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Antiviral Research 52 (2001) 147–152

Antiviral Research

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Extended follow-up of anti-HBe-positive patients with chronic hepatitis B retreated with ribavirin and interferon-α

Vicente Carreño *, Miguel A. Rico, Margarita Pardo, Juan Antonio Quiroga

Institute of Hepatology, Hospital Pardo de Aravaca and Fundación para el Estudio de las Hepatitis Virales, C/Guzmán el Bueno, 72, 28015 Madrid, Spain

Abstract

In a pilot study of combination therapy with ribavirin and IFNα conducted in anti-HBe-positive individuals with chronic hepatitis B, 21% of patients achieved a sustained ALT normalization and clearance of hepatitis B virus (HBV) DNA as measured by PCR. The present work has assessed whether these sustained responses are lasting long-term. In addition, IFNγ levels were tested serially in serum as a measure of the immune system activation during treatment. By extending the post-treatment follow-up period 2 years the occurrence of delayed HBV DNA relapses was observed. A low serum level of IFNγ was detected during and after treatment. IFNγ demonstrated a multiphasic time-course: the amount of IFNγ increased in parallel with reductions in HBV DNA but also with ALT flare-ups. In conclusion, the extended follow-up study of anti-HBe-positive patients after combined treatment with ribavirin and IFNα has shown that sustained responses are lasting in 17% patients but also that a late onset HBV DNA relapse may occur. © 2001 Published by Elsevier Science B.V.

Keywords: Chronic hepatitis B virus infection; Ribavirin therapeutic use; Interferon-α therapeutic use

1. Introduction

Chronic hepatitis B anti-HBe and hepatitis B virus (HBV) DNA-positive patients very rarely go into remission spontaneously (Brunetto et al., 1993) and thus have a high risk of liver disease progression (Fattovich et al., 1995). There are few reports about the efficacy of the retreatment of anti-HBe-positive patients who did not respond to initial interferon (IFN) α therapy. In general, most of the patients relapse after treatment discontinuation (Oliveri et al., 1999).

In a pilot study conducted in anti-HBe-positive patients (Cotonat et al., 2000), we investigated the efficacy of the combination therapy with ribavirin and IFNα for the retreatment of patients who had failed previous IFNα therapy. Five (21%) of the 24 patients enrolled in the pilot study had achieved a sustained ALT normalization and clearance of HBV DNA as measured by PCR 2 years after initiation of therapy. The rate of sustained response observed with ribavirin and IFNα is within the range (15–25%) of the therapeutic efficacy expected in this population of IFN-resistant patients (Alberti et al., 1997). It is noteworthy that in the anti-HBe-positive setting the

* Corresponding author. Tel.: +34-915-446-013; fax: +34-915-449-228.

E-mail address: fehvhpa@fehv.org (V. Carreño).

0166-3542/01/$ - see front matter © 2001 Published by Elsevier Science B.V.

PII: S0166-3542(01)00179-6

existence of the precore HBV mutant variant at position 1896 is very frequent and that its increasingly relative proportion diminishes the response rate to therapy B (Brunetto et al., 1991). In the present work, we have extended the follow-up period of the retreated patients beyond 2 years after the end of treatment in order to assess whether the sustained responses already described (Cotonat et al., 2000) are long-lasting. In addition, IFNγ levels were tested serially in serum as a measure of the immune system activation during treatment with combination therapy with ribavirin and IFNα.

2. Patients and methods

2.1. Patients

Twenty-four anti-HBe and HBV DNA (PCR)-positive patients with chronic hepatitis (Scheuer et al., 1996) who had persisted with ALT elevation after having failed previous interferon (IFN) treatment received combination therapy with daily oral ribavirin (1000–1200 mg according to body weight) plus 5 million units IFNα2b (Intron-A, Schering-Plough, Kenilworth, NJ) thrice weekly for 12 months (Cotonat et al., 2000). Serial blood samples were obtained prospectively in 18 patients (Table 1) who have completed the study period; blood was collected at the time of each visit during treatment and until 24 months after cessation of treatment (extended post-treatment follow-up period). The study was conducted in accordance with the declaration of Helsinki on human experimentation. Informed consent was obtained from the patients.

