anti-HCV results among 86 symptomatic patients with very active CAH (presumably similar in characteristics to those enrolled in the Mayo Clinic steroid therapy trial) and have found 8/86 to be anti-HCV(+) but only 4/86 (5%) to be RIBA positive. Of note, however, 11 of 30 (37%) consecutive patients referred to Mayo Clinic for evaluation of asymptomatic, HBsAg negative, mild or moderately active CAH were anti-HCV(+) and 27% of these patients were RIBA positive (20). Thus, it appears that the clinical criteria used by these two institutions to select patients for steroid therapy of "chronic active liver disease" and in particular the Mayo Clinic requirements for active disease has resulted in patient populations containing very few individuals with specific serologic evidence of chronic HCV infections. Thus, retrospective analysis of therapeutic outcome at these two institutions is unlikely to be very informative about the efficacy of steroids in chronic HCV.

Despite the lack of data from any randomized trial of steroid therapy in chronic HCV, the now generally accepted lack of efficacy of such therapy in chronic hepatitis B virus (HBV) infection, the apparently high morbidity from chronic NANB or chronic HBV hepatitis infections in immunocompromised renal transplant recipients (21-23), and the lack of dramatic biochemical or histologic response to steroids in small series of patients with post-transfusion chronic NANB hepatitis (24) has led to a general lack of enthusiasm for immunosuppressive therapy in chronic viral hepatitis of any etiology.

ANTIVIRAL THERAPY OF CHRONIC HEPATITIS B: INITIAL FAILURES

With increasing appreciation of the lack of benefit from immunosuppressive therapy in chronic HBV, pilot studies of the efficacy of human interferon-alpha and the antiviral compounds adenine arabinoside (Ara-A) and adenine arabinoside monophosphate (Ara-AMP) were initiated in the mid-to-late-1970's (25). Sensitive and specific tests for the presence of chronic HBV infection (serum HBsAg) and for markers of active viral replication (HBeAg, HBV-DNA and DNA polymerase activity) were available and permitted these investigators to readily demonstrate that these agents were capable of in vivo inhibition of HBV replication. A number of investigators described patients in whom therapy with these agents was associated with conversion from HBeAg(+) to HBeAg(-), anti-HBe(+) serologic status. While complete loss of HBsAg expression was seen much less commonly in such cases of apparent therapeutic responses, additional studies demonstrated that spontaneous or therapy induced conversion from HBeAg(+), anti-HBe(-) to HBeAg(-), anti-HBe(+) was associated with loss of infectivity (as demonstrated by direct inoculation of patient sera into chimpanzees) and improvement in biochemical and histologic parameters of liver disease activity (26,27).

Despite these optimistic findings a number of small prospective studies failed to show consistent benefit of either form of therapy when compared to untreated controls (see Table II). In a larger, multicenter U.S. trial published in 1987 (32, detailed in Table II), no benefit of Ara-AMP or Ara-AMP alternating

with interferon-alpha therapy could be demonstrated despite use of doses of these agents which led to very high levels of toxicity. Not only did these dismal results tend to significantly diminish the enthusiasm of many physicians for use of antiviral therapy, but in addition, the very high rate of neuropathic side effects associated with Ara-A or Ara-AMP in this (32) and other studies has since led to the withdrawal of these agents from investigational use in this disease.

TABLE II

RANDOMIZED, PROSPECTIVE CLINICAL TRIALS FAILING TO DEMONSTRATE EFFICACY OF ANTIVIRAL THERAPY OF CHRONIC HBV INFECTION

First Author Year	Therapy	# Patients in Group	HBV-DNA(-),HBeAg(-), anti-HBe(+)[a]	HBsAg(-)[a]
Weimar (28) 1980	Placebo	8	0 (0%)	0
	IFN-α x 6 weeks[b]	8	0 (0%)	0
Schalm (29) 1982	Placebo	10	4 (40%)	0
	IFN-α x 6 weeks[b]	10	2 (20%)	0
Perrillo (30) 1985	Nil	11	0 (0%)	NR[c]
	Ara-AMP x 28d[d]	11	1 (9%)	NR
	Nil	6	0 (0%)	NR
	2 cycles Ara-AMP[d]	7	1 (15%)	NR
Anderson (31) 1987	Nil	16	0 (0%)	NR
	IFN-α x 28d[e]	14	1 (7%)	NR
Garcia (32) 1987	Placebo	27	4 (15%)	0
	Ara-AMP/Placebo[f]	24	2 (8%)	1
	ARA-AMP/IFN-α[f]	13	2 (15%)	0
Lok (33) 1988	Nil	18	0 (0%)	0
	IFN-α 2.5×10^6 u/ml[g]	18	1 (6%)	0
	IFN-α 5×10^6 u/ml[g]	18	3 (17%) 15%[h]	0
	IFN-α 10×10^6 u/ml[g]	18	5 (28%)	0

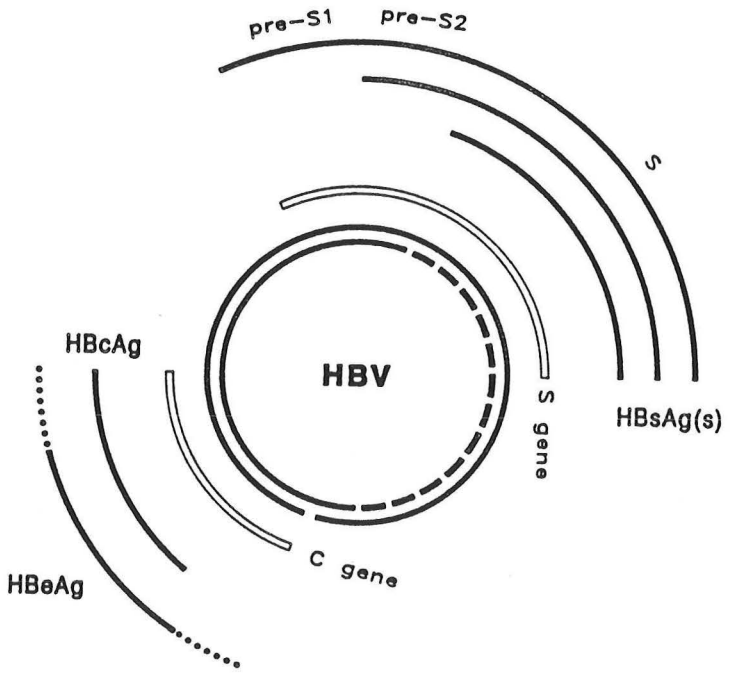
[a]Assessed at 12 months or latest point of follow-up prior to 12 months.
[b]Leukocyte interferon (native interferon α species) was given at initial dose of 12×10^6 units/d I.M. x 1 week, then doses reduced by 50% in each subsequent week of 6 week course.
[c]Not reported
[d]Ara-AMP 5 mg/kg I.M. bid for first 5 days, then 2.5 mg/kg I.M. bid for remaining 23 days. A four week rest interval separated the first and second cycles of therapy.
[e]Lymphoblastoid interferon-α was initiated at a dose of 2.5×10^6 units/m^2 I.M. qd and then advanced to 7.5×10^6 u/m^2 I.M. qd as tolerated.
[f]Three 28 day courses of Ara-AMP, 2.5 mg/kg I.M. bid (or placebo) alternating with 28 day courses of 2.5×10^6 units sc bid of leukocyte interferon α (or placebo).
[g]Doses given I.M. three times per week for 12-24 weeks of treatment.
[h]Not significantly different from control.

Yet, despite nearly a decade of experimental use without demonstrated efficacy, a series of studies published over the last 3-4 years have demonstrated a consistent pattern of efficacy of interferon-alpha therapy in achieving HBeAg(+), HBV-DNA(+), anti-HBe(-) to HBeAg(-), HBV-DNA(-), anti-HBe(+) seroconversions with concomitant resolution of active hepatitis (34-39). Why, after a decade of apparent failure, is this therapy now poised to enter broader clinical application? An understanding of this apparent abrupt improvement in therapeutic success requires some additional insight into selected aspects of HBV-related disease and the in vivo effects of interferon-alpha.

HBV-HOST INTERACTIONS

HBV is one of the best characterized of human viral pathogens. In addition to the present detailed knowledge of the partially double-stranded circularized DNA genome of this agent and related hepadnaviruses (40), major advances have been made in our understanding of the role of a variety of HBV proteins in viral replication, viral structure, and stimulation of the host immune response (40,41). The original discovery of this virus and the earliest clinical tests for HBV infection were based on the propensity of this virus to produce excess quantities of the HBV surface antigen (HBsAg). Thus, the sera of infected individuals contain not only infectious virus particles (Dane particles) but also incomplete viral particles made up entirely of HBsAg. These noninfectious particles that are identified by our clinical tests for "HBsAg" usually greatly outnumber intact viral particles by as much as 10,000,000:1. As schematically detailed in Figure 1, the gene for HBsAg has three start codons and initiation of mRNA synthesis at these three alternate sites produces three different proteins ranging in length from 226 amino acids (S region only) to 400 amino acids (pre-S_1 + pre-S_2 + S). All are recognized in commercially available HBsAg assays.

