TREATMENT OF CHRONIC HEPATITIS B VIRUS INFECTION

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INTRODUCTION

There are probably between 150-200 million carriers of the hepatitis B virus in the world today. These individuals are at risk of developing chronic liver disease and primary liver cell carcinoma. In dealing with a problem of such magnitude, an approach based on the possibility of protecting future generations from infection is attractive. To this end, a vaccine suitable for active immunisation is being developed (reviewed by Zuckerman, 1980). Such a programme will probably reduce infection rates in childhood and adult life (Maupas, 1976) but its application and effectiveness in reducing the considerable number of carriers arising because of perinatal infection is less certain. Indeed, the available data already suggest that passive and active immunisation at birth are only partially effective in protecting the child of the HBs antigen-positive mother (Beasley and Stevens, 1978; Maupas *et al.*, 1981). To prevent perinatal infection (vertical transmission), a programme of treatment designed to abolish or reduce the infectivity of the mother is required. The needs of these mothers are similar, but perhaps more important in epidemiological terms, to those of all existing carriers of the hepatitis B virus.

The therapeutic goal in HBs antigen positive subjects is eradication of the virus. It should be emphasised that at present there is no totally satisfactory method of achieving this objective, and this chapter is an account of experimental methods of therapy.

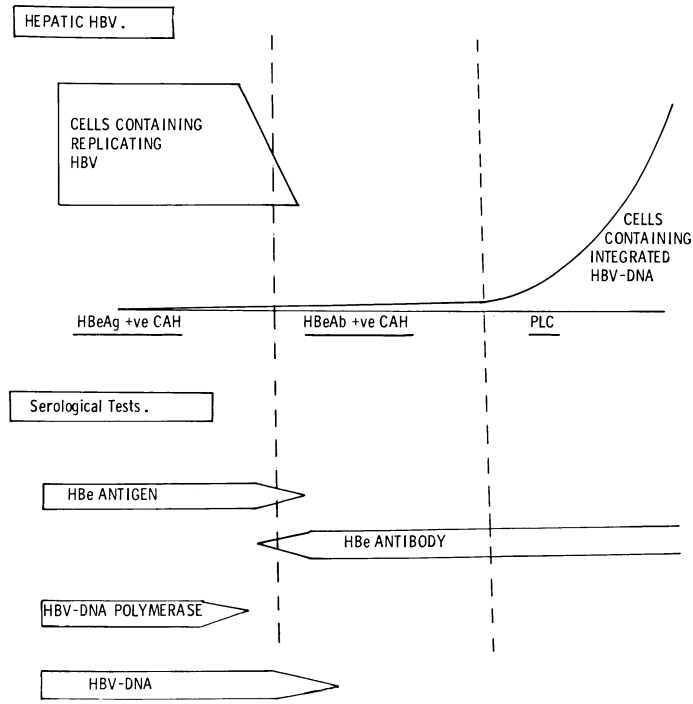

Fig. 1. — Integration of HBV-DNA into hepatocyte DNA.

MECHANISM OF VIRAL REPLICATION

In order to discuss this problem we must first consider the replication of the hepatitis B virus. Hirschman (1975) suggested that on first entering the hepatocyte, the virus DNA is transcribed by viral DNA polymerase (replication of non-integrated virus). The circular viral DNA then attaches to and integrates into the host cell DNA so that both host and viral DNA are transcribed by host DNA polymerase. Transcription of integrated viral DNA may then code the ribosomal "machinery" of the cell to produce viral proteins.

In the early phase of the infection, patients synthesize and secrete into the plasma whole virus particles (HBV). These patients are HBe antigen positive. Later, the patients cease to replicate HBV particles, HBe antigen is cleared and anti-HBe is produced. In these patients, HBV-DNA integrated into the host DNA continues to code for HBs antigen (fig. 1). This latter situation may pertain the majority of carriers whose serum contains small spheres of HBsAg in the absence of infective virus particles.

