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（出國類別：進　修）

慢性病毒性肝炎之診斷與治療

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內容摘要: 慢性病毒性肝炎（主要爲B型及C型）乃國人好發之慢性疾病之一。截至目前爲止，對於慢性B型及C型肝炎之致病機轉及治療仍有許多地方待進一步瞭解或加強之處。職奉淮於於民國八　九年四月赴美國密西根大學醫學中心胃腸科跟隨國際著名之肝炎學者Anna S.F. Lok 教授進行研究，爲期兩年。訓練期間除參加密西根大學醫學中心胃腸科之學術活動外，並積極參與慢性B型及C型肝炎患者之新藥臨床試驗，肝臟移植後病患之照顧以及在實驗室中以分子生物學技術探討慢性病毒性肝炎之致病機轉。受訓過程並參加美國消化疾病週(DDW), 美國肝臟學年會(AASLD)以及歐洲肝臟學年會(IASL/EASL)並發表論文。總計截至目前爲止已針對B型肝炎病毒基因型、以聚合酵素鏈反應定量血清B型肝炎病毒量之臨床應用及B型肝炎肝臟移植患者之預後發表研究成果。回國之後當繼續致力於慢性病毒性肝炎之研究以提昇學術研究及服務之品質。

本文電子檔已上傳至出國報告資訊網

摘要

慢性病毒性肝炎（主要為B型及C型）乃國人好發之慢性疾病之一。截至目前為止，對於慢性B型及C型肝炎之致病機轉及治療仍有許多地方待進一步瞭解或加強之處。職奉淮於於民國八十九年四月赴美國密西根大學醫學中心胃腸科跟隨國際著名之肝炎學者Anna S.F. Lok 教授進行研究，為期兩年。訓練期間除參加密西根大學醫學中心胃腸科之學術活動外，並積極參與慢性B型及C型肝炎患者之新藥臨床試驗，肝臟移植後病患之照顧以及在實驗室中以分子生物學技術探討慢性病毒性肝炎之致病機轉。受訓過程並參加美國消化疾病週(DDW), 美國肝臟學年會(AASLD)以及歐洲肝臟學年會(IASL/EASL)並發表論文。總計截至目前為止已針對B型肝炎病毒基因型、以聚合酵素鏈反應定量血清B型肝炎病毒量之臨床應用及B型肝炎肝臟移植患者之預後發表研究成果。回國之後當繼續致力於慢性病毒性肝炎之研究以提昇學術研究及服務之品質。

正文

目的：

慢性病毒性肝炎（主要為B型及C型）乃國人好發之慢性疾病之一。若不及早加以治療，病毒持續繁殖以及肝臟慢性發炎纖維化將導致肝硬化及其併發症如腹水、肝腦病變、食道靜脈曲張出血之發生，長此以往甚至導致肝細胞癌之形成。

截至目前為止，對於慢性B型及C型肝炎之致病機轉仍有許多地方待進一步瞭解。另一方面慢性病毒性肝炎病患對於目前之治療方法如干擾素或口服抗病毒藥物肝安能（lamivudine）其療效並不十分令人滿意。長期服用甚至有部份病患其病毒產生突變造成肝臟功能惡化(decompensation)。若能針對慢性病毒性肝炎之致病機轉作進一步之研究或使用新開發之抗病毒藥物加以治療，不僅可提高治癒率並有助於提昇病患之生活品質並預防併發症之產生。

過程

經內科部李主任之推薦，職於民國八十九年四月赴美國密西根大學醫學中心胃腸科跟隨國際著名之肝炎學者 Anna S.F. Lok 教授進行研究，為期兩年。第一年帶職帶薪，第二年留職停薪。訓練期間除參加密西根大學醫學中心胃腸科之學術活動外，並積極參與慢性

