ANTIVIRAL COMBINATION THERAPY

IN CHRONIC HEPATITIS B

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ANTIVIRAL COMBINATION THERAPY IN CHRONIC HEPATITIS B

(ANTIVIRALE COMBINATIE THERAPIE BIJ CHRONISCHE HEPATITIS B)

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aan Anne Marijke
aan mijn ouders

Contents

List of abbreviations

ACV	acyclovir
AIDS	acquired immune deficiency syndrome
ALT	alanine aminotransferase
ARA-A	adenine arabinoside
ARA-AMP	adenine arabinoside monophosphate
AST	aspartate aminotransferase
anti-HBc	antibodies against hepatitis B core antigen
anti-HBe	antibodies against hepatitis B e-antigen
anti-HBs	antibodies against hepatitis B surface antigen
anti-HBx	antibodies against hepatitis B x-antigen
anti-preS1	antibodies against hepatitis B pre-S1 antigen
anti-preS2	antibodies against hepatitis B pre-S2 antigen
CAH	chronic active hepatitis
CPH	chronic persistent hepatitis
cpm	counts per minute
DACV	descyclovir
DNA	deoxyribonucleic acid
DNA-p	deoxyribonucleic acid polymerase
ELISA	enzyme linked immunosorbent assay
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B s antigen
HBxAg	hepatitis B x antigen
HDV	hepatitis delta virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IFN	interferon
IgG	immunoglobulin G
IgM	immunoglobulin M
nm	nanometre
RIA	radioimmunoassay
RNA	ribonucleic acid

Chapter one.

Introduction.

As our knowledge increases so does the circumference of darkness.
(A.Einstein)

Chapter one.

Introduction.

1.1 The hepatitis B virus:history.

An outbreak of parenterally transmitted hepatitis was probably first recorded in 1885 by Lurman who reported the occurrence of jaundice among personnel of a Bremen factory after revaccination against smallpox.Of 1289 individuals vaccinated in one day,191 developed jaundice 2 to 8 months after administration of glycerinated human lymph preparations.The illness usually began with fatigue,anorexia and gastrointestinal complaints followed by jaundice and often pruritus;it generally lasted a total of 4 to 6 weeks.Personnel vaccinated on another day with another vaccine preparation as well as those who left the job before revaccination were not affected.Comparison of the water supply, domicile,alcohol abuse and vaccine exposure indicated the latter as the probable cause of the outbreak (1,2,3).In 1945 MacCallum postulated that,on the basis of differences in incubation period and mode of transmission,two different agents cause hepatitis: hepatitis A and hepatitis B.He was not able to isolate the infectious agents (4).In 1967 Krugman and Giles confirmed the existence of two types of hepatitis:one with a short and one with a long incubation period (5).In 1965 Blumberg had already discovered an antigen in the serum of an Australian aboriginal which he called 'Australia antigen' (6).In 1968 Prince identified an antigen in the serum of patients with post-transfusion hepatitis,an antigen which he called SH antigen (7).The antigens discovered by Blumberg and Prince were found to be identical and represent the hepatitis B surface antigen.Between 1968 and 1973 the other principal viral antigens (HBeAg,HBcAg) and their antibodies were identified (8,9).The electron microscopy features of the virus were described by Dane in 1970.In the blood of infected patients the large complete virus particle (diameter 42 nm),small 22 nm spherical surface antigen particles and tubular forms (length 100 nm,diameter 22 nm) were found (10).Infection with the hepatitis B virus is characterized not only by production of infectious complete virus particles (Dane particles) but also by an enhanced production of incomplete viral particles made up entirely of HBsAg without HBcAg,DNA-polymerase activity or HBV-DNA.

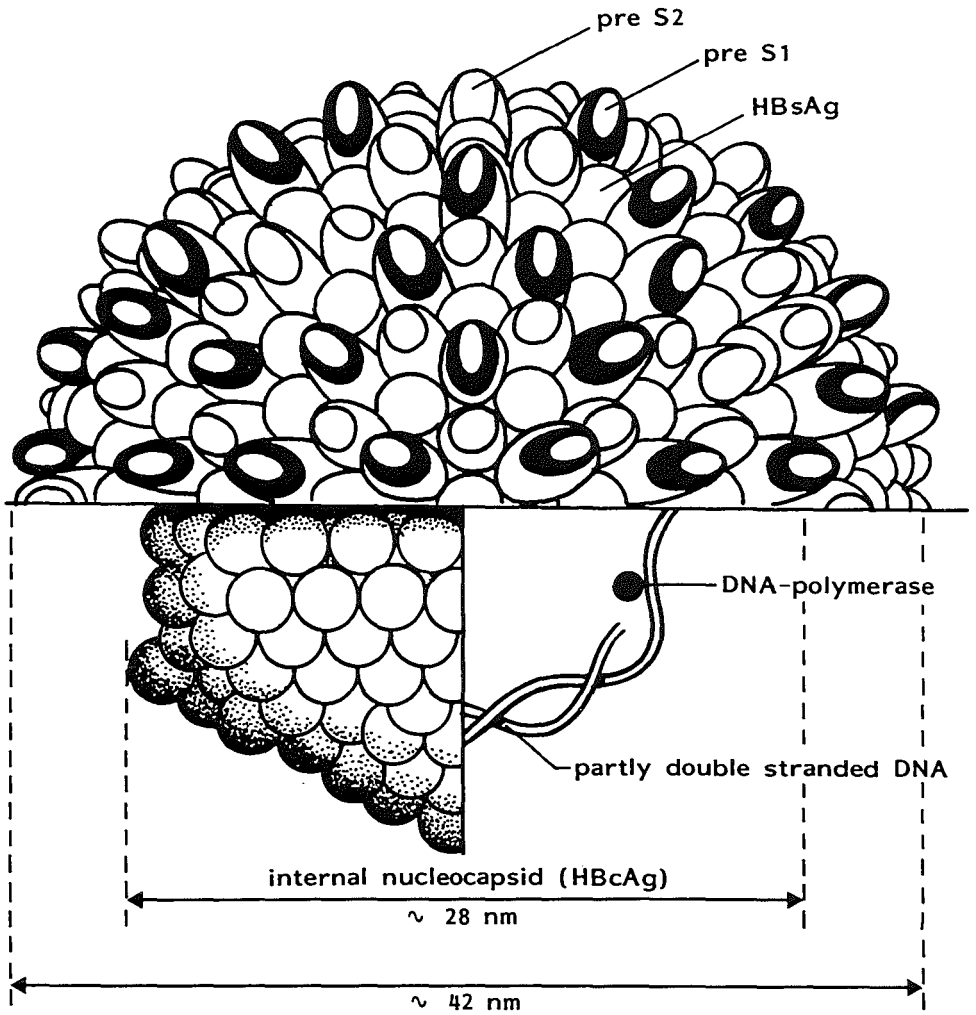


fig 1.Schematic representation of the hepatitis B virus.The outer shell contains the pre-S1,pre-S2 and surface antigens;a core particle contains the associated antigens HBeAg and HBcAg, nucleus-associated enzyme DNA-polymerase and partly double stranded circular DNA (partly from Neurath).

1.2 The hepatitis B virus.

Hepatitis B virus is a DNA virus that belongs to a family of viruses called hepadna viruses (11,12).This family includes the woodchuck hepatitis virus,ground squirrel hepatitis virus,tree squirrel hepatitis virus and Peking duck hepatitis virus (13,14, 15,16).Hepadna viruses are hepatotropic and tend to cause chronic infections;some of them have been associated with the development of hepatocellular carcinoma.HBV can cause infections in humans and certain non-human primates (chimpanzee and gibbon).The animal viruses do not appear to be infectious in man (17).

The stucture of HBV is shown in figure 1.It has an outer envelope composed of three related proteins:hepatitis B surface antigen (HBsAg:Australia antigen),pre-S1 and pre-S2.Compared with HBsAg the pre-S1 and pre-S2 proteins are preferentially incorporated into the complete viral particle.All three forms of HBsAg (HBsAg, pre-S1,pre-S2) can become glycosylated.Protein analysis of HBsAg in serum can therefore yield HBsAg in six different sizes (18).

The inner component of the infectious virion contains the core antigen (HBcAg:core antigen).Inside the core particle is a single molecule of circular,partially double-stranded DNA and an endogenous DNA-polymerase which also possesses reverse transcriptase activity.A third viral antigen associated with HBV is the hepatitis B 'e' antigen (HBeAg).HBeAg is a soluble protein closely related to core antigen.Disruption of core particles by detergents or proteolytic enzymes releases HBeAg reactivity.The presence of HBeAg in blood is associated with high levels of viral replication (19).A fourth viral antigen is the x-protein: the exact function of this polypeptide is still unknown.The antigens HBsAg,pre-S1,pre-S2,HBcAg,HBeAg,HBxAg all can induce a specific antibody response,although the x-protein is not found in hepatitis B particles nor in any other form in blood.

The HBV genome is the smallest of any of the viruses known to infect man.The DNA molecule contains partially double-stranded circular DNA,which is 3200 base pairs in length.A striking feature of the hepatitis B virus is the presence of overlapping gene sequences which enables the virus to produce more unique protein per genome unit than other viruses (20).

1.3 Chronic hepatitis B:impact on public health.

On the basis of longitudinal studies of patients with hepatitis B,chronic hepatitis B has been defined as persistence of hepatitis B surface antigen in the circulation for more than six months.The number of HBsAg-positive patients worldwide is estimated to be 200 million,25 % of whom will die as a result of a liver-related affliction (21).Primary hepatocellular carcinoma is one of the ten most common carcinomas in the world with an estimated yearly incidence of 250,000 cases (22).HBsAg-positive patients run an increased risk of developing hepatocellular carcinoma whereby the development of cirrhosis is an independent risk factor (21,23).

1.4 Chronic hepatitis B:natural history.

The natural history of the infection can be considered to consist of three phases (24):

1.An initial period of high infectivity accompanied by various degrees of inflammatory activity in the liver.Serum AST levels are elevated and HBeAg,HBV-DNA and DNA-polymerase are present in the serum.In liver tissue HBcAg and HBV-DNA can be found.This period lasts for several years (fig 2,left panel).

2.A transitional phase during which viral replication is reduced, HBeAg and often HBV-DNA are lost and increased lobular inflammation is seen in the liver.The loss of HBeAg is often accompanied by a flare in hepatitis activity which can cause severe liver damage if prolonged (25) (fig 2,right panel).

3.A long-term phase of low infectivity with resolution of liver inflammation and detectable hepatitis B e antibody.Subsequently HBsAg may be lost.In phase 3 reactivation of HBV infection may occur with reappearance of replication markers (fig 2,right panel) (26).The incidence of reactivation may be higher for those with acquired immunodeficiency.

These complex aspects of the disease have rendered the differentiation between the healthy HBsAg carrier state and chronic type B hepatitis with the subcategories chronic persistent and chronic active hepatitis outdated (27). Classification of chronic type B hepatitis is now based on the

presence or absence of HBV replication and liver cell inflammation.HBV replication can be assessed in serum by measuring HBV-DNA,DNA-polymerase activity or HBe-antigen and in the liver by immunologic detection of HBc-antigen.Routine measurement of HBV replication is most easily done by measuring HBeAg in serum and HBcAg in liver because of the general availability of these tests.Liver cell inflammation can be assessed by serum aminotransferase activity (AST).HBV replication is usually associated with an elevated AST and absence of HBV replication with normal AST levels (25,28,29).An elevated AST together with a negative HBeAg test points to low-level HBV replication (liver HBcAg positive,serum HBV-DNA positive) or to concomitant disease such as hepatitis D infection (delta agent), hepatocellular carcinoma,autoimmune reactivity,alcoholism,drug toxicity,schistosomiasis,non-A non-B hepatitis or metabolic disease (30,31).

In general chronic hepatitis B can thus be classified as:

1.HBeAg-positive chronic hepatitis B (CHBe+).

2.HBeAg-negative,AST positive chronic hepatitis B (CHBe-/AST+).

3.HBeAg-negative,AST negative chronic hepatitis B (CHBe-/AST-).

HBeAg-positive chronic hepatitis B.

Chronic HBeAg-positive hepatitis,which is characterized by active viral replication,is usually associated with symptoms and liver cell inflammation.The rate of spontaneous seroconversion from HBe-positive to anti-HBe positive has been reported to vary between about 5 % and 15 % per annum. Seroconversion from HBsAg+ to HBsAg- can follow several months to several years later in 10-20% of these patients.In the vast majority of patients HBe-seroconversion is accompanied by clinical,biochemical and histological improvement.However 5 % of patients show continuing disease activity with persistent viral replication,as indicated by the presence of HBV-DNA.The 5-year mortality rate can be less than 10 % (32,33).

HBeAg-negative,AST positive chronic hepatitis B.

Patients with chronic HBeAg-negative hepatitis and an elevated serum aminotransferase activity form a heterogeneous group with

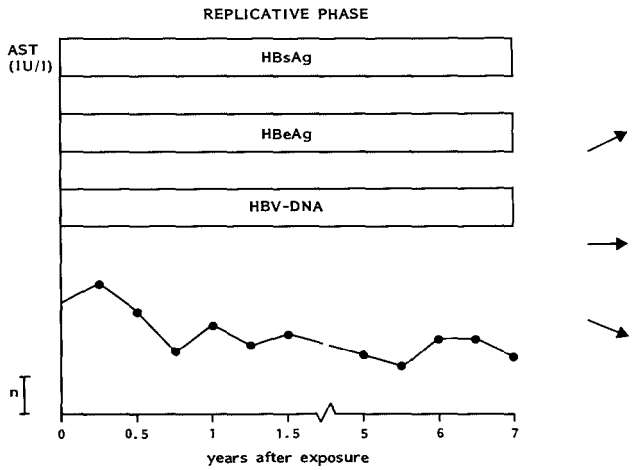
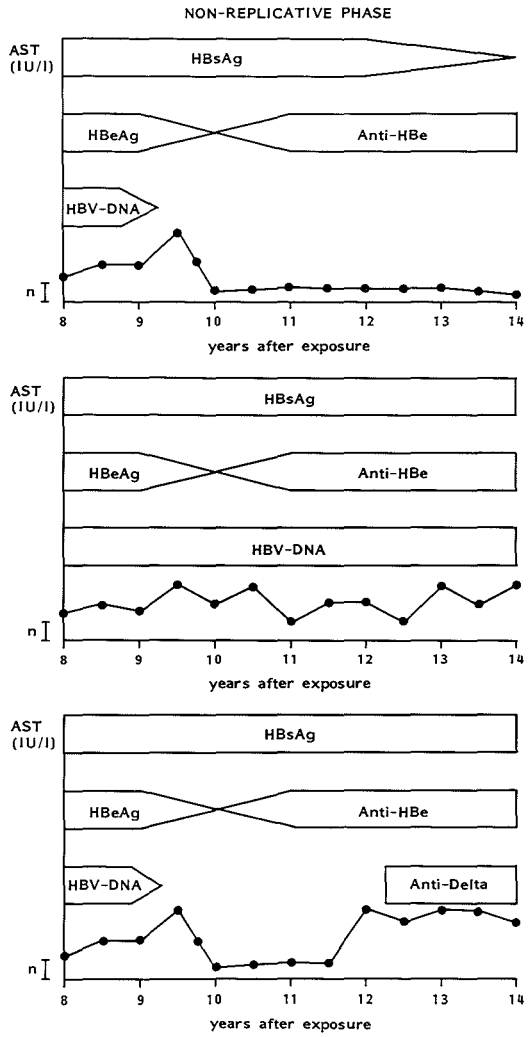


fig 2.Transition from the replicative phase (left panel) to the non-replicative phase of chronic type B hepatitis (right panel) with eventual loss of HBeAg and seroconversion to anti-HBe. Persistence of serum HBV-DNA is associated with progressive liver disease.HBe-negative,AST-positive chronic liver disease can be associated with superinfection with the delta agent.


NON-REPLICATIVE PHASE
AST (IU/l)
HBsAg
HBeAg
Anti-HBe
HBV-DNA
n
8
9
10
11
12
13
14
years after exposure
AST (IU/l)
HBsAg
HBeAg
Anti-HBe
HBV-DNA
n
8
9
10
11
12
13
14
years after exposure
AST (IU/l)
HBsAg
HBeAg
Anti-HBe
HBV-DNA
Anti-Delta
n
8
9
10
11
12
13
14
years after exposure

either ongoing HBV replication or concomitant disease such as hepatitis D infection (delta agent),hepatocellular carcinoma, autoimmune reactivity,alcoholism,drug toxicity,schistosomiasis, non-A non-B hepatitis or metabolic disease.The natural history will vary according to the aetiology.HBs-seroconversion and HBe-reactivation may be 5-10 times more common than in HBeAg-negative,AST negative chronic hepatitis B.The 5-year mortality rate,which is markedly affected by the characteristics of the patients at diagnosis (asymptomatic chronic hepatitis B detected by screening or symptomatic decompensated cirrhosis),is highest (20-30%) for this category of patients with chronic hepatitis B (31,34).

HBeAg-negative,AST negative chronic hepatitis B.

Chronic HBe-negative hepatitis with normal serum aminotransferase activity usually develops without symptoms,liver cell inflammation and active viral replication.HBs-seroconversion occurs in approximately 1 % per year.HBe-reactivation is low,but elevated aminotransferase activity has been observed in 5-15% of cases.Mortality is low (32,35,36).

Prevalence of the 3 types of chronic hepatitis B.

Chronic HBeAg-positive hepatitis usually comprises about 15 % of all cases of chronic hepatitis B,whereas HBeAg-negative,AST negative chronic hepatitis B accounts for about 60 % and HBeAg-negative,AST positive chronic hepatitis B about 25%.The prevalence of the three types of chronic hepatitis B may differ in various regions of the world (33,36,37,38).

1.5 Chronic hepatitis B:goals and strategies for treatment.

The goals of treatment are to alleviate symptoms,lower infectivity,prevent cirrhosis and minimize mortality due to liver failure and hepatocellular carcinoma.Strategies for therapy are required for chronic HBeAg-positive hepatitis and HBeAg-negative,AST positive chronic hepatitis B;no therapy is indicated for HBeAg-negative,AST negative chronic hepatitis B.

Treatment of HBeAg-positive chronic hepatitis B infection.

The relationship between active HBV replication and histological hepatic damage has been well documented;several studies have now shown that disappearance of HBeAg from the serum and the development of anti-HBe lead to low infectivity as well as clinical,biochemical and histological remission in the majority of individuals (25,28,29).Disease progression will then stop; prevention of cirrhosis may also decrease the incidence of hepatocellular carcinoma.

Treatment of HBeAg-negative/AST positive chronic hepatitis B.

In HBeAg-negative,AST positive chronic hepatitis B suppression of liver cell inflammation appears to be crucial.Elimination of aetiological factors (HBV,HDV,alcohol,drugs,schistosomiasis) should be the initial therapeutic approach.Antiviral agents could be beneficial for a subgroup with circulating virus DNA despite the absence of HBeAg (30,33).

1.6 Objectives of the study.

1.To review the main current therapeutic approaches - with a single agent - to HBeAg-positive chronic hepatitis B as they have been reported in literature (chapter 2).

2.To evaluate the inhibitory effect on viral replication as well as the efficacy of acyclovir, descyclovir and alpha-interferon alone or as combination therapy in chronic HBV infection (chapters 3,4,5,6,7,9).

3.To study the mechanisms of a combination therapy-induced transition from the state of active viral replication to the state of virus latency in more detail (chapter 9).In particular we investigated whether the level of serum beta-2 microglobulin either before or during treatment can be used to predict the response to antiviral combination therapy (chapter 8).

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Chapter 2.

Antiviral monotherapy for chronic hepatitis B.

Chapter 2.

2.1 Introduction.

The goals of the treatment of patients with chronic HBV infection are to alleviate symptoms,lower infectivity,prevent cirrhosis and minimize mortality due to liver failure and hepatocellular carcinoma.This chapter will focus on patients in the HBe-positive phase of the disease.Active viral replication in these patients is reflected by the presence of HBeAg,DNA-polymerase activity and HBV-DNA in the serum.Therapy is directed towards inhibition of viral replication,as reflected by the loss of the antigen related to active viral replication (HBe-seroconversion) and elimination of HBV-DNA from the serum.In a complete therapeutic response production of incomplete viral particles will cease (HBs-seroconversion) and antibodies will develop.

Targets for therapy in chronic HBe positive hepatitis.

The host's immune system is responsible for clearance of the virus,which is more likely to occur in patients with low levels of viral replication (1,2).Presentation of viral proteins to the immune system and various steps in the viral replication cycle are targets for antiviral therapy (fig 1).

The major therapeutic approaches to the patient with chronic hepatitis B are summarized in table 1.Although other methods of treatment have been described,those listed in table 1 have been subjected to at least two randomized controlled trials.I will discuss the results of the trials in detail in this chapter.New developments in antiviral combination therapy will be considered in chapter 10.

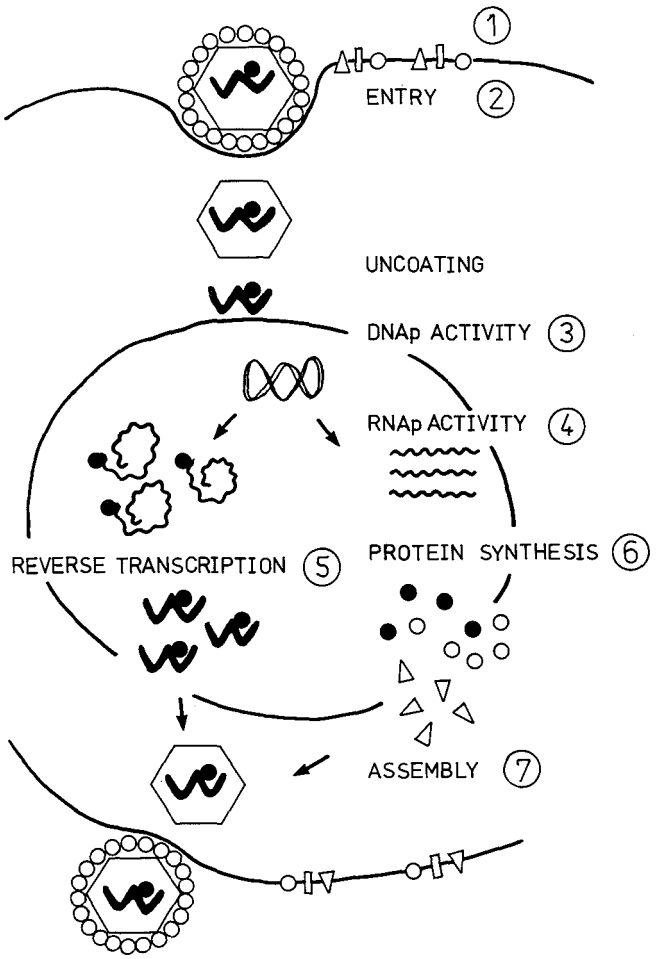
Table 1. Therapeutic approaches to chronic hepatitis B.

1.Synthetic antivirals.
Acyclovir
Adenine Arabinoside (ARA-A,ARA-AMP)

2.Immune modulation.
Alpha-interferon
Corticosteroid withdrawal

3.New developments in antiviral therapy.
Combination therapy
Drug targetting

fig 1.Possible targets for antiviral therapy.
A.Presentation of viral proteins to the immune system
B.Viral replication cycle:viral cell entrance
:viral DNA-polymerase activity
:cellular RNA-polymerase activity
:viral reverse transcriptase activity
:viral protein synthesis
:viral assembly



Inhibition of reverse transcription
Immune modulatory therapy

Possible modes of action.

The main action of synthetic antivirals is inhibition of both chain elongation and viral DNA-polymerase activity.Depending on the selectivity of the action between host and virus,synthetic antivirals may also be immunosuppressive.Interferons have a direct antiviral action as well as an immune modulatory function.Expression of antigens of the MHC class I in combination with viral antigens on the hepatocyte surface leads to cytotoxic immune reactions and elimination of infected cells.Increased expression in combination with inhibition of viral replication is induced by interferon therapy and also plays an important role in the immunologic rebound after corticosteroid withdrawal (3). Interferons may inhibit virus entry as well as subsequent events in the replication cycle within the target cell (4).

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2.2 Interferon.

1.The agent.

In 1957 Isaacs and Lindeman discovered interferon,an agent of viral interference.Interferon is a protein which is released by cells exposed to a virus and enables other cells to resist viral infection (1).It has become clear that the interferons form a large family of species-specific proteins that are produced by virus-infected cells in vivo and in vitro and are important in the natural control of virus infections (2).In addition interferons can be induced by various other stimuli,including other cytokines.Three main types of interferon have now been recognized:interferon-alpha,beta and gamma (3).Alpha-interferon is produced by B-lymphocytes and monocytes,beta-interferon by fibroblasts and gamma-interferon by T-lymphocytes (4).Because of the wide clinical application of alpha-interferon compared to interferon-beta and gamma,I will concentrate on the former.The prototype of human alpha-interferon was leucocyte interferon,the product made when human buffy coat cells are stimulated with Sendai virus (5).Further analysis has shown that preparations of human leucocyte interferon are not homogeneous.Recombinant DNA technology has confirmed that a number of genes each code for an individual subtype of interferon.There are at least 15,and probably more,of these subtypes which although chemically distinct exhibit closely related amino acid sequences (6).In vitro each individual subtype differs from the others in its biological properties (7).The particular mixture of alpha-interferon subtypes depends on the conditions of manufacture and purification.Lymphoblastoid interferon,which consists of a mixture of alpha-interferon subtypes,is made by stimulating cells of the Namalwa cell line with Sendai virus.In our clinical studies we used lymphoblastoid alpha-interferon (Wellferon).
Individual subtypes of alpha-interferon can be produced in significant quantities by recombinant DNA technology.Commercially available recombinant interferons contain alpha-2a (Roferon-A; Hoffman-La Roche),alpha-2b (Intron-A,Schering Corporation) or alpha-2c interferon (Boehringer).Biological activity of interferons is usually measured in terms of the antiviral effects in vitro.Depending on the particular interferon 10-100 pg. correspond to one unit of antiviral activity.

2.Mode of action.

Interferons have a distinct antiviral and immunomodulatory action.Although there is an enormous amount of data on the isolated effects of interferon on cell systems,an overall understanding of the precise mode of action of interferon is lacking.I will discuss the effects of interferon in the normal host,followed by the effects of interferon in patients with chronic hepatitis B virus infection.Finally we will describe a hypothesis for the mode of action and the rationale behind the therapeutic use of interferon in patients with chronic hepatitis B.

After an intramuscular or subcutaneous injection,absorption into the bloodstream is relatively quick and maximum blood levels are obtained 4-6 hours later.Clearance from the blood is rapid:after an intravenous injection,the initially high blood levels drop rapidly to about 10 % of the original value within 1 hour. Interferons are eliminated mainly by glomerular filtration followed by reabsorption by renal tubular cells and catabolism. They are active at extremely low concentrations (fmol/ml) (8).

The antiviral response in cells is elicited by binding to the specific interferon receptor.Interferon-alpha and beta share this receptor while interferon-gamma has a separate receptor.The alpha-interferon receptor consists of two parts:a binding site and an activation site (9,10).After binding intracellular protective mechanisms are activated by a second messenger,not by the direct intracellular injection of interferon (11,12,13). Interferon stimulates the synthesis of intracellular protein kinase and the intracellular enzyme system 2',5'-oligoadenylate synthetase/endonuclease.Activation of this latter system leads to the production of short oligonucleotides which,in the presence of ATP and double-stranded RNA,activate ribonucleases (fig 1.) (14).It has been suggested that the activation of these ribonucleases,which destroy viral messenger RNA and ribosomal RNA within infected cells,is the major mechanism by which interferons inhibit viral replication.The requirement of double-stranded RNA or another activator is one explanation for the selective inhibition of protein synthesis in virus-infected cells.Activation of intracellular protein kinase initiates phosphorylation of an initiating factor essential for protein synthesis.After phosphorylation the initiating factor is inactive and protein synthesis is therefore inhibited (fig 1.) (15,16).However interferons may also inhibit other parts of the

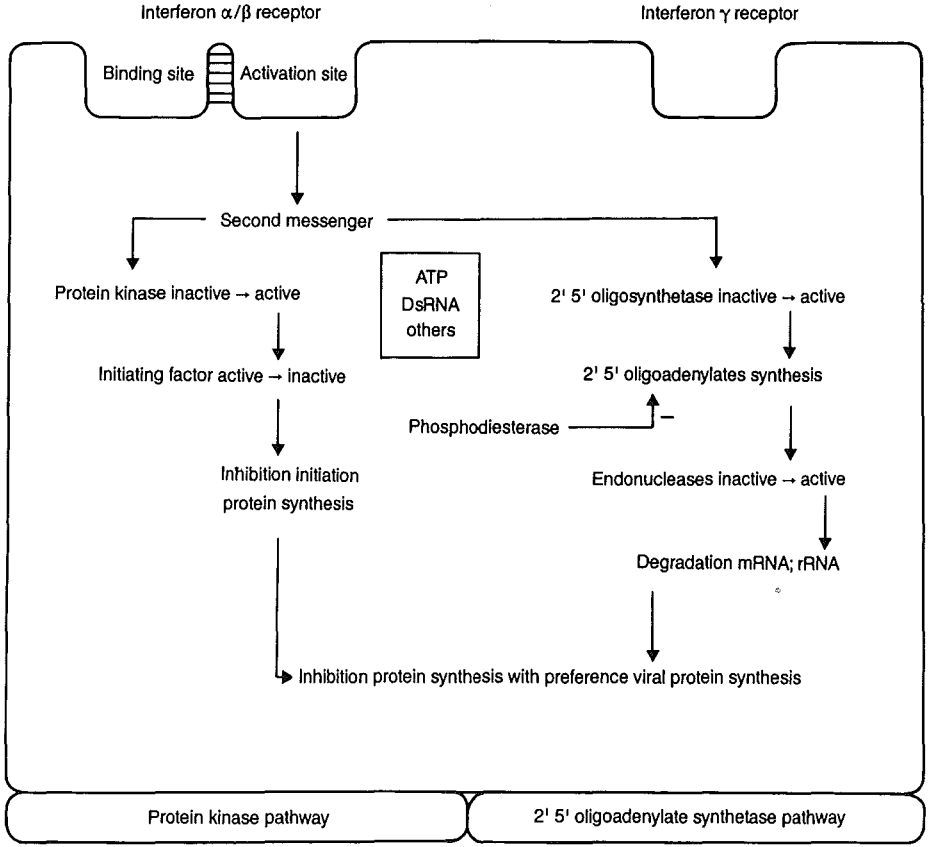

fig 1. Induction of cellular enzyme systems by interferon and their inhibitory effect on protein synthesis.