Table 1
Characteristics of the 18 patients at the start of the extended follow-up

Age (year)	38 (23–53)
Gender (M/F)	17/1
Duration of known HBsAg positivity (year)	8.8 (4.5–20)
ALT (IU/l)[a]	92 (30–287)
HBV DNA in serum (log_{10} copies/ml)[a]	3.28 (<2.60–7.75)
Liver histology:	
Grading (mild/moderate/severe/not available)	3/12/1/2
Staging (mild/moderate/severe/not available)	3/7/6/2

Results are expressed as the mean (range) or the number of cases.

[a] Normal ALT values: <43 IU/l; negative HBV DNA values: 400 copies/ml or <2.6 log_{10} units.

2.2. Laboratory tests

HBsAg, anti-HBe and anti-HBs were tested by commercial assays (Abbott Labs, North Chicago, IL). HBV DNA was quantitated by a PCR-based assay according to the instructions of the supplier (Amplicor HBV Monitor Test, Roche Diagnostic Systems, Branchburg, NJ) with a detection limit of 4.0×10^2 copies/ml (Gerken et al., 1998). Biochemical and hematological parameters were measured by standard methods. Levels of IFNγ were measured by microplate ELISA using reagents and protocols included in the duoset ELISA development system provided by R&D Systems Inc. (Minneapolis, MN). The detection limit of the assay is 1.5 pg/ml.

2.3. Statistical analysis

The data were analysed using the SPSS program (v 7.5) by the χ^2 or Fisher's exact test and Mann–Whitney's or Wilcoxon's signed rank tests for independent or paired samples, respectively. All *P*-values reported are two-tailed.

3. Results

3.1. HBV DNA response

Eighteen anti-HBe-positive patients had been followed-up during 2 years after completion of treatment with ribavirin and IFNα. At the end of the first year of follow-up, 11/18 (61%) patients tested negative for HBV DNA by quantitative PCR. After the second year of follow-up, HBV DNA continued to test negative in four patients, whereas HBV DNA became undetectable in another four patients. However, viremia reappeared

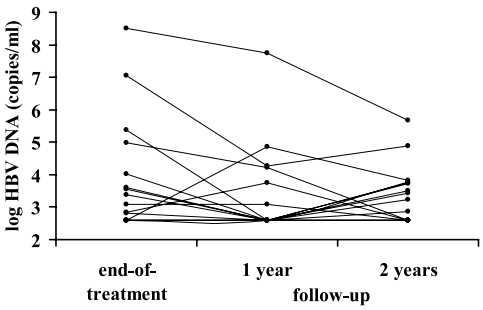

Fig. 1. Changes in HBV DNA concentrations expressed as $\log_{10}$ copies/ml (cut-off for negative values: 400 copies/ml or 2.6 $\log_{10}$ units) during the 2-years follow-up in 18 anti-HBe-positive patients treated with ribavirin and IFNα.

detectable in seven patients and HBV DNA had persisted detectable all the time in the serum of another three patients. Thus, 8/18 (44%) patients were HBV DNA-negative at the end of the extended follow-up period after cessation of treatment.

After completion of treatment with ribavirin and IFNα in anti-HBe-positive patients HBV DNA concentrations were significantly reduced (mean $\log_{10} \pm$ SD: 7.01 ± 1.90 vs 3.80 ± 1.88, $P < 0.001$). During the follow-up period there were fluctuations in HBV DNA levels according to the virological relapses observed in several patients (Fig. 1), although HBV DNA concentrations remained generally low in most of the patients. Thus, viremia levels had been reduced by at least 3 $\log_{10}$ units at the end of treatment, they remained low after 1 year of follow-up (3.28 ± 1.33) and persist reduced 2 years after stop of treatment (3.30 ± 0.88, $P < 0.001$).

3.2. ALT response

ALT levels were within the normal range in five patients after the first year of follow-up. At the end of the extended follow-up period, four patients (22%) had normal ALT values. Of these four patients, three also were HBV DNA-negative. Thus, 2 years after completion of therapy with ribavirin and IFNα 3/18 (17%) patients continued to show a sustained response to retreatment.