From the above observations it can be predicted that the blood of HBe antigen positive patients would be highly infectious, and these patients might benefit from antiviral therapy. Patients without demonstrable viral replication (anti-HBe positive) are assumed to have predominantly integrated virus and be unsuitable for these forms of therapy.

ANTI-VIRAL THERAPY

1. *Interferon*

Interferon is a glycoprotein, produced by virus infected cells, which induces the synthesis in uninfected cells of a protein that selectively inhibits the synthesis of virus-coded protein. This has the effect of reducing the rate of synthesis of virus particles.

Patients with hepatitis B virus infection have low levels of circulating interferon and it has been suggested that decreased interferon production might be responsible for failure of the host to eliminate the virus (Tolentino *et al.*, 1975). This has led to trials of interferon in HBs antigen positive chronic active liver disease (CALD). The interferons used were produced by the stimulation of human leucocytes, of fibroblasts, with certain virus particles or double-stranded RNA. Studies have been largely uncontrolled with variable doses, frequency, route and duration of administration. Human leucocyte-derived material administered for at least 100 days, to patients with HBs antigen-positive CALD suppressed HBV particle production during the period of treatment and in 3 patients gave permanent loss of HBV particles and HBeAg with complete or partial clearance of HBsAg (Greenberg *et al.*, 1976; Merigan and Robinson, 1978). Although these studies are encouraging, in the majority of patients the inhibition of viral replication was incomplete and following cessation of therapy, HBV particles were once again demonsrated. A controlled trial failed to show permanent inhibition of viral replication but the duration of treatment was shorter and total dose smaller than in the uncontrolled studies (Weimar *et al.*, 1980).

In general, reproducible inhibition of viral replication is observed with leucocyte interferon but the results obtained with fibroblast derived material are variable. Desmyter and his colleagues (1976) initially described the partial clearance of HBsAg and HBc antigen from the liver of a patient treated with fibroblast interferon, but subsequent studies have failed to confirm any consistent effect (Weimar *et al.*, 1977). One patient treated for a longer period than in other studies lost serum markers of replication with partial clearance

of HBsAg (Dolen *et al.*, 1979). Lower circulating levels of antiviral activity are achieved with fibroblast than with leucocyte interferon (Edy *et al.*, 1978) and this may explain the different results obtained with the two materials. The loss of *in vivo* antiviral activity may stem from the demonstrably greater lability of the fibroblast-derived material (Harmon *et al.*, 1976; Cesario, 1977; Merigan *et al.*, 1966). The results of clinical trials with lymphoblastoid cell interferon are not yet available.

Interferons also have immunomodulatory properties. When used in subjects without evidence of viral replication, fibroblast-derived interferon produced an immediate fall in HBc antibody titres (Kingham *et al.*, 1978). Similar material used in a patient with primary liver cell cancer also produced an immediate fall in HBc antibody titre and in addition titres of antibody to rubella and measles were noted to fall (pers. obs.). The relevance of this property to the value of interferon as an antiviral agent remains to be determined. *In vivo* induction of interferon has strong stimulatory effects on non-specific killer (NK) cells (Gidlund *et al.*, 1979). These have an important role in the host's defence against virus infection and malignant transformation (Kiessling and Haller, 1978).

Although these studies with interferon are encouraging, the high cost of production and limited availability make the clinical application of such therapy uncertain. The production of interferon by genetic engineering would reduce the cost considerably and might significantly alter the prospects for the clinical use of this glycoprotein in treating virus infections and tumours.

2. *Synthetic antiviral drugs*

Because of the limited availability of interferon, various synthetic antiviral drugs have been considered.

An interferon inducer (polyriboinosinic; polyribocytidylic acid) has been evaluated in two chimpanzees chronically infected with HBV (Purcell *et al.*, 1976). In these animals the drug produced falls in virus-specific DNA polymerase, HBc and HBs antigen concentrations. These agents are, however, of limited usefulness because of their toxicity and further development is needed before this form of therapy can be applied to human infections.