Table 1. Mechanisms of interferon actions (partly from (18)).

EFFECT	Result
Antiviral:	
Inhibition of virus entry into the cell	Protects uninfected cells
Inhibition of virus uncoating	Protects uninfected cells
Activation of cellular ribonucleases	Destroys viral mRNA
Inhibition of RNA translation	Prevents production of viral components
Inhibition of viral assembly	Prevents completion of viral replication
Immunomodulatory:	
Amplification of class I HLA display on virus-infected cells	Makes virus antigen on cell membrane more visible to antigen-processing cells
Activation of antigen-processing cells	Improved recognition of viral antigens
Augmentation of NK cells activity	Nonspecific killing of virus-infected cells
Increase in sensitivity of virus-infected cellls to cytotoxic T-cells attack	Specific killing of virus -infected cells

viral replication cycle (table 1) (17,18).

The interferon system in chronic viral hepatitis.

Interferons possess several potent immunomodulatory characteristics which are important in viral clearance. Interferons amplify HLA class I antigen expression on the membranes of virus-infected cells (19,20).Activation of antigen-processing cells which recognize viral-HLA antigen complexes on cell membranes is increased (21).Studies of patients with chronic HBV infection suggest that the nucleocapsid antigens (HBe and HBc) are the important targets (22,23).Cytotoxicity of T and K-lymphocytes is increased.Natural killer cell activity is enhanced by interferon,although conflicting results have been reported (24,25).Interferons also direct the cytotoxic T-lymphocyte response by decreasing the resistance of virus-infected cells to such an attack,while increasing the threshold of uninfected cells against attack by the virus (26).

In contrast to acute viral hepatitis, the endogenous interferon system does not seem to be stimulated during chronic viral hepatitis.Despite the presence of active viral replication serum interferon is not detectable in patients in the chronic phase of the disease (27).At the hepatocellular level there is evidence of abnormal activation of the hepatocytes by endogenous interferon:the expression of HLA class I antigens on hepatocyte membranes is not enhanced (28,29);cellular 2'5'-oligoadenylate synthetase activity exhibits only a minimal increase (27,30). Using monoclonal and polyclonal antibodies against alpha-2 interferon in liver sections from patients with chronic HBV infection,it was shown that mononuclear cells and fibroblasts are positive in patients with chronic active hepatitis or cirrhosis.Alpha-2 interferon-positive cells were often found adjacent to hepatocytes that contain cytoplasmic core antigen associated with active viral replication.In vivo interferon production was demonstrated at the site of viral replication which implies local and not systemic regulatory mechanisms (31).Peripheral blood mononuclear cells are not primed for interferon production (32,33).In vitro production of alpha and gamma-interferon by stimulated peripheral blood mononuclear cells from patients with chronic hepatitis was lower than that found for healthy controls,although there was considerable overlap (34).

The interferon-induced enzyme 2',5'-oligoadenylate synthetase

reflects the level of in vivo activation of the interferon system.In patients with chronic active hepatitis B a lack of activation has been shown,whereas 50 % of asymptomatic HBsAg carriers and patients with chronic persistent hepatitis exhibit in vivo activation (30).This result was not confirmed in a recent report which showed normal levels of 2',5'-oligoadenylate synthetase in chronic HBV infection (35).These findings have led to the hypothesis that a defect in the ability to produce sufficient amounts of alpha-interferon during acute viral hepatitis may give rise to chronic infection (36).However it is not known whether the defect in interferon production already exists before occurrence of the hepatitis infection or is a consequence of the infection itself.There are several contradictions in this hypothesis:if the basic defect is a lack of interferon production,activation of cytotoxic lymphocytes is not to be expected;moreover low levels of serum interferon may be attributable to insensitive test methodology or inaccurate timing of the test.Interferon production is reduced in vitro but there is considerable overlap with uninfected controls;moreover extrapolation of data from the in vitro to the in vivo situation is always dangerous.Defects that are measured in circulating lymphocytes do not necessarily reflect the activity of the interferon system in the liver because in situ interferon production has been shown in cells adjacent to cells with active viral replication.However it is possible that hepatitis viruses suppress interferon production,because at the cellular level a nucleotide homology exists between HBV and the sequences that regulate the interferon-induced antiviral system (37).

Changes in the cellular response to interferon after hepatitis B virus infection have also been postulated.Recent transfection experiments have indicated that cells transfected with the hepatitis B genome are less sensitive to exogenous interferon (38).In patients with chronic hepatitis exogenous interferon induces the expression of HLA class I and beta-2 microglobulin and activates cytotoxic lymphocytes.However,interferon remains a moderately effective therapeutic agent in the majority of these cases,which might imply that the basic defect is not a lack of interferon;instead the low levels of interferon may be one of the sequela of the chronic viral infection.The rapid increase in ALAT levels after 8-12 weeks of exogenous interferon therapy, reflecting immunologic attack on the hepatocyte after the virus load has been reduced,is compatible with this finding (39,40,41).

3.Clinical trials.

The fact that alpha-interferons exhibit a broad antiviral activity in combination with the observation of possible defects in interferon production in patients gave rise to clinical studies of interferon in chronic hepatitis B.

Open studies of alpha-interferon.

The first open studies on interferon therapy for chronic active hepatitis B infection were published in 1976.The Stanford group reported on the use of Cantell (buffy coat) interferon in man. Four patients were treated with dosages ranging between 6,000 and 170,000 units of interferon/kg bodyweight (specific activity 500,000 units per mg protein) which resulted in a rapid and reproducible decline in HBsAg,HBcAg levels and DNA-polymerase activity.DNA-polymerase activity returned to initial levels when therapy was discontinued within 10 days or less.Prolonged therapy lasting several weeks produced a more permanent suppression of polymerase activity (42).At the same time the group from Leuven reported on the effects of fibroblast interferon in the chimpanzee and man.In the chimpanzee a rapid decrease in intrahepatic core expression was observed,indicating that replication of the hepatitis B virus is sensitive to interferon (43).These studies led to a number of small open studies aimed at defining the appropriate dose,tolerance and possible duration of therapy.Relatively low doses of alpha-interferon have the same antiviral effects as high doses (1.5-5 versus 36-100 megaunits) but fewer side effects (44,45,46).Treatment schedules should last at least 3 to 4 months in order to achieve a therapeutic response,but 6 months of continuous therapy are poorly tolerated; in this respect however conflicting reports have been published (47,48).Interferon can be administered once daily,three times weekly or on alternate days via the intramuscular or subcutaneous route.Although these aspects have not been compared in large patient series the results of various trials show that administration on alternate days or once daily does not produce substantially different results (49).Response to alpha-interferon usually occurred after 6-10 weeks of therapy and was often,but not always,accompanied by a two to three-fold increase in serum aminotransferase activity (18,50,51).

Randomized controlled trials on alpha-interferon.

The first randomized double-blind placebo-controlled trial with

Table 2. Alpha-interferon randomized controlled trials

Author (reference)	type design	dose MU	treatment schedule	cumulative (estimated)	patients (n)	seroconversion HBe(n)	HBe(%)	HBs(n)	HBs(%)
Schalm (52,53)	Cantell (i.m)	12->0.375	once daily, 6 weeks	14	10	2	20	0	0
			albumin		10	4	40	0	0
Anderson (54)	lymphoblastoid (i.m)	2.5->7.5 (sqm)	once daily, 4 weeks.	238	14	2	14	0	0
			no therapy		16	0	0	0	0
Hoofnagle (55)	recombinant (alpha 2b) (s.c)	5	once daily or	560	16	3	19	0	0
		10	every other	560	15	7	47	1	7
			day,16 weeks.		31	10	32	1	3
			no therapy		14	2	14	0	0
Barbara (56)	lymphoblastoid (i.m)	5 sqm	once daily,28 days,3xweekly, 20 weeks.	748	30*	21	70	8	27
			no therapy		31	10	33	0	0
Dusheiko (57)	recombinant (alpha 2a) (i.m)	2.5 sqm	3xweekly,	306					
		5 sqm	3xweekly,	612					
		10 sqm	3xweekly,	1224					
			24 weeks.		14	1	7	0	0
			no therapy		5	0	0	0	0
McDonald (58)	recombinant (alpha 2a) (i.m)	2.5 sqm	3xweekly,	306	9	1	11	1	11
		5 sqm	3xweekly,	612	9	1	11	0	0
		10 sqm	3xweekly, 12-24 weeks.	1224	14	4	29	1	7
			no therapy		9	0	0	0	0
Lok (59)	recombinant (alpha 2a) (i.m)	2.5 sqm	3xweekly,	306	18	1	6	0	0
		5 sqm	3xweekly,	612	18	4	22	0	0
		10 sqm	3xweekly, 12-24 weeks.	1224	18	2	11	0	0
			no therapy		18	1	6	0	0

Author (reference)	type design	dose MU	treatment schedule	cumulative (estimated)	patients (n)	seroconversion HBe(n)	HBe(%)	HBs(n)	HBs(%)
Perez (60)	recombinant (alpha 2a) (i.m)	2.5 sqm	3xweekly,	306	6	4	67	0	0
		5 sqm	3xweekly,	612	6	2	33	0	0
		10 sqm	3xweekly, 12-24 weeks.	1124	6	0	0	0	0
			no therapy		6	1	17	0	0
Lai (61)	recombinant (alpha 2a) (i.m)	10 sqm	3xweekly, 12 weeks.	612	12	1	8	0	0
			vitamin B syrup.		12	1	8	0	0
Alexander (62)	lymphoblastoid (i.m)	5-10 sqm	3xweekly, 24 weeks.	1224	23	6	26	5	22
			no therapy		23	0	0	0	0
Carreno (63)	recombinant (alpha 2c) (i.m)	5.5 sqm	once daily for 28 days, twice a week, 24 weeks.	710	10	2	20	0	0
			no therapy		10	0	0	0	0
Pastore (64)	Cantell (i.m)	0.07-0.1 pro kg.	once daily for 28 days, twice a week, 2 months.	308	14	8	57	0	0
			no therapy		14	4	29	0	0

* 5 patients withdrawn from the trial.

Cantell interferon was published in 1980 (52,53).Although the numbers were small the results of this trial did not demonstrate a clinical relevance or a statistically beneficial effect of interferon treatment in chronic hepatitis B.As of the first quarter of 1989 11 other randomized controlled trials had been published (table 2) (54-64).From these trials several conclusions can be drawn:perhaps the most important concerns trial size:most are small,thereby increasing the risk of a type II error. Therapeutic response,as defined by a statistically significant increase in HBe-seroconversion,was achieved in a minority of the trials.The net increase in seroconversion rate achieved varies between 18 and 37 %.Seroconversion rates for the control groups vary from 0% to 33 %,clearly reflecting the patient populations selected to participate in the various trials.Spontaneous clearance of HBsAg occurs in about 1-3 % of the cases each year.Interferon therapy can,however,also produce complete 'cure':HBsAg clearance occurs in 18 % of patients (62,65).

Meta-analysis of alpha-interferon therapy.

A meta-analysis is the process of combining the results of several related studies.When adressing an important clinical question,such as the value of alpha-interferon therapy for chronic HBV infection,we normally combine pieces of knowledge coming from various sources.In a sense,a meta-analysis is nothing else than a rigorous,quantitative approach to this combination proces.In this chapter the use of meta-analysis is in the combination of small studies,with often conflicting results,so that more reliable conclusions can be obtained.The result of a meta-analysis is expressed as an odds ratio with its 95 % confidence interval.An odds ratio above 1 (and a confidence interval which excludes 1) suggests a positive treatment effect. Meta-analysis of the twelve published randomized controlled trials on alpha-interferon therapy is shown in table 3.For all individual studies,the odds ratio for HBeAg seroconversion with its 95 % confidence interval was calculated.Stratified exact analysis was carried out by the classic Mantel-Haenszel method. The overall odds ratio for HBeAg seroconversion is estimated to be 3.59 (95 % confidence interval:1.94 - 6.92) ($p < 0.001$) for alpha-interferon therapy.No heterogeneity between the studies was shown ($p = 0.398$).In figure 2 the individual contributions of the studies to the overall odds ratio are shown.Meta-analysis suggests that alpha-interferon is an effective drug for treatment

of chronic hepatitis B infection.

Unfortunately the lack of adequate amounts of interferon for the open studies and the need for a further selection of patients for the small studies have led to the introduction of the concept of the 'responder' patient in literature.This is a patient who is considered highly likely to respond to interferon therapy on the basis of a number of interdependent biased characteristics related to the virus,the host and the interaction between the two (66).According to this hypothesis the ideal candidate should posses :

A.Virus-related factors.

1.Low levels of viral replication (low serum HBV-DNA).
2.Recently acquired infection.

B.Interaction between virus and host.

1.Acute icteric hepatitis in the past.
2.No HIV infection.
3.No HDV infection.
4.No immunodeficiency (e.g.agammaglobulinaemia,renal transplant recipients or the use of immunosuppressive drugs).

C.Host-related factors.

1.Caucasian,not infected at birth.
2.Heterosexual.
3.Female.
4.Elevated serum transaminase activity.
5.Liver histology:marked lobular and periportal activity.

As discussed in chapter one,chronic hepatitis B infection can be divided into an early,an active and a late phase.In the early stage response to interferon therapy is unlikely to occur whereas in the active stage response usually takes place.A predictor accepted by most investigators is the initial level of viral replication:the best response is found for patients with low replication activity.This observation is compatible with the predictive value of the stage of the disease since high levels of viral replication characterize early disease and low levels active disease.Furthermore,high levels of viral replication are seen in immunodeficiency syndromes.Other factors said to be predictive,such as race,age at onset of the infection and sexual

Table 3. Alpha-interferon:meta-analysis of HBe-seroconversion in 12 randomized controlled trials.

Author (reference)	Odds ratio (HBe)	95% confidence interval for odds ratio	Bar no. (figure 2)
Schalm (52,53)	0.39	0.03- 3.89	1
Anderson (54)	infinite	0.22-infinite	2
Hoofnagle (55)	2.8	0.47-30.47	3
Barbara (56)	4.76	1.47-16.76	4
Dusheiko (57)	infinite	0.01-infinite	5
McDonald (58)	infinite	0.33-infinite	6
Lok (59)	2.51	0.29-120.72	7
Perez (60)	2.42	0.20-137.65	8
Lai (61)	1	0.01-85.55	9
Alexander (62)	infinite	1.35-infinite	10
Carreno (63)	infinite	0.19-infinite	11
Pastore (64)	3.19	0.55-21.66	12
All studies	3.59	1.94- 6.92	13

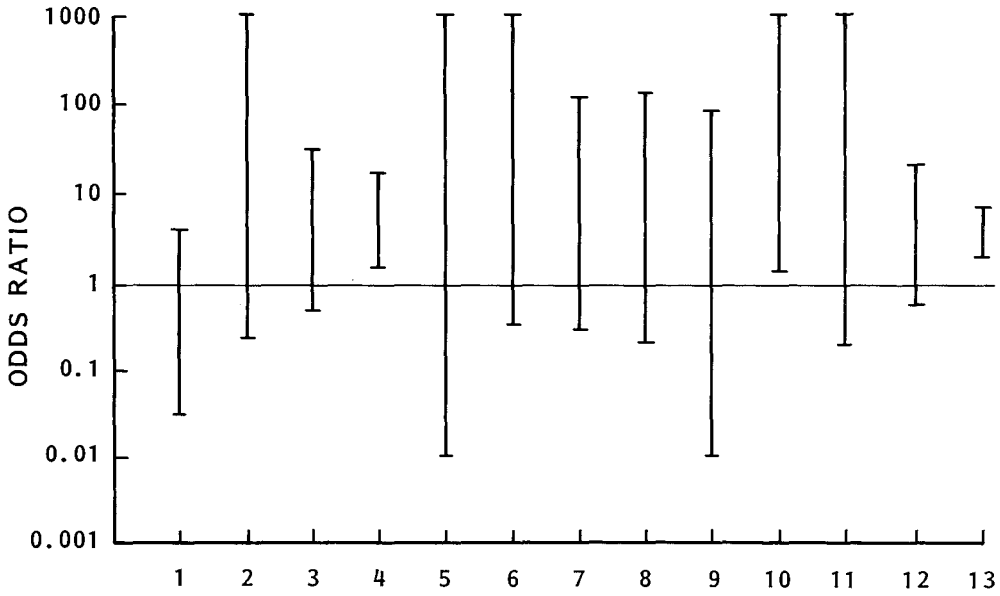

fig 2.Therapeutic efficacy (HBe-seroconversion) for 12 individual studies on alpha-interferon and estimated 'overall'efficacy.Odds ratios are expressed as 95 % confidence limits.Numbers on x-axis: see table 3.

preference,are probably related to the above mentioned factors. Therefore one should interprete the data on predictive factors with extreme caution.

Side effects.

Antiviral therapy for chronic hepatitis has advanced tremendously in the past ten years,with interferon rapidly becoming the cornerstone of treatment schedules.Therefore controlling the side effects of long-term interferon therapy has become important in view of patient compliance.Side effects are related to route of administration,treatment schedule and dosage.We will discuss the most frequently encountered side effects and possible solutions to these problems during alpha-interferon treatment.

Injection technique.

In our hospital the patients themselves inject interferon via the subcutaneous route,like diabetics who administer their own insulin.Patients are provided with written instructions which describe how to prepare and administer an injection and how to cope with frequently encountered problems.To familiarize them with the use of sterile vials and syringes they receive a vial of sterile water to practise preparing a syringe for injection. The patient is instructed on an outpatient basis by a specially trained nurse.We advocate rotating the site of injection between the right and left upper leg and the abdomen;of special importance is the use of small needles to minimize tissue trauma. Disposable syringes without a dead space (Monoject 0.4 x 12 mm) are particularly suitable.Local irritation at the site of injection is often due to insufficient variation of the injection site.One patient developed a traumatic lesion of the lateral cutaneous nerve of the thigh,which resolved spontaneously within five months.Most minor problems can be solved by returning to the 'interferon nurse' for instruction.In some cases we teach the partner to administer the interferon injection.

Systemic side effects:the first week.

During the first week of treatment most patients complain of an influenza-like syndrome with malaise,myalgia and high fever. Without treatment tachyphylaxis will develop in the first week and most symptoms will subside.First-week symptoms can be adequately suppressed by acetaminophen (500 mg six times daily) or indomethacin (Indocid Retard:75 mg twice daily).HLA-antigen

presentation,as reflected by serum beta-2 microglobulin levels, and depression of viral replication are not affected by either of these drugs (67).Acetaminophen and indomethacin can be discontinued after the first three days of interferon treatment; mild flu-like symptoms may persist.

Systemic side effects:after the first week.

During a course of 5 megaunits of alpha-interferon daily or thrice weekly systemic manifestations of the drug,such as fatigue,malaise,arthralgia,myalgia,anorexia,weight loss and hair loss,have been observed in up to 50 per cent of patients.Hair loss can persist for as long as two months after cessation of the drug.Leucocytopenia (granulocytopenia) is always observed;anaemia and thrombocytopenia occur in a minority of patients (59,68). Higher doses of alpha-interferon (36-100 MU/day) cause increased bone marrow toxicity.The antiviral action is not enhanced by elevating the dose in this dose range (44).Central nervous system side effects may occur,including an organic personality syndrome characterized by irritability and short temper and an organic affective syndrome marked by extreme emotional lability, depression and tearfulness.The organic nature of these changes is confirmed by electroencephalographic changes during interferon therapy,which remit after treatment is discontinued (69,70).Loss of tendon reflexes,paraesthesia and peripheral sensory neuropathy have been observed,also when the EMG is normal.Liver functions (SGOT) can increase during interferon treatment without being indicative of imminent HBe-seroconversion (71).In the animal model interferon depresses cytochrome p450 activity in liver tissue (72).In man inhibition of antipyrine metabolism by exogenous interferon has been shown.Changes in the pharmacokinetics of drugs metabolized by cytochrome p450 can therefore be expected (73,74,75).

Management of interferon-related side effects.

Our patients used to give themselves the injection in the morning.It appears that such side effects as malaise and myalgia have decreased since our recommendation that the drug be injected in the evening.Most side effects reverse quickly on dose reduction or cessation of treatment.In case of clinically important side effects a 50 % dose reduction is prescribed.If side effects subside the dose is increased to 75 % of the original dose.In our experience with 75 patients treatment was

discontinued only once because of severe malaise.If adequate monitoring of therapy is provided alpha-interferon is a safe and well-tolerated drug.

4.Conclusions.

Single drug therapy with alpha-interferon is clearly a starting point for antiviral therapy for chronic hepatitis B.In contrast to other drugs alpha-interferon can promote HBsAg clearance. Clearly efficacy has to be increased either by selection of patients,on the basis of a model which describes the complex host-virus interaction more adequately,or by combination of alpha-interferon either with other immunomodulatory agents or with nucleoside analogues.

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2.3 Adenine Arabinoside and Adenine Arabinoside Monophosphate.

1.The agent.

Adenine arabinoside is a purine analogue with potent antiviral activity.It inhibits the synthesis of HBV nucleic acid by acting as a faulty substrate,thus preventing DNA synthesis.It inhibits DNA-polymerase activity selectively,being more potent against viral than against mammalian cell polymerases.Due to the effect on host cell DNA synthesis and lymphocyte proliferation it is immunosuppressive (1).Because the solubility of the drug in water is very low,it must be administered by slow intravenous infusion in rather large volumes of fluid.The monophosphorylated analogue adenine arabinoside monophosphate (ARA-AMP) has a similar spectrum of antiviral activity but is at least a thousand-fold more soluble in water.ARA-AMP can be given as an intravenous bolus or an intramuscular injection.The more recent studies focus mainly on ARA-AMP.

2.Mode of action.

The clinical efficacy of ARA-A in systemic herpes infections has been proven.Before acyclovir became available ARA-A was the treatment of choice for systemic herpes infections and herpes simplex encephalitis (2,3).Antiviral activity against hepatitis DNA viruses has been shown in vitro and in vivo in woodchucks. Potent inhibition of endogenous and woodchuck DNA-polymerase activity by ARA-A triphosphate has also been demonstrated (4).In the woodchuck model,inhibition of viral replication by ARA-AMP was shown (5).

3.Clinical trials.

Results of open studies.

The first use of ARA-A for treatment of chronic type B hepatitis was reported by Stanford University (6).Two patients with active viral replication (HBsAg,DNA-polymerase positive) received two courses of intravenous ARA-A in a dosage of 15 mg/kg/day for 9-14 days.DNA-polymerase inhibition was approximately 50 % on day 3, 75 % on day 7 and 90 % on day 14.When ARA-A was discontinued DNA-polymerase activity returned to pre-treatment levels in one patient but remained negative in the other.This patient subsequently became HBsAg negative.Reviews of the use of ARA-A in open studies at Stanford have been presented by Scullard et al.(7,8,9).In the opinion of this group ARA-A is a more potent inhibitor of hepatitis B virus replication than human leucocyte

Table 1. Adenine arabinoside (ARA-A;ARA-AMP),randomized controlled trials

Author (reference)	type design	dose mg/kg/day	treatment schedule	cumulative (mg/kg)	patients (n)	seroconversion HBe(n)	HBe(%)
Bassendine (13)	ARA-A	10	iv,10 days	100	7	3	42
	no therapy				6	0	0
Hoofnagle (14)	ARA-AMP	10	iv,5 days	165	10	4*	40
	ARA-AMP	5	im,23 days				
	no therapy				10	2	20
Yokosuka (15)	ARA-A	10	iv,28 days	280	7	1	10
			iv,56 days	560	3		
	no therapy				10	2	20
Ouzan (16)	ARA-A	15	iv,7 days	210	15	3	20
		7.5	iv,14 days				
	placebo				15	1	7
Weller (17)	ARA-AMP	10	im,5 days	165	15	6	40
		5	im,23 days				
	no therapy				14	0	0
Perillo (18)	ARA-AMP	10	im,5 days	165	11	0	0
		5	im,23 days				
	no therapy				11	1	9
	ARA-AMP	10	im,5 days	330	7	1	14
		5	im,23 days				
			2 cycles				
	no therapy				6	0	0
Trépo (20,21)	ARA-AMP	10	im,5 days	165	19	10++	53
		5	im,23 days				
	NaCl 0.9%		im,28 days		18	5	28
Garcia (22)	ARA-AMP	5	im,28 days	420	24(<>)	2	9
			3 cycles				
	Albumin		sc,28 days		27	4	15
			3 cycles				

<> 2 patients lost to follow up.
++ 2 patients reactivated; 42 patients entered the trial, reports on 37 patients.

interferon but permanent loss of hepatitis B virus replication is less likely with ARA-A than with interferon therapy.
The first pilot studies of ARA-AMP were reported by Weller and Hoofnagle (10,11).ARA-AMP therapy resulted in a prompt decrease in HBV-DNA serum levels and DNA-polymerase activity when administered twice daily.Persistent response to therapy,however, was observed in only a minority of patients.The overall beneficial response to ARA-AMP therapy in seven uncontrolled studies is estimated at 11% (12).

Randomized controlled trials.

When randomized controlled trials were initiated ARA-A was already being used by several investigators to treat patients with chronic type B hepatitis.Although adenine arabinoside markedly reduced DNA-polymerase activity during treatment,in the majority of cases DNA-polymerase activity and other HBV markers returned to pre-treatment levels when ARA-A(MP) was discontinued.A matched controlled study showed an initial 40 % response rate (HBe-seroconversion) after a ten-day course of ARA-A (13).A summary of the main randomized placebo-controlled trials is given in table 1.Hoofnagle initially found a beneficial effect of 4 weeks of ARA-AMP compared to no therapy.Extensive follow-up revealed reactivation in two patients who had initially responded.Ultimately,the authors could not demonstrate any benefit of 4 weeks of ARA-AMP therapy (14).Weller observed a beneficial response in 6/15 treated patients compared to none in 14 untreated control patients.Interpretation of this trial is difficult because of concomitant viral infections.Two patients were infected with the Delta agent and one with the hepatitis A virus.Both viruses are known to interfere with hepatitis B replication and make interpretation of a therapeutic modality aimed at hepatitis B difficult (17).In France Trepo reported a 25% increase in seroconversion rate after 28 days of ARA-AMP.A considerable number of patients dropped out of this study which makes interpretation hazardous (20,21).Comparable results were reported by Ouzan who found that intravenous ARA-A led to a 13 % increase in seroconversion rate compared to controls (16).In these French studies patients who did not respond to therapy were given another course of ARA-A(MP) 6 months later.In both studies the net response rate increased after two courses to around 40 % compared to the initial control group.In a three-part randomized trial Lok compared short (4-week) and long

Table 2. ARA-A/ARA-AMP:meta-analysis of HBe-seroconversion in 9 randomized controlled trials.

Author (reference)	Odds Ratio (HBe)	95% confidence interval odds ratio	Bar no. (figure 1)
Bassendine (13)	infinite	0.39-infinite	1
Hoofnagle (14)	2.54	0.26-37.18	2
Yokosuka (15)	0.46	0.10-10.51	3
Ouzan (16)	3.36	0.23-196.60	4
Weller (17)	infinite	1.38-infinite	5
Perillo (18)	0.00	0.00-39.00	6
	infinite	0.02-infinite	7
Trepo (20,21)	2.8	0.61-14.44	8
Garcia (22)	0.53	0.04-4.13	9
All studies	2.37	1.12-5.19	10

(initially 12,later 7-week) courses of ARA-AMP to interferon treatment.Sustained inhibition of viral replication was found for the patients receiving short courses of ARA-AMP or interferon but not in those on long courses of ARA-AMP.Side effects increased dramatically in the latter group (19).

Meta-analysis of adenine arabinoside (ARA-A/ARA-AMP) therapy.

Meta-analysis of the eight published randomized controlled trials on ARA-A(MP) therapy is shown in table 2.For all individual studies,the odds ratio for HBe-seroconversion was calculated with its 95 % confidence interval.Stratified exact analysis was performed by the classic Mantel-Haenszel method.The overall odds ratio for HBeAg seroconversion is estimated to be 2.37 (95 % confidence interval 1.12 - 5.19) ($p < 0.017$) for ARA-A therapy. No heterogeneity between the studies was demonstrated ($p = 0.1$).In figure 1 the relative contributions of the 8 trials to the overall odds ratio are shown.Meta-analysis suggests that ARA-A is an effective drug for the treatment of chronic hepatitis type B.

Side effects.

ARA-A and ARA-AMP have significant toxic side effects which are dose-related.In the case of long-term low-dose therapy,toxicity is related to the cumulative dose.The safe period of treatment probably does not exceed 4 weeks (23).Side effects can be grouped into three main categories:gastrointestinal,bone marrow and neurologic.Prolonged or high-dose therapy causes anorexia,nausea and fatigue;vomiting and diarrhoea can also occur.Reversible bone marrow depression with leucopenia and thrombocytopenia has been reported.The most troublesome problem is the neuromuscular toxicity of ARA-A and ARA-AMP;at dosages in excess of 10 mg/kg/day headaches,lethargy,stupor and tremors may develop. These symptoms can last several weeks and may be associated with EEG changes (24).A neuromuscular pain syndrome which affects mainly the nerves and the muscles of the back and lower extremities has been descibed (25,26).This syndrome,which can be severely disabling and may persist for months after therapy is discontinued,is probably due to axonal neuropathy.It has been suggested that patients with preexisting electromyographic (EMG) abnormalities are more likely to develop this syndrome (27,28). It rarely occurs when ARA-A is used to treat viral infections other than hepatitis.Toxicity is enhanced by the combination of

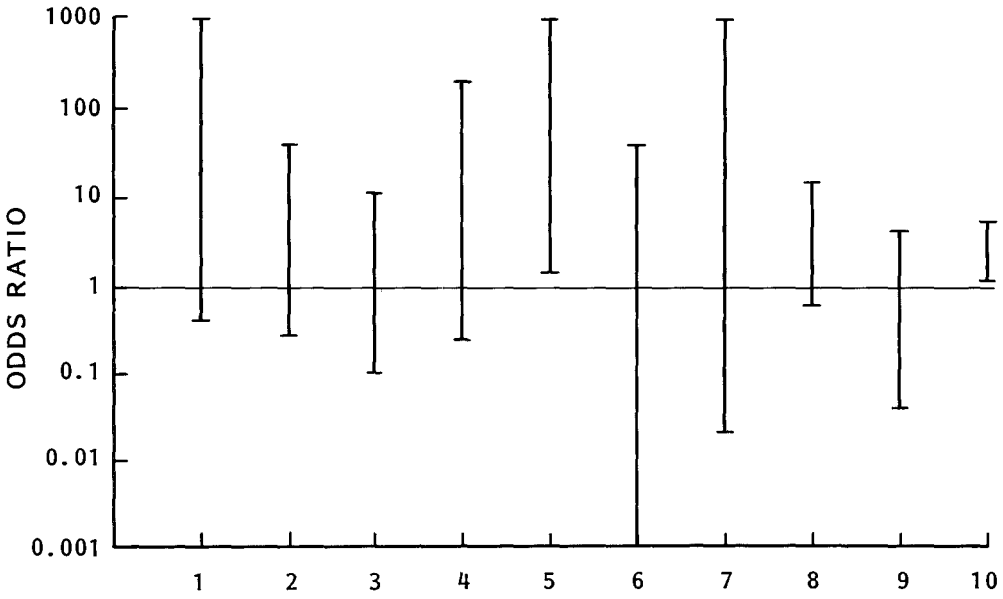

fig 1. Therapeutic efficacy (HBe-seroconversion) in 8 individual studies on ARA-A(MP) and estimated 'overall' efficacy.Odds ratios are expressed as 95 % confidence limits.Numbers on x-axis:see table 2.

interferon and ARA-AMP (22).