B 型及 C 型肝炎患者之新藥臨床試驗，肝臟移植後病患之照顧以及在實驗室中以分子生物學技術探討慢性病毒性肝炎之致病機轉。受訓過程並參加美國消化疾病週(DDW), 美國肝臟學年會(AASLD)以及歐洲肝臟學年會(IASL/EASL)並發表論文。總計截至目前為止已發表五篇論文（見附錄）並有二至三篇論文接近完成階段。

心得

茲將受訓兩年之心得依各主題分段敘述如下：

1. B 型肝炎：

截至目前為止，美國食品藥物衛生管理局所核准之慢性 B 型肝炎用藥僅干擾素（interferon）及口服抗病毒藥物肝安能(lamivudine)兩大類。干擾素之缺點在於副作用較大且病患若有非代償性肝硬化則不適合使用。Lamivudine 之缺點在於病患若長期服用則病毒容易產生突變種(YMDD mutation)，此一突變種可造成部份病患肝功能惡化甚至產生肝衰竭。而新開發的口服抗病毒藥物如 adefovir, entecavir 等於臨床試驗結果初步看來不僅可用於未經治療之 B 型肝炎患者，病患若產生 YMDD mutation 此藥亦可有效抑制突變種之複製且其療效較原有藥物更好。這些新的口服抗病毒藥物應是治療慢性 B 型肝炎之明日之星。 此外，目前

亦有許多研究探討合併療法（如使用干擾素加口服抗病毒藥物或兩種抗病毒藥物）用於慢性B型肝炎之治療。

致病機轉方面近年來最為熱門之研究課題為B型肝炎病毒基因型(genotype) 之研究。截至目前為止B型肝炎病毒可依其nucleotide sequence 之不同分為七種不同之基因型(A-G)。基因型A主要分布於北美、西北歐、中非；基因型B及C分布於亞洲；基因型D主要分布於南歐、中東及印度。截至目前為止的報告認為B 型肝炎病毒基因型可能和 e 抗原陰轉(HBeAg seroconversion)、肝病嚴重度、不同之突變型態（precore and core promotor mutations）以及治療之成功率有關。針對此主題職與指導教授撰寫一review article 刊登於肝臟學雜誌(Hepatology, 附錄一)並深入研究發現與基因型C相比，基因型B之患者其e抗原陰轉較早。於追蹤過程中罹患基因型C之患者雖有ALT 之fluctuation 但卻無法達到e抗原陰轉，這可以解釋為何近來研究顯示罹患基因型C之患者其肝病嚴重度較重且肝硬化比例較高。此一研究成果已於今年六月份發表於胃腸學雜誌上(Gastroenterology, 附錄二)。另與全美其他十六個醫學中心合作有關不同B型病毒基因型在美國各地區之盛行率以及與出生地區，人種，肝病嚴重度、及不

同突變型態關係之研究亦已接近完成將於最近投稿。目前 B 型肝炎之診斷已進入分子生物技術階段，運用聚合酵素鏈反應(polymerase chain reaction, PCR)我們可精確得知患者体內 B 型肝炎病毒之病毒量（copies/mL)，但也因此使得慢性 B 型肝炎患者之診斷或治療進入一個新的領域。針對以聚合酵素鏈反應定量血清 B 型肝炎病毒量之臨床應用，職有幸與指導教授發表一 editorial 於歐洲肝臟學雜誌上(Journal of Hepatology, 附錄三)。另外對於在不同階段 B 型肝炎患者病毒量之研究，吾等亦發現並無一特定之血清病毒量（threshold HBV DNA level）伴隨著 e 抗原消失(HBeAg clearance)但病患於 e 抗原消失前後其病毒量約減少 $3\log_{10}$ 。e 抗原消失時其病毒量之高低並無法預測此反應為暫時或是持續（超過一年)。此外，e 抗原消失時其病毒量之高低與突變種（precore or core promotor mutation）之產生並無絕對關係。此一研究成果已發表於今年度之美國消化疾病週(DDW)並已投稿國外雜誌。