Virazole is a nucleoside analogue which will inhibit viral DNA synthesis *in vitro*, but in short term trials no significant effect on DNA polymerase, HBs antigen concentrations or liver function was noted in six HBs antigen-positive patients (Jain *et al.*, 1978).

Adenine Arabinoside (ARA-A) is a purine nucleoside which is a potent inhibitor of viral DNA synthesis and has been shown to be effective in the treatment of herpes encephalitis (Whitely *et al.*, 1977). Preliminary studies in patients with HBs-positive CALD demonstrated a fall in HBV-DNA polymerase and HBs antigen concentrations during treatment (Chadwick *et al.*, 1978; Pollard *et al.*, 1978). A randomised controlled study in HBsAg-positive CALD has confirmed that ARA-A consistently induces a fall in HBV-DNA polymerase activity during treatment (Bassendine, 1980) and in a third of patients this effect was maintained after cessation of treatment.

The fall in DNA polymerase activity was accompanied by a similar reduction in hepatitis B viral DNA. ARA-A thus induced a permanent inhibition of viral replication in some patients, and this was followed by loss of HBeAg with development of anti-HBe (fig. 2). In other patients ARA-A produced only transient inhibition of DNA polymerase activity and no change in HBeAg status. No fall in DNA polymerase activity or loss of HBeAg was seen in control patients over a similar period of time.

In the HBeAg-positive patients in whom ARA-A produced a permanent inhibition of HBV replication, there was a significant fall in HBsAg concentrations as compared with controls. All patients, however, remained HBsAg-positive. In the HBe antibody-positive patients, ARA-A produced no change in HBsAg concentrations. The continued production of HBsAg after permanent inhibition of HBV-DNA polymerase activity with ARA-A is compatible with the presence of viral DNA which has integrated into the patient's liver cell DNA.

Although ARA-A is of low toxicity relative to other antiviral agents, its potential usefulness is limited by its insolubility and the need for continuous intravenous infusions. ARA-AMP is the synthetic monophosphate ester of ARA-A and is at least 400 times more water soluble. It can, therefore, be given by intermittent intramuscular or intravenous administration. In patients with HBe antigen positive chronic HBV infection, a 10 day course of ARA-AMP given intravenously or intramuscularly 12 or 6 hourly, produced inhibition of HBV-DNA polymerase activity, but replication returned on cessation of therapy (Weller *et al.*, 1982). A more prolonged course (21-34 days) at a reduced dosage has, in three consecutive patients, produced permanent inhibition of HBV-DNA polymerase activity, clearance of HBe antigen and production of anti-HBe (Weller *et al.*, 1982). This promising protocol is now being evaluated in a randomised controlled trial. The long term side effects of adenine arabinoside must also be further studied before the drug is considered for widespread use, particularly in women of childbearing age.