4.Conclusions.

Adenine arabinoside and adenine arabinoside 5'monophosphate are effective in inhibiting hepatitis B virus replication.Meta-analysis of randomized controlled trials shows enhancement of HBe-seroconversion rates compared to untreated control patients. Neurotoxicity is a major problem which can be managed partly by pre-treatment EMG monitoring.Since drug toxicity is a dose-related problem,limitation of the cumulative dose and avoidance of high peak plasma concentrations can partly prevent ARA-A toxicity.A relatively safe therapeutic regimen consists of a 4-week course followed by a six-month rest period before the course is repeated.The study by Lok suggests that if ARA-AMP is used for a period of more than 4 weeks,its immunosuppressive properties become more dominant in relation to its antiviral properties.This may result in a lower therapeutic response.In the event of treatment failure after one course of ARA-A,the patient can be treated again with the same agent (16,19,20).A substantial number of these patients will respond to this second course of therapy.In some studies a first course of ARA-A(MP) did not induce seroconversion of DNA-polymerase or HBeAg although post-treatment levels of viral replication were substantially lower in treated patients compared to untreated controls. Insufficient activity of the drug in patients with high levels of viral replication could possibly explain the favourable response to a second treatment course.

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2.4 Corticosteroid withdrawal.

The observation that discontinuation of corticosteroid therapy in patients with chronic hepatitis type B often results in remission of the disease has led to the hypothesis that abrupt withdrawal of treatment causes an immune rebound effect followed by clearance of the virus,the mechanism being enhanced hepatocyte expression of viral antigens during steroid therapy (1).After steroid withdrawal immunocompetence is restored and parenchymal cells which express target viral antigens are destroyed (2).In uncontrolled pilot studies this hypothesis was further explored: 20 patients followed a tapered dose schedule consisting of 60,30, 10 and 5 mg prednisone per day,each dose for 2 weeks and a total of 10 weeks of treatment.Ten of 20 treated patients became HBe-negative.In addition there was a significant drop in HBsAg titre and transaminase activity in the prednisone-treated group.However 5/20 patients had a period of hepatic decompensation (4 with ascites-two of whom also had encephalopathy-,one haemorrhage from oesophageal varices with encephalopathy).Hepatic decompensation was closely related to the presence of chronic active hepatitis with cirrhosis (3).In a study of 12 patients discontinuation of immunosuppressive therapy was associated with a bout of hepatitis four weeks after withdrawal.Interestingly the flare of hepatitis only occurred in HBeAg-positive patients.HBeAg was eliminated in 8/12 patients.However one patient died of liver failure and 2 patients experienced a deterioration of the liver disease (increasing bilirubin,ascites) (4).The steroid withdrawal hypothesis was further investigated in a double-blind randomized placebo-controlled study (5).Fifteen patients without a history of decompensated liver disease participated in this study. Histologically all had chronic persistent or mild to moderate chronic active hepatitis.Ten patients received prednisolone (60 mg/day) for two weeks,followed by 30 mg/day for another two weeks with sudden withdrawal.No significant effects on viral replication were observed during prednisolone therapy. Aminotransferase activity decreased significantly during treatment but exhibited a severe rebound to above-initial levels after termination of the drug.Follow-up at one year showed no improvement in biochemical or serological features of the disease.Follow-up liver biopsies revealed deterioration in 4/7 treated patients vs 0/5 untreated control patients.In conclusion this treatment has no beneficial effect and may in fact be harmful.

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2.5 Acyclovir.

1.The agent.

Acyclovir or 9-(2-hydroxyethoxymethyl)guanine is a non-cyclic guanosine analogue that specifically inhibits viral DNA synthesis but barely interferes with host cell DNA synthesis.A systematic programme which was initiated at Burroughs Wellcome to prepare synthetic nucleoside analogues with a non-cyclic side chain led to the discovery of acyclovir in 1974 (1,2).Acyclovir can be administered via the topical,oral and intravenous routes.In the case of oral administration absorption of acyclovir is slow and incomplete,with a bioavailability of 15 to 30%.Steady state levels of about 2.5 uM are achieved with 200 mg acyclovir every 4 hours (3).Five mg acyclovir/kg body weight given intravenously yielded peak levels of about 35 uM;peak serum concentrations increased to 90 uM after 15 mg/kg body weight.Renal excretion is by filtration and active secretion (4).

Descyclovir (6-deoxyacyclovir) is an oral pro-drug of acyclovir without antiviral activity.Descyclovir,which is almost totally absorbed from the gastrointestinal tract,is converted by xanthine oxidase to acyclovir and by aldehyde oxidase to the inactive metabolites 8-hydroxy-6-deoxyacyclovir and 8-hydroxy-acyclovir. Since the human liver contains low levels of aldehyde oxidase, conversion of 6-deoxyacyclovir to acyclovir is the principal metabolic pathway in man.Peak plasma levels of acyclovir after 250 mg descyclovir orally every six hours are around 40 uM (5,6).

2.Mode of action.

The acyclic structure of acyclovir explains its effect as a terminator of DNA synthesis because it lacks the 3'hydroxyl group essential for DNA elongation.For acyclovir to act as an inhibitor of viral DNA synthesis,it must be phosphorylated to the monophosphate by a viral thymidine kinase.The selective antiviral effect of acyclovir on viral DNA-polymerase is due to its preferential phosphorylation by cells expressing a viral thymidine kinase (7).Acyclovir enters the infected cell by passive diffusion;after monophosphorylation redistribution to the blood is hampered because the monophosphate derivative does not easily cross the cell membrane.Acyclovir binds 200-fold better to and is phosphorylated 3.10^6 times more easily by the viral than the cellular thymidine kinases (8,9).The acyclovir monophosphate is further metabolized to the triphosphate by cellular enzymes (10).

Acyclovir triphosphate inhibits all human herpes viral DNA polymerases in vitro (11,12,13).Viral thymidine kinase is not encoded by the human cytomegalovirus,which is relatively insensitive to acyclovir (14,15).Still some inhibitory effects of acyclovir on cytomegalovirus have been shown in vitro (16, 17).Like cytomegalovirus the human hepatitis B virus does not encode for a thymidine kinase in the infected cell although there is evidence for protein kinase activity associated with the complete virion (18).Some authors have suggested that in certain settings cellular enzymes may be able to phosphorylate acyclovir sufficiently to allow its activation in the absence of a virus-specified thymidine kinase (19,20).In vitro acyclovir triphosphate inhibits human hepatitis B virus and a closely related virus in woodchucks (21).In chronically infected Peking ducks acyclovir inhibits DNA-polymerase activity as long as the drug is administered (22).

3.Clinical trials.

Open studies of intravenous acyclovir.

In a dose-response study six patients were treated with acyclovir (5-15 mg/kg every 8 hours) given as an intravenous bolus or one-hour intravenous infusion for up to seven days.Two patients receiving 10 and 15 mg acyclovir/kg showed 50 % inhibition of DNA-polymerase activity.The mean plasma acyclovir concentrations were 5.0 and 13.2 uM,respectively;the latter was associated with a more marked effect,suggesting a dose-response relation (23). Three patients received doses that increased from 15 mg/kg/day to 45 mg/kg/day for 1-2 weeks.Inhibition of DNA-polymerase activity was 60 - 80 % during all courses;in fact,DNA-polymerase activity could not be detected for four months in one patient.A dose-response relationship in the dose range 15 to 45 mg/kg/day was less evident in these three patients (24).Trepo treated 10 patients with acyclovir (45 mg/kg/day administered via a continuous infusion) for 21 days and found a transient drop in DNA-polymerase activity in 9 patients.In two patients a trend towards a prolonged 50 % reduction in DNA-polymerase activity was observed after therapy.Both patients had low pre-treatment DNA polymerase activity.In a follow-up study six patients with low grade virus replication (HBV-DNA positive,low DNA-polymerase activity) were evaluated.DNA-polymerase activity decreased during therapy and exhibited a further reduction over the next two months.However in five patients DNA-polymerase activity

reactivated in the course of the extended follow-up (25,26).

Open studies of oral acyclovir and descyclovir.

Oral acyclovir was ineffective in depressing DNA-polymerase activity (26,27).Four patients received 250 mg descyclovir orally every 6 hours for 10 days.No depression of DNA-polymerase activity during therapy was noted in three patients.In one patient HBe-seroconversion was observed,but retrospectively this patient had unstable disease at the start of the study (28).

Randomized controlled trials with acyclovir and descyclovir.

Two small randomized controlled studies on the effect of intravenous acyclovir in chronic type B hepatitis have been performed (29,30,31).Results and treatment schedules are summarized in table 1.

Continuous infusion of acyclovir (45 mg/kg/day) resulted in a mean decrease in DNA-polymerase activity and serum HBV-DNA of 12 and 35 per cent,respectively,after 28 days.Therapy was associated with seroconversion of HBe in 33% of patients (5/15),HBV-DNA in 16% (2/12) and DNA-polymerase in 33% (5/15).Seroconversion rates for controls were 13 % (2/15),7 % (1/13) and 0 % (0/11), respectively;these differences did not reach statistical significance (30).

In a study by Guarescio the effect of a continuous or intermittent infusion (three times daily) of 45 mg/kg/day for 28 days was compared to that of no therapy.No effect on viral replication was found after the continuous infusion but there was a 20 % inhibition of DNA-polymerase activity after the intermittent infusions of 45 mg/kg/day acyclovir,although the difference was not statistically significant.Compared to untreated control patients no apparent beneficial effect on HBe-seroconversion was observed (31).

Meta-analysis of acyclovir therapy.

Meta-analysis of the two randomized controlled trials on the use of acyclovir in chronic hepatitis type B is shown in table 2.For the individual studies,the odds ratio for HBeAg seroconversion was calculated with its 95 % confidence interval.Stratified exact analysis was done by the classic Mantel-Haenszel method.Overall odds ratio with regard to HBe-seroconversion is estimated to be 1.38 (95 % confidence interval:0.32 - 6.46) ($p = 0.43$) for acyclovir therapy.In figure 1 the contributions of the two

Table 1. Acyclovir, randomized controlled trials.

Author (reference)	dose mg/kg/day	treatment schedule	days (n)	cumulative (mg/kg)	patients (n)	seroconversion HBe(n)	HBe(%)
Alexander (30)	45	continuous, infusion	28	1260	15	5	33
		no therapy			15	2	13
Guarescio (31)	45	continuous or 8-hour infusion	28	1260	11	2	18
		placebo			9	3	33

Table 2. Acyclovir:meta-analysis of HBe-seroconversion in 2 randomized controlled trials.

Author (reference)	Odds Ratio (HBe)	95% confidence interval for odds ratio	Bar no. (figure 1)
Alexander (30)	3.12	0.40-39.97	1
Guarescio (31)	0.46	0.03-5.39	2
All Studies	1.38	0.32-6.46	3

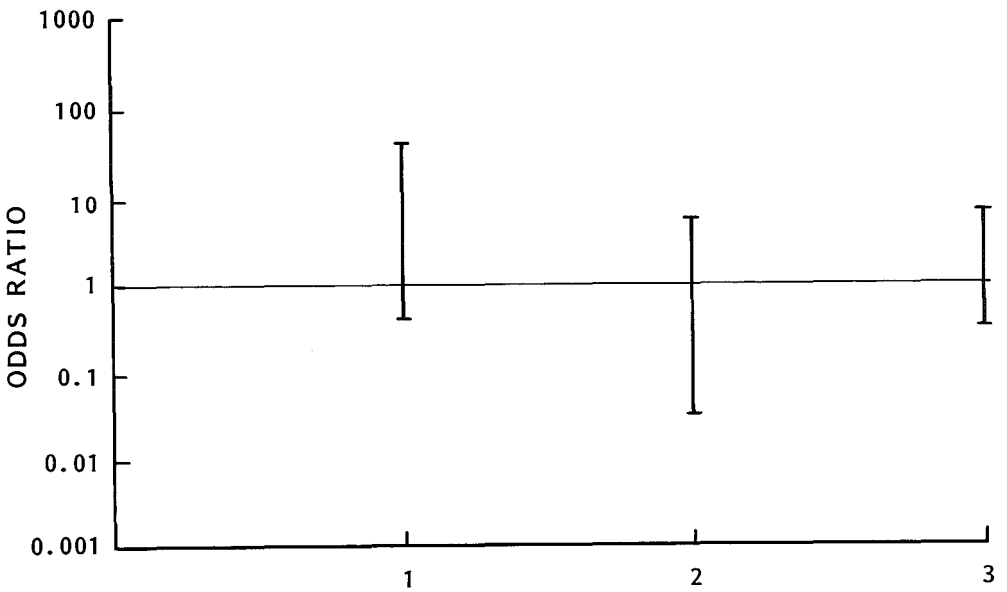

fig 1.Therapeutic efficacy (HBe-seroconversion) determined in 2 individual studies on acyclovir and estimated 'overall' efficacy. Odds ratios are expressed as 95 % confidence limits.Numbers on x-axis:see table 2.

studies to the overall odds ratio are shown.Although there are only two studies meta-analysis does not suggest beneficial effects of single drug therapy with acyclovir.

Side effects.

Acyclovir is a relatively non-toxic drug compared to,for instance,adenine arabinoside.Local irritation and phlebitis at the site of infusion have occurred after high dose intravenous administration and were probably due to the high pH of the acyclovir solutions (32,33).The major adverse effect of acyclovir involves renal function.Reversible elevation of serum creatinine has been found,especially when doses exceeding 5 mg/kg are given intravenously every 8 hours.Dehydration,pre-existing renal insufficiency and high dose bolus infusions can enhance the risk of crystallization of acyclovir in renal tubules or collecting ducts,causing a reversible crystalline nephropathy (1,34,35). Case reports suggest a relation between the use of acyclovir and central nervous system toxicity,including delirium,tremors, coma and abnormal electroencephalograms as well as acute psychiatric disturbances.Slight increases in aminotransferase levels, nausea,abdominal discomfort,diarrhoea,dry skin,rashes and decreased haematological indices during high-dose intravenous acyclovir treatment have been reported (36,37,38,39).

4.Conclusions.

Acyclovir triphosphate inhibits woodchuck and human hepatitis B virus DNA-polymerase activity in vitro.In vivo inhibition by acyclovir has been shown for Peking duck and human hepatitis B virus DNA-polymerase activity.In contrast to the herpes viruses the hepatitis B virus does not encode for a specific thymidine kinase.Therefore acyclovir is phosphorylated by cellular kinases to active acyclovir triphosphate.Because of this lack of specificity the drug is only moderately effective at relatively high doses;one small study indicated that intermittent infusions are preferable to continuous infusions,suggesting that peak serum levels are more important than steady state serum levels (32).No apparent long-term effect on HBe-seroconversion has been shown in randomized controlled studies.The drug is non-toxic if adequate precautions against renal toxicity are taken.Single drug therapy with acyclovir or descyclovir does not seem to be justifiable for chronic type B hepatitis.

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2.6 Conclusions.

Single drug therapy with alpha-interferon is clearly a starting point for antiviral therapy for chronic hepatitis B.In contrast to other drugs alpha-interferon can promote HBsAg clearance. Efficacy has to be increased either by selection of patients,on the basis of a model which describes the complex host-virus interaction more adequately,or by combining alpha-interferon with either other immunomodulatory agents or nucleoside analogues.Of the available nucleoside analogues acyclovir and adenine arabinoside are effective in inhibiting hepatitis B virus replication,but the use of ARA-A is limited because of severe neurotoxicity.Acyclovir is non-toxic if adequate precautions against renal toxicity are taken.Our studies concentrated on the combination of alpha-lymphoblastoid interferon and acyclovir.

Chapter 3.

Antiviral effect of acyclovir (oral and intravenous) and descyclovir in chronic hepatitis B.

Chapter 3.

Introduction.

Single drug treatment with alpha-interferon,adenine arabinoside or its monophosphate derivative has resulted in predictable but often only partial suppression of HBV-associated DNA-polymerase in patients with chronic hepatitis B and active virus replication (1).The virustatic agent acyclovir was also found to inhibit HBV-DNA-polymerase activity (2,3).In view of the fact that acyclovir is relatively non-toxic and allegedly does not interfere with the immune system as well as the availability of an oral prodrug that is easily absorbed,we investigated the effect of this drug more fully in patients with chronic HBe-positive hepatitis.

Methods.

Patients with chronic hepatitis B and stable HBeAg and DNA-polymerase serum levels for at least six months participated in the study.Patients who had undergone a liver biopsy prior to entry exhibited HBcAg in the nuclei of the hepatocytes.Six patients,including one individual with chronic renal failure on dialysis,received acyclovir orally in a dose of 800 mg four times daily for four weeks (study I).Intravenous acyclovir (15 mg/kg body weight in a 1-hour infusion of 0.5 l of fluid,twice daily) was administered to 11 patients for a period of 4 weeks (study II).Twelve patients were selected to participate in study III:six patients received deoxy-acyclovir orally in a dose of 250,375 or 500 mg 3 times daily for two weeks.In each case the three treatment periods were separated by a two-week wash-out period.Six control patients received no treatment;they were followed simultaneously.

In all studies blood samples were taken twice weekly for monitoring of virus replication (DNA-polymerase activity,HBeAg) ,AST and serum creatinine levels.DNA-polymerase activity in serum was measured by standard methods and expressed as the P/N ratio (cpm patient sample/cpm normal control sample) (4).HBsAg and HBeAg were determined by radioimmunoassay (Abbott,Ill,USA).HBeAg was assessed in a dilution of serum that was constant for each patient;this dilution was chosen such that pre-treatment sera had a P/N ratio of 10 +/- 2.A P/N ratio below 2.1,in undiluted serum, was considered a negative result.For studies I and II,data

Table 1.Features of patients prior to acyclovir and descyclovir treatment.

	ACV(oral) (n=6)	ACV(iv) (n=11)	DCV(oral) (n=6)	Control (n=6)
Sex,male	6	10	6	4
Homosexual	4	4	4	1
Duration of HBsAg (yr)*	5	3.3	2.7	3.0
HBsAg titre (10^{-3})+	5.7	2.2	nt	nt
HBeAg (P/N ratio)+	7.2	8.5	8.3	6.7
DNA-p (P/N ratio)+	4.9	25.1	14.7	6.7
HBcAg in nuclei (%)*	20	39	24	20
Bilirubin (µmol/l)* (normal < 17)	9	18	12	11
AST (IU/l)* (normal < 30)	58	119	63	40
Cirrhosis	2	1	2	1

nt = not tested
* = mean
\+ = geometric mean

before,during and after treatment were analysed by the Wilcoxon matched pairs test.For study III the difference between the first and last days of treatment was calculated for the three dose groups and compared to the difference found for the control patients followed in the same period by means of the Wilcoxon/ Mann-Whitney rank-sum test.Differences between the first and last days of treatment within the groups were analysed by a Wilcoxon matched pairs test.Before analysis the DNA-polymerase ratios underwent log transformation.

Results.

Features of patients at entry into the study are summarized in table 1.No major differences were observed between the groups, although the level of virus replication and the activity of the liver disease tended to be higher in patients receiving intravenous acyclovir.

Oral acyclovir did not influence levels of viral replication. Intravenous acyclovir had an inhibitory effect on serum DNA-polymerase activity during the first three weeks of treatment ($p < 0.05$) but this effect was not sustained during the fourth treatment week and disappeared after treatment was discontinued. No effect on serum HBeAg was observed.The effects of oral and intravenous acyclovir on HBeAg and DNA-polymerase activity are shown in figure 1.

Figure 2 shows the effects of 250, 375 and 500 mg of descyclovir,administered 3 times daily for two weeks,on markers of viral replication in individual patients.Therapy was associated with 3%,19% and 9% inhibition of HBeAg in the 250,375 and 500 mg groups,respectively.In untreated controls HBeAg increased approximately 10 %. DNA-polymerase inhibition was 7%, 32% and 20%,respectively in the treatment groups compared to an increase ranging between 5 and 55 % in the control group.These differences did not reach statistical significance.No significant effects on either DNA-polymerase activity or HBe-seroconversion were shown. Tolerance for oral acyclovir and descyclovir was good.The main complication of intravenous therapy was thrombophlebitis.No patients were withdrawn from the study because of suspected side effects.

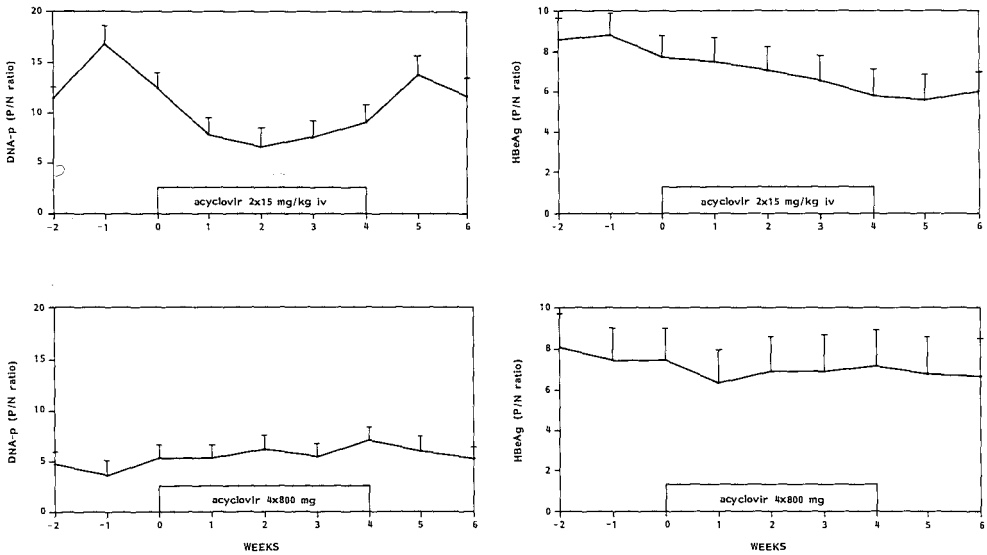

fig 1. Inhibitory effect of oral acyclovir (800 mg four times daily) and intravenous acyclovir (15 mg/kg twice daily) on DNA-polymerase activity (left panel) and HBeAg (right panel).Data are expressed as GMT +/- SEM.

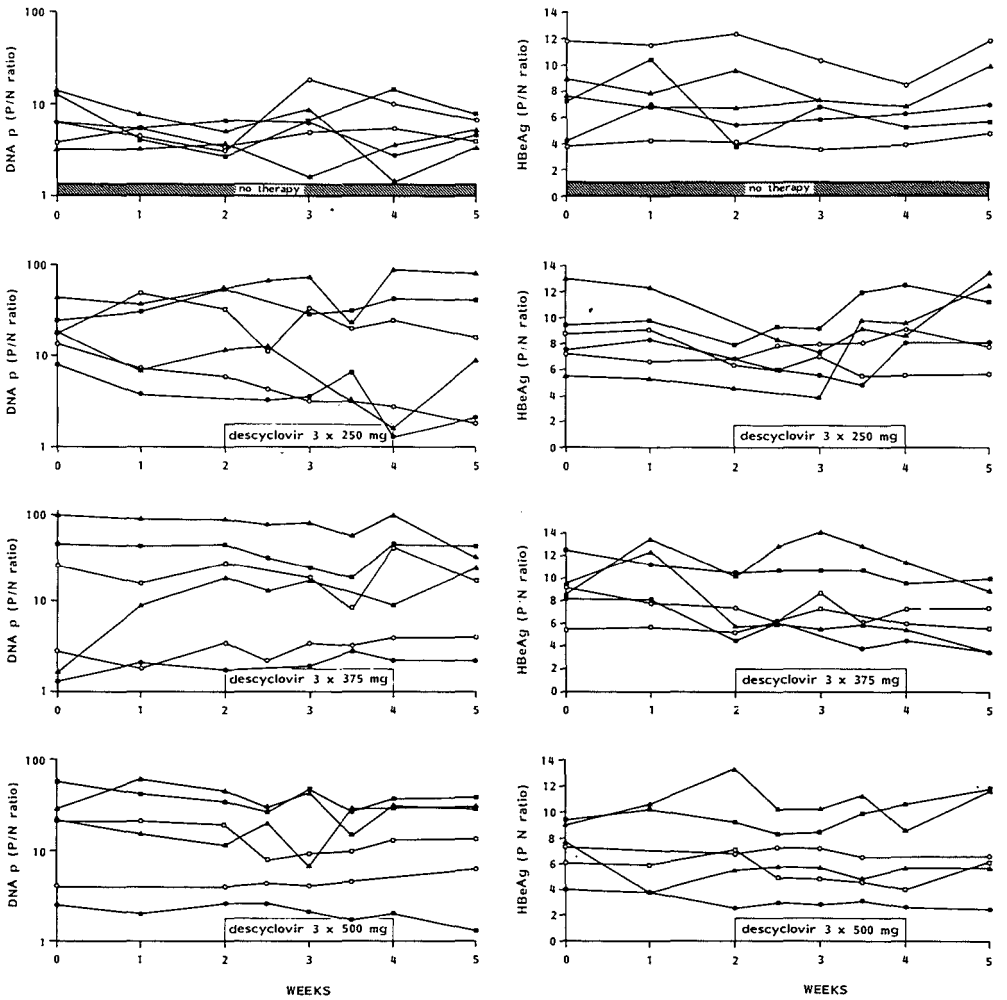

fig 2. Inhibitory effect of oral descyclovir (250 mg, 375 mg or 500 mg three times daily) on DNA-polymerase activity (left panel) and HBeAg (right panel) in individual treated patients compared to untreated control patients.

Discussion.

Oral acyclovir is ineffective in chronic hepatitis B,probably because of inadequate serum levels as a result of poor absorption of the drug.In pharmacokinetic studies of patients without liver disease serum levels of about 100 umol were found after an intravenous infusion of 15 mg/kg body weight in one hour,whereas serum levels after oral administration were usually below 20 umol (5).In our study four weeks of intravenous acyclovir had a transient inhibitory effect on serum DNA-polymerase activity and no effect on serum HBeAg.Serum levels of acyclovir after descyclovir were higher than those after oral acyclovir but approximately four times lower than those after high-dose intravenous acyclovir (6).Descyclovir has only a modest effect on hepatitis B virus replication and no effect on seroconversion. In conclusion single drug treatment with oral acyclovir or descyclovir is not a valid option for patients with chronic hepatitis type B.

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Chapter 4.

Acyclovir enhances the antiviral effect of interferon in chronic hepatitis B.

The contents of this chapter have been published in the Lancet 1985;2:358-360,under the same title and with the following authors:S.W.Schalm,H.R.van Buuren,R.A.Heijtink,R.A.de Man.

Chapter 4.

Introduction.

Antiviral agents may become the treatment of choice for chronic hepatitis B with active virus replication,since induction of virus latency - characterised by the disappearance of hepatitis-B e antigen (HBeAg)- is associated with virtual disappearance of symptoms and infectivity as well as inactivation of the liver disease (1,2).

Single-drug treatment with interferon,adenine arabinoside (ARA-A),or ARA-A monophosphate has resulted in predictable,but often only partial,suppression of hepatitis-B-virus (HBV) associated DNA-polymerase;after treatment a state of virus latency occurs only occasionally.Severe side-effects appear to prohibit the use of large doses or combinations of drugs (3).

The observation that the relatively non-toxic virustatic agent, acyclovir,inhibited HBV-DNA-polymerase prompted us to compare acyclovir with interferon and subsequently to evaluate combined treatment with acyclovir and interferon (4).

Our preliminary results suggest that combined treatment strongly suppresses not only DNA-polymerase but also HBeAg;such therapy appears promising for induction of a state of virus latency.

Patients and methods.

Twelve patients with chronic hepatitis B and active virus replication participated in the study.All had HBeAg and DNA-polymerase in their serum for at least 6 months,and the 11 who had had liver biopsies before the study had HBcAg in the nuclei of hepatocytes.

Six patients were given a 4-week course of intramuscular alpha-interferon and,after a month without treatment,a 4-week course of intravenous acyclovir.The remaining 6 patients initially received acyclovir and then interferon.The dose of acyclovir administered was 15 mg/kg in 0.5 litres of fluid twice daily. Interferon was given intramuscularly in a dose of 2.5 MU/m^2 once daily;the dose was adjusted downwards according to tolerance.

Fluid intake during acyclovir treatment was kept above 3 litres/24 h to prevent renal toxicity.Paracetamol 500-1000 mg was given 1 h before interferon to minimise its side-effects.

Blood was taken twice a week for monitoring virus replication (DNA-p, HBeAg), renal function (creatinine), leucocyte and

Table 1.Features of patients before specific therapy.

	Acyclovir (n=11)*	Interferon (n=10)*	Interferon +acyclovir (n=5)
Male,sex	10	9	5
Homosexual	4	4	1
Duration of HBsAg (yr)+	3.3	4	3.2
HBsAg (titre.10^{-3})++	2.2	2.3	5.2
HBeAg (P/N)++	8.5	8.8	9.2
DNA-polymerase (P/N)++	25.1	34	28.7
HBcAg in nuclei (%)+	39	44	55
Bilirubin (µmol/l)+	18	9	17
Aspartate aminotransferase (IU/l)+	119	71	117
Cirrhosis	1	1	1

* 3 Patients received only one treatment course because they became DNA-polymerase negative after the first treatment.
\+ Mean.
++ Geometric mean

granulocyte counts.DNA-polymerase was measured in serum with a standard method and expressed as P/N ratio (cpm patient sample /cpm normal control serum).HBeAg was measured with radio-immunoassay (Abbott) in a serum dilution constant for each patient;this serum dilution (5, 25, 125, or 625) was chosen so that pretreatment sera had a P/N ratio of 10±2.A P/N ratio of less than 2.1 was considered a negative result.