Figure 1



Following identification of HBsAg in the circulation of acutely or chronically infected individuals, another HBV viral protein was found to be present in sera of individuals with active, transmissible HBV infections. Like HBsAg this HBeAg is present largely (if not entirely) outside of complete viral particles and is actually a non-particulate soluble protein that appears to be directly secreted from infected host cells into the serum during active viral replication. It has been subsequently determined that HBeAg is encoded by the HBV

C gene which also encodes the nucleocapsid or core protein of the virus (40). As shown schematically in Figure 1, the amino acid sequence of HBeAg and HBcAg have significant identity which results from the fact the HBeAg and HBcAg synthesis derives from initiation of translation at the first and second start codon, respectively, of the C gene. The HBe mRNA contains a signal sequence and the newly synthesized polypeptide product is directed to the host endoplasmic reticulum prior to additional removal of N and C terminal sequences. The secreted HBeAg contains an additional 10 N-terminal amino acids absent from HBcAg but lacks the 34 C-terminal residues of HBcAg. These structural differences lead to major differences in the tertiary structures of these proteins such that the antibody responses to these two antigens are quite distinct. While HBsAg and HBcAg play obvious structural roles in the HBV virus, the function of the HBeAg and the large quantities of excess HBsAg synthesized by this virus have been less clearly defined. In particular, HBeAg production appears not to be required for viral replication (42).

A number of clinical correlations between the activity of HBV related liver disease and immune responses to HBcAg, HBeAg and HBsAg have demonstrated a primary role for the host immune response in initiating liver injury during infection by this virus. Thus, investigators from Taiwan describe three phases of neonatally acquired chronic HBV infection that are summarized in Table III (43,44). In the first phase that typically covers the first 20 years of life, high levels of serum viral particles (as measured by assessments of HBV-DNA and DNA polymerase activity), HBeAg and HBsAg are present. Yet serum transaminases are normal or near normal and liver histology is normal except for high levels of HBcAg staining in hepatic nuclei and minimal histologic changes in some patients. While such individuals mount brisk antibody responses to HBcAg, they appear to have deficient cell-mediated immune responses to this antigen and seem to be completely tolerant to HBeAg and HBsAg. The lack of biochemical, histologic or symptomatic disease in such individuals has led to the conclusion that HBV is not directly cytopathic.

TABLE III

THREE PHASES OF CHRONIC HBV INFECTION IN CHINESE PATIENTS

	Phase 1	Phase 2	Phase 3
Serologic Markers			
HBsAg	+	+	+
HBeAg	+	+	-
HBV DNA	+++	+	-
anti-HBc	+	+	+
anti-HBe	-	-	+
Histologic Abnormalities	Minimal	Active Hepatitis ± Cirrhosis	Minimal Hepatitis ± Cirrhosis

In the second phase of HBV infection which usually occurs between ages 20 and 40 years in such Asian patients, increasing biochemical and histologic signs of active hepatitis are apparent despite decreased levels of HBV-DNA in the serum. A variety of evidence suggests that the predominate cause of liver injury in patients with chronic HBV is mediated by anti-HBcAg specific T cell responses directed at infected hepatocytes (45a). Patients at this stage of infection have no evidence of B or T cell responses to HBsAg and have yet to mount an effective antibody response to HBeAg. Nevertheless, they appear to have B and T cell responses to HBcAg including anti-HBcAg specific cytotoxic T cells present in hepatic lymphocytic infiltrates. The course of this phase of disease in Chinese patients tends to be characterized by a series of remissions and relapses and variable degrees of hepatic histologic abnormality. Some individuals progress to liver failure and death during this phase, and overall adult HBeAg(+) patients progress to cirrhosis at a rate of 2.4% per year (44).

Development of anti-HBe responses and loss of HBeAg reactivity from the serum is correlated with transition from this second phase of active disease to a third "non-replicative" phase in which histologic activity of liver disease subsides, HBV-DNA, DNA polymerase activity and HBeAg disappear from the serum, and intra-hepatic viral replication is undetectable, or nearly so. These phase 3 Chinese HBV patients are typically >40 years of age and are usually found to have HBV-DNA integrated into the host genome. Most are still HBsAg(+), but some have lost this marker as well. Some such patients already have an underlying cirrhosis but many do not. Despite much less evidence of disease activity by conventional histologic and biochemical parameters, a recent prospective study of 175 HBsAg(+), HBeAg(-), anti-HBe(+) patients in Taiwan found that such patients still progressed to cirrhosis at a rate of 1.3% per year (44). However, progression to cirrhosis in > 2/3 of such initially HBeAg(-) cases was preceded by a "reactivation" of viral replication and reversion to HBeAg(+) serologic status (44).

While there are many disparities between the chronological course of chronic HBV infections that are typically acquired in adulthood among Western patients and those acquired neonatally or in early childhood in Asian patients, clinical correlates of these three phases of disease have clearly been well described in U.S. or European HBV carriers. In addition, it has been shown repeatedly that a variety of agents capable of suppressing the host immune response will typically induce a transient suppression of biochemical and histologic markers of hepatic necrosis despite leading to apparent increases in viral replication (45b,46). Inevitably, either later in the course of presumably incomplete suppression of the host immune response or following cessation of immunosuppression, a rebound rise in hepatitis activity is commonly seen (46). In some individuals, actual clearance of active viral replication and loss of HBeAg has been noted to occur during this apparent immunologic rebound following short courses of therapy with corticosteroids or cytotoxic agents (47). Of note, it was observed that in the few responders to interferon or antiviral therapy in early trials, clearance of circulating HBeAg and HBV-DNA was commonly associated with a transient exacerbation of hepatitis marked by sharp rises in serum transaminases and often the typical symptomatology of acute viral hepatitis (34). Such events also have been observed during the course of spontaneous conversion from HBeAg(+) active HBV liver disease to HBeAg(-), anti-HBe(+) inactive HBV carrier status (34).

These clinical observations have led most investigators in this field to conclude that while the host immune response is a major cause of hepatic injury during chronic active HBV disease, evolution of this response is essential in finally removing the bulk of actively infected hepatocytes and making the transition from "phase 2" histologically active disease to "phase 3" inactive disease. While the nature of the immune events that occur as part of this transition have not been clearly demonstrated, the correlation with development of anti-HBe responses has posed a number of intriguing questions. As HBeAg is not a HBV structural protein and does not appear to play a role in viral replication, how can an anti-HBe response play a role in controlling viral replication? One potential answer is of course that no such role exists and that loss of HBeAg is merely an epiphenomenon associated with lower levels of viral replication. However, a variety of findings suggests a potential crucial role for T cell responses to this antigen in the evolution of HBV infection.

Thus, HBV is but one of a family of hepadnaviruses capable of infecting a variety of animal species. Among all such hepadnaviruses, production of excessive surface antigen particles (HBsAg) and retention of the precore domain giving rise to HBeAg appear to be highly conserved (40,42). These observations have led many investigators to suggest that production of forms of HBsAg and HBeAg that appear in large excess in the host circulation may constitute an evolutionarily conserved strategy for evading a protective host immune response either via "diversion" of the immune response or by directly tolerizing the host to viral antigenic epitopes that would be the normal target of the host immune response (48). As shown in Figure 1, HBeAg and HBcAg have significant sequence identity. While the antibody response to these antigens appears largely directed at tertiary structures not common to the native structures of these two proteins, the T cell responses of multiple strains of mice have recently been shown to be directed at immunodominant peptide epitopes that reside within the shared sequence of these two proteins (49). Moreover, cross-tolerance to such shared epitopes has been demonstrated in a murine model of T cell tolerance (48). Such studies also demonstrated that transplacental passage of HBeAg is capable of tolerizing newborn mice to HBeAg and HBcAg (48). Thus an intriguing hypothesis to explain the association of acquisition of anti-HBe responses with the transition from HBeAg(+), serum HBV-DNA(+) active liver disease to HBeAg(-), serum HBV-DNA negative inactive HBV carrier status has been proposed. It seems likely that development of antibody responses to this antigen correlates with acquisition of HBeAg-specific T cell responses which, because of sequence identity, implies acquisition of an expanded repertoire of anti-HBc specific T cells. This more vigorous T cell response to HBc epitopes seems to be a candidate mechanisms for more aggressive clearance of virally infected hepatocytes (and hence an acute hepatitis picture) that in turn results in lower levels of viral replication and hence a subsequent fall in disease activity. Clearly, additional studies need to be performed to confirm such hypotheses, but this putative mechanism appears to be the best current explanation of the clinical phenomenon observed during acquisition of anti-HBe response (48,50).

Evolving concepts of the human immune response to HBV proteins has led to better understanding of the clinical course of this disease. Realization that the chronic HBV carrier state was not a static state became abruptly clear to a number of investigators when they first began to compare their therapy induced response rates to those observed in prospectively randomized controls. To their

surprise, spontaneous HBeAg(+)→anti-HBe seroconversions occurred far more frequently than initially predicted. Analysis of the clinical characteristics of such spontaneous HBeAg→anti-HBe convertors revealed that they typically had high ALT and AST levels and low HBV-DNA levels (34). As there seemed to be no rationale for treating patients poised to undergo a spontaneous conversion, most investigators have tended to require a 6-12 month waiting period between presentation and enrollment in a clinical trial to verify that patients had stable levels of disease activity. Alternatively, they have used very high ALT values to identify such patients and exclude them from therapy trials. An example of the benefits of this approach is given by the results of a trial conducted by Alexander et al in London (34), a trial which led to the first published results of a large randomized, prospective study demonstrating significant efficacy of interferon-alpha therapy. During selection of patients for this trial, 13 were excluded because they had ALT values >4 times the upper limit of normal. During 6-12 month follow-up of these patients 7 became HBeAg negative with 5 (38%) developing anti-HBe responses. In contrast, loss of HBeAg was observed in 0/23 patients accepted into the trial and randomized to the control group. This lowering of the "background" response rate was almost certainly useful in demonstration of efficacy of interferon administered to control patients as only 6/23 patients (26%) with moderately active hepatitis responded with loss of anti-HBeAg.