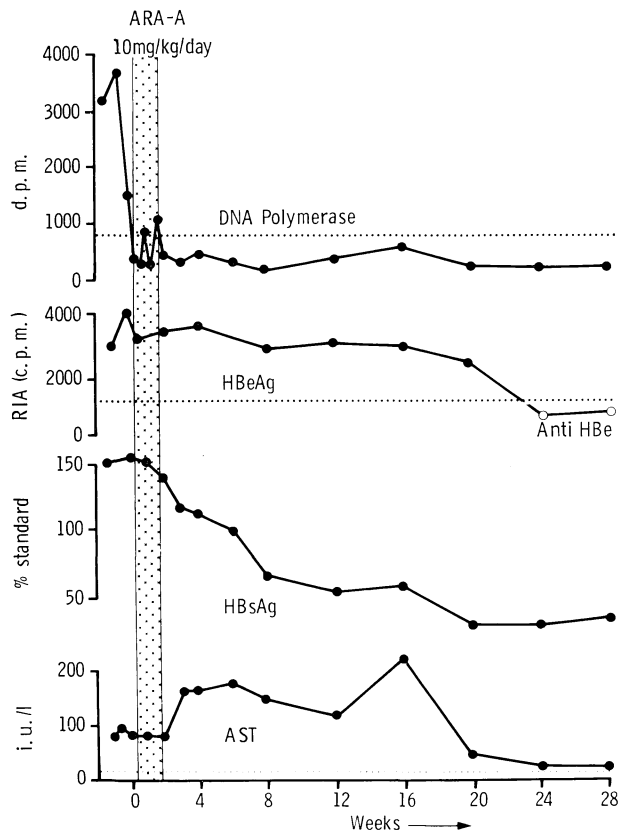

Fig. 2. — Permanent inhibition of HBV replication, with loss of HBe antigen and appearance of anti-HBe. Note the transient increase in transaminases during HBe antigen/antibody conversion followed by return of the normal range.

Intercalating agents. Chloroquine, quinacrine and chlorpromazine, can inhibit the hepatitis B DNA polymerase reaction *in vitro*, probably by acting as a template blocker (Hirschman and Garfunkel, 1978). Preliminary studies *in vivo* with 200 mg of chloroquine base daily have, however, shown that this agent is unsuccessful in maintaining suppression of HBV-DNA polymerase following intravenous adenine arabinoside (fig. 3) (personal observations).

Phosphonoformate. Trisodium phosphonoformate, like phosphonoacetic acid, is a pyrophosphate analogue. It inhibits hepatitis B viral DNA polymerase activity at low concentrations *in vitro* (Nordenfelt *et al.*, 1979). Preliminary studies indicate that it is of low toxicity, but, as illustrated by the intercalating agents, *in vivo* studies are necessary.

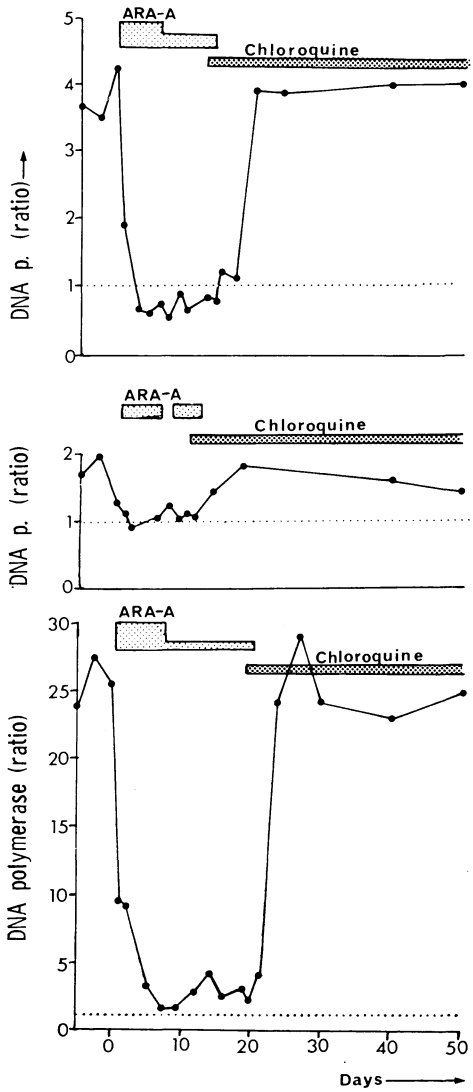

Fig. 3. — Effect of ARA-A, followed by chloroquine on HBV replication.

Acycloguanosine. This drug is active against herpes viruses. It is phosphorylated by the thymidine kinase specified by the herpes virus, and in the form of the triphosphate, it inhibits herpesvirus DNA polymerase. These actions take place to a very limited extent in uninfected cells and the drug is therefore, relatively selective and non-toxic.