Five patients with persisting active viral replication after two treatment courses received the combined treatment of interferon and acyclovir.Interferon was given for at least 8 weeks at the maximum tolerable dose used in the first study.Acyclovir was started 2 days after interferon,when fever and malaise had subsided;the dose was also the same as that given in the first course (15 mg/kg twice daily).

For analysis,all P/N ratios were log-transformed.Means were taken of two consecutive values before treatment (x_1),for the 1st and 2nd weeks of treatment (x_2),and 3rd and 4th weeks of treatment (x_3),and for the 2nd and 3rd weeks after treatment (x_4).For each patient,differences between x_1 and x_2,x_1 and x_3 and x_1 and x_4 were calculated.For comparison within the group, Student's t-test was used to test the significance of these differences.Comparison of the combination treatment with acyclovir alone or interferon alone was done for the 5 patients who received all three treatments.

Results.

Features of the patients at entry into the study are summarised in table 1.No major differences in virus markers were observed between the groups.The activity of the liver disease tended to be lower in patients assigned to interferon,but the difference was not significant.

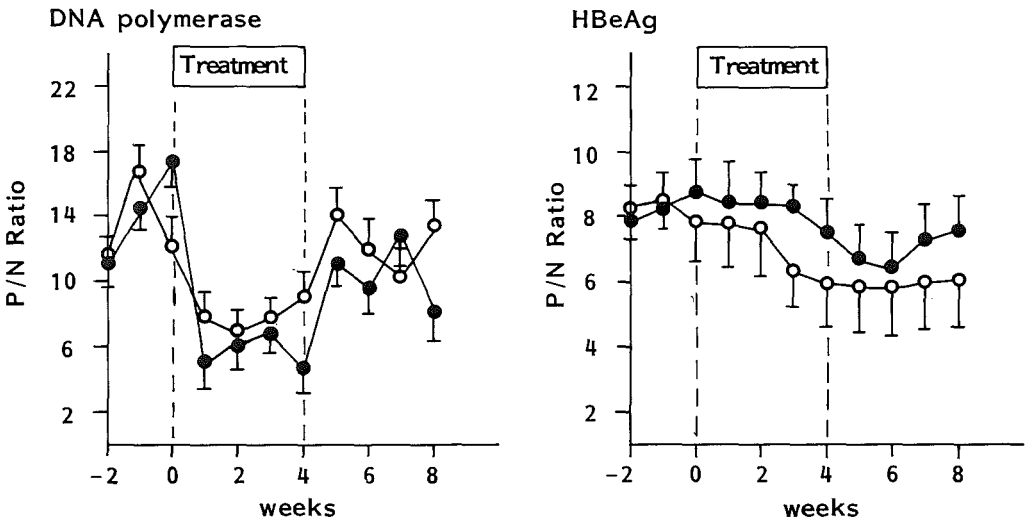

fig 1.Effects of interferon (●----●) and acyclovir (0----0) on serum HBV-DNA-polymerase and HBeAg.Both drugs induce a significant depression in DNA polymerase; the effect on HBeAg is significant only for acyclovir.

Figure 1 summarises the effects of single-drug treatment on hepatitis-B virus markers.Both interferon and acyclovir significantly depressed DNA-polymerase;this effect was achieved in the first 2 weeks of treatment.DNA-polymerase levels tended to rise after discontinuation of treatment.In 6 patients (4 with initial acyclovir,and 2 who started with interferon),however, DNA-polymerase remained negative 6 months after initial therapy. In addition,acyclovir treatment was associated with a significant fall in HBeAg (apparent in the 3rd and 4th weeks),which persisted in the month following treatment.Six months after treatment 5 patients (3 acyclovir,2 interferon) had become HBeAg negative.
Side-effects necessitated lowering the dose below 80% of the original dose in 3 instances of each therapy.For interferon, malaise and fatigue were the reasons for dose adjustment; difficulties with the intravenous administration of acyclovir caused suboptimum dosage in outpatients.Treatment had to be discontinued prematurely once with interferon (extreme fatigue) and once with acyclovir (severe thrombophlebitis).Apart from leucopenia,no major abnormalities in laboratory-test results were observed during antiviral therapy.
The effects of combination treatment with interferon and acyclovir are shown in figure 2.In addition to a rapid fall in DNA-polymerase,a significant depression in HBeAg levels was apparent in the first 2 weeks of treatment,with a further fall thereafter under continued interferon treatment.In patients who received all three treatments,the combination therapy was significantly more effective in depressing DNA-polymerase and HBeAg in the 3rd and 4th weeks of treatment than either interferon or acyclovir alone.Four patients became DNA-polymerase negative,and subsequently 3 of them became HBeAg negative.The absence of these markers of active viral replication have persisted for a mean period of follow-up of 6 months (range 1-12 months).
Tolerance of the combination therapy when administered as described above was similar to that seen with single-drug therapy,with fatigue and thrombophlebitis as major complications. No patient,however,failed to complete the full combination treatment because of side-effects.

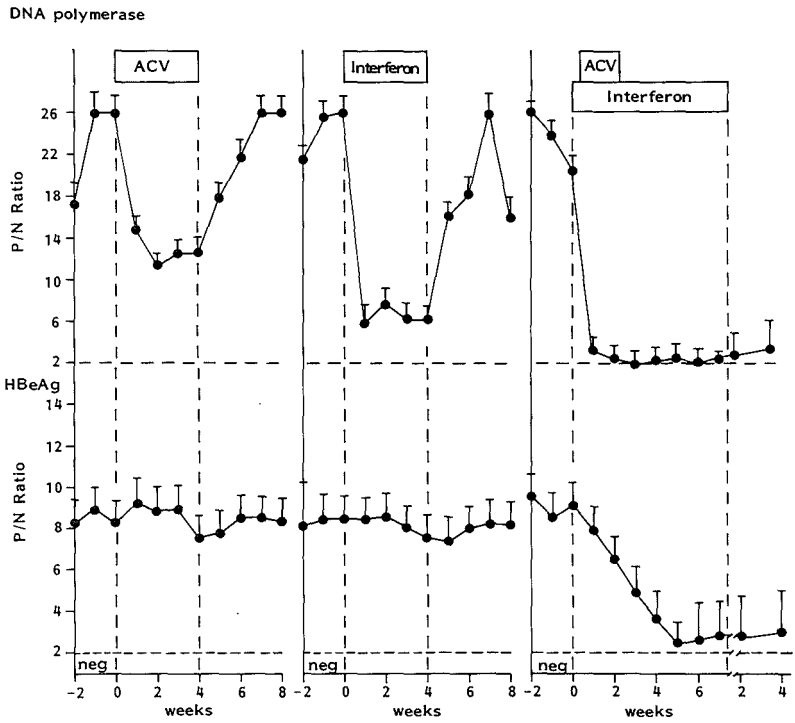

fig 2. Effects of the combination interferon/acyclovir on DNA-polymerase (upper panel) and HBeAg (lower panel).Note the more profound and persistent effect of combination therapy,compared to single-drug treatment,in 5 patients receiving all three treatments.

Discussion.

These findings confirm the reported antiviral effect of acyclovir on hepatitis B virus replication in a larger group of patients and suggest that acyclovir also depresses HBeAg synthesis (4). Moreover,a combination of acyclovir and interferon was significantly more effective in depressing HBV-DNA-polymerase and HBeAg.

Induction of long-term negativity of DNA-polymerase and HBeAg may be a prerequisite for an antiviral regimen that converts a state of active viral replication into virus latency in a high proportion of patients.Single-drug therapy with interferon,ARA-A,or acyclovir usually results in only partial depression of DNA-polymerase and HBeAg.The results of this study suggest that combination therapy is more effective in suppressing viral replication;this may well improve the chance that transition of active viral replication to virus latency will follow.

Tolerance to the combination treatment was remarkably good, provided that acyclovir was started after the initial side-effects of interferon therapy had worn off.The excellent tolerance of the combination of acyclovir and interferon is in striking contrast to that reported of the combination of interferon and ARA-A;clinical investigation of the latter regimen was stopped because of unacceptable toxicity (3).

Our encouraging results still require confirmation in a larger, controlled trial.The need to give acyclovir intravenously twice daily for at least 2 weeks makes such a study difficult.However, the recent development of an oral prodrug of acyclovir (5),which is well absorbed and gives rises to acyclovir blood levels in the range of intravenous acyclovir,will probably overcome this difficulty.

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CHAPTER 5.

Interferon plus descyclovir in chronic hepatitis type B: incidence of virus marker elimination and reactivation.

The contents of this chapter have been published in: Viral hepatitis and liver disease; Zuckerman AJ (ed.); Alan R.Liss, Inc.; New York 1988:913-916 under the same title with the following authors: R.A. de Man, S.W.Schalm, R.A.Heijtink, R.A.F.M.Chamuleau, H.W.Reesink, J.den Ouden, R.Grijm, M. de Jong, J.T.M.van der Heijden, F.J.W.ten Kate.

Introduction.

The prognosis of a patient with chronic hepatitis type B is mainly determined by the long-term complications of the infection: the development of liver cirrhosis and hepatocellular carcinoma.Therapeutic intervention can be indicated not only to improve long term prognosis but also to treat clinically important complaints such as fatigue and blood contagiousness.

Theoretically therapy aimed at eradication of the virus came available by the discovery of interferon by Isaacs and Lindeman in 1957 (1).Approximately fifteen years later, sufficient quantities of the drug are now available for clinical evaluation. In practice,treatment with interferon alone appears effective in only a minority of patients with chronic hepatitis B (2).

However combination therapy of interferon with another antiviral agent may lead to synergistic effects compared to either drug alone as has been shown for several viral infections in vitro (3,4).Inhibition of hepatitis B replication was shown for interferon and acyclovir respectively in earlier studies;however markers of viral replication often relapsed to their initial levels after therapy (5-9).In a pilot study we found that the combination of interferon and acyclovir could induce a state of virus latency (HBe-seroconversion) in 80 % of treated patients (10).HBe-seroconversion was accompanied by an improvement in clinical, biochemical and histological manifestations of the disease.The prognosis of these patients probably can become excellent if cirrhosis has not developed and no HBV-DNA can be detected in serum and no free or integrated HBV-DNA can be detected in the liver.

To document the validity of these encouraging therapeutic results we have performed a randomised controlled study using the combination of alpha-lymfoblastoid interferon (Wellferon) and descyclovir (BW 515), an oral prodrug of acyclovir (11). Descyclovir does not have any intrinsic antiviral activity of its own but is converted by hepatic xanthine oxidase to acyclovir. This paper reports on virus elimination and reactivation till 32 weeks of observation.

Methods.

Patients.

Patients who participated in this study all had chronic HBsAg positive liver disease and showed active viral replication as shown by the presence of HBsAg and HBeAg and/or the presence of DNA-polymerase activity in serum.

The main exclusion criteria for participation in the trial were: age under 18 years or above 65 years,recent alcohol or drug addiction,decompensated liver disease (ascites,encephalopathy, variceal bleeding),serious other diseases,recent antiviral or immunosuppressive therapy.Patients who were positive for HIV antibodies were accepted if they belonged to class I or II of the Walter Reed classification,which implies normal immune reactivity (12).All patients signed an informed consent form after which they were randomised in blocks of six to either treatment or control.Follow-up during therapy was weekly;after therapy had stoppped and in untreated control patients,monthly monitoring was done till one year after entry to the study.

The study was appproved by the Medical Ethics Committee of the participating hospitals.

Therapy.

Treated patients received alpha-lymphoblastoid interferon (Wellferon) 5 MU once daily subcutaneously for sixteen weeks in combination with descyclovir (BW515) twice daily 1 gram orally for 8-16 weeks;during the first three days of therapy all treated patients received indomethacine (Indocid Retard^{R}) 75 mg twice daily orally.Patients administered themselves interferon like diabetics insulin.Treatment was stopped if the HBeAg test was repeatedly negative (HBe-seroconversion) or if side effects were not tolerated.Control patients received no treatment.

During therapy patients were tested weekly for HBeAg,SGOT, kreatinin,leucocytes and granulocytes.After therapy and in control patients,blood testing was done four-weekly.At each visit serum was prepared and stored at -20°C for HBV-DNA analysis.

Laboratory methods.

HBsAg and HBeAg were measured with a standard radioimmunoassay (Abbott,Ill.).For quantification,HBeAg was measured in a constant serum dilution for each patient.This dilution was chosen so that pre-treatment sera showed a P/N ratio (cpm patient sample/cpm negative control sample) of approximately 12.A P/N ratio below

2,1 in undiluted serum was considered a negative test.DNA-polymerase activity was measured by the method of Howard as modified by Fang (13),and also expressed as P/N ratio.HBV-DNA in serum was measured by dot blot hybridisation (14).Liver biopsy samples were scored blindly according to the criteria of an international group (15).
Patient compliance with interferon therapy was estimated by the reduction in blood leucocyte count.Acyclovir levels in serum were measured using a radioimmunoasssay as described by Quinn (16).
Differences between the populations were analysed using the non-paired Wilcoxon test for continuous data and Fisher Exact test for discontinuous data.

Results.

The main characteristics of the 36 patients who participated in this trial are shown in table 1.There were no significant differences between treated patients and untreated controls on entry to the study.

Effects.

The effects of therapy are shown in figure 1-5.A significant decrease in markers of viral replication (HBeAg,HBV-DNA,DNA-p) was observed in treatment responders compared to non-responders and untreated control patients ($p<0.01$).Seven treated patients lost HBeAg (responders),but none of the 18 controls.Five of seven responders also developed antibodies to HBeAg.Two treated patients lost HBsAg,one of them developed antiHBs antibodies.
In patients with HBe-seroconversion clinical improvement (admittedly a subjective but important criterium) was noticed,and liver function became normal.Histologic evaluation will be done after one year follow-up.
An analysis of factors that discriminates responders to antiviral therapy from non-responders showed significantly lower DNA-polymerase activity and serum HBV-DNA in responders (table 2).
During therapy reduction of DNA-polymerase activity,HBV-DNA, HBeAg and HBsAg was observed in all treated patients.However only patients with initial low levels of DNA-polymerase activity and HBV-DNA accomplished HBeAg seroconversion (fig 2,3,4).

Table 1. FEATURES OF PATIENTS ON ENTRY TO THE STUDY

	Therapy (n =18)	Control (n =18)
DEMOGRAFIC DATA		
sex (m/f)	18/0	14/4
age,yr	39.9+ (1.9)*	32.9+ (3.0)*
country of origin:		
Western Europe	17	13
Meditterranean	1	2
Asia/Far East	0	3
VIROLOGY		
HBsAg (titer)	83602+ (23681)*	81055+ (43142)*
HBeAg (P/N ratio)	13.9+ (1.3)*	13.7+ (1.4)*
DNA-p (P/N ratio)	22.7+ (7.7)*	48.8+ (22.9)*
HBV-DNA (pg/ml)	676+ (228)*	3267+ (2014)*
HIV antibodies	3	5
LIVER		
cirrhosis	5	5
varices	3	3
SGOT:normal	4	6
SGOT:1-2 x raised	12	10
SGOT > 3 x raised	2	2

* = SEM
\+ = mean.

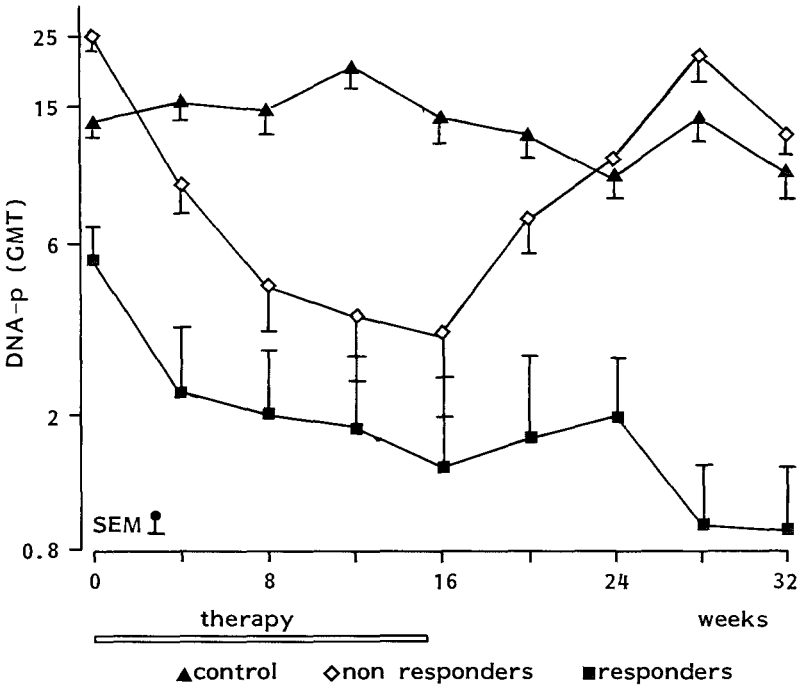

fig 1.DNA-polymerase activity before, during and after treatment with interferon and descyclovir.Viral markers decreased in responders and non-responders, but responders had lower levels of viral markers at the start of therapy.Viral markers in control patients showed no change.

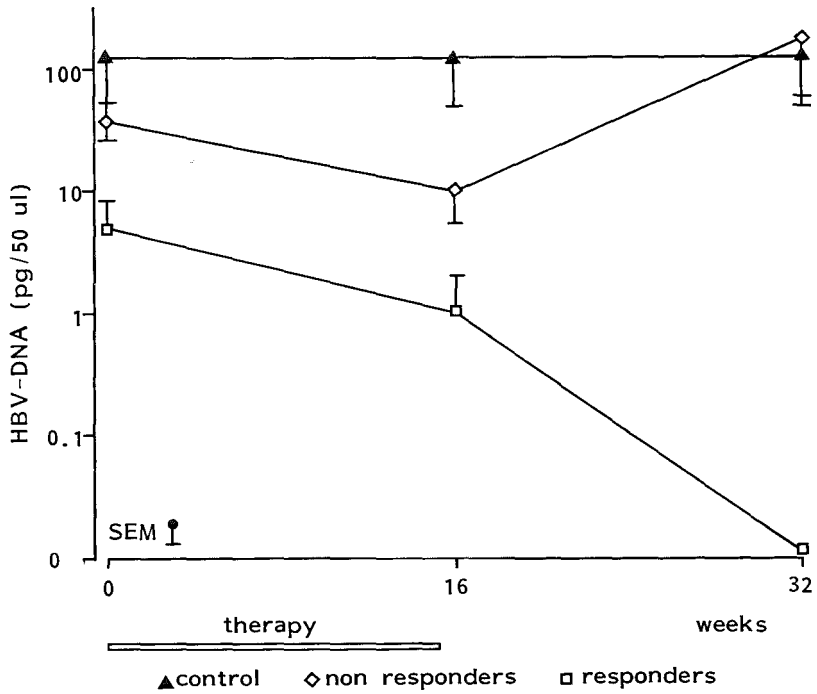

fig 2. Serum HBV-DNA before, during and after treatment with interferon and descyclovir.Viral markers decreased in responders and non-responders, but responders had lower levels of viral markers at the start of therapy. Viral markers in control patients showed no change.

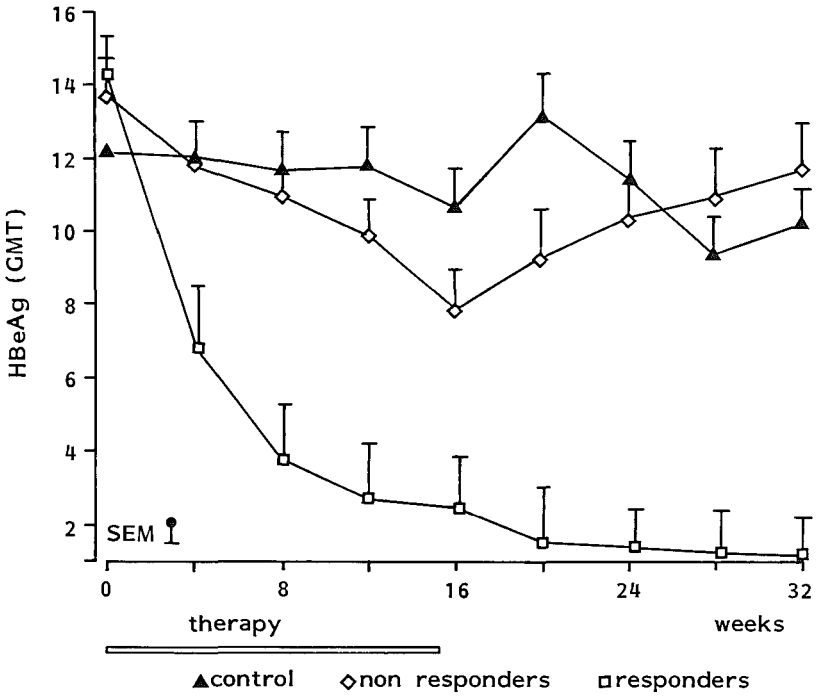

fig 3.HBeAg before, during and after treatment with interferon and descyclovir.

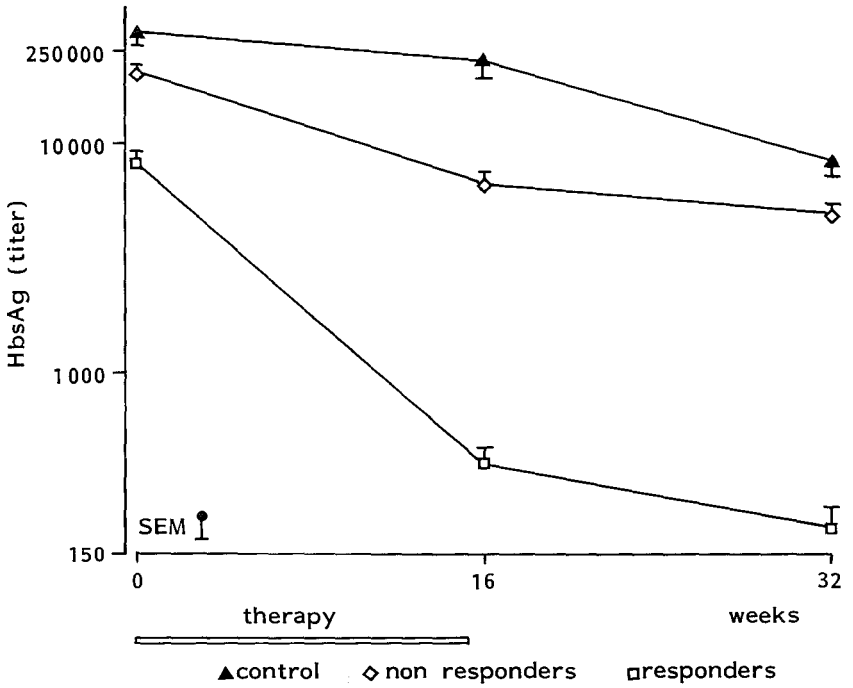

fig 4.HBsAg before,during and after treatment with interferon and descyclovir.

Table 2. VIROLOGICAL PROFILE OF TREATED PATIENTS ON ENTRY TO THE STUDY

	Responders n = 7	Non-Responders n = 11	p.value
HBsAg (titer)	977714+	74622+	n.s.
HBeAg (P/N ratio)	13.2+	14.3	n.s.
DNA-p (P/N ratio)	9.4+	31.1+	n.s.
DNA-p P/N ratio < 3	4*	0*	p<0.02
HBcAg (%)	18+	38+	n.s.
HBV-DNA (pg/ml)	135+	1019+	p<0.05
HIV antibodies	2*	1*	n.s.

* = number of patients
\+ = mean
n.s. = not significant.

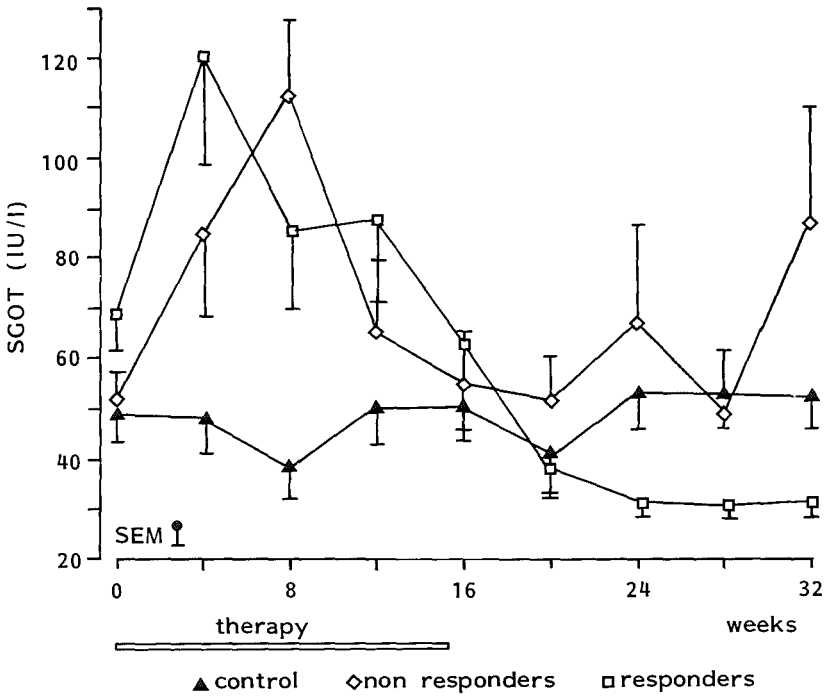

fig 5.Aminotransferase-levels in patients with chronic hepatitis B before, during and after antiviral combination therapy.Elevation of SGOT occurred in the treatment group, without a difference between responders and non-responders.

Side Effects.

In general therapy was tolerated well.The main side effects consisted of fatigue and malaise (often described as a flu like illness),transient leuco/trombopenia and loss of scalp hair.
All side effects dissappeared after dose reduction or discontinuation of therapy.In one patient therapy was stopped because of severe fatigue after 14 weeks.In the majority of patients (n = 12) the dose of interferon and descyclovir was reduced till 50-75 percent of the original dose.In 5 patients no dose adjustment was required.

Compliance.

Acyclovir plasma levels measured 2-4 hours after ingestion varied between 20 and 40 umol/l.Only 2.9% (3/103) of the samples tested showed no acyclovir when this should have been present according to treatment schedule.
All patients had marked leucopenia during interferon therapy.

Discussion.

This study is among the first randomized controlled trials that shows significantly increased HBe-seroconversion in treated patients compared to untreated control patients.The HBeAg seroconversion rate observed was about 40% above that of the control group.

Review.

Our initial trial performed in 1979 with leucocyte interferon for 6 weeks (dosage now considered adequate were used for 4 weeks) showed no difference between therapy and control (17).Recently, preliminary reports were published on 8 controlled trials with lymphoblastoid or recombinant alpha-interferon;the duration of treatment was 3-6 months with dosage of 15-35 MU per week (18-25).Seven trials did not show a significant difference for HBe-seroconversion between treatment and control,although a 20 % higher HBe-seroconversion rate was observed with inferferon in several studies (18,21,23,24,25).
The beneficial results of our recent controlled trial might be related to the use of the combination therapy,or be influenced by the selection of patients.
To clear the hepatitis B virus an intact host defence system is thought to be of importance.Previous studies identified patients

with HIV antibody as non-responders to antiviral therapy.However details of their immune status were not presented.In our study three HIV antibody positive patients with apparantly normal immunity were treated;two responded with HBe-seroconversion. Immune reactivity rather than the presence of HIV antibodies may be important for a therapeutic response.

Favourable prognostic factors regarding the outcome of therapy in our population were low values of serum HBV-DNA and low DNA-polymerase activity.In patients with high levels of DNA-polymerase activity or HBV-DNA activity only transient depression of viral markers was observed.

The 80% HBe-seroconversion rate observed in our pilot study with interferon and intravenous acyclovir could not be confirmed in this study with the oral prodrug of acyclovir.A possible explanation could be the lower plasma level of acyclovir that was obtained with the oral produg compared to intravenous acyclovir.A large randomised study using the combination of interferon and intravenous acyclovir is in progress now.

Our results justify an optimistic view regarding antiviral treatment of chronic hepatitis B patients.Special attention should be given to initial levels of viral replication for identification of patients who could benefit from current antiviral therapy.

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CHAPTER 6.

Long-term follow up of antiviral combination therapy in chronic hepatitis B.

The contents of this chapter have been published in the American Journal of Medicine 1988;85:150-154,under the same title with the following authors:R.A.de Man,S.W.Schalm,R.A.Heijtink,L.Berk, J den Ouden,F.J.W.ten Kate,R.A.F.M.Chamuleau,H.W.Reesink.

Chapter 6.

Introduction.

Antiviral combination therapy for chronic hepatitis type B has been used in our hospital since 1983.In this report we present the long-term follow-up of patients treated with alpha-lymphoblastoid interferon in combination with acyclovir or the oral prodrug descyclovir (Burroughs Wellcome 515).
We have re-analyzed the outcome for two groups of patients,who were the subject of previous reports (1,2).From 1983-1984,in an uncontrolled pilot study 12 patients were treated with acyclovir, interferon or - if both drugs were unsuccesfull - a combination of both (study I).In 1985-1986 a randomized controlled study was performed in 36 patients comparing the combination of interferon and descyclovir to "no therapy" (study II).Results are presented as of December 1987 after a follow-up of 1-4 years.

Methods.

Patients and treatment.

Patients who participated in the studies all had active viral replication as shown by the presence of stable levels of HBsAg and HBeAg in the serum for at least six months.
Exclusion criteria for experimental antiviral therapy were:age under 18 or above 65 years,alcohol or drug addiction, decompensated liver disease (ascites, encephalopathy, variceal bleeding),serious other diseases or recent immunosupppressive therapy.Patients with cirrhosis all belonged to group A according to the Child-Pugh classification.Patients who were positive for HIV antibodies could enter the trial if they belonged to class 1 or 2 of the Walter Reed classification (3).

Study I.

Twelve patients entered this study.Six patients were given a 4-week course of alpha-lymphoblastoid interferon (Wellferon^{R}), and in case of no HBe-seroconversion after a month without treatment,a 4-week course of acyclovir (Zovirax^{R}).The remaining six patients received acyclovir initially and non-responders subsequently interferon.Assignment to treatment schedule was determined by random numbers.Acyclovir was administered intravenously in a dose of 15 mg/kg in 0.5 l of fluid twice daily.Interferon was given intramuscularly in a dose of 2.5 MU/m^2 once daily;the dose was adjusted downwards according to tolerance.