3.3. HBsAg response

None of the patients had lost HBsAg 2 years after cessation of treatment (extended follow-up at the end of the study).

3.4. Serum levels of IFNγ

The levels of IFNγ were longitudinally measured in the serum samples collected during the period of treatment and follow-up. Serum IFNγ levels were low in general and there were no significant differences in IFNγ levels between responders and nonresponders to therapy during the treatment period (data not shown). Serum IFNγ levels showed a multiphasic course throughout the study period (Fig. 2). Thus, IFNγ levels increased initially during reductions in HBV DNA concentrations but then started to decrease in parallel with an augment in the rate of ALT normalization until the end of the treatment period. During the follow-up a rise in IFNγ levels was observed reaching a peak which coincided with the maximum rate of HBV DNA clearance (Fig. 2) but, at the same time, with ALT relapses. Fig. 3 shows the time course of serum levels of IFNγ in a patient who demonstrated HBV DNA clearance and ALT normalization at the end of treatment but who subsequently relapsed the ALT levels and ultimately HBV DNA reappeared detectable. Because there were fluctuations in the serum levels of IFNγ, the total amount of IFNγ

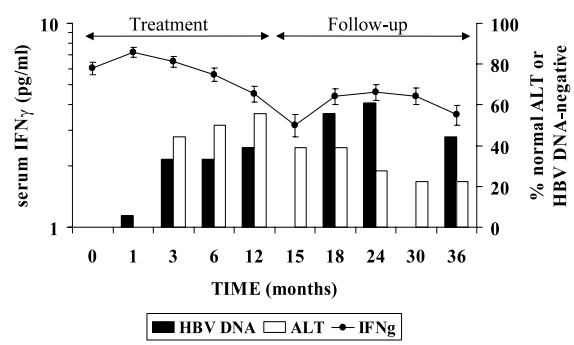

Fig. 2. Serum IFNγ levels (mean ± SD) in 18 patients treated with ribavirin and IFNα in relation to the kinetics of HBV DNA clearance and ALT normalization throughout the study period.

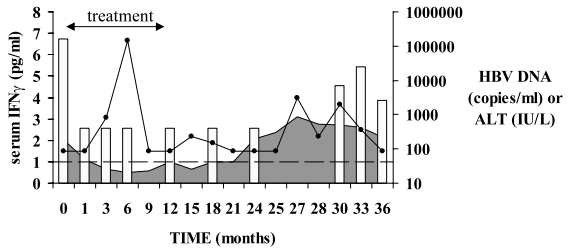

Fig. 3. Time course of serum levels of IFNγ (●) in a patient treated with ribavirin and IFNα who demonstrated HBV DNA clearance and normalization of ALT levels (shaded area) at the end of treatment but who subsequently relapsed the ALT levels and ultimately HBV DNA reappeared detectable. Vertical bars represent HBV DNA concentrations expressed as copies/ml (cut-off for negative values: 400 copies/ml or 2.6 $\log_{10}$ units). Horizontal line refers to the upper limit (43 IU/l) of the normal range for ALT values.

detected in the three serum samples taken 6 months apart during the extended follow-up period was analysed. Thus, IFNγ levels were significantly lower in eight patients who had been able to clear HBV (including the three patients who also entered in disease remission) compared with the ten patients who remained viremic (mean pg/ml IFNγ $\pm$ SEM: 6.06 ± 0.44 vs 13.53 ± 0.84, $P < 0.01$ by Mann–Whitney's test).

4. Discussion

In this work, we have investigated whether the sustained response observed in anti-HBe-positive patients to combined treatment with ribavirin and IFNα is long-lasting by extending the post-treatment follow-up period to 2 years and beyond. Thus, besides ALT relapses which are found within the first year of follow-up, the occurrence of delayed HBV DNA relapses was now observed. This finding may account for differences in the virologic outcome following administration of ribavirin and other nucleoside analogues (Torresi and Locarnini, 2000). For comparison, relapses in viremia levels occur shortly after drug withdrawal by using lamivudine (Tassopoulos et al., 1999; Santantonio et al., 2000; Honkoop et al., 2000; Liaw et al., 2000), ganciclovir (Hadziyannis et al., 1999) or famciclovir (de Man et al., 2000), even when using their combinations with IFNα (Mutimer et al., 1998; Marques et al., 1998; Hadziyannis et al., 2000). Nevertheless, in spite of the virologic relapse, most of the patients have remained with a low level of viremia.