The drug is likely to have similar effectiveness against other viruses which have thymidine kinase enzymes. At concentration similar to those used in treating herpes encephalitis, the drug has no activity against HBV infection. However, when doses of 10-15 mg/kg/8 hourly by i.v. infusion are used, inhibition of HBV-DNA polymerase activity is observed (Weller *et al.*, 1982). Further studies are in progress to evolve and evaluate a safe regimen.

3. *Manipulation of the immune response*

Cells containing replicating or integrated HB virus (see fig. 1) may be destroyed by the immune system of the infected subject. Such lysis is dependent on the recognition of foreign or altered liver membrane determinants by the cells of the immune system.

In acute hepatitis the immune response to either viral antigens or virus-modified host antigens results in elimination of the infected hepatocytes. The destruction of these cells appears to be mainly T-cell mediated but antibody may play an important role in neutralisation of extracellular virus (Good and Page, 1960; Thomas *et al.*, 1979).

In chronically infected subjects, various immunostimulants have been used to assist the immune system in clearing infected cells. Our knowledge of the immune status of patients with chronic active or persistent hepatitis and carriers with normal hepatic histology is incomplete, and therefore manipulations of the immune system must be cautious.

Initial attempts at immunotherapy have been made in patients with HBsAg-positive CALD. The various agents used are classified in terms of their influence on humoral or cell-mediated immunity respectively. Many also stimulate mononuclear phagocytic cell function, e.g. levamisole, *C. parvum* and mycobacterial adjuvants.

Enhancement of cell mediated immunity. Non-specific stimulation with levamisole has been tried. This agent exerts its effect by modulating the membrane ratio of cyclic AMP and GMP (Hadden *et al.*, 1975). In limited trials the drug produced increased T-cell concentrations (DeCree, 1974; Thomas *et al.*, 1977), which increased the rate of destruction of infected cells in some patients (Thomas *et al.*, 1977; Thomas *et al.*, 1979). However, all patients remained HBs antigen-positive at the end of the trial.

This drug has also been used in acute hepatitis, in an attempt to accelerate clearance of the virus and to prevent development of the carrier state (Par *et al.*, 1977). In this study transaminase concentrations were higher in the levamisole treated group and the HBs antigen was cleared more quickly.

The Authors recognised the possible danger of precipitating a fulminant course and this therapy is not recommended at the present time.

Antigen-specific stimulation was achieved in a small group of patients with transfer factor derived from the leucocytes of patients who had recovered from type B hepatitis. In one controlled study this produced a transient rise in transaminase which was interpreted as evidence of increased cell-mediated immunity and increased destruction of infected cells (Jain *et al.*, 1977). In another uncontrolled study no effect was seen (Tong *et al.*, 1976).

Enhancement of humoral immunity. Passive immunisation with HBs antibody produced no effect, other than a transient fall in HBs antigen titre, in 6 patients with HBsAg positive CALD (Reed *et al.*, 1973).

Monoclonal antiviral and anti-tumour antibodies, both native and toxin-conjugated, are currently being assessed to determine their value in neutralisation of infectious viral particles and in elimination of potentially malignant clones of cells containing the integrated HBV-DNA.

Enhancement of humoral and cell-mediated immunity. Complete Freund's adjuvant was used to immunise a group of 16 patients who had HBs antigen-positive CALD. In 3 cases the HBs antigen disappeared from the peripheral blood and in a further 5 there was a significant reduction (Kassur *et al.*, 1977). The Authors report no significant deterioration in liver function and whether the virus was eliminated from the liver or merely suppressed remains to be determined. These findings suggest that stimulation of both humoral and cell-mediated immunity is necessary to clear the virus. The side effect of treatment with complete Freund's adjuvant make further use of this agent undesirable.

Similar results were obtained in 20 children with chronic active hepatitis treated by BCG immunostimulation, 7 showing elimination of HBsAg and all 20 showing a return to normal liver function tests and 11 decreased hepatic inflammatory activity one year after completion of the course of BCG injections (Brzosko *et al.*, 1978).