Seven patients with persisting active viral replication after two treatment courses received the combination treatment of interferon and acyclovir.Interferon was given for at least 8 weeks at the maximum tolerable dose used in the first study. Acyclovir was started 2 days after interferon,when fever and malaise had subsided;the dose was also the same as that given in the first course (15 mg/kg twice daily).Acyclovir infusions were given on an outpatient basis;two patients living more than 20 kilometers outside the hospital area did not complete the acyclovir part of the combination therapy.If no seroconversion had occurred after two months,the combination treatment was repeated in some patients.To suppress side effects of interferon therapy 500 mg paracetamol was given qid.

Study II.

Thirty-six patients were randomized in blocks of six to either combination therapy or control.Treatment patients received interferon (Wellferon^{R}) 5 MU once daily subcutaneously for 16 weeks in combination with descyclovir 1 gram orally twice daily for 8-16 weeks (4);during the first three days of therapy patients received slow-release indomethacin 75 mg orally twice daily to suppress fever and the flu-like syndrome.Patients administered themselves interferon like diabetics insulin. Treatment was stopped if a HBeAg test was negative on two consecutive occasions (HBe-seroconversion) or if side effects were intolerable.Control patients received no treatment.

Assessment and laboratory methodology.

Before treatment,and 4-monthly thereafter,all patients completed a diary score concerning quality of life,symptoms and signs;the maximum score was 120 points (5).During therapy patients were tested weekly for HBeAg, SGOT, creatinin, leukocytes and granulocytes.After therapy had been stopped and in control patients,monitoring was done every four weeks.At each visit serum was prepared and stored for HBV-DNA analysis.HBsAg was measured by a standard radioimmunoassay (Abbott,Ill).HBeAg was measured by a radioimmunoassay (Abbott,Ill) in a constant serum dilution for each patient.The dilution was chosen so that pre-treatment sera showed a P/N ratio of approximately 12.A P/N ratio below 2,1 in undiluted serum was considered a negative test.DNA-polymerase activity was measured by the method of Howard as modified by Fang (6),and expressed as P/N ratios (cpm

patient sample/cpm negative control sample).HBV-DNA in serum was measured by dot blot hybridisation (7).All liver biopsy samples were scored blindly according to the criteria of an international group (8).Patient compliance with interferon therapy was estimated by the decrease in blood leukocyte count;acyclovir levels in serum were measured by a radioimmunoasssay as described by de Miranda (9).Differences between the populations were analysed by the non paired Wilcoxon test for continuous data and Fisher Exact test for non-continuous data.
Both studies were approved by the Medical Ethics Committee's of the participating hospitals.

Results.

Table I shows general,biochemical,virological and histologic entry data for patients in both the pilot and controlled study.No significant differences were observed - for study II - between treated patients and control patients in demografic data,mode of transmission,activity of the liver disease,degree of active viral replication or liver histology.Patients in study II however were in comparison to study I more frequently young homosexuals with a shorter duration of HBs antigenemia,higher levels of viral replication and less liver cell inflammation.
Virologic response rates are shown in table II.Re-evaluation of the data from the pilot study made us realize more clearly that during the development of the antiviral combination treatment program repeated courses of therapy had been used in several patients.Therefore we also related response to the number of antiviral treatment courses.HBe-seroconversion related to the number of courses of antiviral therapy is now estimated at 42% for interferon/acyclovir (5/12), 27% for interferon alone (3/11) and 18% for acyclovir alone (2/11).An example of a patient from study I who lost DNA-polymerase activity,HBeAg,HBV-DNA and HBsAg,is shown in figure 1.
Long-term follow up showed HBs-seroconversion in 4 out of 10 study I patients with HBe-seroconversion.Three of those 4 patients developed antiHBe antibodies and subsequently antiHBs after HBs-seroconversion.AntiHBe antibodies were observed in 3 out of 6 patients with isolated HBe-seroconversion.HBe-reactivation was observed in two patients.Both patients had cirrhosis and did not develop antibodies to either HBeAg or HBsAg at any time,although one patient had no evidence of HBV-DNA and

Table I: Entry features of patients

	Study I	Study II	
General		treatment	control
no of patients	12	18	18
sex (male)	11	18	14
age (yrs,mean)	43.6	39.9	32.9
origin: West-Europe	11	17	13
Mediterranean	0	1	2
East Asia	1	0	3
Transmission			
bloodproducts	1	0	3
medical profession	2	3	1
homosexual	4	7	10
heterosexual	1	2	1
tattoo/iv drug abuse	0	2	0
unknown	4	4	3
Virology			
HBsAg positive (yrs,mean)	6.0	2.5	3.0
HBsAg (titre,mean)	33360	83602	81055
HBeAg (P/N,mean)	9.1	14.8	13.7
DNA-p (P/N,mean)	14.3	31.7	45.5
HBV-DNA (pg/ml,mean)	nt	676	3267
HBcAg in nuclei,% (mean)	28	31	23
antiHIV,positive	nt	3	5
Biochemistry			
SGOT (IU/l,mean;n < 30)	115	59	49
Beta-2 microglobulin (mg/L,mean)	nt	2.0	2.5
Liver histology			
CPH/CAH mild	6	9	10
CAH (moderate,severe)	2	4	1
Cirrhosis	4	5	5
not tested	0	0	2

Table II: **Number of patients (percentage) with therapeutic responses after antiviral therapy in chronic hepatitis B.**

	Study I			*Study II*	
Therapy	ACV	IFN	ACV/IFN	DACV/IFN	CONTROL
Response					
HBeAg(+), DNA-p(+)	8 (73%)	6 (60%)	2 (29%)	10 (55%)	11 (61%)
HBeAg(+), DNA-p(-)	1 (9%)	1 (10%)	0 (0%)	1 (5%)	7 (39%)
HBeAg(-), DNA-p(-)	2 (18%)	3 (30%)	5 (71%)	7 (40%)	0 (0%)
number of patients	11 (100%)	10 (100%)	7 (100%)	18 (100%)	18 (100%)

ACV: acyclovir, IFN: interferon, ACV/IFN: combination of acyclovir and interferon, DACV/IFN: combination of descyclovir and interferon.

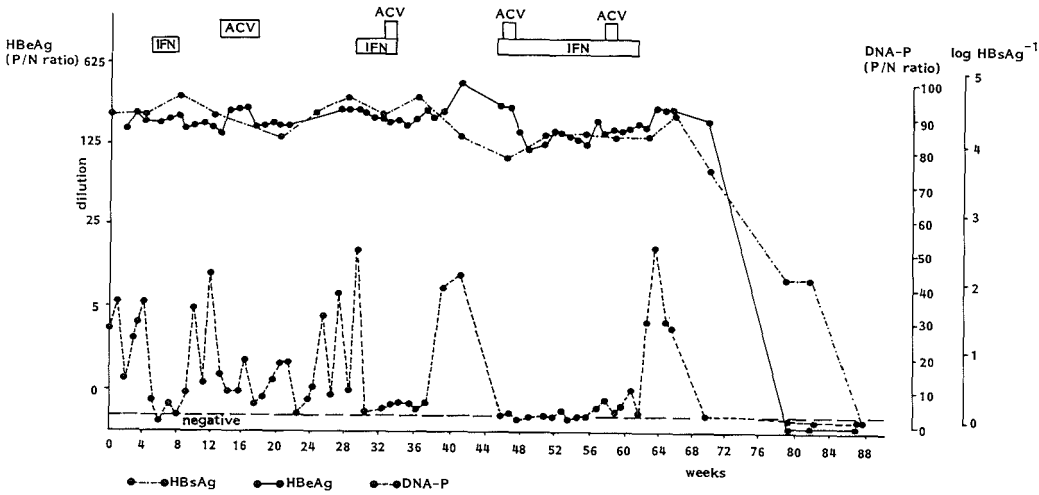

fig 1. Repeated courses of antiviral therapy (ACV, IFN, ACV/IFN) ultimately leading to successive clearance of DNA-polymerase activity, HBV-DNA, HBeAg, HBsAg and development of antibodies.

HBsAg in the serum for more than 1 year.Two of the 12 patients from study I never had any persistent effect on the virus replication after antiviral therapy.

In study II seven of 18 treated patients cleared HBeAg in contrast to none of 18 control patients.No reactivation has been observed.Six of 7 responders developed antibodies to HBeAg.HBsAg clearance with development of antiHBs antibodies was observed in two of 7 responders.DNA-polymerase and HBV-DNA was not detectable at entry in 2 patients of both treatment and control group. During follow-up DNA-polymerase disappeared in 6 treated and 5 control patients respectively,whereas HBV-DNA became undetectable in 5 treated and 2 control patients.

In both studies transition from the state of active viral replication (HBe-positive) to the state of virus latency (HBe-negative) was associated with clinical improvement as reflected by increasing diary score and decreasing biochemical abnormalities in SGOT.

In study II histological evaluation was done at entry and repeated after 1 year in both treated patients and controls. Twenty-eight patients had paired liver biopsies (13 controls,15 treated,5 responders,10 non-responders).In all 7 patients who cleared HBeAg and HBV-DNA,HBcAg could no longer be detected in the liver.Significant structural improvement however was not observed,since the degree of fibrosis remained similar.

In study II analysis of factors that could predict response to antiviral therapy,showed low initial serum DNA-polymerase and HBV-DNA in responders.During therapy depression of DNA-polymerase activity,HBV-DNA,HBeAg and HBsAg was observed in all treated patients.However only patients with initial low levels of DNA-polymerase activity and HBV-DNA had HBeAg seroconversion. Response was not related to anti-HIV status or sexual preference. No differences in mode of transmission,SGOT-levels or HLA turnover assessed by serum beta-2 microglobulin levels at entry could be shown.

Also,a non-specific rise in SGOT-levels during therapy was not a prognostic factor for HBe-seroconversion.However a specific (monofasic spike during the 8-12th week of interferon therapy) rise in SGOT was observed in seven of 7 responders,while such a rise in SGOT occurred in 6/11 non-responders ($p < 0.001$).The SGOT spike preceded HBeAg clearance in six out of seven responders. Figure 2 shows examples of specific and non-specific rises in

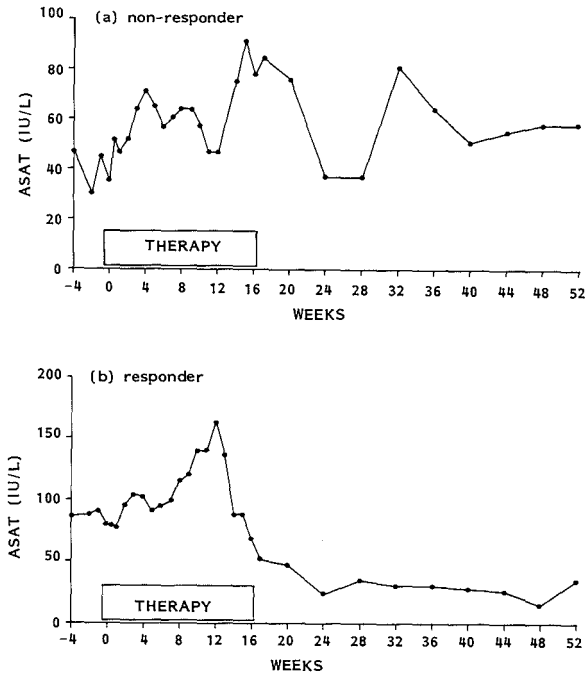

fig 2.In relation to HBe-seroconversion specific and non-specific rises in SGOT in the second part of treatment are shown in panel A and B respectively.The early rise in SGOT is probably a toxic effect of interferon.

serum transaminases.
A lag time between the period of drug therapy and HBe-seroconversion was observed frequently.In study II HBe-seroconversion occurred twice during therapy but after therapy in five patients.In study I HBe-seroconversion was observed during therapy in five patients and after therapy in others.

Discussion.
Treatment with the combination of interferon/acyclovir or interferon/descyclovir showed HBe-seroconversion in more than 40% (12/25),whereas none of the untreated control patients lost HBeAg during the observation period.Treatment with a single drug was associated with a lower percentage of HBe-seroconversion (27% for interferon and 18% for acyclovir).These results suggests the enhanced efficacy of combination therapy,but proof can only be obtained in a randomized controlled study comparing single agent and combination therapy.The overall high HBe-seroconversion rate (10/12,83%) of study I is in all likelihood influenced by patient-selection and repeated courses of treatment.Other investigators have found that symptomatic patients with a high degree of liver cell inflammation are prone to HBe-seroconversion (10);also repeating a course of antiviral therapy can boost the percentage HBe-seroconversion (11).
During long-term follow up (1-4 years) reactivation of hepatitis B virus replication occurred in two patients.One of them had no HBsAg detectable in the serum for more than 1 year.Both patients had macronodular cirrhosis and did not develop antibodies after clearance of HBeAg or HBsAg.These observations made clear,that the concept of cure from chronic viral hepatitis B can only be applied to patients with loss of HBsAg and HBeAg,development of antiHBe and antiHBs antibodies and absence of HBcAg and HBV-DNA in the liver.
Long-term follow up also showed an unexpected high rate of HBs-seroconversion in patients with loss of HBeAg (6/17,36%) confirming observations by others (12,13).Apparently liver cells containing the hepatitis B viral genome are being distroyed or synthesis of HBsAg stops by an undefined mechanism.Disappearance of HBsAg does not imply cure of chronic hepetitis B since both development of hepatocellular carcinoma and reactivation can occur.
Virus clearance and HBe-seroconversion occurred mainly in patients with initial low levels of viral replication and most

often after therapy had been stopped.Clearance of the virus was usually accompanied by a spike-like rise in SGOT levels during the third month of treatment.These observations,also found by others (12),suggest that antiviral agents initially suppress viral replication,thereby allowing the host immune response to recover and to eliminate virally infected cells.

HBe-seroconversion was associated with clinical and biochemical improvement in almost all patients which persisted during follow up.However we did not observe a significant improvement in liver histology 1 year after start of treatment.In 5 study II patients liver fibrosis had not been affected.These findings underline the concept that antiviral therapy should now be applied early in the course of chronic infection to prevent structural damage of the liver as well as to minimize the risk of viral integration.

Who should be offered this rather expensive therapy? We studied a predominantly Westeuropean population with an adult life acquired HBV infection.We did not observe any relationship between therapeutic response and sexual preference,HIV status,SGOT levels and the histological stage of the liver disease.The only predictors of HBe-seroconversion were initially low levels of viral replication.We therefore think that such patients should be candidates for antiviral combination therapy,which holds promise - albeit small - of a cure of the HBV infection.

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Chapter 7.

Long-term follow up of alpha-interferon descyclovir combination therapy.

The contents of this chapter have been submitted for publication.

Chapter 7.

Introduction.

Since the loss of viral HBeAg and HBV-DNA from the blood has been associated with improvement in liver disease (1,2),elimination of the hepatitis B virus,and not immunosuppresion,has become the key to effective treatment of chronic hepatitis B virus infection (3).In a pilot study we found that a combination of alpha-interferon and acyclovir could induce a state of virus latency (HBe-seroconversion with loss of HBV-DNA) in 4 out of 5 treated patients (4).To document the validity of these encouraging therapeutic results,we performed a randomized controlled study using the combination of alpha-lymphoblastoid interferon (Wellferon) and descyclovir,an oral prodrug of acyclovir (5).The aim of the controlled study was to determine whether combination therapy consisting of interferon with descyclovir induces virus latency as indicated by disappearance of HBeAg.In addition we wanted to assess whether the induced loss of HBeAg persists,how persistent absence of this marker correlates with absence of HBV-DNA and whether a change from active hepatitis B virus replication to a state of virus latency is associated with disappearance of the histological characteristics of hepatic inflammation.We have already reported on virus elimination and reactivation after 32 weeks of observation (6).This paper concerns the long-term efficacy of antiviral combination therapy with special emphasis on the results of serum virology and liver histology till 2 years of observation.

Patients and Methods.

Study design.

Patients who participated in this study all had histologically proven chronic liver disease and showed active viral replication as indicated by the presence of HBsAg,HBeAg and HBV-DNA or DNA-polymerase activity in serum.The main exclusion criteria were:age under 18 or above 65 years,recent alcohol or drug addiction, decompensated liver disease (ascites,encephalopathy,variceal bleeding),clinical or histological suspicion of concomitant non-A non-B hepatitis,other serious diseases including active concomitant viral infection (CMV,Delta or EBV) and recent (<1 year before entry) antiviral or immunosuppressive therapy. Patients who were positive for HIV-antibodies were accepted if class I or II of the Walter Reed classification,which implies normal immune reactivity (7).Patients were randomized in blocks

of six either to undergo treatment or to be controls.During the 16 weeks of therapy patients were monitored weekly;for patients who had completed therapy and untreated controls,follow up was monthly until one year after entrance into the study. Subsequently,treated patients were examined every three months until two years after entry into the study.Nine patients who were initially in the 'no treatment' group received alpha- interferon after one year of follow up.Follow up for these patients was identical to that for treated patients during the first year of the study.Key-point evaluation was performed after 4,8,12 and 24 months of follow up (fig 1.).All patients gave written informed consent to the trial.The study was approved by the Medical Ethics Committee of the participating hospitals.

Therapy.

Treated patients received 5 MU alpha-lymphoblastoid interferon (Wellferon) once daily subcutaneously for sixteen weeks in combination with 1 gram descyclovir twice daily orally for 8-16 weeks;during the first three days of therapy all treated patients received 75 mg of indomethacin (Indocid Retard^{R}) twice daily orally.Patients administered the interferon themselves, as diabetics do insulin.Treatment was stopped if the quantitative HBeAg test was negative twice or side effects were not tolerated. Control patients received no treatment during the first year of the study:subsequently they were offered 5 MU alpha-interferon (Wellferon) once daily subcutaneously for sixteen weeks (descyclovir was no longer available).During therapy patient sera were tested weekly to determine HBeAg,AST, creatinine,leucocyte and granulocyte levels.Sera from patients who had completed therapy and all control patients were assessed every four weeks for one year and then every three months (fig 1).Each serum sample was prepared and stored at -20 ^{o}C for beta-2 microglobulin and HBV-DNA analysis.

Laboratory methods.

HBsAg was measured using a standard radioimmunoassay (Abbott, North Chicago,Ill.);HBeAg was measured using a radioimmunoassay (Abbott,North Chicago,Ill).For quantification,HBeAg was measured in a dilution of serum that was constant for each patient.This dilution was chosen such that pre treatment sera showed a P/N ratio (cpm patient sample/cpm negative control sample) of approximately 12 +/- 2.A P/N ratio below 2.1 in undiluted serum

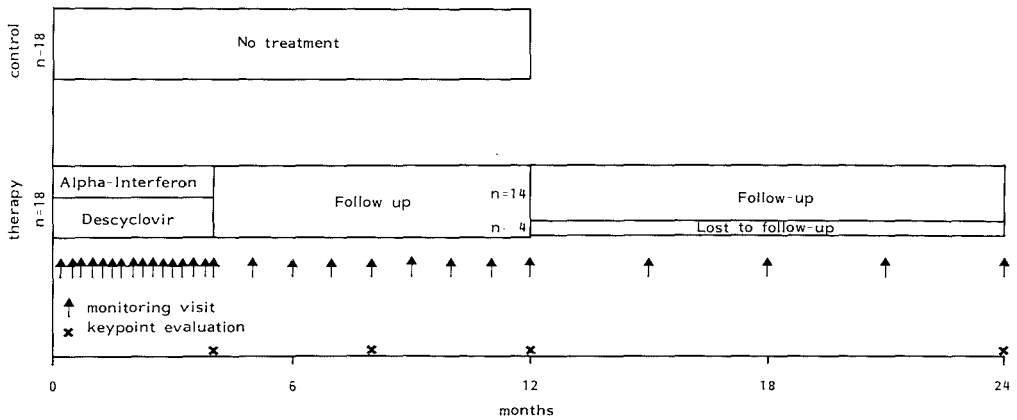

fig 1.Treatment design and follow-up schedule.

was considered a negative result.DNA-polymerase activity was measured by the method of Howard,as modified by Fang,and was expressed as a P/N ratio (8).HBV-DNA in serum was measured by dot blot hybridisation (9).IgM anti-HBc was measured using a standard Elisa (Abbott,North Chicago,Ill.)

At entry and after one year all patients were tested for the presence of IgM-CMV,IgM-EBV(VCA),HIV and Delta antibodies.A positive HIV enzyme immunoassay antibody test (Wellcozyme) was confirmed by Western blot analysis.Routine serum biochemical tests were done using automated techniques (Coulter,SMAC, Technicon, NY).Serum beta-2 microglobulin concentrations were measured in duplicate with the commercially available beta-2 microglobulin radioimmunoassay (Pharmacia Diagnostics AB,Uppsala, Sweden).In addition in all cases an ultrasound examination of the liver and oesophagogram according to Muller's procedure were performed to detect portal hypertension and to exclude patients with possible hepatocellular carcinoma.In the event of suspected oesophageal varices on the oesophagogram, gastroscopy was carried out for confirmation.

Two liver biopsies were obtained from all patients at the beginning of the study and again one year later:one was snap frozen in liquid nitrogen for immunofluorescence studies,the other was processed for routine microscopy.Liver biopsies were taken by the percutaneous Tru-cut technique.To reduce risks and sampling error in patients clinically suspected of cirrhosis,the liver biopsies were obtained by laparoscopy.Liver biopsy specimens were graded (under code) by one pathologist (FJWtK) according to the criteria of an international group (10).Each biopsy received a score for:classification, fibrosis, portal infiltrate density,portal infiltrate penetration and HBc in hepatocyte nuclei.Classification ranged from 0-6 (0 = no or non-specific abnormalities, 1 = chronic persistent hepatitis, 2 = mild chronic active hepatitis, 3 = moderate chronic active hepatitis, 4 = severe chronic active hepatitis, 5 = probable cirrhosis, 6 = cirrhosis).Fibrosis ranged from 0-5 (0 = none,1 = extension from portal tracts, 2 = sporadic portoportal septa,3 = portoportal septa, 4 = broad fibrous bands distorted architecture ,5 = two nodules surrounded by fibrous bands).Portal infiltrate density ranged from 0-3 (0 = none, 1 = mild, 2 = moderate, 3 = dense).Portal infiltrate penetration ranged from 0-3 (0 = none, 1 = mild, 2 = moderate, 3 = severe).

HBcAg was expressed as the estimated percentage of nuclei

positive for HBcAg in the snap frozen specimen.In the event of insufficient frozen material the biopsy specimens were stained for core antigen by the peroxidase anti-peroxidase technique.

Statistics.

Group frequencies were compared by the chi-square test and group medians by the Wilcoxon-rank sum test.Laboratory features of patients before treatment and at last follow up were compared by the Wilcoxon sign test.All data were analysed according to the 'intention to treat' principle.

Results.

The main characteristics of the 36 patients who participated in this trial are shown in table 1.There were no significant differences between treated patients and untreated controls at entry into the study).All patients were followed for at least one year after randomization.One treated patient (non-responder) died of Kahler's disease in the second year of the study;one control patient and 3 treated patients (one responder,two non-responders) were lost to follow up in the second year of the study.

Responses to combination therapy.

Changes in HBeAg.

At the end of therapy (16 weeks) 3 patients had responded,while in the interval between 16 and 32 weeks another 4 patients exhibited loss of HBeAg (fig 2a).After one year of follow up HBeAg seroconversion had occurred in 0% of control patients (95 % confidence interval 0-18 %) and 39% of treated patients (95% confidence interval 20-60%).This result is statistically significant ($p<0.01$).In the second year of the study one initial non-responder became HBeAg negative.Since no reactivation had occurred at two years,8/18 patients (44 % ;95 % confidence interval 22-69 %) were HBeAg negative after two years.

Changes in DNA-polymerase activity.

Table 2 shows the number of patients with a positive test for DNA-p at each evaluation point.Figure 2 displays the percentage of patients who lost DNA-polymerase activity.During the treatment period 7/18 (38%) treated patients and 1/18 (5%) control patients exhibited disappearance of DNA-polymerase activity ($p<0.01$).After 32 weeks of observation reactivation had occurred in 3 treated patients all of whom were HBeAg positive;DNA-polymerase activity

Table 1. Pretreatment characteristics of the study groups.

	Therapy (n=18)	Control (n=18)
Age (yrs; median, range)	36 (23-64)	33 (19-49)
Sex (% males)	100	77
Homosexual (n, %)	7 (39)	10 (28)
Anti HIV-positive (n, %)	3 (17)	5 (28)
Area of origin		
Western Europe	17	13
Mediterranean	1	2
Asia/Far East	0	3
AST: Normal (n)	4	6
1-2 x raised (n)	12	10
> 3 x raised (n)	2	2
Bilirubin (µmol/l, n < 12)	11 (5.5)	7 (3.9)
IgG (g/l, median, range, n < 17)	14.1 (6.9-14.2)	16.3 (9.6-34.7)
HBsAg (median titre,range)	10,000 (800-640,000)	20,000 (200-320,000)
HBeAg (median P/N ratio,range)	12.3 (5.7-25.6)	12.4 (2.5-26.8)
DNA-p (median P/N ratio,range)	10.9 (1.1-139)	13.9 (1.2-390)
HBV-DNA (median pg/ml,range)	233 (0-4,000)	133 (0-26,700)
Liver biopsy classification		
CPH/CAH (mild)	9	10
CAH (moderate, severe)	4	1
Cirrhosis	5	5
Not tested	0	2
Child-Pugh classification A	18	18
Oesophageal varices	3	3

Table 2. Outcome of therapy for treated patients compared to untreated controls.

Group	Number of patients in follow-up	Number of patients HBe-negative			
		Entry	16 weeks	32 weeks	52 weeks
Control	18	0	0	0	0
Therapy	18	0	3	7	7

Group	Number of patients in folow-up	Number of patients DNA-polymerase negative			
		Entry	16 weeks	32 weeks	52 weeks
Control	18	2	3	3	7
Therapy	18	3	10	7	8

Group	Number of patients in follow-up	Number of patients HBV-DNA negative			
		Entry	16 weeks	32 weeks	52 weeks
Control	18	2	3	3	5
Therapy	18	1	9	7	7

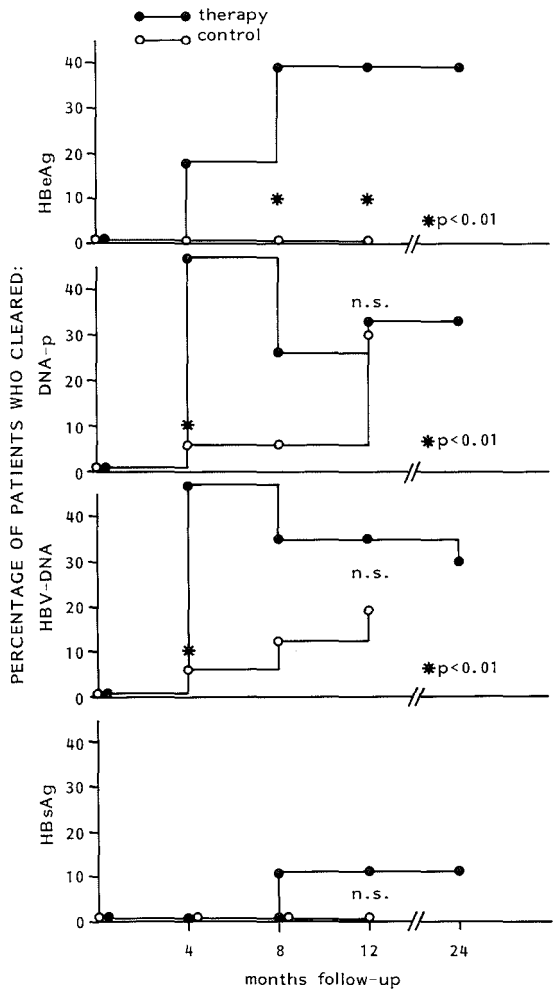

fig 2. Loss of serum viral markers in treated patients compared to untreated control patients.(* = p < 0.01 ; ns = not significant).

in control patients remained stable.After one year of follow up one treated patient who initially exhibited reactivation again showed absence of DNA-polymerase activity.In four control patients DNA-polymerase activity had disappeared at one year.The difference between treated and untreated control patients at 32 and 52 weeks was not statistically significant.

Changes in HBV-DNA.

After 16 weeks 8/18 (44%) treated patients cleared HBV-DNA compared to 1/18 (5%) control patients ($p<0.01$).After 32 weeks of observation reactivation was demonstrated in 3 treated patients; all three patients had failed to clear HBeAg.One patient,who had already cleared HBeAg,showed loss of HBV-DNA at 32 weeks.In one patient (HBeAg positive after treatment) HBV-DNA had disappeared from the serum at 32 weeks but reappeared at 52 weeks.Three control patients exhibited loss of HBV-DNA after 52 weeks of observation (table 3;fig 2).The difference between treated and untreated control patients at 32 and 52 weeks was not statistically significant.

Loss of HBsAg and antibody development.

At one year two responders who had become HBsAg-negative had developed approximately 75 IU/l anti-HBs.Five of 7 responders had developed anti-HBe after one year of follow up.This result still applied after two years of follow up.

Other virological tests.

At entry one control patient had IgM-EBV(VCA) antibodies;after one year no such antibodies were found.No Delta or IgM-CMV antibodies were found .

Follow up of control patients after one year.

Of the 18 control patients,one was lost to follow up and 9 control patients received alpha-interferon treatment 1 year after randomization.Three of the nine responded (HBeAg, HBV-DNA negative).The 8 control patients who were not treated after one year either refused treatment (n = 5) or were considered to have unstable disease (n = 3).Four of these patients became HBeAg, HBV-DNA negative.At two years 7/18 (39 %) control patients lost HBeAg with single alpha-interferon therapy in 9 patients.