In addition to acquiring a better appreciation of the potential for spontaneous resolution of the active replicative phase of chronic HBV infections, investigators have acquired a good deal of information about the clinical characteristics of patients who did respond to therapy. Table IV lists a variety of patient characteristics that have been associated with high or low rates of response to interferon-alpha or Ara-A/Ara-AMP therapy (50-52). These characteristics fall into three broad categories: length or mode of acquisition of infection (i.e. vertical transmission with neonatal tolerance in Asian patients), histologic and biochemical activity of disease, and sex/homosexuality/anti-HIV status. While subsequent more refined statistical analysis has shown that these variables are not all independent predictors of response (39,51), virtually all of these factors can be integrated into a unified concept regarding the role of the host immune response in control of active HBV infections.

TABLE IV

CHARACTERISTICS ASSOCIATED WITH HIGH OR LOW RATES OF RESPONSE TO ANTI-VIRAL THERAPY OF CHRONIC HBV INFECTION

High Responder	Low Responder
Chronic Active Hepatitis	Chronic Persistent Hepatitis
High ALT, AST	Low ALT, AST
Female	Homosexual
Low Serum HBV-DNA	Anti-HIV(+)
Recent Onset Infection	Age <12 Years
History of Acute Icteric Hepatitis	High Serum HBV-DNA
	Anti-Delta(+)
	Chinese

see references 39, 50, 51 and 52

Thus, responders tend to be immunocompetent hosts with pre-existing immune responses to HBV infected hepatocytes (as indicated by the presence of active hepatitis). Of note, the three largest trials with negative results (detailed in Table II) were heavily populated with "low responder" patients. Thus, the trial conducted by Lok et al examined an exclusively Chinese patient population in which the mean ALT was only 1.5 times the upper limit of normal. The studies of Garcia et al and Anderson et al contained 75% and 90% homosexual male patient populations, respectively, and in the trial conducted by Garcia et al fully 32% of enrollees were anti-HIV(+), and 61% of patients had only chronic persistent hepatitis on initial liver biopsy.

IN VIVO EFFECTS OF INTERFERON AND ARA-AMP

With the accumulation of clinical data from early, often unsuccessful trials of antiviral therapy in HBV infections and with experience gained during interferon-alpha therapy of a variety of malignancies, a better understanding of the pharmacokinetics, toxicity and in vivo antiviral effects of these agents was accumulated. The net result of this accumulated knowledge was that investigational use of Ara-A/Ara-AMP was abandoned and dosage schedules for interferon therapy were greatly modified.

Ara-A and the related derivative Ara-AMP are potent anti-viral agents that have been shown to have inhibitory effects on HBV replication comparable to those achieved with interferon-alpha therapy (53-55). One small randomized prospective trial showed significant benefit of a one month course of Ara-AMP with 4/15 treated patients and 0/14 untreated controls converting to HBeAg(-), anti-HBe(+) status within twelve months of onset of therapy (54, see Table V). Of note, in follow-up studies an interferon-alpha regimen subsequently shown to be effective (34-39) was not superior to one month cycles of Ara-AMP in frequency of HBeAg(+)→HBeAg(-) or HBsAg(+)→HBsAg(-) seroconversion.

Moreover, in responders to both a four week course of Ara-AMP and a 12 week course of lymphoblastoid interferon, clearance of HBeAg was associated often with a transient many-fold increase in AST (55). Thus, response following both forms of therapy has been associated with a presumably immunologically mediated flare in hepatitis. However, in a later study by this same group of investigators (see Table V), a paradoxic decreased rate of response was seen when Ara-AMP therapy was extended to an 8 week course. Moreover, as in the case of the study of Garcia et al (32, Table II), occurrence of a disquieting frequency of severe neuropathy was seen in recipients of extended courses of Ara-AMP. While the investigators were at a loss to explain the lack of efficacy of prolonged courses of Ara-AMP at the time these trials were reported, they have subsequently hypothesized that the anti-proliferative effects of Ara-A/Ara-AMP are likely to impart an immunosuppressive effect capable of interfering with the host immune response necessary to mediate an anti-HBeAg(+)→HBeAg(-), anti-HBe(+) seroconversion (56).

TABLE V

PROSPECTIVE, RANDOMIZED TRIALS OF ARA-AMP THERAPY OF CHRONIC HBV INFECTION CONDUCTED AT THE ROYAL FREE HOSPITAL IN LONDON

Report	Therapy	# of Patients	HBeAg(+), HBeAg(-) Anti-HBe(+)	Loss of HBsAg
			# patients at 12 months	
Weller et al (54)	Nil	14	0	0
	Ara-AMP[a] x 28d	15	4[b]	1
Lok et al (55)	Ara-AMP[a] x 28d	15	3	1
	Ara-AMP[a] x 49-56d	14	0	0
	IFN-α[c] x 84d	16	5	2

[a]Ara-AMP 5 mg/kg I.M. bid x 5 days, then 2.5 mg/kg I.M. bid
[b]$p<0.03$
[c]Lymphoblastoid interferon-α, 5-10 x 10^6 u/m^2/day I.M. for 5 days, then 7.5-10 x 10^6 units I.M. three times per week.

Interferon therapy of human diseases on a large scale first became a reality in the early 1980's with the availability of large quantities of these proteins produced by recombinant DNA technology. The 3 major classes of interferons are summarized in Table VI. While in early human trials hepatologists were hoping to utilize the anti-viral effects of these agents, hematologists and oncologists were assessing these agents for capacity to evoke anti-tumor immune responses. In reality the in vivo and in vitro effects of these agents are protean (see Table VII), and the mechanism whereby in vivo efficacy has been achieved has often not derived from initially hypothesized modes of action. Thus, as detailed in Table VII, both the interferon-alpha/interferon-beta and the interferon-gamma classes of interferon have inhibitory effects on viral replication, regulate growth and differentiation of a variety of human cells, enhance expression of HLA antigens, and modulate NK, B and T lymphocyte responses (57-60).

TABLE VI

DISTINGUISHING CHARACTERISTICS OF HUMAN INTERFERONS

Characteristic	α	β	γ
Source	Monocytes, B Cells	Fibroblasts	T,NK Cells
Genes			
Number	≥24[a]	1[b]	1
Chromosome	9	9	12
Sequence homology to α	70-100%	29%	Insignificant
Introns	0	0	3
Number of amino acids	165-166	166	146
Glycosylation	some species	Yes	Yes
Acid stability	Yes	Yes	No
Receptor			
Subunit Mr	130 kd, 110 kd	130 kd, 110 kd	70-80 kd
Chromosome	21	21	6
Specific antiviral activity	1.5x10^8 u/mg	1.1x10^8 u/mg	1-2x10^7 u/mg

See references 57, 58, 59.
[a]Encode ≥14 functional problems
[b]A second protein, initially designated interferon β_2, has been renamed Interleukin 6 and appears to have little intrinsic direct anti-viral activity.

TABLE VII

SELECTED ACTIONS OF INTERFERONS[a]

Functions	α	β	γ
Immunomodulatory Effects			
Boost NK cytotoxicity	+++	+++	+
Modulate B cell responses	++	++	++
Augment T cell functions	+	+	++
Macrophage activation	-	-	+++
Fever induction	++	++	++
Enhance MHC Class I expression	+	+	+++
Enhance MHC Class II expression	-	-	++
Anti-Viral Actions			
Degradation of viral mRNA	+++	+++	+
Inhibit viral mRNA and protein synthesis	+++	+++	+
Inhibit viral/cell membrane interactions	++	++	+
MX protein induction	++	++	-
Regulation of Cell Growth and Differentiation			
Inhibit oncogene expression	++	++	+
Inhibit cell proliferation	++	++	++
Regulate differentiation of hematopoietic cells	++	++	++

[a]See references 57-60.