Fluctuations in HBV DNA concentrations occurred and thus viremia levels were up and down the threshold for detection of HBV DNA by PCR (Gerken et al., 1998). These changes were likely associated with ALT flare-ups. It is suspected that reappearance of HBV DNA in the serum means virus release due to HBV persistence (Pichoud et al., 2000) and replication in the liver (Cacciola et al., 1999), albeit at a reduced level. Alternatively, this event may reflect ongoing immune elimination of HBV and release from the infected cell during hepatocytolysis (Tsai et al., 1992). However, none of the patients had lost HBsAg 2 years after cessation of treatment. This finding does not support eradication of HBV and cure. Instead, it appears that variations in HBV DNA concentrations and ALT values may correlate with the outcome of the infection. As already reported in the anti-HBe-positive population with chronic hepatitis B (Brunetto et al., 1991), the course of the disease is accompanied by persistently abnormal ALT values in half of the patients but is characterized by flare-ups interspersed with periods of normal ALT levels in the remaining patients. Thus, only three patients continued to show a sustained response to treatment 2 years after completion of therapy with ribavirin and IFNα. Therefore, it remains to be confirmed that such responses are long-lasting in a more extended survey.

Another finding of the study is the low level of IFNγ measured in the serum of anti-HBe-positive chronic hepatitis B patients during and after treatment, compared with the levels reported in HBeAg-positive individuals (Kakumu et al., 1994; Rossol et al., 1997). IFNγ levels are a measure of the immune system function and correlate with T-cell function. Indeed, HBV-specific $CD4^+$ T-cell responses are rare among patients with anti-HBe-positive chronic hepatitis B compared with HBeAg-positive carriers (Ferrari et al., 1990). Even though the amount was low in the sera of the patients, IFNγ demonstrated a multiphasic

time-course with levels rising and falling according to viremia and ALT values. The amount of IFNγ appeared to increase timely with reductions in HBV DNA but also in parallel with ALT flare-ups.

Although the precise mechanism of HBV elimination is not completely known in the anti-HBe-positive patients, it is tempting to speculate that immune elimination plays an important role (Jung et al., 1995). Both ribavirin and IFNα are drugs with known immunomodulatory properties. In fact, ribavirin treatment has the ability to increase in vitro IFNγ production in transgenic mice (Hultgren et al., 1998). T-cell responsiveness may be enhanced following significant reductions in viremia levels by treating chronic hepatitis B with IFNα, lamivudine, famciclovir or with combination therapy with lamivudine and IFNα (Lohr et al., 1995; Marinos et al., 1996; Boni et al., 1998). In this way, we have recently shown that combined therapy with ribavirin and interferon-α for chronic hepatitis B not only significantly reduces viremia levels but also induces lasting $CD4^+$ T-cell responses and Th1 cytokine release at the site of infection (Rico et al., 2001), which may lead to sustained eradication of the HBV.

In summary, the extended follow-up study of anti-HBe-positive patients after combined treatment with ribavirin and IFNα has demonstrated that sustained responses are long-lasting in some patients but also that a late onset HBV DNA relapse may occur in other cases. There is a need to investigate novel drugs (Torresi and Locarnini, 2000) or different schedules of combination therapy with more than one nucleoside analogue (Lau et al., 2000) to achieve a long-term sustained eradication of HBV in this difficult-to-treat population of patients with chronic hepatitis B.

References

Alberti, A., Chemello, L., Pontisso, P., Benvegnù, L., Bernardinello, E., Cavalletto, L., 1997. Interferon therapy for chronic viral hepatitis: status report. In: Rizzetto, M., Purcell, R.H., Gerin, J.L., Verme, G. (Eds.), Viral Hepatitis and Liver Disease. Edizioni Minerva Medica, Turin, Italy, pp. 727–735.