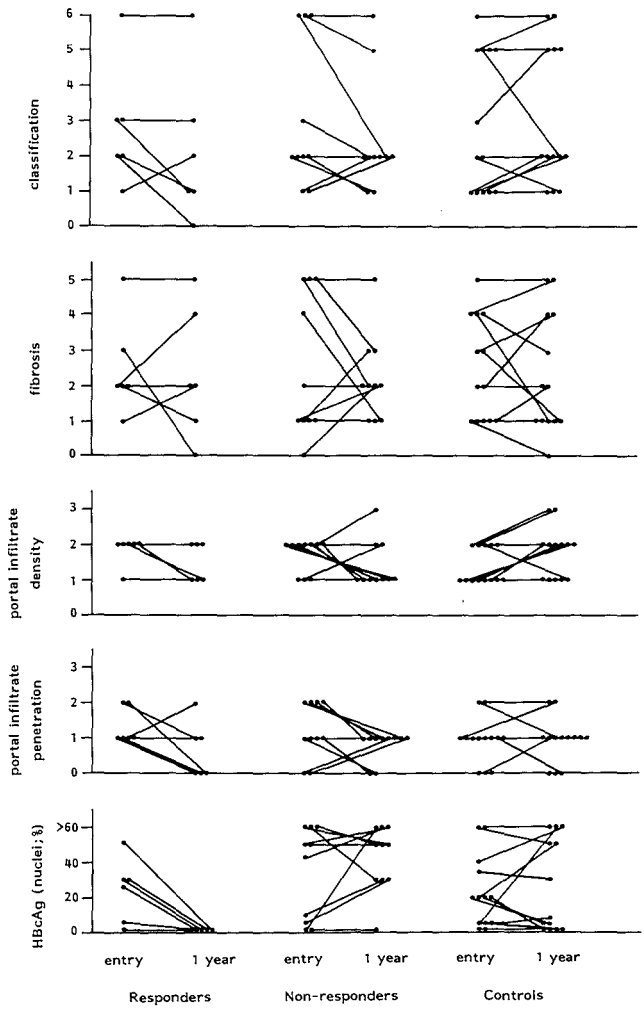

fig 3.Results of paired liver histology for responders (n = 6), non-responders (n = 10) and control patients (n = 13).

Histology.

Paired liver histology was available for 16 treated and 13 control patients.Analysis at entry and during follow up within this group was performed.Paired results before therapy and at one year for the items classification,fibrosis,portal infiltrate density and penetration and HBcAg are shown in figure 3. Histological classification improved significantly in 50% of treated patients compared to 15% of control patients (p=0.05;95% confidence interval for the difference: 2.7 - 66.5%).Portal infiltrate density decreased significantly in 44% of treated patients compared to 8% of control patients (p = 0.03;95% confidence interval for the difference 7.2-64%).Penetration of portal infiltrate decreased significantly in 63% of treated patients compared to 15% of control patients (p = 0.01;95% confidence interval for the difference 16-78%).Fibrosis did not change significantly.Among responding patients a significant decrease in HBcAg expression was observed (p = 0.03).

Changes in liver enzymes.

As shown in figure 4 AST levels in patients responding to therapy improved while levels in non-responders and untreated controls did not change.Improvement in liver enzymes did not reach statistical significance.

Beta-2 microglobulin.

Results of the beta-2 microglobulin assay have been described in detail elsewhere (11).Pretreatment levels of beta-2 microglobulin were elevated in 39 % of patients.Significant differences in beta-2 microglobulin between treated patients and untreated controls were observed after 4 and 8 weeks of treatment ($p < 0.05$).Prior to and during therapy the mean elevation of beta-2 microglobulin was similar in responders (n = 7) and non-responders (n = 11).

Because of the observations of Pignatelli (12) we also tested the serum of seven responders and eight non-responders after 3, 7, 14, 21 and 28 days of treatment.Beta-2 microglobulin in responders showed a mean increase of 20, 99, 74, 82 and 52% at 3, 7, 14, 21, 28 days,respectively.In non-responders the mean increase in beta-2 microglobulin was 177, 121, 133, 104, 82 percent,respectively.Four of seven responders and six of eight non-responders showed a rise in beta-2 microglobulin at 2 weeks.

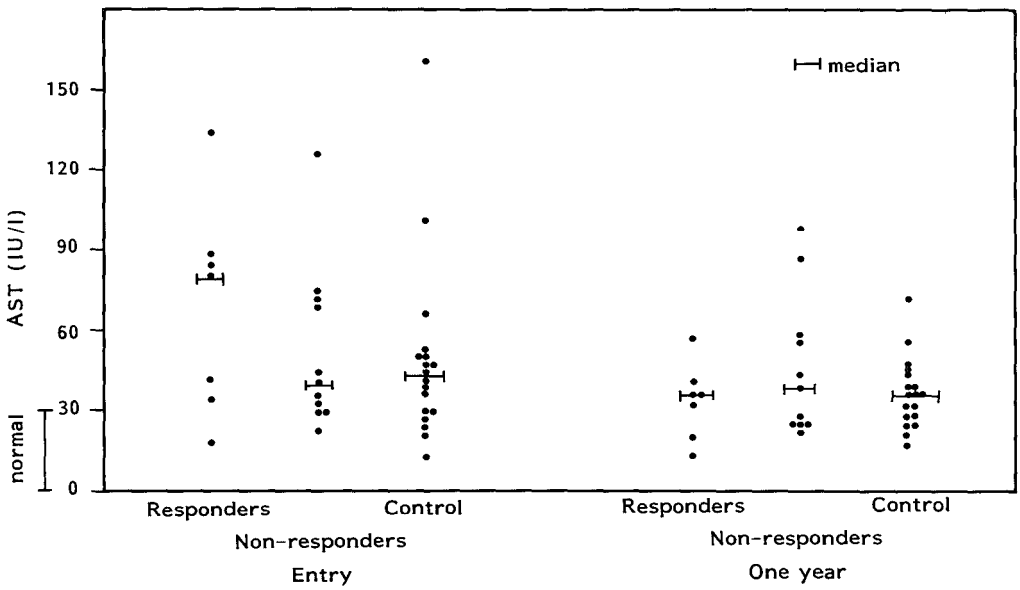

fig 4.AST levels in treated responders, non-responders and control patients at entry and after one year of follow-up.

Table 3. Individual characteristic of patients responding to antiviral combination therapy

Patient no.	8	14	29	36	201	205	212
Sex (m/f)	m	m	m	m	m	m	m
Homosexual (+/-)	-	+	+	-	-	+	-
HIV antibodies (+/-)	-	-	-	+	-	+	-
AST (IU/l, n < 30)	34	84	81	133	88	18	41
Bilirubin (µmol/l, n < 12)	8	16	9	12	6	6	13
Liver Histology:							
Biopsy Class (0-6)	2	6	2	3	1	3	3
Fibrosis (0-3)	2	5	3	2	1	2	2
Portal infiltrate density (0-3)	2	2	1	2	2	2	3
Portal infiltrate penetration (0-3)	1	2	1	1	1	2	2
HBcAg (%)	50	0	30	25	30	5	0
Virology:							
HBsAg (titre.10^2)	64	32	64	256	640	16	8
HBeAg (P/N ratio)	6.8	7.1	18.7	12.4	25.6	15.8	5.7
(dilution)	25	0	25	125	25	0	0
DNA-polymerase (P/N ratio)	2.7	1.6	21.2	29.3	3.1	1.9	1.5
HBV-DNA (pg/ml)	67	0	667	400	67	133	13

Characteristics of patients responding to combination therapy.
Individual characteristics of patients responding to antiviral combination therapy are shown in table 3.In an earlier report we analysed factors that might determine the outcome (6).Responding patients differed from non-responding patients only in lower pretreatment levels of viral replication (HBV-DNA,DNA-polymerase activity).

Discussion.

The principal aim of this randomized controlled study was to determine whether combination therapy consisting of alpha-interferon and descyclovir induces virus latency,as indicated by the disappearance of HBeAg.Our results show that approximately 40 % of patients responded to a schedule of alpha-interferon and descyclovir combination therapy.This result is statistically significant.At the time this study was designed no statistical difference had been shown in studies using alpha-interferon alone,since then this has been shown in a large study (13). Therefore the additional effect of descyclovir is probably small.

None of the patients who exhibited loss of HBeAg underwent reactivation,while in all patients persistent absence of HBeAg correlated with persistent absence of HBV-DNA.In contrast 5 patients with persistent HBe-antigenaemia but loss of HBV-DNA (n = 3) or DNA-polymerase activity (n = 2) after therapy exhibited reactivation.In our study a negative HBeAg-test on two successive occasions correlated with a persistent favourable outcome of antiviral combination therapy.Responding patients differed from non-responding patients only in lower pretreatment levels of viral replication (HBV-DNA,DNA-polymerase activity).

If we analyse the HBeAg results after 2 years of follow up on the basis of the 'intention to treat' principle the difference at one year disappears: 8/18 treated patients (44 %) compared to 7/18 (38%) control patients lost HBeAg.This observation can be explained partly by elective alpha-interferon treatment of control patients but is also due to changes in untreated control patients in the second year of the study.Whereas in most studies follow up ends after one year of observation,our observations strongly indicate the need for prolonged follow up to evaluate the real efficacy of antiviral therapy.

A change from active hepatitis B virus replication to a state of virus latency was associated with disappearance of histological

characteristics of hepatic inflammation and loss of expression of HBcAg in the liver (fig 3).An interesting finding of our study is the statistically significant improvement in liver histology for all treated patients compared to untreated control patients as far as portal infiltrate penetration and density are concerned.This result conflicts with the findings of Hoofnagle (14).In his study improvement in degree of inflammation and necrosis was observed in the one year follow up liver biopsy for responders and not for treated non-responders.Possible explanations for these differences are selection bias (29/36 patients had paired liver biopsies in our study compared to 41/45 in Hoofnagle's study) or our use of a more detailed histological grading system.Therapy-induced improvement in liver histology could be an important argument in support of treatment of patients with chronic HBV infection.

In patients responding to antiviral combination therapy AST levels normalized;this did not reach statistical significance.One possible explanation is the inclusion of patients with normal pretreatment AST levels in this study (fig 4).

At entry 1 non-responder and 2 control patients had IgM-antiHBc antibodies.After one year IgM-antiHBc antibodies had developed in 1 non-responder and had disappeared in one control patient.Both patients with IgM-antiHBc antibodies at one year were HBe- negative in the second year of the study.

At entry to the study 8 patients had HIV antibodies.Two of 3 treated HIV-positive patients responded while none of 5 control patients exhibited loss of HBeAg,HBV-DNA or DNA-polymerase activity.After one year HIV-antibodies had developed in one non-responder.The presence of HIV-antibodies was not related to the outcome of antiviral therapy.

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Chapter 8.

Beta-2 microglobulin and antiviral therapy for chronic hepatitis type B.

The contents of this chapter have been published in Antiviral Research 1989;11:191-190, under the same title with the following authors: R.A.de Man, J.Lindemans, S.W.Schalm, F.J.W.ten Kate.

Chapter 8.

Introduction.

Beta-2 microglobulin is a low molecular weight (11.8 kD) protein that is found in low concentrations in serum, saliva and cerebrospinal fluid.Serum levels increase with age,possibly reflecting decreased renal clearance.Elevated beta-2 microglobulin levels are found in patients with renal disorders,autoimmune diseases,various viral infections (including HIV) and lymphoproliferative diseases.Of the hepatic disorders beta-2 microglobulin was found to be elevated in chronic active hepatitis,chronic hepatitis type B,alcoholic liver disease and primary biliary cirrhosis but could not be used to discriminate between these diseases (1-7).

In serum beta-2 microglobulin occurs as a small protein,while in tissue it is present as part of an HLA class I glycoprotein. Expression of HLA antigens on the cell membrane depends on the presence of beta-2 microglobulin;the latter ensures that the three-dimensional configuration of the antigens is retained. Normal parenchymal liver cells do not express either HLA class I antigens or beta-2 microglobulin on their membranes.Recently however it was found that expression of HLA antigens in the liver of chronic hepatitis B patients coincided with the presence of cytotoxic T-cells in periportal infiltrates (8).It is postulated that hepatocytes infected with hepatitis B-virus are destroyed by these cytotoxic T-cells,which respond to the complex of viral antigens and HLA class I antigens on the hepatocyte cell membrane.

Interferon has been shown to enhance the concentration of HLA class I antigens on the surface of infected cells.The therapeutic action of these proteins therefore may be related to an increased presentation of infected hepatocytes to the immune system. Recently a mean increase in serum beta-2 microglobulin levels was observed after two weeks of interferon therapy in patients who exhibited a loss of active viral replication as a result of this therapy (9).In a randomized controlled trial we studied the serum levels of beta-2 microglobulin as well as markers of viral replication and liver cell necrosis (SGOT) in 36 chronic HBe-positive patients,18 of whom received alpha-lymphoblastoid interferon and descyclovir (BW 515).We tried to relate these findings to initial data at the start of the study as well as the therapeutic response in an attempt to evaluate whether beta-2 microglobulin could be of any use in identifying patients who are

likely to benefit from antiviral therapy.

Patients and methods.

Patients.

Thirty-six patients who participated in a randomized controlled trial of antiviral combination therapy for chronic hepatitis type B were studied.In nearly all cases active viral replication was reflected by HBeAg,HBV-DNA and DNA-polymerase activity in serum (patients without DNA-polymerase activity had stable HBeAg levels for three months prior to the trial).Patients who participated in this study had no history or signs of recent alcohol abuse, drug addiction or non-A non-B hepatitis.

In addition those with decompensated liver disease (ascites, variceal bleeding,encephalopathy,hepatoma),a malignancy (other than basocellular type skin cancer) within the past 5 years or impairment of renal function were excluded from this study.All patients were evaluated on day 0 with a standardized history, physical examination,laboratory investigations,esophagogram and liver biopsy.Randomization was done in blocks of six.In the treatment group,there were 18 males with a median age of 36 years (range 23-64 yrs).The control group consisted of 14 males and 4 females with a median age of 33 years (19-49 yrs).Ten controls and seven treated patients were homosexuals.All patients were tested for the presence of HIV antibodies by a commercially available ELISA (Wellcozyme);positive results were confirmed by Western blotting.The HIV antibody-positive patients who participated in this trial (3 treated,5 control) belonged to class I or II of the Walter Reed classification system which implies normal immunology (10).In addition all patients were tested for the presence of concomitant viral infections using IgM CMV,IgM EBV (VCA) and anti-HDV (delta) antibodies at the beginning and the end of the study.

The study was approved by the Medical Ethics Committee of the participating hospitals and written informed consent was obtained.

Study design and follow-up.

Eighteen patients received 5 Megaunits of alpha-lymphoblastoid interferon (Wellferon) once daily by subcutaneous injection in combination with 1 gr of the oral prodrug of acyclovir, descyclovir (BW 515) twice daily,for sixteen weeks.Treated patients were instructed to maintain fluid intake above 2.0

l/day.Descyclovir was started three days after interferon therapy had commenced.Control patients did not receive any treatment.Treated patients were seen after three days and then weekly during treatment.Control patients and those who had completed the sixteen-week course of therapy were seen monthly.Blood was sampled at each visit for assessment of the haematological,biochemical and viral parameters;part of the serum was prepared and stored immediately at -20°C for determination of beta-2 microglobulin.Treatment response was defined as absence of HBe antigen on two successive occasions with absence of serum HBV-DNA.

In study **A**,we compared beta-2 microglobulin with entry features,liver biochemistry,liver histology and virology.
In study **B** we determined beta-2 microglobulin,viral markers, SGOT,creatinine and creatinine clearance every 4 weeks and compared the results obtained for 18 treated and 18 untreated control patients.
In addition,we compared these markers after 3,7,14,21,28 days of interferon therapy in 7 treatment responders and 8 non-responders.(For 3 non-responders insufficient serum was available for testing.)

Beta-2 Microglobulin assay.

Serum beta-2 microglobulin concentrations were measured in duplicate with the commercially available beta-2 microglobulin radioimmunoassay (Pharmacia Diagnostics AB,Uppsala,Sweden).The assay was carried out according to the manufacturer's instructions.The mean and upper limits of normal(+ 2 SD) for serum beta-2 microglobulin levels for normal subjects under the age of 60 are 1.7 and 2.4 mg/l and for subjects over the age of 60 2.0 and 3.0 mg/l,respectively.The initial standard curve was repeated after 50% and 100% of the samples had been analyzed to test for trend.No trend was observed.Pooled serum samples were used to determine the intra-assay coefficient of variation.This coefficient equaled 6% when the beta-2 microglobulin concentration was 2.8 mg/l.

Laboratory evaluation of the liver disease.

HbeAg was measured by radioimmunoassay (Abbot,Ill,USA).DNA-polymerase activity was measured by standard methodology as described by Fang (11).Results are expressed as a ratio of the

activity in patient serum to that in normal control serum.The serum amino-transferases,alkaline phosphatase,bilirubin,serum albumin and serum creatinine were measured in our clinical chemistry laboratory using an automated system (SMA-12). Immunological activity was indicated by the immunoglobulin G and smooth muscle autoantibody concentrations as well as the C1q binding activity.Liver biopsy samples were obtained from eighteen treated patients and sixteen controls.One sample was processed for routine histological determinations while the other was snap-frozen in liquid nitrogen for immunofluorescence studies.The liver biopsy samples were scored blindly on the basis of the criteria established by an international group.Both the density and the penetration into liver tissue of the portal inflammatory infiltrate were scored on a scale of 0 to 3 (12).

Statistical Analyses.

Beta-2 microglobulin levels at entry to this study were analyzed in relation to age,HIV antibody status,immunological measurements,liver function and liver biopsy score using a multiple matrix plot and calculation of linear correlation. Significance was tested with a Spearman rank correlation test.Changes in beta-2 microglobulin levels in treated patients and controls were analyzed by the method of variance using age and HIV status as covariates.Differences in SGOT,DNA-polymerase and HBeAg levels were tested using a Mann Whitney U test for non-paired samples.All statistical analyses were carried out with STATA (Computing Resource Center,Los Angeles,U.S.A.) on an Olivetti M24 personal computer.

Results.

Antiviral treatment.

Baseline characteristics are shown in table I.No significant differences were observed between control patients,treatment responders and treatment non-responders with regard to age, sex, HIV status, histology, transaminase activity or IgG levels.

Seven treated patients responded (seroconversion from the HBe-positive to the HBe-negative state with absence of HBV-DNA) to therapy.Antibodies against HBe developed in five of these patients.No serum HBV-DNA could be detected in any of the seven responders after one year of follow-up.Liver histology after one year of follow-up showed clearance of HBcAg from the liver.In control patients HBe-seroconversion was not observed.The results

Table I: Characteristics of patients participating in our studies.

	Control	Therapy	Responders	Non-responders
Number of patients (n)	18	18	7	11
Age (years, median, range)	33 (19-49)	36 (23-64)	35 (23-64)	42 (26-63)
Male/female (n)	14/4	18/0	7/0	11/0
Homosexual (n, %)	10 (28)	7 (39)	3 (43)	4 (36)
anti-HIV positive (n, %)	5 (28)	3 (17)	2 (28)	1 (9)
IgG (g/l, median, range)	16,3 (9,6-34,7)	14,1 (6,9-14,2)	20,4 (6,9-26,2)	12,6 (9,3-27,2)
SGOT (IU/l, median, range)	43 (12-161)	43 (18-133)	81 (18-133)	40 (22-127)
Beta-2 microglobulin (mg/l, median, range)	2,4 (1,6-4,9	1,8 (0,3-4,0)	1,89 (1,5-2,4)	1,6 (0,3-4,0)
Beta-2 microglobulin, mean increase. (t = 2 weeks vs. t = 0 weeks; %, range)	nt*	nt*	+74% (-47 - +320)	+133% (-19 - +294)
Cirrhosis (n, %)	5 (28)	5 (28)	1 (14)	4 (36)

of antiviral treatment have been described in more detail elsewhere (13-15).

Serum beta-2 microglobulin levels in chronic hepatitis type B.

In study A,serum beta-2 microglobulin levels were elevated in 14 of the 36 patients (39%).

The initial levels of beta-2 microglobulin could not be related to hepatitis activity, as indicated by the SGOT levels (table I),nor was there a relation with alkaline phosphatase or bilirubin levels,liver histology or the penetration or density of the portal inflammatory infiltrate (table II).There was no correlation between the levels of beta-2 microglobulin and either immuno-globulin-G concentrations or the presence of smooth muscle antibodies.Of the eight patients with HIV antibodies,four showed elevated beta-2 microglobulin levels (50%).Renal function, assessed by serum creatinine levels and creatinine clearance, was normal in all patients.

Serum beta-2 microglobulin during treatment.

In study B the beta-2 microglobulin serum levels at the start of therapy found for patients who responded to treatment (responders),patients who did not respond to treatment (non-responders) and untreated control patients were comparable.During treatment mean beta-2 microglobulin levels rose in responders and non-responders,while the levels remained stable in untreated controls (fig.1).Significant differences were observed at week 4 and week 8 between treated patients and control patients ($p<0.05$).No significant differences however were observed between responders and non-responders.Serum transaminases followed the same pattern with significant differences between treated and non-treated patients at week 4 and week 8 ($p<0.05$) but not between responders and non-responders.Renal function did not change significantly during therapy ($p>0.10$).

Because of the observations of Pignatelli (9) we also tested the serum of seven responders and eight non-responders after 3,7, 14,21 and 28 days of treatment.Beta-2 microglobulin in responders showed a mean increase of 20,99,74,82 and 52% at 3,7,14,21,28 days,respectively.In non-responders the mean increase in beta-2 microglobulin was 177,121,133,104,82 percent,respectively.Four of seven responders and six of eight non-responders showed a rise in beta-2 microglobulin at two weeks (fig.2).

Table II: Beta-2 microglobulin levels in relation to portal infiltrate and histological classification of liver biopsy.

Histological classification	n	Infiltrate Density				Infiltrate Penetration				Beta-2 microglobulin (mg/l)	
		0	1	2	3	0	1	2	3	range	no. of patients > 2.4
CPH	11	7	4	-	-	8	3	-	-	1.5 - 4.9	5 (45%)
CAH	13	3	6	3	1	-	9	4	-	1.3 - 5.0	4 (30%)
Cirrhosis	10	2	8	-	-	-	5	5	-	0.3 - 2.8	5 (50%)

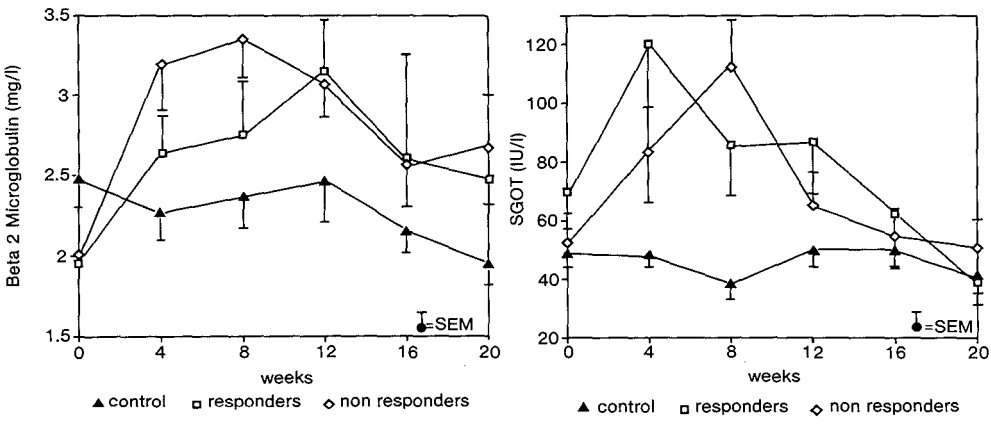

fig 1. Beta-2 microglobulin (mean) and SGOT (mean) in responders, non-responders and controls.Treatment was given from week 0 to week 16.

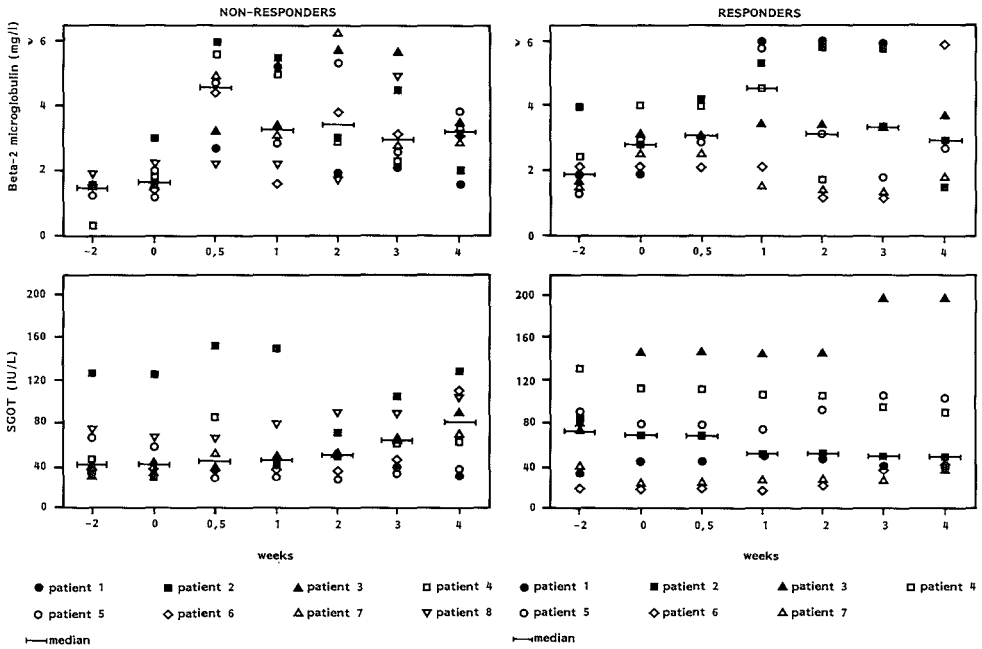

fig 2.Beta-2 microglobulin and SGOT in non-responders (left panel) and responders (right panel) during the first 28 days of treatment.

Discussion.

Recent studies suggest possible therapeutic efficacy of long-term interferon therapy for chronic hepatitis B.However,HBe-seroconversion with development of antibodies occurred in only a minority of the treated patients (16-21).Early identification of patients who could benefit from treatment is therefore important.Various factors related to the outcome of treatment have been proposed retrospectively:oriental race,positive HIV antibody status and homosexuality are characteristics of those who show little response whereas an elevated SGOT level or a histology compatible with chronic active hepatitis is said to identify patients who could benefit from treatment (22).In a matched controlled study a mean increase in beta-2 microglobulin after 14 days of interferon therapy was reported to be indicative of a favorable response to therapy,the mechanism being enhanced expression of HLA class I antigens leading to increased presentation of infected hepatocytes to the immune system (9). Our study indeed showed a mean increase in beta-2 microglobulin levels in patients during interferon therapy.This rise in beta-2 microglobulin could not be explained by age or HIV status because both were used as covariates in our analysis,nor could it be attributed to either decreased renal tubular function related to descyclovir therapy or other viral infections (23).Possibly concomitant cell lysis induced by interferon after 4 weeks of therapy can explain the rise in beta-2 microglobulin after 4 weeks.However,elevation of beta-2 microglobulin in our studies was not related to imminent HBe-seroconversion,but probably reflects interferon-induced hepatitis.Lok also found interferon therapy-related hepatitis (16).Beta-2 microglobulin levels after two weeks of interferon therapy increase in 73% of patients without discrimination between responders and non-responders (fig.2).

We conclude therefore that exogenous alpha-lymphoblastoid interferon increases serum levels of beta-2 microglobulin, possibly by enhanced HLA class I expression.However we could not distinguish responders from non-responders by means of beta-2 microglobulin serum levels alone,even if assessed in the first 4 weeks of therapy before SGOT rises.

In the complex interaction between the virus,the host and the antiviral agent beta-2 microglobulin reflects HLA antigen expression.We could not use beta-2 microglobulin alone for the selection of individual patients for antiviral therapy but it may

be of use in a multifactorial model which describes this interaction.

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Chapter 9.

Improvement of hepatitis B associated glomerulonephritis after treatment with antiviral combination therapy.

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Chapter 9.

Introduction.

Infection with the hepatitis B virus can be associated with extrahepatic manifestations such as polyarthralgia,skin lesions (Gianotti-Crosti syndrome),polyarteritis nodosa and certain types of glomerulopathy,all of which are thought to be immune complex-mediated (1,2).In patients with hepatitis B-related glomerulonephritis,gross proteinuria is related to the presence of the viral HBe-antigen in the blood (3).The important role of HBe-immune complexes in the pathogenesis of kidney disease is often emphasized (4).Improvement of kidney disease after spontaneous clearance of the virus has been described although favorable effects of antiviral therapy in hepatitis-B associated glomerulonephritis have also been reported (5,6,7,8).In four patients the nephrotic syndrome disappeared after antiviral therapy.Relapse of the nephrotic syndrome was associated with persistence of the HBe-antigen.The present report concerns a patient with a progressive nephrotic syndrome associated with active hepatitis B virus replication for a period of at least four years and deposition of HBc and HBe-immune complexes in the kidney;antiviral combination therapy induced virus latency, followed by disappearance of the nephrotic syndrome.

Materials and methods.

Hematology and routine chemistry were determined by the autoanalyzer using standard methods.HBsAg was measured using a standard radioimmunoassay (Abbott,Ill).HBeAg was measured by radioimmunoassay (Abbott,Ill) in a constant serum dilution.This dilution was chosen such that a P/N ratio of approximately 12 +/- 2 would be obtained in pretreatment sera.A P/N ratio of less than 2.1 in undiluted serum was considered HBeAg negative. DNA-polymerase activity was measured by the method of Howard as modified by Fang (9).Results were expressed as P/N ratios (cpm patient sample/cpm negative control sample).HBV-DNA in serum was measured by dot blot hybridization (10).Liver and kidney immunofluorescence studies for detection of viral antigens were performed using monoclonal antibodies (anti-HBe antibody courtesy of Prof.H.C.Thomas;anti-HBs,anti-HBc antibody courtesy of Organon,Oss).The specificities of the antiHBs,anti-HBc and anti-HBe monoclonal antibodies were confirmed with separate

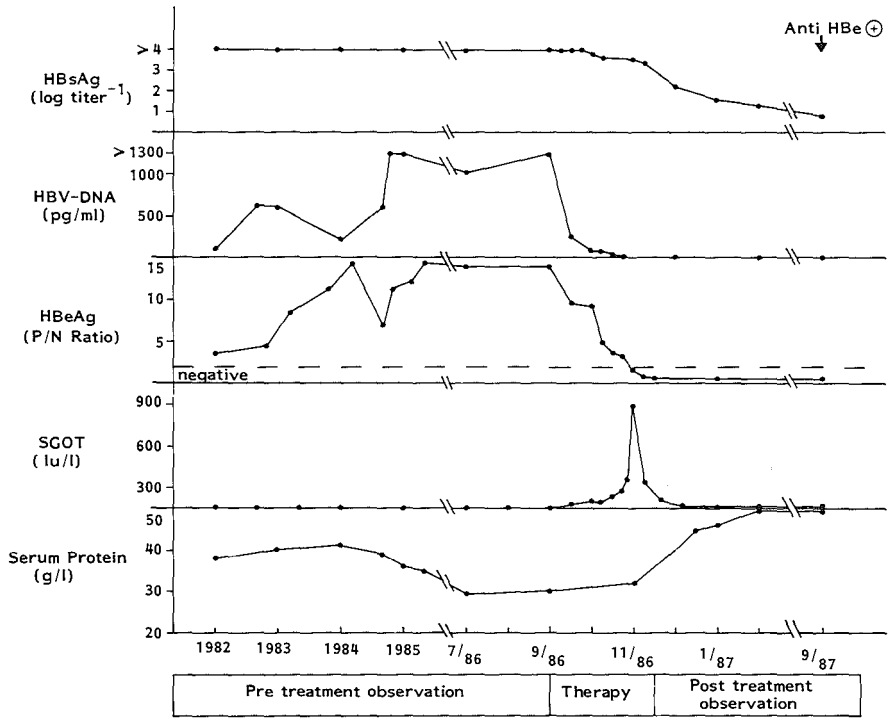

fig 1. Stable pretreatment markers of hepatitis B-associated nephrotic syndrome, improving after therapy-induced HBe-seroconversion.Note the sequence of events after start of treatment: drop in HBV-DNA and HBeAg, rise in SGOT and subsequent rise in serum total protein.Apparently treatment induces suppression of virus DNA and protein synthesis, allowing recovery of immune response with clearance of virally infected hepatocytes.

positive and negative control slides.All biopsies were scored according to the criteria of an international group (11,12).