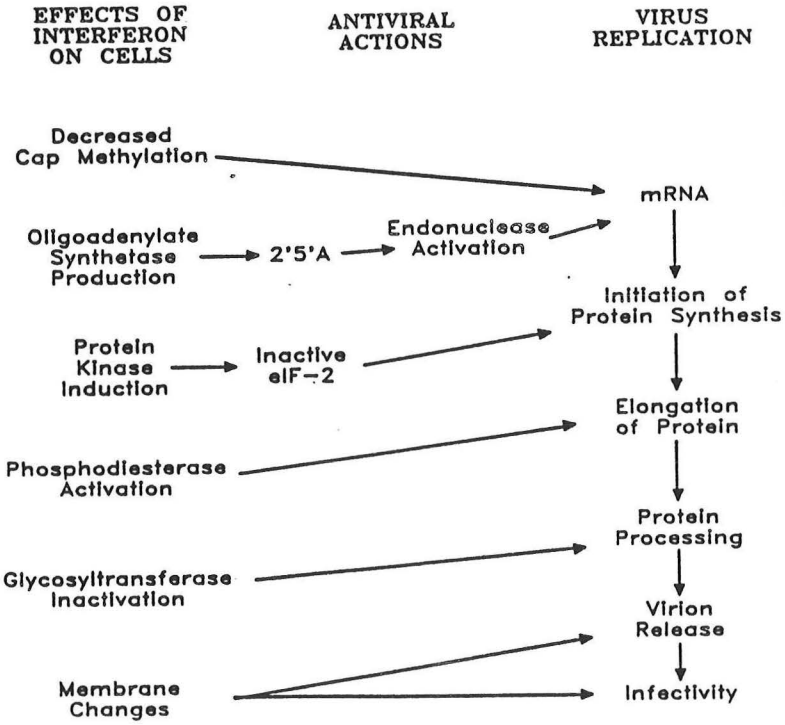
Interferons were initially defined by their capacity to inhibit viral replication, but as indicated by the data summarized in Table VI and VII, the multiple alpha interferons and the single beta-interferon gene are encoded by homologous genes clustered on the same chromosome, interact with the same cell receptor(s) and mediate virtually identical actions. While some minor species of interferon-alpha selectively lack some of the activities detailed in Table VII, the major alpha-2 species (including alpha-2a and alpha-2b) closely mimic the activity of more heterogeneous preparations of interferon-alpha produced from mixed leukocyte populations or lymphoblastoid cell lines. From a therapeutic viewpoint the major disadvantage of interferon-beta is that its pharmacokinetics are quite distinct such that, unlike interferon-alpha and interferon-gamma, it does not achieve detectable levels in the circulation after subcutaneous or intramuscular injection. In contrast to the similarities between interferons-alpha and beta, interferon-gamma has no sequence homology to the other interferons and is encoded on a different chromosome and interacts with a different receptor. While units of activity have been conventionally defined by effects on viral replication, interferon-alpha/beta species typically have 1-$2x10^8$ antiviral units of activity per milligram whereas the specific activity of interferon-gamma is 10-fold less. In contrast, the specific activity of interferon-gamma is 100-fold greater than that of interferon-alpha/beta when assessed for capacity to induce Class I MHC antigens (HLA-A,B,C).

Despite the uniform augmentation of in vitro NK cell mediated anti-tumor responses induced by a broad range of interferon-alpha doses, initial results of in vivo therapy indicated that NK tumoricidal activity was usually inhibited rather than augmented. Careful dose response studies indicate that NK function is augmented after single injections of 1-10 million units of human interferon-alpha but not after injection of $30x10^6$ units (61). However, when repetitive doses of interferon-alpha have been administered three times per week, doses of

1-10x10^6 units/m^2 have been suppressive of NK function (62,63), whereas augmentation was seen after administration of 0.1x10^6 units/m^2 t.i.w. Thus, in the dosages commonly used clinically, interferon therapy has an apparent inhibitory effect on NK function.

Similar effects of interferon-alpha on human B cell responses have been seen. Whereas lower doses of interferon-alpha augment in vitro human B cell responses, higher doses have been shown to be inhibitory (64,65). Following in vivo administration of 5x10^6 u/day or 10x10^6 u qod, both serum immunoglobulin levels and in vitro pokeweed mitogen-induced immunoglobulin production has been found to be significantly and uniformly inhibited (64-67). Similar effects on T cell responses to mitogens have been observed (66,67). However, in contrast to the apparent suppressive effects of high dose in vivo interferon-alpha administration on various measures of lymphocyte response, reproducible evidence of in vivo interferon-alpha induced enhancement of Class I MHC molecules has been accumulated. Thus, not only do serum levels of the Class I MHC associated molecule, $beta_2$-microglobulin, uniformly increase in HBV patients treated with daily or every other day doses of interferon-alpha, but hepatocyte HLA-A,B,C expression is enhanced as well (68). In one study of interferon-alpha treated chronic HBV patients, "responders" had significantly higher serum $beta^2$-microglobulin levels at week 2 but not at any of the other three time points at which this parameter was assessed during therapy (68).

Figure 2



In contrast to the generally poor correlation between efficacy of in vivo interferon therapy and capacity to stimulate lymphocyte responses, more predictable and uniform in vivo anti-viral activity has been observed. The anti-viral effects of in vivo interferon administration have been measured by a variety of direct and indirect measures. Thus, as detailed in Figure 2, the antiviral effects of interferons are mediated via a large number of mechanisms. However, two of the best characterized pathways are mediated indirectly via induction of 2′,5′-oligoadenylate synthetase activity. While all HBV infected patients treated with $>3x10^6$ unit/day of interferon-alpha have significant induction 2′,5′-oligoadenylate synthetase in peripheral blood leukocytes, the magnitude of this induction has been found to be significantly higher in HBV "responders" than in non-responders (69,70). Differences in the degree of interferon-induced reduction in serum HBV-DNA or DNA polymerase levels have also been observed between responders and non-responders (38). However, these latter differences are most marked only late in therapy at a time when putative immune-mediated activation of hepatitis is observed whereas responder/non-responder differences in 2′,5′-oligoadenylate synthetase have been observed within 24 hours after the first injection.

Despite increased belief in the need for "immunomodulation" of anti-HBV responses to achieve satisfactory responses to interferon therapy, virtually all dose modifications have been based on observed effects on markers of HBV replication and need to keep toxicity within acceptable ranges. Final modifications have been based on empiric observations of responses to various regimens. Thus, in very early studies it was observed that little additional decrease in serum HBV DNA levels or DNA polymerase activity could be achieved by administering doses in excess of $5\text{-}10x10^6$ u/day. In contrast, a variety of disabling side effects become much more prevalent following administration of higher doses. Not surprising, in light of the pleiotropic biological actions of the interferons the toxicities of these agents are legion. A summary of such toxicities is summarized in Table VIII.

Fortunately, most of the side effects of interferon therapy are reversible and dose dependent. Thus, adverse effects often subside with dose reduction and tend to completely disappear after therapy is discontinued (71). Fever and an influenza-like syndrome are observed almost uniformly after initial interferon-alpha doses of $>2x10^6$ units. However, these symptoms largely resolve within 2 weeks of onset of therapy without reduction in dose. Some degree of leukopenia (granulocytopenia > lymphopenia) and thrombocytopenia is observed in virtually all recipients of $10\text{-}30x10^6$ u/week of therapy for more than a few weeks duration, but in only a minority of patients is dose reduction required. However, cirrhotic HBV infected patients were noted to suffer a number of major bacterial infections during early trials (70). The appearance of a broad spectrum of neuropsychiatric complications including deficits in attention span and memory coupled with irritability, emotional lability, and insomnia are very frequent and evolve into major depressive or even psychotic illness in some patients (74-76). Depression has been a major cause for discontinuation of therapy (71). Frank seizures and coma fortunately have been observed largely in patients on unusually high dose regimens. Rare exacerbations of autoimmune diseases and especially Graves' disease have been observed in trials of interferon treatment of a variety of diseases and have occurred in recipients of relatively low doses of this drug (72,77). In at least some cases, retrospective analysis of stored sera revealed

TABLE VIII

SELECTED TOXICITIES OF PHARMACOLOGIC DOSES OF INTERFERON-α

Complication	Early (<2 weeks)	Late (>2 weeks)	Incidence on $9\text{-}30\times10^6$ units/week Rare (<5%)	Common (5-35%)	Frequent (35-100%)
Systemic					
Fever	+				+
Flu-like syndrome	+				+
Weight loss*		+			+
Fatigue*	+	+			+
Hematologic					
Leukopenia*		+			+
Thrombocytopenia*		+			+
Gastrointestinal					
Nausea	+			+	
Diarrhea*	+	+		+	
Hepatic					
Ascites, Variceal Bleeding, Encephalopathy**		+	+		
P450 enzyme inhibition	+	+			+
Neuropsychiatric					
Insomnia	+	+			+
Irritability*	+	+			+
Decreased Mental Acuity*		+		+	
Depression**		+		+	
Seizures, Coma**		+	+		
Autoimmune					
Graves Disease**		+	+		
Polyarthritis**		+	+		
Non-specific Autoantibodies		+			+
Other					
Alopecia		+		+	
Bacterial Infection**		+	+		
Cardiomyopathy**		+	+		

See references 34-39, 71-81
* Occurrence occasionally requires dose reduction
** Occurrence usually requires dose reduction or discontinuation of therapy

pre-existing evidence for subclinical autoimmune disease (77). However, newly detectable titers of a variety of autoantibodies as well as antibodies directed towards recombinant interferon-alpha species have been shown to develop in a number of patients, especially late in the course of prolonged therapy (78-80). However, in the majority of patients no abnormality in clinical symptomatology appears to be associated with appearance of these autoantibodies. Even the appearance of neutralizing anti-interferon antibodies has often not interfered with response to therapy in HBV infected patients, presumably because they often arise only late in therapy when an immunologically mediated seroconversion is already underway. Finally, in a number of HBV patients with advanced cirrhosis, the flare of hepatitis commonly seen with "response" to therapy has been associated with major new complications of liver disease that have been occasionally fatal. In light of these findings, patients with decompensated liver disease (jaundice, variceal bleeding, ascites, encephalopathy, coagulopathy) have been excluded from recent trials of interferon therapy.