Boni, C., Bertoletti, A., Penna, A., Cavalli, A., Pilli, M., Urbani, S., Scognamiglio, P., Boehme, R., Panebianco, R., Fiaccadori, F., Ferrari, C., 1998. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J. Clin. Invest. 102, 968–975.

Brunetto, M.R., Giarin, M.M., Oliveri, F., Chiaberge, E., Baldi, M., Alfarano, A., Serra, A., Saracco, G., Verme, G., Will, H., Bonino, F., 1991. Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. Proc. Natl. Acad. Sci. USA 88, 4186–4190.

Brunetto, M.R., Giarin, M., Saracco, G., Oliveri, F., Calvo, P., Capra, G., Randone, A., Abate, M.L., Manzini, P., Capalbo, M., 1993. Hepatitis B virus unable to secrete e antigen and response to interferon in chronic hepatitis B. Gastroenterology 105, 845–850.

Cacciola, I., Pollicino, T., Squadrito, G., Cerenzia, G., Villari, D., de Franchis, R., Santantonio, T., Brancatelli, S., Colucci, G., Raimondo, G., 1999. Quantification of intrahepatic hepatitis B virus (HBV) DNA in patients with chronic HBV infection. Hepatology 31, 507–512.

Cotonat, T., Quiroga, J.A., Lopez-Alcorocho, J.M., Clouet, R., Pardo, M., Manzarbeitia, F., Carreño, V., 2000. Pilot study of combination therapy with ribavirin and interferon alfa for the retreatment of chronic hepatitis B e antibody-positive patients. Hepatology 31, 502–506.

de Man, R.A., Marcellin, P., Habal, F., Desmond, P., Wright, T., Rose, T., Jurewicz, R., Young, C., 2000. A randomized, placebo-controlled study to evaluate the efficacy of 12-month famciclovir treatment in patients with chronic hepatitis B e antigen-positive hepatitis B, Hepatology, pp. 413–417.

Fattovich, G., Giustina, G., Schalm, S.W., Hadziyannis, S., Sanchez-Tapias, J., Almasio, P., Christensen, E., Krogsgaard, K., Degos, F., Carneiro de Moura, M., 1995. Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis type B. The EUROHEP study group on hepatitis B virus and cirrhosis. Hepatology 21, 77–82.

Ferrari, C., Penna, A., Bertoletti, A., Valli, A., Antoni, A.D., Giuberti, T., Cavalli, A., Petit, M.A., Fiaccadori, F., 1990. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J. Immunol. 145, 3442–3449.

Gerken, G., Gomes, J., Lampertico, P., Colombo, M., Rothaar, T., Trippler, M., Colucci, G., 1998. Clinical evaluation and applications of the Amplicor HBV Monitor test, a quantitative HBV DNA PCR assay. J. Virol. Meth. 74, 155–165.

Hadziyannis, S.J., Manesis, E.K., Papakonstantinou, A., 1999. Oral ganciclovir treatment in chronic hepatitis B virus infection: a pilot study. J. Hepatol. 31, 210–214.

Hadziyannis, S., Alexopoulou, A., Papakonstantinou, A., Petraki, K., Manesis, E., 2000. Interferon treatment with or without oral ganciclovir in HBeAg-negative chronic hepatitis B: a randomized study. J. Viral Hepat. 7, 235–240.

Honkoop, P., de Man, R.A., Niesters, H.G., Zondervan, P.E., Schalm, S.W., 2000. Acute exacerbation of chronic hepati-

tis B virus infection after withdrawal of lamivudine therapy. Hepatology 32, 635–639.

Hultgren, C., Milich, D.R., Weiland, O., Sallberg, M., 1998. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. J. Gen. Virol. 79, 2381–2391.

Jung, M.C., Diepolder, H.M., Spengler, U., Wierenga, E.A., Zachoval, R., Hoffmann, R.M., Eichenlaub, D., Frosner, G., Will, H., Pape, G.R., 1995. Activation of a heterogeneous hepatitis B (HB) core and e antigen-specific $CD4^+$ T-cell population during seroconversion to anti-HBe and anti-HBs in hepatitis B virus infection. J. Virol. 69, 3358–3368.