Four adult male patients with HBsAg-positive CALD were treated with intradermal BCG vaccine injections at the Royal Free Hospital, London. These patients had high HBV-DNA polymerase activity before treatment. In 2 patients BCG therapy was associated with complete inhibition of viral replication as indicated by lowering of DNA polymerase activity and a fall in hepatitis B viral DNA. There was a transient increase in aspartate transaminase levels, presumably reflecting lysis of HBV-DNA infected hepatocytes.

Although these studies of immunostimulant therapy are incomplete, they are of considerable theoretical interest because this treatment is potentially effective against both replicating and integrated virus. Thus, the infectivity of a patient stemming from active viral replication and the risk of developing

carcinoma – possibly related to the presence of integrated virus – are both adequately dealt with. The effectiveness of therapy directed to eliminating both non-integrated and integrated virus is dependent on the rate of infection of regenerating hepatocytes versus the rate of destruction of cells containing episomal and integrated viral DNA. The former may be limited by adding inhibitors of viral replication to immunostimulant therapeutic regimes and further studies along these lines seem justified.

IMMUNOSUPPRESSION

The rationale for using immunosuppressive therapy in HBsAg-positive CALD arose from beneficial effect of these agents in HBsAg-negative CALD (Cook *et al.*, 1971; Soloway *et al.*, 1972; Kirk *et al.*, 1979), and the absence of effective alternative therapy. The initial reports of immunosuppressive treatment in HBsAg-positive CALD showed that these patients entered remission less frequently and failed therapy more often than HBsAg-negative patients (Schlam *et al.*, 1976). This lack of objective improvement in HBsAg-positive CALD patients on immunosuppressive therapy has since been confirmed in two controlled trials (Meyer zum Buschenfelde, 1978; Lam *et al.*, 1981).

More recently it has become apparent that HBe antibody positive patients may respond differently from HBe antigen positive patients. When steroids are given to HBe-antigen positive patients the level of HBV-DNA polymerase increases significantly and transaminases are unchanged. Similar treatment of HBe antibody positive cases results in a fall in transaminase without return of active viral replication (Weller *et al.*, 1982). Thus immunosuppressant therapy is contra-indicated in HBe antigen positive cases but may be beneficial in the HBe antibody positive cases. If patients are HBe antigen positive on prednisolone therapy, cessation of treatment usually results in conversion to anti-HBe, a transient rise in transaminase followed by reduced activity (Weller *et al.*, 1982).

PREVENTION OF PRIMARY HEPATOCELLULAR CARCINOMA

The mechanism by which the HB virus induces neoplasia is unknown. One obvious analogy can be drawn to SV40 virus infection in which integration of the viral genome into the infected cell eventually results in malignant transformation. The evidence for a similar chain of events in HBV-induced liver carcinoma is at the moment controversial (Robinson, 1978; Summers *et al.*, 1978; Brechot *et al.*, 1981; Shafritz *et al.*, 1981). However if the risk of

developing carcinoma is related to the presence of the integrated HBV genome, the prevention of this complication of the carrier state must be aimed at prevention of integration of the HB virus or elimination of the clone of cells containing the integrated viral genome. The potential of immunotherapy in this sphere has already been discussed.

CONCLUSIONS

Although we have reached the stage when we can influence viral replication and some aspects of the immune response to the viral antigens, no practical and effective therapeutic regimen has been evolved. The next few years should, however, see considerable advances in this field, perhaps with combined antiviral immunostimulant regimens. The need to consider the infectivity of the patient, the risk of developing clinically significant chronic liver disease and the risk of developing primary liver cell carcinoma in each patient, may dictate that different regimens will be needed for HBe antigen-positive and negative carriers and for patients with chronic active or chronic persistent hepatitis and carriers with normal liver histology.

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