After nearly a decade of experience with interferon therapy it became clear that interferon regimens in which patients received >30-40x10^6 units per week were rarely tolerated for prolonged time intervals. Moreover, as one month or shorter courses of 5-100x10^6 units/day of interferon-alpha were not found to be efficacious, it also became clear that longer therapeutic course needed to be examined. Finally, it came to be appreciated that interferon-alpha doses of >5x10^6 units/m^2/day offered little in the way of enhanced inhibition of HBV viral replication. In light of these findings a number of trials of more prolonged therapy of interferon-alpha were performed in patient populations in which at least some attempts had been made to exclude patients unlikely to respond.

Figure 3A

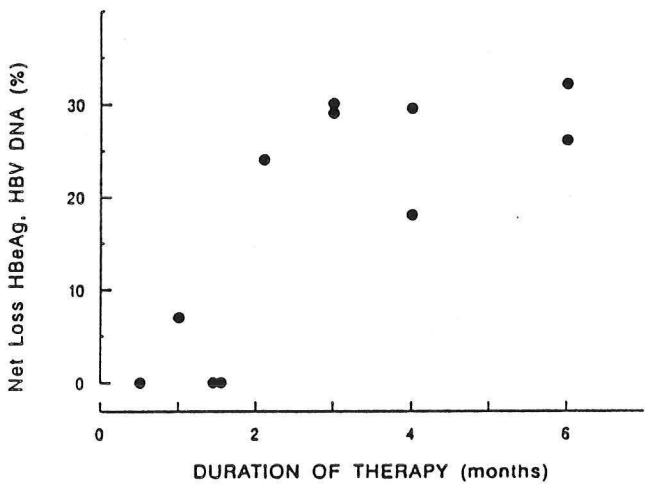
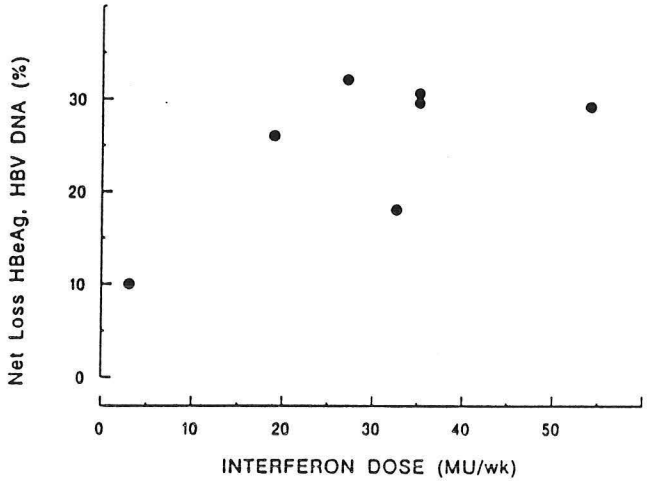


Figure 3B



The results of major large randomized prospective trials representing the "modern era" of anti-viral therapy of HBV infection are summarized in Table IX. The results of these studies, those in Table II and those of several additional studies (83-85) are plotted in Figures 3A and 3B to better illustrate the effect of dose and duration of therapy on interferon response. In particular, these results indicate that in American or European adult carriers of active hepatitis B, interferon-alpha therapy at a dose of 19-54x10^6 units per week for 3-6 months induces a net 27% mean increase in HBV DNA(+), HBeAg(+)→HBV-DNA(-), HBeAg(-), anti-HBe(+) seroconversions and a net 13% increase in HBsAg(+)→HBsAg(-) seroconversions. While this level of efficacy was relatively uniform from trial to trial, statistical significance was achieved only in trials containing >20 patients per experimental group. Of note, in two trials a steroid taper was employed prior to interferon therapy in an attempt to induce a post-steroid immunological rebound. No overall benefit relative to interferon-alpha therapy alone was noted (except perhaps in patients with relatively low pre-therapy ALT, see reference 39). All trials were able to show significant biochemical and/or histologic resolution of active hepatitis in responders that lost serum HBV DNA and HBeAg whereas spontaneous improvement in activity of hepatitis rarely occurred in untreated controls. Thus, by these criteria approximately 1/4 of treated patients appeared to derive net benefit from therapy. By examination of the data displayed in Figures 3A and 3B, this degree of benefit is seen in patients treated for $\geq$3 months with at least 20-30x10^6 units per week of interferon-alpha.

TABLE IX

RANDOMIZED, PROSPECTIVE CLINICAL TRIALS OF >3 MONTHS OF THERAPY WITH INTERFERON-α THERAPY IN CHRONIC HBV HEPATITIS

Author Year	Duration of IFN-α Therapy	IFN-α Therapy (mean dose per 1.8 m^2 pt)	# Patients in Group	HBV-DNA(-) HBeAg(-), anti-HBe(+)[a]	HBsAg(-)[a]
Alexander et al, 1987 (34)	6 mo	Nil	23	0 (0%)	0 (0%)*
		6.3 MU tiw	23	6 (26%)*	5 (22%)
Hoofnagle et al, 1988 (35)	4 mo	Nil	14	2 (14%)	0 (0%)
		5 MU qd	16	3 (32%)	0 (3%)
		10 MU qod	15	7 (32%)	1 (3%)
Perrillo et al, 1988 (36)	3 mo	Nil	21	3 (14%)	0 (0%)
		Pred.[b], 5 MU qd	18	8 (44%)	4 (22%)*
Saracco et al, 1989 (37)	6 mo	Nil	31	9 (29%)	1 (3%)
		9 MU tiw	33	20 (61%)*	8 (24%)*
Brook et al 1989 (38)	3 mo	Nil	34	1 (3%)	1 (3%)
		18 MU tiw	37	12 (32%)*	4 (11%)
Perrillo et al 1990 (39)	4 mo	Nil	43	3 (7%)	0
		1 MU qd	41	7 (17%)	1 (2%)
		5 MU qd	41	15 (37%)*	5 (12%)*
		Pred[b], 5 MU qd	44	16 (36%)*	5 (11%)*
Composite		No Therapy	166	18 (11%)	2 (1%)
		IFN-α	227	87 (38%)	32 (14%)
		Net Response		27%	13%

[a]Assessed at 12-18 months after onset of therapy
[b]Prednisone given at dose of 60 mg qd x 2 weeks, then 40 mg qd x 2 weeks, then 20 mg qd x 2 weeks during 6 weeks prior to IFN-α in first trial; and in same manner with additional 2 week rest prior to start of IFN-α in second trial.
*$p<0.05$

This level of response is clearly suboptimal with the majority of treated HBV carriers continuing to have active hepatitis and viral replication. However, it should be noted that in the trials listed in Table IX, not all predictably poor responders to therapy had been excluded. Thus, only the last trial was initiated after anti-HIV screening was readily available and this was the only trial in which all anti-HIV(+) patients were prospectively excluded. Therefore, if more selective criteria area used in selecting HBV infected patients for interferon-alpha therapy, high rates of response can be anticipated. Of note, however, in the trials of Hoofnagle et al (35) and the first Perrillo trial (36), some anti-HIV(+) patients were noted to respond to interferon therapy. In the latter trial (36) in which peripheral blood CD4(+) T cell counts were available, the two anti-HIV(+) responders were found to be asymptomatic patients with CD4(+) T cell counts >400/mm^3. Thus, no single criterion listed in Table IV can be used to absolutely predict failure to respond.

The results of these recent trials again have been analyzed in an effort to better determine predictors of response to interferon therapy. Although in the most extensive of such analyses, such parameters as being anti-HIV(-) and having CAH on liver biopsy were found to be associated with higher rates of response by univariate analysis (51), only low levels of serum HBV DNA, high AST levels and a history of acute hepatitis (at the onset of chronic HBV infection) were found

to be independent predictors of response when step-wise logistic regression analysis was performed. In a similar analysis of the exclusively anti-HIV(-) patients in the large multicenter U.S. trial reported in 1990 (39), only low serum HBV DNA levels and duration of chronic hepatitis were found to be independent predictors of response while by univariate analysis such additional factors as sexual orientation (homosexuals responded at less than half the rate of heterosexuals) and ALT levels at entry were also found to be associated with response to treatment. As summarized in Table X, Brook et al (51) have proposed that the most reliable combination of predictive factors in their patient population was negative anti-HIV status coupled either with a history of acute icteric hepatitis and AST >45 IU or no history of acute hepatitis and AST >85 IU. These criteria retrospectively predict response with 77% sensitivity and 79% specificity in this British patient population. Hoofnagle has proposed that ALT levels >2-fold the upper limit of normal and serum HBV DNA levels in the low-to-moderate range (<200 pg/ml by the Abbott assay used in the most recent trial of Perrillo et al) be used to identify candidates for interferon-alpha therapy of chronic HBV infection (see Table X). Thus, while the majority of chronic HBV carriers can be predicted not to benefit from this therapy, sufficient data is now available to better identify the subpopulation of potential responders to this therapy.