Case report.

A boy born in 1976 on the Cape Verde Islands came to the Netherlands in 1982.In that year he visited the Children's Hospital because of scrotal swelling.Nephrotic syndrome with generalized edema,hypertension (RR 130/80),hypoproteinemia (38 g/l,normal 62-75 g/l),hypocomplementemia (C1q 0.09 g/l,normal 0.14-0.26 g/l; C3 0.7 g/l, normal 0.9-1.6 g/l; C4 0.11 g/l, normal 0.17-0.37 g/l; CH50 50 %,normal 80-155%),microscopic erythrocyturia and proteinuria (9 g/l) was diagnosed.A kidney biopsy showed membranous glomerulonephritis with deposition of immune complexes.Subsequently his serum was found to contain HBsAg (titer > 1:10.000),HBeAg,HBV-DNA and DNA-polymerase activity.His mother was HBsAg positive and HBeAg negative without antibodies to HBe.The nephrotic syndrome continued for four years.In total twelve hospital admissions took place because of exacerbation of the nephrotic syndrome and the need for albumin infusions.Anti-hypertensive treatment was started in 1985 with hydrochlorthiazide,dihydralazine and propanolol. Hepatitis B markers showed stable active viral replication during this four-year period (fig 1.).In May 1986 the patient underwent renewed assessment because antiviral combination therapy was being considered.Clinical problems included fatigue which,in combination with the frequent hospitalization,had impaired his psychosocial development.On physical examination he was found to have generalized edema; there were no cutaneous signs of chronic liver disease.Blood pressure was 100/70 mm Hg (with therapy).

Laboratory studies yielded the following data: blood urea nitrogen 9.3 mmol/l (normal 3.3-5.6 mmol/l),creatinine 42 umol/l (normal 50-90 umol/l),total serum protein 32 g/l and albumin below 9%.Bilirubin was below 5 umol/l (normal below 9 umol/l), AST was 23 IU/L,ALT was 29 IU/L (AST/ALT normally below 30 IU/L).The urinary sediment showed microscopic erythrocyturia and gross proteinuria which equaled 13.8 g/l.Corrected creatinine clearance was 67 ml/min (normal for age and body surface).Further immunologic evaluation revealed low serum levels of C1q (0.07 g/l),C3 (0.51 g/l),C4 (0.14 g/l) and CH50 (53%).Serum IgG was

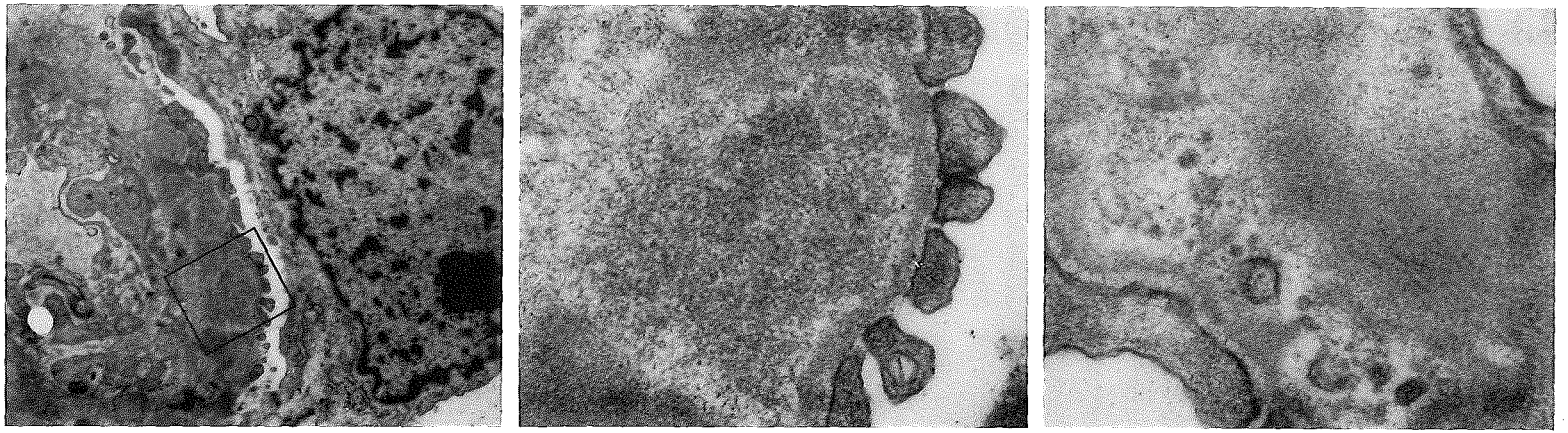

fig 2. Electron microscopy of kidney before treatment showing HBc like particles along the glomerular basement membrane (left panel, overview EM 7000x; middle panel EM 50.000x).
After therapy-induced HBe-seroconversion only an amorphous protein-like substance persists (right panel EM 50.000x).

5.55 g/l (normal 7.5-16.0 g/l).HBsAg was positive (titer > 1:10.000),HBeAg was positive (P/N ratio 14.6,dilution 1:5),DNA-polymerase activity was strongly positive (P/N ratio 77.1) and the HBV-DNA level in serum was 50 pg/ml.An esophagus X-ray showed no signs of esophageal varices.Liver histology demonstrated chronic persistent hepatitis,with positive immunofluorescence for HBsAg,HBcAg (80% hepatocyte nuclei) and HBeAg.A kidney biopsy taken before treatment revealed membranous glomerulonephritis with spikes (Churg type III) and extensive granular deposits of IgG,C3,C1q,HBcAg and HBeAg along the glomerular basement membrane.HBsAg was absent.Electron microscopy of the kidney showed virus-like particles resembling HBcAg along the basement membrane (fig 2).After approval of our treatment protocol by the Medical Ethics Committee and the boy's parents,he was given 2.5 Megaunits alpha-lymphoblastoid interferon (Wellferon) subcutaneously once daily for 10 weeks and 450 mg of intravenous acyclovir twice daily for the first two weeks.Therapy proceeded without complications.In the 8th week,a severe hepatitis (ALT 30x upper limit of normal) developed without evidence of hepatic decompensation (normal bilirubin, normal clotting factors).HBeAg became negative in the 8th week; therapy was stopped after 10 weeks.Antibodies against HBe developed while HBsAg (titer 1:8) persisted.No HBV-DNA could be detected in serum after HBe-seroconversion had occurred.The nephrotic syndrome disappeared after HBe-seroconversion. Reevaluation one year after treatment showed marked clinical improvement with disappearance of edema,hypoproteinemia and hypocomplementemia.The antihypertensive treatment had been discontinued.In the urine microscopic erythrocyturia had disapppeared while slight proteinuria (serum protein 60 gr/l,proteinuria 1- 2 gr/l/24h) remained.A kidney biopsy still showed membranous glomerulonephritis (Churg type III) with mild mesangial cell proliferation,and immunofluorescence indicated granular deposits of IgG (mainly IgG subclass four),C3 and C1q along the basement membrane.HBsAg,HBcAg and HBeAg were absent. Electron microscopy did not reveal any virus-like particles.In the blood a state of virus latency (HBsAg positive: titer 1:8, HBeAg/HBV-DNA negative,antiHBe positive) had developed.In the liver a minimal portal infiltrate persisted,without piecemeal necrosis or ground glass cells.No HBcAg,HBeAg or ground glass

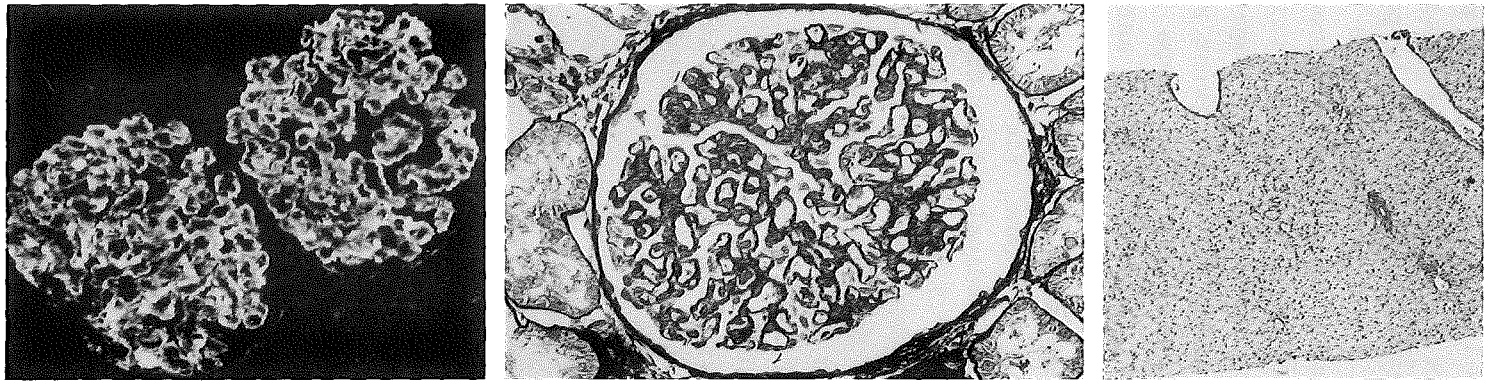

fig 3. Pretreatment liver and kidney biopsies showing minimal chronic persistent hepatitis (left panel, LM 150x), membranous glomerulonephritis with extensive IgG deposits (middle and right panel, LM 380x).

cells could be detected by immunofluorescence; membrane-bound HBsAg remained present in low quantities.

Discussion.

We have described a patient who suffered from a hepatitis B-related membranous glomerulonephritis.Pretreatment observation during a four-year period showed a stable nephrotic syndrome with markers of active viral replication in the blood but no signs of hepatitis activity.Antiviral treatment induced a state of virus latency reflected by clearance from the blood of HBeAg,HBV-DNA and DNA-polymerase and development of antiHBe antibodies. Induction of virus latency was associated with an immediate improvement in the clinical condition of the patient and disappearance of the nephrotic syndrome.Complement levels in serum became normal and anti-hypertensive treatment could be stopped.The liver and kidney biopsies showed clearance of viral antigens,although appreciable kidney damage remained.

Hepatitis B associated glomerulopathy in children occurs predominantly in boys.The morphologic lesion is usually membranous glomerulonephritis (3,16) but IgA nephropathy,a mixed form between IgA nephropathy and membranoproliferative glomerulonephritis,and mesangioproliferative glomerulonephritis also appear to be associated with hepatitis B markers (13,14). Associated liver histology can be chronic active hepatitis but chronic persistent hepatitis and liver cirrhosis have also been described (15).Our patient presented with extensive granular immune complex deposits,containing HBcAg,HBeAg but not HBsAg, along the glomerular capillary wall.Electron microscopy showed core-like particles within the basement membrane and in the subendothelial space.The deposition of HBsAg,HBeAg and HBcAg has been described previously (4,13,15,17),but the specificity of the polyclonal antibodies used in the studies has been questioned (18).If the deposits in the kidney are related to particle size, one would expect HBeAg deposits.At blood pH both HBsAg and HBeAg have a net negative charge which may favor deposition along the glomerular basement membrane.

HBeAg is present in the blood in two forms,the smaller form having a molecular weight of 19,000 D and the larger a molecular

weight of 300,000 D; the latter consists of circulating HBeAg-immune globulin complexes (4).During the stage of active viral replication HBeAg is present in excess and both forms can precipitate on the basement membrane.In a state of virus latency the continuous influx of antigen stops and the kidney can clear antigens already precipitated.In our patient,as in other studies,HBcAg was also demonstrated by immunofluorescence while electron microscopy showed core-like particles (13).Primary deposits of HBeAg may have induced structural changes with increased permeability paving the way for the deposition of HBcAg.After HBeAg seroconversion and disappearance of the viral markers from the kidney,residual damage to the kidney was still found to be marked.Partly,the damage must be structural since it is attributable to a chronic infection of several years duration; however inflammation with immune deposits has persisted, even after the disappearance of viral antigens.In the animal model renal tubular antigens have been identified in immune deposits along glomerular capillary walls (19).Possibly deposition of hepatitis B antigens initiated the kidney inflammation with exposure of kidney antigens ultimately leading to postinfectious glomerulonephritis.

Was antiviral therapy successful in curing the nephrotic syndrome or have we just observed a spontaneous remission? Marked proteinuria appears to be associated with the presence of HBsAg and HBeAg in serum (3,20).Improvement of the kidney disease after spontaneous clearance of the virus has been described (5). Our pretreatment observation data with quantitative hepatitis B virology and histologic documentation of the disease over a 4-year period point to unabating virus replication until the start of treatment and therefore make spontaneous seroconversion highly unlikely.

We treated our patient because conservative therapy failed and frequent hospitalization was hampering his psychosocial development.Should all of our patients with hepatitis B-associated membranous glomerulonephritis now undergo experimental antiviral combination therapy? We believe this approach should be reserved for HBeAg-positive patients who are not likely to undergo spontaneous seroconversion with an associated remission

of their nephrotic syndrome.The incidence of spontaneous remission of nephrotic syndrome drops after approximately one year of observation (20).We suggest a kidney biopsy for detection of viral antigen and/or particles by immunofluorescence and electron microscopy after an observation period of one year. If strong evidence for viral-induced disease is present antiviral combination therapy should be considered since,at that time,the chance of spontaneous remission is low and marked post-infectious damage usually has not yet occurred.

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Chapter 10.

Future strategies for treatment of hepatitis B virus infections.

Chapter 10.

Introduction.

The studies described in this thesis focussed on a combination of acyclovir and alpha-lymphoblastoid interferon for treatment of chronic HBV infection.In this chapter results obtained with other combination therapies will be discussed.Using the new information that has evolved from our clinical research as well as that reported in the literature, I will try to predict future strategies for antiviral therapy.The model aims at a differential approach to antiviral treatment for the various categories of cases, since it is likely that the pathobiology of HBV-induced liver damage differs between these categories.

Combination therapy.

Combination of single drugs in order to improve the results obtained with alpha-interferon, nucleoside analogues or corticosteroids alone seems to be a rational approach.A problem is the selection of the drugs to be included in a combination therapy.What is more important: more profound suppression of viral replication than can be achieved with alpha-interferon alone,or enhancement of the immune response ? Nucleoside analogues suppress viral replication, but may also suppress immune reactivity.Prednisone withdrawal enhances immune reactivity,but may also increase viral replication.Nearly all combinations have been tested in the last 5 years.

Alpha-interferon + adenine arabinoside.

The combination of alpha-interferon with the nucleoside analogue adenine arabinoside or its monophosphate derivative has been evaluated by the Stanford group in two open studies and one large randomized double-blind placebo-controlled trial (1-4).In the first open study sixteen patients received ARA-A intravenously (5-15 mg/kg/day) for 7-14 days at intervals of four to six weeks in combination with 2.5-7.5 MU leucocyte alpha-interferon daily.Later in the trial interferon was given in 14-day cycles of a dose of 5 MU daily overlapping with the last 5 days of ARA-A therapy.Six of sixteen patients had received prior antiviral therapy.HBe-seroconversion occurred in 7/16 (43%) patients;one patient became HBsAg negative.From this pilot study it was concluded that combination therapy increases the therapeutic response to ARA-A but also severely enhances ARA-A toxicity.

In the second open study ten patients were treated with ARA-AMP in combination with alpha-interferon as out-patients.The purpose of this study was to evaluate regimens that could be used in a larger controlled study.Three regimens were evaluated in a non-randomized fashion:

a.7-day cycles of 5 mg/kg ARA-AMP alternating with 5 MU alpha-interferon daily,starting with ARA-AMP and lasting 28 days for a total of four courses.

b.28-day cycles of 5 mg/kg ARA-AMP alternating with 5 MU alpha-interferon daily for 28 days for a total of three courses.

c.28-day cycles of 7.5 mg/kg ARA-AMP alternating with 5 MU alpha-interferon daily for 28 days for a total of three courses.

The most important finding of this study was patient non-compliance for scheme 'c' because of severe ARA-A toxicity.One patient of the first group 'a' became HBeAg negative.

The randomized placebo-controlled trial evaluated the efficacy of sequential courses of ARA-AMP with or without sequential courses of human leucocyte interferon (table 1).The study was severely compromised by toxicity problems:enrollment in the group receiving ARA-AMP sequentially with human leucocyte interferon had to be stopped because of painful paraesthesias of the legs.The study could not show any significant therapeutic effect of either regimens compared to placebo-treated controls.In conclusion,it was shown that alternating regimens of interferon and ARA-AMP are associated with significant toxicity and that repeated ARA-AMP cycles had no demonstrable effect,as had been suggested by other studies (5).

Corticosteroid withdrawal + ARA-AMP.

In a pilot study Perillo treated 11 patients with prednisone (40 mg/day tapered off to zero over 8 weeks) followed by a 4-week rest interval and a single 28-day cycle of ARA-AMP (6).ARA-AMP therapy consisted of intramuscular injections of 10 mg/kg twice daily for the first 5 days followed by 5 mg/kg twice daily for the next 23 days.The majority of patients participating were non-responders to a single course of ARA-AMP (n = 7) one year before.Five of 11 patients (45%) exhibited a loss of HBeAg and DNA-polymerase activity.Side effects during steroid withdrawal did not occur.

A randomized controlled trial was performed by Yokosuka (table 1) (7).The response of patients on prednisolone withdrawal or a single course of ARA-A was comparable to that found for untreated

Table 1. Antiviral combination therapy,randomized controlled trials.

Author (reference)	trial design	treatment schedule	patients (n)	Seroconversion HBe(n)	(HBe(%)
Perillo (9)	prednisolone	60 mg/d, 2 weeks	18	8	44
		40 mg/d, 2 weeks			
		20 mg/d, 2 weeks			
		0 mg/d, 2 weeks			
	followed by				
	rec.alpfa 2 b. interferon	5 MU,once daily 90 days			
	no therapy		21	4	19
Yokosuka (7)	prednisolone	40 mg/d, 4 weeks	14	3	21
	prednisolone	40 mg/d, 4 weeks			
	followed by	0 mg/d, 2 weeks			
	ARA-A iv.	10 mg/d, 4 weeks	5		
		10 mg/d, 8 weeks	4	6	67
	ARA-A iv.	10 mg/d, 4 weeks	7		
		10 mg/d, 8 weeks	3	1	10
	control	no therapy	10	2	20
Garcia (4)	ARA-AMP im.	2.5 mg/kg, twice daily,28 days.	13	3	23
	alternated with Cantell interferon sc. for six months.	5 MU, twice daily daily 28 days,			
	ARA-AMP im.	2.5 mg/kg, twice daily 28 days.	24	1	4
	alternated with albumin im/sc. for six months.	daily 28 days.			
	albumin im/sc. alternated with saline im for six months.	0.5 cc. twice daily	27	5	19
De Man (10)	lymfoblastoid alpha-interferon	5 MU, once daily 16 weeks	18	7	39
	descyclovir	2 g daily			
		no therapy	18	0	0

controls (HBe-seroconversion about 20 %), in contrast patients treated with prednisolone withdrawal followed by a single course of ARA-A responded with 67 % HBe-seroconversion.Although the numbers were small this study confirmed the lack of efficacy of prednisolone withdrawal therapy alone,whereas the combination with a nucleoside analogue may have influenced the response favourably.A large controlled study comparing corticosteroid withdrawal and ARA-AMP with ARA-AMP alone was never performed, probably because ARA-AMP became unavailable after toxicity reports in the USA study.

Corticosteroid withdrawal + alpha-interferon.

In one small uncontrolled study of 5 patients treatment with steroids followed by alpha-interferon led to HBe-seroconversion in three patients (8).The logical follow-up to this initial study on steroid withdrawal was a small randomized controlled study on the combination of steroid withdrawal and alpha-interferon (table 1) (9).This study included only asymptomatic patients with preserved hepatic synthetic function and normal haematological indices.The study could not prove a significant effect on HBe-seroconversion although the number of patients who became HBV-DNA (9/18 vs. 3/21) and HBsAg (4/18 vs. 0/21) negative was higher in the treatment group.Possibly because of careful patient selection no hepatic decompensation was observed.Whether this treatment approach is superior to single drug therapy with alpha-interferon has to be proven by large comparative studies,that are now in progress.

Meta-analysis of antiviral combination therapy.

Meta-analysis of the four published randomized controlled trials on various forms of combination therapy is shown in table 2.For all individual studies,the odds ratio for HBeAg seroconversion was calculated with its 95 % confidence interval.Stratified exact analysis was done by the classic Mantel-Haenszel method. The overall odds ratio for HBeAg seroconversion is estimated at 4.1 (95 % confidence interval:1.67 - 10.61) ($p < 0.001$) for combination therapy.No heterogeneity between the studies was shown ($p = 0.164$).In figure 1 the individual contributions of the studies to the overall efficacy of antiviral combination therapy are shown.In figure 2 the results of meta-analysis of combination therapy are compared with the results of meta-analysis of single drug therapy.Combination therapy seems to be superior to single

Table 2. Antiviral combination therapy:meta-analysis of HBe-seroconversion in 4 randomized controlled trials

Author (reference)	odds ratio	95% confidence interval for odds ratio	Bar no. (fig 1)
Perillo (9)	3.29	0.67- 19.06	1
Yokosuka (7)	7.03	0.73-111.81	2
Garcia (4)	1.31	0.17- 8.40	3
De Man (10)	1.000	1.85-1000	4
All studies	4.1	1.67- 10.61	5

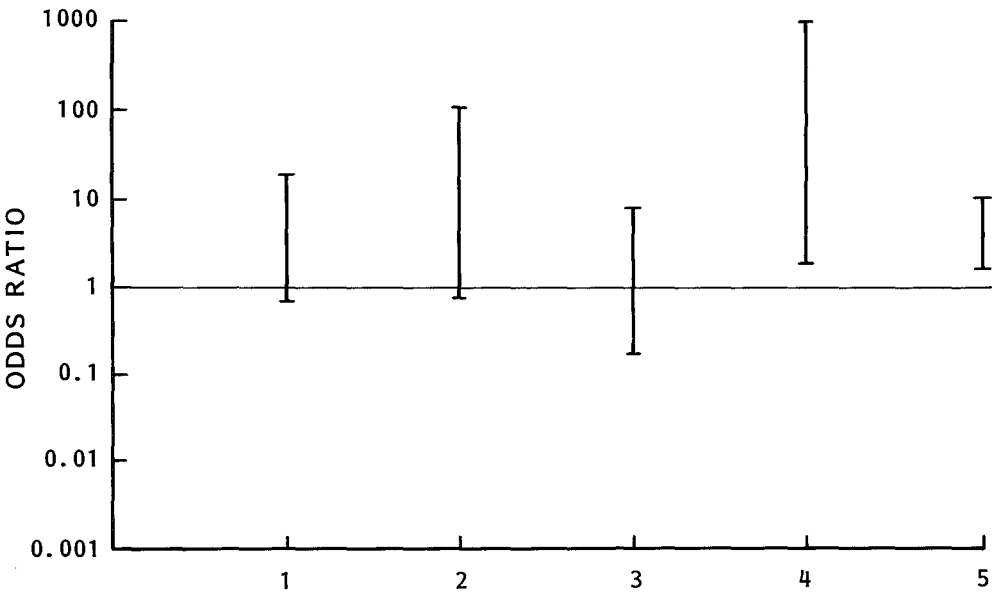

fig 1.Therapeutic efficacy (HBe-seroconversion) in 4 individual studies on antiviral combination therapy,and estimated 'overall' efficacy.Odds ratios are expressed as 95 % confidence limits. Numbers on x-axis:see table 2.

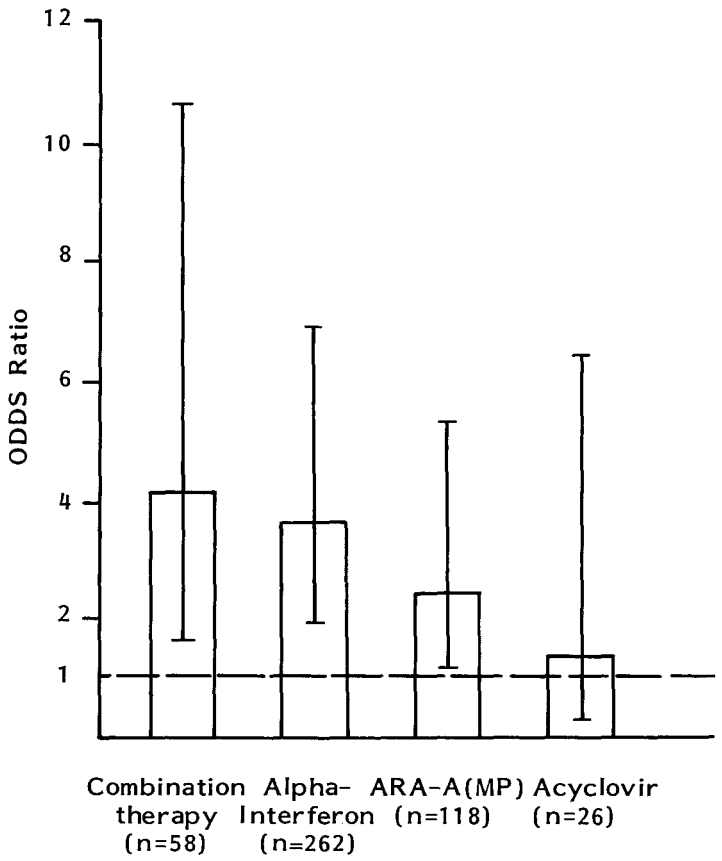

fig 2.Estimated overall efficacy (HBe-seroconversion) of 4 treatment regimens for chronic hepatitis type B.Each bar represents the odds ratio with 95 % confidence limits.Number of treated patients is shown on the x-axis.

Table 3. Antiviral therapy:summary of meta-analysis of randomized controlled trials.

Treatment	patients treated (n)	odds ratio	95% confidence interval for odds ratio
Combination therapy	58	4.10	1.67-10.61
Alpha-interferon	262	3.59	1.94- 6.92
ARA-A/ARA-AMP	118	2.37	1.12- 5.19
Acyclovir	26	1.38	0.32- 6.46

drug treatment with acyclovir or ARA-A(MP).Meta-analysis suggests combination therapy to be somewhat better to single drug treatment with alpha-interferon,but the differences are small and the confidence intervals clearly overlap (figure 2,table 3).In conclusion the therapeutic result with combination therapy has not come up to expectations compared to the result obtained with alpha-interferon alone.

A differential approach to antiviral therapy for chronic HBV infection.

Current approaches to antiviral therapy for chronic HBV infection have been aimed at developing one treatment strategy for the cure of all infected patients.However if we study specific groups of HBV infected patients it will probably appear that the mechanism by which HBV has caused the disease differs between them.To illustrate this,the pathobiology of chronic HBV infection in patients with a 'normal' immune response, immune-compromised patients and patients with immune complex-mediated disease,will be described.Subsequently data which are not sufficiently explained by the classical hypothesis for HBV-induced liver cell necrosis will be discussed.Finally possible modifications of this hypothesis and resulting ideas for future treatment strategies will be considered.

<u>The 'normal' host.</u>

Chronic HBV infection is characterized by a broad range of outcomes,which are thought to reflect differences in the cellular immune response of the host to HBV-infected hepatocytes (11). According to the classical hypothesis the hepatitis B virus is not cytopathic; liver cell necrosis is attributed to dual recognition of HBV and HLA antigens on the liver cell membrane by cytotoxic T-cells (figure 3).Treatment for this group of patients should be aimed at clearance of HBV.

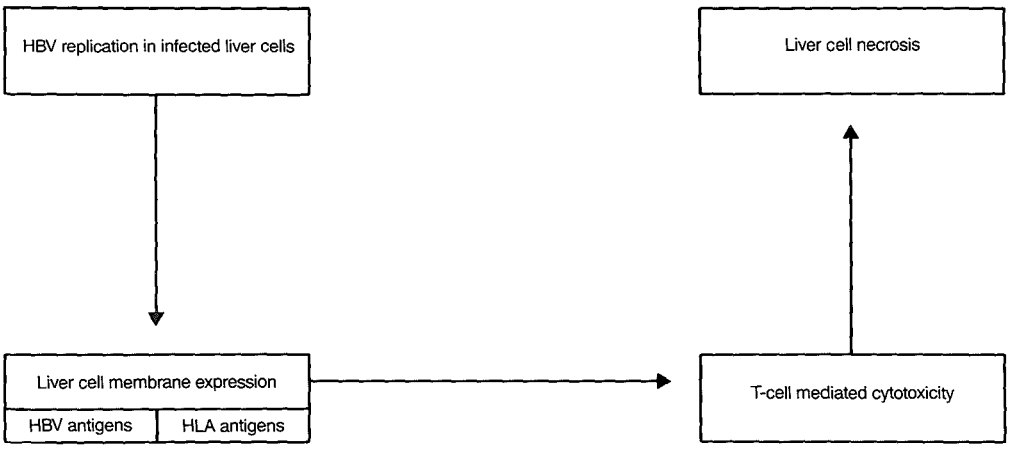

fig 3.Mechanism of liver cell necrosis according to the classical hypothesis.

The immune-compromised host.

Infection with the human immunodeficiency virus leads primarily to destruction of T-helper cells.Levels of HBV replication are higher in HIV-infected patients than in HBsAg-positive HIV antibody-negative patients.Liver lesions in the former often lack both necrosis and inflammatory infiltrates compared to those in HIV antibody-negative,HBsAg-positive patients (12).