2. C 型肝炎：

慢性 C 型肝炎目前之標準療法為使用干擾素加上 ribavirin 之合併療法。近年來治療之新趨勢乃使用長效干擾素(pegylated interferon) 每週皮下注射

一次再加上口服 ribavirin 800-1200 mg/day。長效干擾素不僅較方便，其優異之藥物動力學特性使得治癒率有效的加以提昇。病患若感染 C 型肝炎病毒第二或第三基因型（genotype 2 or 3），使用此方法治療六個月其成功率甚至可達 80%。而第一基因型(genotype 1)患者使用此療法一年其成功率約可達 50% 左右。

病患若感染 C 型肝炎病毒則約有 50%-80%會進展至慢性肝炎進而產生肝硬化。因此，如何減緩肝臟纖維化之程度乃是目前研究之重要課題。在美國衛生總署（National Institute of Health，NIH）之主導下，全美共有約十個醫學中心參與一個以長效干擾素(pegylated interferon)長期治療以往治療失敗之慢性 C 型肝炎合併肝臟纖維化患者之研究(HALT-C trial)，密西根大學即為其中之一。此研究之目的在探討長期干擾素治療是否能減緩纖維化之產生。此研究目前仍在進行當中，其結果將對慢性 C 型肝炎患者之治療提供重要參考。

致病機轉方面，以往來自日本之研究報告認為 C 型肝炎病毒有一段基因可能和干擾素治療之感受性有關（interferon sensitivity determining region, ISDR)。然而，此項推論卻未能被來自歐美國家之研究結果所認同。職於進修期間曾參與 ISDR 胺基酸變異數

目之多寡與干擾素治療成功與否之相關性研究，結果發現患C型肝炎病毒第一型患者接受干擾素治療其成功率與ISDR胺基酸變異數目無明顯相關。此外對於細胞激素(cytokine)以及基因變異性(gene polymorphism)在慢性 C 型肝炎致病機轉及纖維化所扮演之角色將是吾等:未來重要之研究課題。

3. 肝臟移植：

密西根大學醫學中心每年約有一百位患者接受肝臟移植，其中以C型肝炎及酒精性肝硬化患者佔大多數。職受訓期間除學習肝移植患者手術前後之照顧外，並分析自1985年來於密西根大學因B型肝炎接受肝移植患者之預後及肝炎復發後藥物治療之效果，結果顯示肝臟移植後 B 型肝炎之復發大多產生術後一至兩年內，復發後預後大多不佳。手術後持續給予靜脈注射B型肝炎免疫球蛋白(hepatitis B immune globulin, HBIG 10,000 IU/month)較肌肉注射低劑量或不使用組可有效降低B型肝炎術後復發。復發之患者若能及時給予 lamivudine 100 mg/day 則可有效改善肝功能、防止肝衰竭之發生。此項成果已於去年發表於肝臟移植雜誌(Liver Transplantation，附錄四)。另針對使用免疫抑制劑OKT3對於B型肝炎移植患者之影響亦有專文發表於肝臟移植雜誌(Liver Transplantation，

附錄五)。

長期使用靜脈注射高劑量 B 型肝炎免疫球蛋白(10,000 IU/month)雖然有效但費用高昂，lamivudine與 HBIG 屬不同機轉防止 B 型肝炎術後復發，若能合併使用不僅可能有加成效果亦可降低 HBIG 之劑量達到 cost-effective 之效果。在 Anna Lok 教授指導下，經密西根大學人體試驗委員會同意後，我們選擇肝臟移植超過一年以上之 B 型肝炎患者在其同意下將 intravenous HBIG 10,000 IU/month ± lamivudine 100 mg/day 調整為 intramuscular HBIG 1,000 IU/month + lamivudine 100 mg/day，一年後若無復發則停止 HBIG 之注射而使用 lamivudine 單一療法以預防 B 型肝炎復發。此項研究目前已進入第二年而初步結果已投稿至今年底之美國肝臟學年會(AASLD)。