TABLE X

STRATEGIES FOR SELECTING HBsAg(+), HBeAg(+) PATIENTS LIKELY TO RESPOND TO INTERFERON-α THERAPY

Author(s)	Recommendations
Brook, et al (51)	Anti-HIV(-), (+) history of acute hepatitis, and AST >45 IU
	or
	Anti-HIV(-), (-) history of acute hepatitis, and AST >85 IU
Hoofnagle (50)	ALT >2-fold upper limit of normal, and HBV-DNA <200 pg/ml

What of other alternatives in the non-interferon-alpha responders? Trials of steroid withdrawal followed by interferon therapy are currently underway in Taiwan and Korea to determine whether this alternative approach will benefit Asian patients who currently constitute >80% of the world's HBV carriers and have yet to be shown to have significant response rates to interferon alone (43). Acyclovir is being studied in a therapeutic combination with interferon-alpha, but preliminary results indicate no impressive benefit over historical controls treated with interferon alone (82). Both interferon-beta and interferon-gamma have been shown to have in vivo efficacy in decreasing rates of HBV viral replication (86-88). However, interferon-beta therapy has been limited to relatively short courses (≤1 month) by requirements for I.V. administration. Interferon-gamma appears to offer no advantage over interferon-alpha in direct anti-viral effects when used alone or in combination with interferon-alpha and may have greater in vivo side effects when used in equivalently effective doses (88). It remains to be determined whether the theoretically distinct immuno-

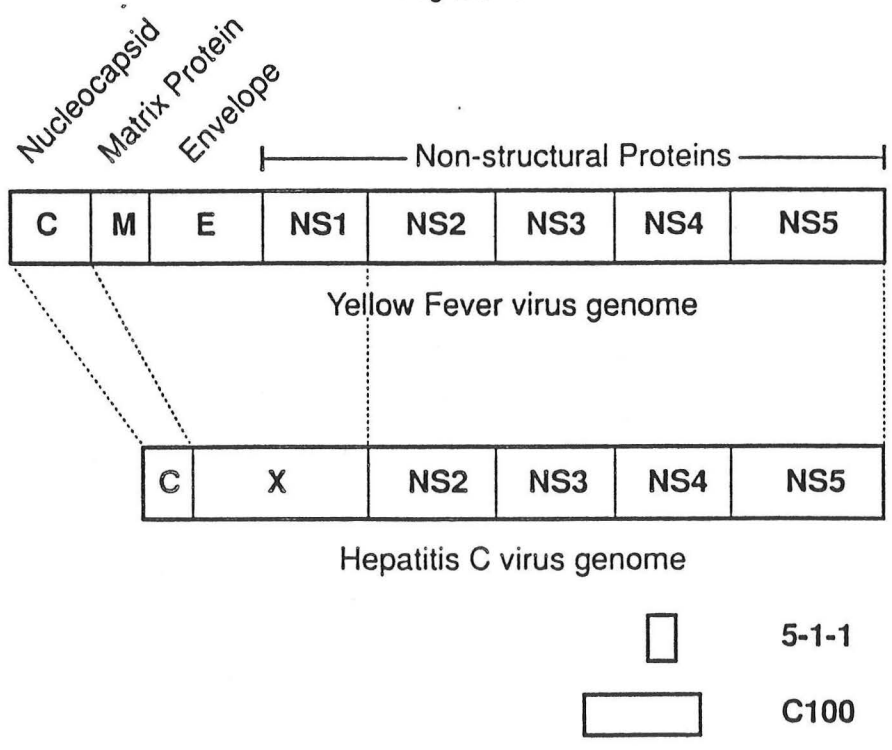
modulatory capacity of interferon-gamma will be of any benefit in this disease. A variety of other agents such as thymosin alpha$_1$ (89), a thymic peptide with a variety of immunomodulatory properties including enhancement of interferon-gamma production, and a variety of bacterial preparations with proposed capacity for in vivo induction of interferon production have been suggested to be potentially effect agents in chronic hepatitis B infections on the basis of results of either uncontrolled trials or very small controlled trials (90). Results of larger randomized, prospective control trials are needed before any of these regimens can be considered as a therapeutic option.

What of HBsAg(+) but HBeAg(-) patients who have abnormal ALT levels or other evidence of active hepatitis? The majority of HBsAg(+) but HBeAg(-) patients continues to have detectable HBV-DNA in serum by polymerase chain reaction (PCR) based assays (90a), but only a minority have detectable HBV-DNA by non-PCR assays. These latter patients can have active hepatitis and progressive liver disease but appear not to respond to interferon therapy (90b).

INTERFERON-ALPHA AND CHRONIC HEPATITIS C

In contrast to our extensive knowledge of the HBV genome and the human immune response to this agent, our level of understanding of the biology of the hepatitis C virus (HCV) is still quite rudimentary, albeit far advanced relative to that of 2-3 years ago. This single-stranded RNA virus accounts for the bulk of the sporadic or parenterally transmitted cases of acute non-A, non-B hepatitis in this country and probably is the single most common etiology of chronic hepatitis in the U.S (91). Figure 4 schematically details our current knowledge of the HCV genome, its homology to a member of the flavivirus family, and the genetic source of two of the recombinant viral antigens used in early development of serodiagnostic assays for HCV.

Figure 4



The HCV virus appears to be a very distant relative of the flavivirus family which includes such agents as the Yellow Fever virus and the Dengue Fever virus. These viruses possess a positive-stranded RNA genome that encodes a polyprotein precursor of viral proteins that are produced by enzymatic cleavage mediated by host-encoded and virus-encoded proteases. The N-terminus of this polyprotein precursor of flaviviruses includes 3 structural proteins including the envelope protein to which neutralizing antibodies are directed. A small domain of the N-terminus of the HCV polyprotein contains significant homology to the nucleocapsid (C) proteins of flaviviruses. However, the adjacent "X" region of the HCV polyprotein precursor appears quite different both in size and hydrophobicity from the matrix, envelope and non-structural protein 1 that occupy similar positions in flaviviruses. However, the non-structural proteins 2-5 of the Yellow Fever virus appear to have homologous counterparts in the HCV polyprotein.

The currently available anti-HCV ELISA assays seek to detect antibody responses directed at the C-100 polypeptide spanning portions of two putative nonstructural HCV proteins (91). This viral antigen is produced as a fusion product of human superoxide dismutase (SOD) and C-100. Initial experience with this test indicates that it will almost certainly be of significant value as a screening test for eliminating potentially infectious blood products. However, this assay has major specificity and sensitivity problems. Patients with well documented chronic post-transfusion hepatitis and HCV-RNA detected in liver and or serum by PCR assays have been found to be anti-HCV negative (92). The developers of this test now estimate that it detects only 80% of cases of HCV infection (91). In addition to apparent false positive results in hypergammaglobulinemic patients (19,93), half or more of anti-HCV(+) volunteer blood donors do not react positively in a second generation confirmatory test (94). This test, termed the Recombinant Immunoblot Assay (RIBA), screens for reactivity to the SOD/C-100 fusion protein expressed in yeast, a highly antigenic subsequence of C-100 (5-1-1) expressed in Escherichia Coli as a fusion protein with SOD and finally human SOD alone. Antibody binding to SOD/C-100 and SOD/5-1-1 and not to SOD are considered true positives whereas binding to SOD suggests false positivity. The RIBA confirmatory test has been shown to discriminate between infectious and noninfectious anti-C-100 positive blood donor units (95) and is reactive in >90% of multitransfused ELISA anti-HCV positive hemophiliacs (96). Yet less than half of Midwestern U.S. blood donors excluded on the basis of anti-HCV ELISA reactivity have been found to be RIBA (+) (94). Thus, this test is of high predictive value only when applied to subjects with clinical or historical factors suggesting reasonable likelihood of HCV infection. Hopefully, a more specific commercially available confirmatory test will soon be on the market.

However, the clinical experience with interferon therapy began before the availability of the anti-HCV test. In the three randomized prospective trials detailed in Table XI, patients were selected on the basis of historical, biochemical and histologic criteria for post-transfusion chronic non-A, non-B hepatitis. However, the majority were found subsequently to be anti-HCV(+), and thus the results appear indicative of the effects of this therapy in disease caused by HCV infection. Of note, response rates for anti-HCV(+) and anti-HCV(-) individuals in these trials were quite similar. However, as anti-HCV(-) patients

viral hepatitis, such patients could either represent cases of HCV infection missed by the insensitive anti-C-100 test, or could have another form of non-A, non-B, non-C viral hepatitis.

TABLE XI

RESULTS OF RANDOMIZED, PROSPECTIVE TRIALS OF INTERFERON-α_{2b} THERAPY OF CHRONIC NON-A, NON-B HEPATITIS (HEPATITIS C)

Authors	% Patients Anti-HCV(+)	IFN-α_{2b} Therapy	Patients in Group	Response During Therapy: Complete[a]	Response During Therapy: Partial[a]	Sustaining Normal ALT off Therapy[b]
Di Bisceglie et al (72)	90	Placebo	20	<10%[c]	<10%[c]	NT[d]
		2 MU tiw x 24 wk	21	16 (48%)	3 (14%)	2 (10%)
Davis et al (73)	86	Nil	51	2 (4%)	2 (2%)	NT
		1 MU tiw x 24 wk	57	9 (16%)	7 (12%)	12%[e]
		3 MU tiw x 24 wk	58	22 (38%)	4 (7%)	22%[e]
Schvarcz et al (97)	67	Nil	12	0 (0%)	4 (33%)	0 (0%)
		3 MU tiw x 36 wk	19	11 (58%)	4 (21%)	4 (21%)

[a]Complete response defined as normal ALT at end of therapy or mean ALT normal during therapy. Partial response defined as >50% reduction in ALT to values <1.5 times upper limit of normal.
[b]Assessed after 6 months off therapy
[c]These results not explicitly stated; complete response rate inferred from graph in manuscript.
[d]Control patients not followed for 6 months after end of therapy
[e]Six month response rate estimated by statistical analysis of patients followed 2 to 46 weeks after therapy.