In HBsAg-positive renal transplant recipients reactivation of viral replication occurs during immunosuppressive therapy.In addition liver histology shows progressive fibrosis without much evidence of liver cell necrosis and minimal signs of inflammation.Compared to HBsAg-negative renal transplant recipients,survival is shorter (13).

In HBsAg-positive liver transplant recipients,a mild clinical, biochemical and morphological hepatitis occurs within three months of liver transplantation.This hepatitis coincides with cytoplasmic expression of HBcAg in the liver biopsy.HBV infection of the graft can be associated with rapid (within one year) development of cirrhosis in the graft.Although adequate immunosuppression prevents allograft rejection,rapidly progressive liver damage does occur in spite of immunologic incompetence (14,15).

Other factors,in particular virus-induced antibody,also play a role in liver cell necrosis since HBV infection in patients with congenital agammaglobulinaemia often has a rapidly progressive course.This observation suggests that virus-induced antibody plays a role in limiting liver cell destruction (16).

Treatment of immune-compromised patients should be aimed at decreasing the level of viral replication since it is unlikely that clearance of HBV is an attainable goal in these patients.

Immune complex-mediated disease.

In patients with HBV-associated periarteritis nodosa or glomerulo -nephritis,progressive extrahepatic damage may be associated with mild liver disease.The extrahepatic lesions are due to immune complex deposits in vessel walls or the kidney;apparently liver damage in these patients is not immune complex-mediated (17).

Treatment for this group of patients should be aimed at clearance of the immune complexes,either by decreasing the production or increasing the rate of elimination of the immune complexes.

A modification of the classical hypothesis for HBV-induced liver cell necrosis.

There are several important findings that cannot be explained by the classical hypothesis for HBV-induced liver cell necrosis,for instance the discrepancy between high levels of viral replication and the absence of liver damage in healthy carriers.Therefore it has been suggested that excess production of HBeAg and HBsAg impairs the cellular immune response to clear HBV-infected hepatocytes (18).The first modification of the classical hypothesis therefore is to include a regulatory role for HBV-induced antigens in T cell-mediated immune mechanisms (fig 4.)

Other unexplained findings are progressive HBV-induced liver damage in liver and renal transplant recipients with adequate suppression of T cell-mediated cytotoxicity (no graft rejection). In addition,in our study the density and penetration of the inflammatory infiltrate in the livers of patients receiving exogenous alpha-interferon remained stable or decreased,even in the absence of HBe-seroconversion.

Because of these unexplained findings we propose an additional modification of the classical hypothesis:in addition to T-cell-mediated cytotoxicity we postulate a direct HBV replication-associated cytopathic effect.Furthermore,cytokines that are evoked by the infection are presumed to modulate the disease process at various points,for instance virus replication may be suppressed.The progressive liver disease in transplant recipients can then be explained as follows:cyclosporin-A selectively inhibits cytokine production,both the absence of cytokines and the presence of steroids enhance HBV replication and direct cytopathic effects.The proposal of a direct replication-associated cytopathic effect is supported by in vitro findings: cytopathic effects of HBV have been found in vitro in transfected hep-g2 cells with high cytoplasmic expression of HBcAg (19).In patients treated with alpha-interferon liver histology improves, which could support the theory of a protective role of cytokines like alpha-interferon (fig.5).

In summary the mechanism by which HBV causes liver disease in the 'normal'host is related to the cytotoxic T-cell response on liver cells expressing HBV and HLA antigens.The T-cell response may be modified by HBV antigens.Especially in immune-compromised hosts with high levels of viral replication the presence of a direct replication-associated cytopathic effect is likely to exist.The selective inhibition of cytokine production by cyclosporin-A in

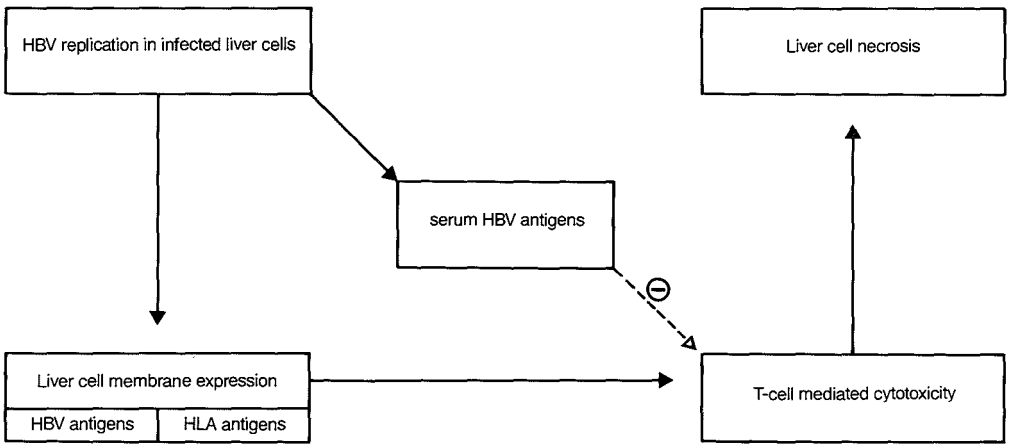

fig 4.Mechanism of liver cell necrosis:T-cell-mediated cytotoxicity is regulated by the presence of serum HBV-antigens.

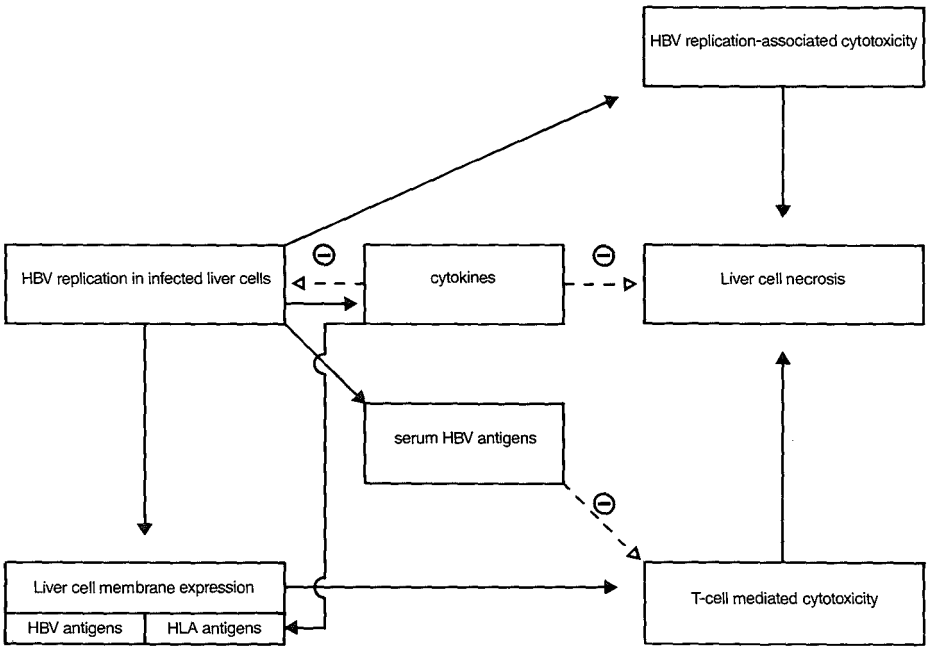

fig 5.A new hypothesis for the pathobiology of hepatitis B virus replication.

liver transplant recipients in combination with improvement in liver histology in patients receiving exogenous alpha-interferon suggests a protective role for cytokines.

Future treatment strategies.

Treatment aims.

Treatment strategies for the 'normal' host should aim toward loss of active viral replication (HBe-seroconversion),preferably followed by HBsAg seroconversion.In the immune-compromised host clearance of HBV is not an attainable goal but if our hypothesis is valid,a substantial decrease in the level of viral replication will result in a decrease in liver cell necrosis.Treatment of patients with immune complex-mediated diseases should be aimed at clearance of the immune complexes,by either decreasing the production or increasing the rate of elimination of the immune complexes.

Nucleoside analoques.

The principal action of nucleoside analogues is inhibition of viral replication.In theory they are excellent candidates for treatment of chronic hepatitis type B especially in immune-compromised patients.A profile of the nucleoside analogue needed for new treatment strategies encompasses the following elements: inhibition of viral DNA-polymerase/reverse transcriptase activity and HBV-DNA synthesis,low toxicity with a view to long-term administration,no immunosuppressive effects and oral route of administration.This ideal nucleoside analogue is not available yet.The most promising developments in this field are drug-targeting and inhibition of reverse transcription.

Drug-targeting.

Intramuscular ARA-AMP inhibits approximately 70 % of DNA-polymerase activity and HBV-DNA.The use of the drug is limited by dose-related toxicity.The toxicity problem might possibly be solved by creating conjugates of carrier molecules and ARA-AMP. The use of conjugated molecules might make it possible to target the drug almost exclusively to the liver,with minimal systemic toxicity (20).The use of ARA-A conjugated to lactosaminated albumin, a neoglycoprotein which specifically penetrates hepatocytes,looks particularly promising in this respect (21).

Inhibition of reverse transcription.

Ongoing extensive research on active drugs against the human immunodeficiency virus should produce a number of reverse transcriptase inhibitors.In vitro the triphosphate derivative of the reverse transcriptase inhibitor 3'-azido-3'deoxythymidine (zidovudine^{R}) inhibits hepatitis B DNA-polymerase activity (22).In a pilot study zidovudine (4 x 200 mg daily) was shown to inhibit DNA-polymerase activity and decrease HBV-DNA.This drug is now being further evaluated in a dose-response study (23). Initial inhibition of DNA-polymerase activity did not exceed 50%.This incomplete inhibition and the expected toxicity during long-term use imply that single drug therapy with zidovudine is unlikely to be of great benefit.However it is the first oral reverse transcriptase inhibitor available that is active against HBV.

Immunomodulatory therapy.

Cytokines.

Alpha-interferon inhibits 90 % of DNA-polymerase activity after one week and can promote clearance of HBsAg; this effect has not been reproduced by any other drug.In addition liver histology improves.Therefore exogenous administration of alpha-interferon appears to be a rational approach for the 'normal' host.Treatment strategies based on short 4-week courses of alpha-interferon in order to induce lower levels of viral replication before long-term interferon therapy starts look of interest.

A theoretical reason for concern is the development of neutralizing interferon antibodies during recombinant alpha-interferon therapy.The antibody renders recombinant interferon ineffective,which could imply that preparations consisting of several different interferon subtypes may be preferred in the future (24).However in more recent studies anti-alpha interferon antibodies developed in one out of every ten patients without correlation with the outcome of therapy (25).

Interleukin-2 therapy inhibits DNA-polymerase activity although pilot studies have produced contradictory results (26,27,28). Recently several cytokines including tumour necrosis factor and interleukins 1 and 2 were found to act in vitro synergistically with interferon (29).A serious dilemma at present is the choice of strategies worthy of further evaluation,because a clear insight into the interactions between the different cytokines is as yet lacking.A safe approach would be to use alpha-interferon

as a basic therapeutic regimen and to compare all other strategies with alpha-interferon.

Monoclonal antibodies.

The use of monoclonal antibodies for treatment of chronic viral infections in man is becoming a reality.Monoclonal anti-HBs can stop HBV replication in chimpanzees,probably by limiting the spread of the infection (30).In some liver transplant recipients large quantities of polyclonal anti-HBs can prevent reinfection of the graft liver (31). Patients with congenital agammaglobulinaemia and chronic HBV infection are now being treated successfully with repeated high dosages of murine monoclonal anti-HBs (32).In theory administration of human monoclonal antibodies to patients with chronic HBV infection to reduce the initial virus load before initiation of long-term interferon therapy seems an attractive option,especially because the result of antiviral therapy is related to pre-treatment levels of viral replication.

Anti-idiotype antibodies.

Cytotoxic T-cells are directed against viral antigens (HBcAg, HBeAg) on the liver cell membrane as well as HLA antigens.The anti-HBc antibody may mask expression of HBcAg,thereby preventing clearance of virus-infected hepatocytes.In theory down-regulation of anti-HBc production,by anti-idiotype antibodies,increases exposure of infected hepatocytes to the immune system and may enhance clearance of infected hepatocytes (33).This strategy is not aimed at inhibition of viral replication but depends entirely on the integrity of the patient's immune system for eradication of the virus.

Plasmapheresis and nucleoside anlogues.

Treatment of patients with immune complex-mediated diseases should be aimed at clearance of the immune complexes either by decreasing the production or increasing the rate of elimination of the immune complexes.The former approach has been shown to be successful in glomerulonephritis caused by chronic HBV infection. In these patients a 4-month course of alpha-interferon can induce clinical,biochemical and histologic remission (34,35).The combination of decreasing the production and increasing the rate of elimination of immune complexes has been successful in patients with hepatitis B-associated periarteritis nodosa.

Promising results were obtained with the combination of short term corticosteroids,repeated courses of ARA-A and plasmapheresis (36).This therapy is now being compared with standard immunosuppressive therapy for periarteritis nodosa in a randomized controlled trial.

Conclusions.

The therapeutic results of combination therapy have not come up to expectations compared to the results obtained with alpha-interferon alone.On the basis of the results of our studies and recent data from literature we developed a new hypothesis for HBV-induced liver damage.The central premise of this hypothesis is the co-existence of the replication-associated cytopathic effect and T cell-mediated cytotoxicity modulated by cytokines. Treatment strategies can be directed toward suppression of virus replication,viral clearance or clearance of virus-related immune complexes.In 'normal' hosts alpha-interferon can promote HBsAg clearance and is now considered the standard regimen for chronic hepatitis B.Improvement of treatment results has to be obtained by repeated courses of alpha-interferon or combination therapy with nucleoside analogues or other cytokines.Immune-compromised patients are likely to benefit from a substantial decrease in the level of viral replication either by long term nucleoside analogues or antibody therapy.For patients with immune complex-mediated disease treatment should be aimed at clearance of the immune complexes either by decreasing the production or increasing the rate of elimination of the immune complexes; alpha-interferon alone or the combination of plasmapheresis and corticosteroids followed by a nucleoside analogue may be beneficial in these cases.

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Summary.
The number of patients with chronic hepatitis B virus (HBV) infection worldwide is estimated to be 200 million, 25 % of whom will eventually die of an HBV-related disease.Elimination of the hepatitis B virus is now considered the key to effective treatment of chronic HBV infection.In this thesis clinical studies on antiviral therapy for patients with chronic HBV infection are described.The studies were designed to evaluate the inhibitory effect on HBV replication of acyclovir,descyclovir and alpha-interferon,alone or in combination.

A review of the history of the hepatitis B virus and the discovery of virus-related antigens and antibodies is presented in **chapter 1**.The natural histories of the clinical,biochemical and histological sequelae of HBV infection are discussed.Goals and possible strategies for therapy are outlined.The aim of the studies is defined.

In **chapter 2** the effects of acyclovir,adenine arabinoside,alpha-interferon or corticosteroid withdrawal as single therapeutic modality for chronic HBV infection are reviewed.We compared the loss of active viral replication achieved.Meta-analysis of the individual studies showed that therapy with alpha-interferon is superior to both adenine arabinoside and acyclovir,as far as persistent suppression of active HBV replication is concerned.

In **chapter 3** the effects of oral and intravenous acyclovir and descyclovir on markers of HBV replication are described.Oral acyclovir appears to be ineffective in chronic HBV infection, whereas high-dose intravenous acyclovir transiently inhibits HBV replication.Descyclovir has no significant inhibitory effect on HBV replication.Single drug therapy with acyclovir or descyclovir is not a valid option for chronic HBV infection.

A pilot study on intravenous acyclovir,alpha-interferon and a combination of the two drugs (**chapter 4**) indicated enhancement of the antiviral effect of alpha-interferon by acyclovir,compared to either drug alone.

In a randomized controlled study **(chapter 5)** a four-month course of a combination of alpha-interferon and descyclovir was compared with no therapy.Combination therapy was shown to inhibit viral replication.Loss of active viral replication (HBe-seroconversion) was significantly higher in treated patients (40 per cent) compared to untreated patients (0 per cent).Patients with low levels of viral replication before treatment responded to therapy with loss of active viral replication;in others only a transient inhibitory effect on HBV replication was seen.

The long-term efficacy,incidence of viral reactivation and improvement in liver disease for patients treated with intravenous acyclovir,alpha-interferon or a combination of the two drugs were evaluated **(chapter 6)**.Results indicate persistent loss of active viral replication in 40 % of patients treated with combination therapy compared to 30 % on alpha-interferon therapy, 18 % on acyclovir therapy and 0 % without treatment.Clinical improvement and prolonged normalization of liver tests were found for all patients who exhibited loss of active HBV replication.

In **chapter 7**, long-term follow-up studies, with emphasis on biochemical and histological improvement of the liver disease,are reported;all patients participated in a randomized controlled study on alpha-interferon and descyclovir.After 16 weeks of therapy,there were significant differences in the number of treated patients who became HBV-DNA and DNA-polymerase negative compared to the untreated patients.After one year of observation, the significant difference in the number of HBeAg-negative patients observed at 32 weeks persisted,but the differences in serum HBV-DNA and DNA-polymerase activity observed at 16 weeks had disappeared due to reactivation in treated patients and spontaneous loss of these markers in untreated patients.Absence of HBeAg,as indicated by a quantatative assay,represents a reliable marker of therapeutic success: patients who lost this marker of active viral replication did not exhibit reactivation. Patients who became HBeAg negative usually showed normalization of liver function tests.In treated patients histology improved compared to that found for untreated control patients,i.e.there was a significant decrease in density and penetration of the inflammatory infiltrate in the liver biopsy.In all cases disappearance of HBeAg and HBV-DNA from the blood was

accompanied by the disappearance of HBcAg in the liver.

In **chapter 8** beta-2 microglobulin levels in the patients with chronic HBV infection participating in our study on alpha-interferon and descyclovir combination therapy were assessed to determine whether the beta-2 microglobulin level is prognostic for HBe-seroconversion.Pretreatment elevation of beta-2 microglobulin was observed in 39 % of patients.Significant differences in the mean beta-2 microglobulin levels between treated patients and untreated controls were observed after 4 and 8 weeks of treatment.Levels in control patients remained stable.The outcome of antiviral therapy in our patients was not dependent on beta-2 microglobulin levels measured before or during antiviral combination therapy.

The treatment of a young patient with an incapacitating nephrotic syndrome and membranous glomerulonephritis,possibly related to active HBV replication,is discussed **(chapter 9)**.Signs of liver disease were minimal:serum aminotransferases were normal and liver histology showed mild chronic persistent hepatitis with HBsAg,HBeAg and HBcAg positivity.In the kidney deposits of HBeAg,HBcAg,IgG,c3 and c1q were observed.After 8 weeks of antiviral combination therapy,replication ceased and urinary protein loss decreased.One year after treatment the boy was asymptomatic.No HBV markers could be detected in the kidney although low grade membranous glomerulonephritis persisted.It appears that antiviral therapy can be beneficial for patients with active viral replication and deposits of HBc,HBe immune complexes in the kidney,even in the absence of active liver disease.

New approaches to and possible future developments in antiviral therapy are also considered **(chapter 10)**.Meta-analysis of 27 randomized controlled trials showed that the therapeutic results with combination therapy have not come up to expectations compared to the results obtained with alpha-interferon alone.
On the basis of the results of our studies and recent data from literature we developed a new hypothesis for HBV-induced liver damage,aimed at a differential appoach to antiviral treatment dependent upon specific circumstances.The central premise of this hypothesis is the co-existence of the replication-associated cytopathic effect and T-cell mediated cytotoxicity.

Treatment strategies can be directed toward suppression of virus replication,viral clearance or clearance of virus-related immune complexes.In 'normal' hosts alpha-interferon can promote HBsAg clearance and is now considered the standard regimen for chronic hepatitis B.Improvement of treatment results has to be obtained by repeated courses of alpha-interferon or combination therapy with nucleoside analogues or other cytokines.Immune compromised patients are likely to benefit from a substantial decrease in the level of viral replication either by long term nucleoside analogues or antibody therapy.In patients with immune complex-mediated disease treatment should be aimed at clearance of the immune complexes either by decreasing the production or increasing the rate of elimination of the immune complexes; alpha-interferon alone or in combination with a nucleoside analogue and plasmapheresis may be beneficial in selected cases.

Samenvatting.

Het totaal aantal patienten met een chronische hepatitis B virus infectie op de wereld wordt geschat op 200 miljoen,25 % van hen zal uiteindelijk overlijden als direct gevolg van de infectie of een eraan gerelateerde complicatie.Klaring van het hepatitis B virus (HBV) is daarom de kern van een effectieve behandeling van een chronische HBV infectie.In dit proefschrift worden klinische studies over antivirale therapie bij patienten met een chronische HBV infectie beschreven.De studies werden uitgevoerd om inzicht te krijgen in het effect op virale replicatie van acyclovir, descyclovir en alfa-interferon als monotherapie of in combinatie.

Het inleidend **hoofdstuk 1** bevat een overzicht van de geschiedenis van het hepatitis B virus en de ontdekking van de aan het virus gerelateerde antigenen en antilichamen.Het natuurlijk beloop van de klinische,biochemische en histologische gevolgen van een HBV infectie wordt besproken.Aan de hand van de replicatie cyclus van het virus wordt nader ingegaan op mogelijke ingangen voor antivirale therapie.Het doel van onze studies wordt gedefinieerd.

In **hoofdstuk 2** wordt een overzicht gegeven van de werkings-mechanismen en resultaten van antivirale behandeling met acyclovir, adenine arabinoside, alfa-interferon en cortico-steroiden toegepast als monotherapie.Door middel van meta-analyse van de individuele studies wordt het verlies van actieve virale replicatie vergeleken.Blijvende onderdrukking van virus replicatie wordt met alfa-interferon vaker bereikt dan met adenine arabinoside of acyclovir.

In **hoofdstuk 3** worden de resultaten van acyclovir (oraal en intraveneus) op het niveau van HBV replicatie beschreven.Oraal acyclovir lijkt niet effectief bij de onderdrukking van HBV replicatie, terwijl hoge dosis intraveneus acyclovir HBV replicatie tijdelijk kan remmen.Descyclovir in lage dosering heeft geen significant remmend effect op HBV replicatie.Mono-therapie met acyclovir of descyclovir als behandeling van een chronische HBV infectie lijkt niet zinvol.

In **hoofdstuk 4** worden klinische studies beschreven met intraveneus acyclovir,alfa-interferon of de combinatie van deze twee geneesmiddelen.De resultaten wijzen op een versterking van het antivirale effect van alfa-interferon door acyclovir in vergelijking met het effect van de afzonderlijke geneesmiddelen toegepast als monotherapie.

In **hoofdstuk** 5 worden de resultaten beschreven van een gerandomiseerd gecontroleerd onderzoek naar de effectiviteit van vier maanden behandeling met alfa-interferon en descyclovir.Het verlies van actieve virale replicatie (HBeAg seroconversie) was significant hoger in behandelde patienten (40 procent) in vergelijking met onbehandelde patienten (0 procent).Patienten die een laag niveau van virale replicatie hadden bij aanvang van de studie reageerden op therapie met verlies van actieve virale replicatie;anderen toonden alleen tijdens therapie een remming van de HBV replicatie.

Hoofdstuk 6 beschrijft de effectiviteit op lange termijn,het voorkomen van reactivatie van de virus infectie en de verbetering van de leverziekte bij patienten behandeld met intraveneus acyclovir, alfa-interferon of de combinatie van deze twee geneesmiddelen.De resultaten wijzen op een blijvend verlies van actieve virale replicatie in 40 % van de patienten behandeld met combinatie therapie in vergelijking tot 30 % met alfa-interferon, 18 % met acyclovir en 0 % zonder behandeling.Klinische verbetering en blijvende normalisering van de leverfunties werd waargenomen bij alle patienten die actieve HBV replicatie verloren.

In **hoofdstuk** 7 wordt een lange termijn vervolgonderzoek beschreven, met nadruk op biochemische en histologische verbetering van de leverziekte,bij patienten die deelnamen aan een gerandomiseerd gecontroleerd onderzoek over de combinatie alfa-interferon en descyclovir.Na 16 weken therapie bestaan er significante verschillen tussen het aantal patienten dat HBV-DNA en DNA-polymerase verliest in de behandelde groep in vergelijking met de onbehandelde controle groep.Na 32 weken is dit verschil ook significant voor HBeAg.Na een jaar observatie blijft het verschil in het aantal HBeAg negatieve patienten significant, maar de verschillen in HBV-DNA en DNA-polymerase activiteit zoals die op 16 weken bestaan verdwijnen als gevolg van reactivatie in

behandelde patienten en het natuurlijk beloop in onbehandelde patienten.Afwezigheid van HBeAg gemeten in een kwantitatieve test is een betrouwbare maat voor therapeutisch succes: bij patienten die deze parameter van actieve virale replicatie verloren werden geen reactivaties gezien.Tevens toonden zij verbetering en meestal normalisering van leverfuncties.Bij alle behandelde patienten toonde de lever histologie verbetering in vergelijking met onbehandelde patienten: een significante afname van de dichtheid en penetratie van het portaal onstekings infiltraat in de biopsie.Bij patienten die HBeAg en HBV-DNA uit het bloed verloren werd geen HBcAg meer in de lever gevonden.

In **hoofdstuk 8** worden de resultaten van het onderzoek naar serum spiegels van beta-2 microglobuline bij patienten met een chronische HBV infectie besproken.Alle patienten namen deel aan ons gerandomiseerd gecontroleerd onderzoek naar de effectiviteit van alfa-interferon en descyclovir.Onderzocht werden beta-2 microglobuline spiegels voor en tijdens antivirale therapie om uit te maken of beta-2 microglobuline een prognostische waarde heeft ten aanzien van een gunstige therapeutische respons op therapie.Voor aanvang van therapie werd een verhoogd serum beta-2 microglobuline gevonden bij 39 % van de patienten.Significante verschillen in beta-2 microglobuline ontstonden tijdens de behandeling tussen behandelde en onbehandelde patienten na 4 en 8 weken behandeling.Het niveau bij onbehandelde controle patienten bleef stabiel.Het eindresultaat van de antivirale therapie was bij onze patienten niet afhankelijk van het niveau van beta-2 microglobuline gemeten voor of tijdens de antivirale therapie.

In **hoofdstuk 9** wordt de behandeling van een jonge patient met een invaliderend nefrotisch syndroom en membraneuze glomerulonephritis, mogelijk gerelateerd aan actieve HBV replicatie, besproken.Bij deze patient waren de tekenen van een actieve leverziekte minimaal: er waren geen biochemische tekenen van hepatitis,terwijl de lever histologie een milde chronisch persisterende hepatitis toonde met de aanwezigheid van HBsAg, HBeAg en HBcAg.In de nierbiopsie werden deposities van HBeAg, HBcAg,IgG,c3,c1q aangetoond.Na 8 weken antivirale therapie nam de virale replicatie af, gevolgd door een afname van het eiwit verlies in de urine.Een jaar na behandeling was de patient klachten vrij.In de nier konden geen HBV-antigenen meer worden aangetoond,er persisteerde echter een milde membraneuze

glomerulonephritis.Antivirale therapie kan van waarde zijn bij patienten met actieve HBV replicatie en deposities van HBc,HBe immuun-complexen in de nier,zelfs als er geen actieve leverziekte aanwezig is.

In **hoofdstuk 10** worden nieuwe benaderingen en de mogelijke toekomstige ontwikkelingen in antivirale therapie besproken.Meta-analyse van 27 gerandomiseerde gecontroleerde onderzoeken toonde aan dat antivirale combinatie therapie misschien een klein additioneel effect heeft,maar niet heeft kunnen voldoen aan de hoog gespannen verwachtingen ten opzichte van therapie met alfa-interferon alleen.
Uitgaande van de nieuwe gegevens voortgekomen uit onze studies en uit de literatuur,wordt een model ontwikkeld gericht op een gedifferentieerde aanpak voor antivirale therapie in verschillende groepen patienten.Vooral in de immuun gecompromitteerde patienten met zeer hoge virale replicatie (HBV positieve transplantatie patienten, anti-HIV positieve patienten) is het waarschijnlijk, dat naast de door de cytotoxische T-cel veroorzaakte schade er sprake is van een HBV-replicatie geassocieerd cytopathogeen effect.
Antivirale therapie kan zich richten op volledige virus klaring (HBsAg seroconversie), verlagen van het niveau van virus replicatie of het omlaag brengen van de met het virus geassocieerde immuuncomplexen.Bij de 'normale' gastheer bestaat antivirale therapie uit alpha-interferon,temeer daar hierdoor HBsAg klaring versneld wordt.Verbetering van de behandelings resultaten moet komen van herhaalde interferon kuren of de combinatie met andere cytokines of nucleoside analogen.Het model ondersteunt de causale rol van antivirale therapie bij immuun gecompromitteerde patienten:remming van virale replicatie om progressieve lever schade te voorkomen.Bij patienten met door HBV immuun complexen veroorzaakte ziekten kan alfa-interferon of een combinatie van een nucleoside analoog met plasmaferese nuttig zijn.Toekomstige ontwikkelingen in antivirale therapie zullen afhangen van de identificatie van nieuwe aangrijpingspunten voor antivirale therapie (remming van 'reverse transcriptase' activiteit, monoclonale of anti-idiotype antilichamen), verbeterde toedienings vormen voor bestaande geneesmiddelen door koppeling aan specifieke drager eiwitten of de ontwikkeling van nieuwe antivirale geneesmiddelen.

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Curriculum vitae.

De auteur van dit proefschrift werd geboren in 1957 te Rotterdam. Hij bezocht de Caland Scholengemeenschap te Rotterdam,alwaar hij in 1975 het Atheneum-B diploma behaalde.In 1975 werd aangevangen met de studie economie aan de Erasmus Universiteit te Rotterdam. Januari 1978 werd het kandidaatsexamen algemene economie behaald. In september 1978 werd gestart met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam.Gedurende deze jaren was hij actief als wedstrijdroeier en wedstrijdcoach bij de ARSR SKADI. Januari 1985 werd het artsexamen behaald.Aansluitend werkte hij op de afdeling Inwendige Geneeskunde II (hoofd Prof.J.H.P.Wilson) van het Academisch Ziekenhuis Rotterdam Dijkzigt;hier werd onder leiding van Prof.dr.S.W.Schalm de basis gelegd voor dit proefschrift.In april 1987 werd begonnen met de opleiding tot internist in het Academisch Ziekenhuis Rotterdam Dijkzigt (opleider Prof.dr.J.C.Birkenhäger).Hij is gehuwd met Anne Marijke Breimer,zij hebben een zoon Auke Jeroen.