Kakumu, S., Ishikawa, T., Wakita, T., Yoshioka, K., Takayanagi, M., Tahara, H., Kusakabe, A., 1994. Interferon-gamma production specific for hepatitis B virus antigen by intrahepatic T lymphocytes in patients with acute and chronic hepatitis B. Am. J. Gastroenterol. 89, 92–96.

Lau, G.K., Tsiang, M., Hou, J., Yuen, S., Carman, W.F., Zhang, L., Gibbs, C.S., Lam, S., 2000. Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study. Hepatology 32, 394–399.

Liaw, Y.F., Leung, N.W., Chang, T.T., Guan, R., Tai, D.I., Ng, K.Y., Chien, R.N., Dent, J., Roman, L., Edmundson, S., Lai, C.L., 2000. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia hepatitis lamivudine study group. Gastroenterology 119, 172–180.

Lohr, H.F., Weber, W., Schlaak, J., Goergen, B., Meyer zum Buschenfelde, K.H., Gerken, G., 1995. Proliferative response of $CD4^+$ T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B e-minus hepatitis B virus mutants. Hepatology 22, 61–68.

Marinos, G., Naoumov, N.V., Williams, R., 1996. Impact of complete inhibition of viral replication on the cellular immune response in chronic hepatitis B virus infection. Hepatology 24, 991–995.

Marques, A.R., Lau, D.T., McKenzie, R., Straus, S.E., Hoofnagle, J.H., 1998. Combination therapy with famciclovir and interferon-alpha for the treatment of chronic hepatitis B. J. Infect. Dis. 178, 1483–1487.

Mutimer, D., Naoumov, N., Honkoop, P., Marinos, G., Ahmed, M., de Man, R., McPhillips, P., Johnson, M., Williams, R., Elias, E., Schalm, S., 1998. Combination alpha-interferon and lamivudine therapy for alpha-interferon-resistant chronic hepatitis B infection: results of a pilot study. J. Hepatol. 28, 923–929.

Oliveri, F., Santantonio, T., Bellati, G., Colombatto, P., Mels, G.C., Carriero, L., Dastoli, G., Pastore, G., Ideo, G., Bonino, F., Brunetto, M.R., 1999. Long term response to therapy of chronic anti-HBe-positive hepatitis B is poor independent of type and schedule of interferon. Am. J. Gastroenterol. 94, 1366–1372.

Pichoud, C., Berby, F., Stuyver, L., Petit, M.A., Trepo, C., Zoulim, F., 2000. Persistence of viral replication after anti-HBe seroconversion during antiviral therapy for chronic hepatitis B. J. Hepatol. 32, 307–316.

Rico, M.A., Quiroga, J.A., Subirá, D., Castañón, S., Esteban, J.M., Pardo, M., Carreño, V., 2001. Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon-α. Hepatology 33, 295–300.

Rossol, S., Marinos, G., Carucci, P., Singer, M.V., Williams, R., Naoumov, N.V., 1997. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J. Clin. Invest. 99, 3025–3033.

Santantonio, T., Mazzola, M., Iacovazzi, T., Miglietta, A., Guastadisegni, A., Pastore, G., 2000. Long-term follow-up of patients with anti-HBe/HBV DNA-positive chronic hepatitis B treated for 12 months with lamivudine. J. Hepatol. 32, 300–306.

Scheuer, P.J., Davies, S.E., Dhillon, A.P., 1996. Histopathological aspects of viral hepatitis. J. Viral Hepat. 3, 277–283.

Tassopoulos, N.C., Volpes, R., Pastore, G., Heathcote, J., Buti, M., Goldin, R.D., Hawley, S., Barber, J., Condreay, L., Gray, D.F., 1999. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B lamivudine precore mutant study group. Hepatology 29, 889–896.

Torresi, J., Locarnini, S., 2000. Antiviral chemotherapy for the treatment of hepatitis B virus infections. Gastroenterology 118, S83–S103.

Tsai, S.L., Chen, P.J., Lai, M.Y., Yang, P.M., Sung, J.L., Huang, J.H., Hwang, L.H., Chang, T.H., Chen, D.S., 1992. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J. Clin. Invest. 89, 87–96.