Candidates for these trials were selected on the basis of sustained >1.5-2-fold elevations of serum ALT and were found to have only rare spontaneous normalization of ALT values during the ensuing 6-15 months of follow-up. In contrast, 38-60% of recipients of 2-3x10^6 units tiw of interferon-alpha were noted to have complete normalization of ALT values on therapy. Unfortunately 80% of responders to 2x10^6 units tiw and approximately 50% of those treated with 3x10^6 units tiw relapsed during the first 6 months after therapy was discontinued. In all 3 of these trials, at the end of therapy, the groups of patients treated with 2-3x10^6 units tiw of interferon-alpha had statistically significant decreases in serum ALT levels and improvement in histologic parameters of acute hepatic injury (piecemeal necrosis and lobular inflammation). In addition to these published results of completed randomized trials, a large number of preliminary reports of trials with similar results have appeared in the literature. The results of some of these ongoing studies (114,115) in combination with those detailed in Table XI are displayed graphically in Figures 5A-B. These results indicate that recipients of $\geq$2x10^6 units tiw of interferon-alpha have a relatively uniform 40-60% rate of complete ALT normalization while on therapy. Following discontinuation of therapy relapse rates are approximately 50% in recipients of $\geq$3x10^6 u tiw and even higher in recipients of lower doses. Early results do not indicate significant improvement in sustained remission rates by extending initial therapy to 9-12 months (116). It remains to be determined whether increasing the daily dose of interferon-alpha or other modifications of dosage schedules will induce increased rates of apparent remissions in HCV hepatitis. Of note, however, patients that relapse following discontinuation of therapy have been reported to uniformly respond to reinstitution of interferon (98).

Figure 5A

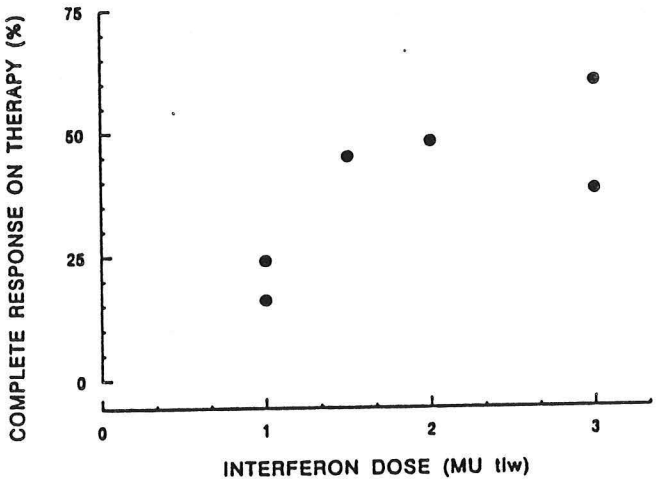
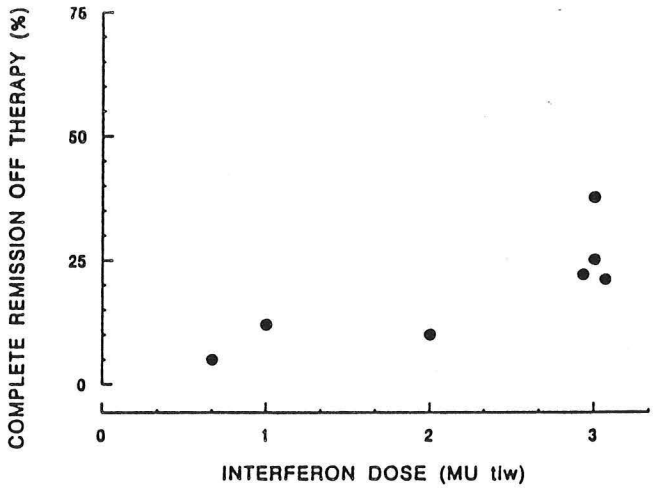


Figure 5B



The pattern of response to interferon in HCV infected patients is quite different than that described in cases of chronic hepatitis B. As shown schematically in Figures 6A and 6B, HBV infected responders to interferon characteristically have flares of hepatitis as indicated by up to 10-20 fold elevations in ALT values. These episodes typically occur between the 6th and 12th week of therapy and herald clearance of HBeAg and HBV-DNA. In contrast, as detailed in Figure 6, the typical non-A, non-B (HCV) viral hepatitis patient either has only a modest decrease in ALT following institution of interferon therapy (partial or non-responders) or within the first 4-12 weeks has a progressive decline of ALT levels into the normal range (72,73).

Figure 6A

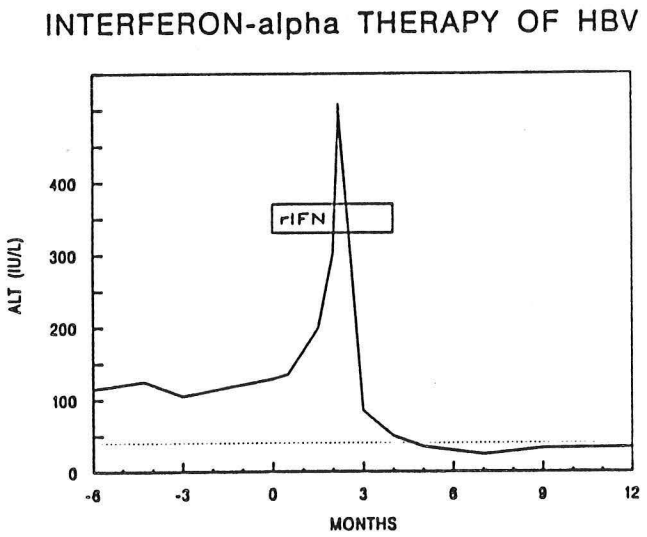
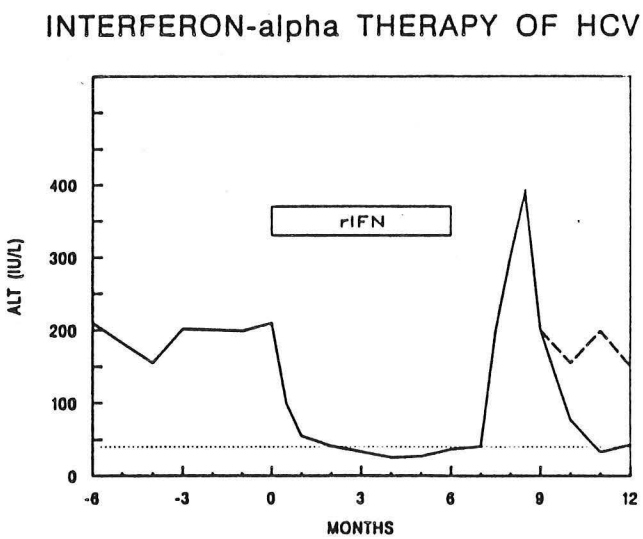


Figure 6B



Following cessation of therapy, ALT values either remain normal (sustained response), gradually rise to pre-therapy levels (relapse), or in 35-40% of initial responders an abrupt flare of hepatitis occurs with elevation in transaminases to levels >8-fold the upper limit of normal (see Figure 6B). Some of these hepatitis flares are following by return to pre-therapy levels of hypertransaminasemia, while in other patients ALT values again normalize. In a French study (99), 2 of 16 patients with post-interferon flares experienced a first episode of ascites (both with underlying cirrhosis). While the precise mechanism of such post-interferon flares is unclear, the early fall in ALT levels following initiation of interferon therapy in hepatitis C has suggested that a cell-mediated immune response, such as has been proposed in HBV, is unlikely to play a role in the initial response to interferon in HCV infected patients. Rather, it has been suggested that unlike HBV, HCV is a directly cytopathic virus that elicits only a very modest host immune response and thus therapy induced declines in ALT levels directly reflect interferon inhibition of viral replication. Early results of semi-quantitative PCR assays of serum HCV RNA during the course of interferon therapy seem in agreement with this hypothesis as levels fall as ALT levels decline during therapy and reappear or rise during post-therapy relapses (100-101). At least three severe episodes of hepatitis have now been reported to occur 4-10 weeks after onset of interferon-alpha therapy for putative non-A, non-B hepatitis. One of these patients (102) progressed to hepatic coma and death (a 17 year old anti-HCV(+) female) while the other two patients recovered after institution of steroid therapy (103). No such episodes were reported in the trials detailed in Table XI.

IMPLICATIONS FOR CURRENT PATIENT MANAGEMENT

Interferon-alpha is licensed in the U.S. for treatment of condylomata acuminata (Alferon N, human leukocyte derived IFN-n3; and Intron-A, recombinant IFN-2b), hairy cell leukemia (Intron-A and Roferon-A, IFN-2a) and Kaposi's sarcoma (Intron-A, Roferon-A), but at the time that this protocol was written, these agents have not been licensed for use in viral hepatitis though this situation seems likely to change in the near future. Aside from these considerations, what conclusions can the individual physician draw from the currently available data regarding use of interferon-alpha in chronic hepatitis? Certainly there are ample and consistent data indicating that approximately 25% of patients with either chronic hepatitis B or C will respond to a 4-6 month course of interferon-alpha therapy with a sustained improvement in markers of active hepatitis. However, there is as yet no direct evidence that these patients will benefit with a reduction in frequency of complications of chronic liver disease or prolongation of life. In the design of recent trials of antiviral therapy of chronic hepatitis, the follow-up of untreated controls has typically been terminated after 6-12 months, at which time control patients have been offered interferon therapy. Therefore, there is unlikely to be meaningful follow-up information from these studies that will give direct answers to these questions. Even follow-up comparisons of the clinical courses of responders versus non-responders may not be very informative. Thus, there are clear-cut biological differences between HBV interferon responders and non-responders that can be identified before therapy (15) and which may play as much of a role in determining long-term outcome as does the effect of interferon. A similar

argument can be made regarding the scientific validity of such analyses of interferon treated HCV patients. Thus, at present and for the foreseeable immediate future, recommendations regarding use of interferon therapy must be based on a variety of inferences.

Proponents of aggressive antiviral therapy of hepatitis B point out that in HBV infected Chinese patients, an estimated 50% of males and 15% of females die of a complication of their liver disease (110). Moreover, in a large study of U.S. patients with hepatitis B related histologic lesions of chronic persistent hepatitis (CPH), CAH, or CAH + cirrhosis, 5 year survival rates were 97%, 86% and 55%, respectively, with 50%, 75% and 70% of deaths in these patient groups being clearly related to complications of liver disease (104). However, it should be pointed out the much of the excess mortality in HBV(+) Chinese males relates to the high rate of hepatocellular carcinoma (HCC) in this population, and it has been estimated that even after HBeAg(+)→HBeAg(-) conversion the rate of HCC development in this population is 2% per year (43). Nevertheless, the rate of death from liver failure alone in U.S. HBV infected individuals is such that even if interferon prevents only a fraction of such deaths, treatment of patients with HBV related CAH +/- cirrhosis is quite likely to lead to improved survival from this disease. Fortuitously, it is these patients with active disease that are among those most likely to respond. In contrast, the patients with low transaminases who are the least likely to respond to therapy are also unlikely to progress to major complications of liver disease during short-term follow-up. Therefore, therapy should be deferred in such patients until such time as their disease evolves into a more active stage, at which time there is both a better indication for therapy and a higher likelihood of response.

A more distressing group of patients are those with decompensated liver disease. All recent controlled trials have excluded such patients (34-39, 72,73,97). While these patients may seem to be the group in greatest need of therapy, they have also been observed to suffer the highest frequency of iatrogenic complications. In a recent report of the outcome after interferon therapy in a small group of such patients (105), it was noted that despite a very high rate of complications (mainly bacterial infections, decompensation of liver disease, and neuropsychiatric problems) that required frequent modifications in interferon dosage, long-standing remissions were achieved in 5 of 12 such patients in whom more than one year of follow-up was available. Moreover, these patients exhibited significant recovery of liver function after clearing HBeAg and HBV-DNA. In contrast, 6 of the other patients either have died or received liver transplants. Hopefully, additional published experience in treatment of such patients will provide meaningful recommendations regarding use of interferon therapy in this patient population.

In contrast to the wealth of data regarding the subgroups of HBV patients likely to benefit from interferon therapy, much less information is available regarding factors predicting response to interferon in HCV related liver disease. Moreover, the implications of responses defined only by normalization of ALT and decreased necroinflammatory activity on liver biopsy are less clear than those that can be drawn from the serologically defined responses in HBV patients. Hopefully, in the near future more data regarding hepatic HCV-RNA levels in putative responders will become available. This is particularly important because the natural history of HCV suggests that this may be a more indolent, insidiously

progressive disease than is either AICAH or chronic active hepatitis B. Long-term follow-up studies of patients with post-transfusion non-A, non-B hepatitis have shown that 20-50% progress to cirrhosis within 5-10 years of follow-up (106,107), yet at 10-15 years of follow-up, only 5-15% develop symptomatic liver disease (107-108). In preliminary reports from an ongoing analysis of 3 large, prospective studies of non-A, non-B hepatitis in transfusion recipients (109), no increase in mortality associated with post-transfusion hepatitis has yet been demonstrated after 10-20 years of follow-up. Yet it is quite clear that individual patients do die of liver failure or hepatocellular carcinoma associated with chronic HCV infection. As shown by the results of a recent retrospective analysis of Japanese hepatitis C patients, a mean of 10, 21 and 29 years elapsed between blood transfusion and development of clinically apparent chronic non-A, non-B hepatitis, cirrhosis, or hepatocellular carcinoma, respectively. In this study, as in previously referenced U. S. studies of chronic non-A, non-B post-transfusion hepatitis, stored sera revealed that >80% of patients were anti-HCV(+), and thus these natural history data appear representative of hepatitis C. Thus, a major problem in interpreting survival data accumulated from post-transfusion studies is that transfused patients generally represent an older patient population with pre-existing diseases. Therefore, mortality rates from non-liver diseases are high (107) and crude mortality rates may not reflect the impact of HCV disease. In addition, data from a variety of studies suggest that risk of progression to cirrhosis is much higher in older patients. In one study in which patients with chronic non-A, non-B viral hepatitis have been followed for a mean of nearly 5 years (106), only 16% of patients with disease onset prior to age 30 progressed to cirrhosis whereas cirrhosis was present in 47% of patients contracting the disease after age 30. Whatever the inadequacies of present clinical data, in all studies of this disease, a significant fraction of patients have relatively benign non-progressive disease over at least 5-10 years of follow-up. Thus, it has yet to be demonstrated that HCV infection actually causes a significant disease in many of the individuals chronically infected with this agent. Yet, other individuals clearly do progress to cirrhosis and/or hepatocellular carcinoma and have symptomatic and/or fatal complications of this infection.

At present, outside of factors such as age and histologic evidence of advanced or progressive liver disease, there are few guidelines for determining which patients are likely to develop major complications from chronic infection. Thus, degree of ALT elevation has not been found to be a good predictor of severity of histologic lesions or risk of progression to cirrhosis (107). Prior histologic classification systems in which biopsies are classified simply as showing CPH versus CAH have also proven of less value in chronic hepatitis C than in autoimmune liver disease. Thus, some patients initially classified as having lesions of CPH have been found to occasionally progress to cirrhosis, thus indicating that this is not a totally benign lesion (113). Use of more quantitative numerical scoring systems such as that described by Knodell et al (112), however, have proven to be of greater value both in predicting risk of progression to cirrhosis and in assessing course of disease in individual patients (113).

Thus, chronic hepatitis C is an insidiously progressive disease that often presents as symptomatic liver disease only after 10-20 years or more of chronic infection. For this reason, while interferon therapy appears to be effective in inducing biochemical and histologic remissions in a subset of patients, it is difficult to determine whether all patients truly need therapy. In particular, in asymptomatic patients with mild non-fibrotic histological lesions, it is not clear that there is a clear-cut rationale for use of interferon therapy outside of clinical trials at the present time. There are clearly other patients, however, in whom current use of interferon therapy seems to be the most reasonable approach. For instance, older adults with severe CAH or fibrotic lesions and therefore clear-cut evidence of risk of progression to complications of liver disease would seem to be a population in which intervention is most likely to be of benefit. Clearly, recommendations regarding the use of interferon therapy in this disease are likely to continue to evolve as more data regarding its efficacy and the natural course of HCV infections becomes available.

When contemplating use of interferon in patients with putative chronic hepatitis C, it is important to remember that the presence or absence of a positive anti-HCV test hardly confirms or refutes this diagnosis. While non-invasive diagnostic criteria may be adequate to confirm the presence of chronic HBV infections and assess indications for interferon therapy, in most patients with presumed chronic hepatitis C, a liver biopsy is at present one of the best diagnostic aides in both confirming that the patient actually has hepatitis and in assessing the risk for progressing to symptomatic liver disease. In addition, not only is it important to screen for other potential etiologies of liver disease in general, it is also important to consider the possibility of AICAH in such patients. Finally, unlike treatment of hepatitis B in which well defined 3-4 week courses of therapy seem most appropriate, the current data suggest that the duration of therapy in chronic hepatitis C should be modified to fit individual patient responses. In particular, as non-responders generally become apparent by persistence of abnormal ALT's beyond the first 2-3 months of therapy (73), therapy should probably be terminated at that point in non-responders, as continuation will only increase the risk of side effects without significant chance of benefit. In patients who appear to respond to 6 month courses of therapy, the next major decision regarding therapy will come if a relapse occurs. Because some patients have a post-therapy rebound in transaminases followed by return to normal ALT values, these cases should be followed for some period of time before considering re-institution of therapy. In those patients who clearly have a sustained relapse, decisions regarding re-institution of therapy must again be determined by such factors as perceived "need to treat" and the patient's tolerance of interferon therapy. Clearly, there is still a great need to learn more about the role of interferon therapy in this disease, and whenever possible it is important that physicians continue to enroll their patients in controlled trials.

SUMMARY

Interferon-alpha therapy now represents a major new therapeutic option for patients with chronic hepatitis. As has been previously demonstrated in therapy of AICAH, a good deal of clinical discretion must be exercised in selecting patients for this therapy with its many systemic side effects. Such decisions are more straightforward in HBV infected patients because of the availability of superior diagnostic tests. In contrast, in patients with HBsAg(-) chronic hepatitis, significant difficulties remain in determining whether a patient is a more appropriate candidate for interferon or immunosuppressive therapy, or whether such a patient needs any therapy. Finally, despite encouraging results obtained in "responders" to interferon, present findings indicate that the majority of patients with chronic viral hepatitis will not receive significant sustained benefit from this form of therapy.

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