建議

1. 本院乃國家級醫學中心，然而各主治醫師由於臨床工作繁忙以及硬體設備或經費不足對於學術研究經常力有未逮。建議院方於經費及編制員額許可下，加強學術研究並提昇醫療水準。
2. 基礎醫學（如分子生物技術）與臨床相結合乃未來醫學研究之必然趨勢，本院教研部與臨床各部科之橫向溝通及整合似不如國外先進研究機構完備。希

望往後能在此方面多加強以維持本院之競爭優勢。

3. 國外先進機構其研究經費多由專業會計人員負責，各項研究計畫多半限制總額對於細目則不嚴加限制由計畫主持人權宜調度。院方並負責常用研究物料之統一採買以增加效率、避免經費濫用及不實申報。
4. 參與臨床長期藥物試驗之病患其回診追蹤應與一般門診患者加以區別於特定時段或特定診間進行。

EDITORIALS

Clinical Significance of Hepatitis B Virus Genotypes

See Article in HEPATOLOGY 2002;35:922-929

Traditionally, hepatitis B virus (HBV) is classified into 4 subtypes or serotypes (adr, adw, ayr, and ayw) based on antigenic determinants of the hepatitis B surface antigen.[1] These subtypes can be further classified into 9 serotypes (ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+, and adrq−).[2] Epidemiologic studies found that the prevalence of these serotypes varies in different parts of the world. In addition, antibody to the common determinant, "a," confers protection against all serotypes. To date, there has been very little data on the clinical significance of HBV serotypes.

Advances in molecular biology techniques revealed significant diversities in sequences of HBV isolates, accounting for the allelic differences among the 4 major HBV serotypes. Based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into 7 genotypes A-G.[3-5] However, genotyping can be accomplished based on a partial sequence of the HBV genome such as the pre-S or S gene. Several methods have been used for HBV genotyping including direct sequencing, restriction fragment length polymorphism, line probe assay, and enzyme-linked immunoassay.

Contrary to hepatitis C virus genotyping, HBV genotyping is a research tool that is only beginning to gain popularity among researchers in hepatitis B. Whether HBV genotyping will constitute part of the clinical evaluation of hepatitis B patients depends on the availability of simple and inexpensive tests and the relevance of the information gained. Currently, restriction fragment length polymorphism is the most commonly used method for HBV genotyping. A line probe assay similar to that used for hepatitis C virus genotyping is also available. These assays can be easily applied in clinical diagnostic laboratories. The key issue is, does knowledge of the HBV genotype help in patient management? The specific questions include, (1) Is there a correlation between HBV genotype and HBV replication, activity of liver disease, clinical outcome, and treatment response? (2) What is the predominant HBV genotype in each country? Is the geographical distribution of HBV genotypes related to the endemicity of HBV infection? (3) Is there a correlation between HBV genotype and risk of progression to chronic infection? (4) Does infection with one HBV genotype confer protection against infection with other HBV genotypes?

Answers to some of the questions raised are beginning to emerge but many of the answers are based on a few studies in selected patient populations. Current information on the geographical distribution of HBV genotypes is summarized in Table 1. However, existing information is incomplete. As an example, earlier studies suggested that HBV genotype A is predominant in the United States. A recent study indicated that HBV genotype G is also prevalent because it was present in 11 of 82 patients from the state of Georgia.[5] However, in an ongoing study involving 17 liver centers across the United States, we found all 7 HBV genotypes: A (33%), B (21%), C (34%), D (9%), E (1%), F (1%), and G (1%) (personal observations). HBV genotype A was more common among whites and African Americans, whereas genotypes B and C were predominantly found in Asian Americans.

The high prevalence of HBV genotypes B and C among Asians raise the possibility that HBV genotype may be related to the endemicity of HBV infection. To date, there has been no study on the relationship between HBV genotype and mode of transmission. One study in Switzerland found that genotype A was more common among patients with chronic hepatitis B, whereas genotype D was more prevalent among patients with resolving acute hepatitis B suggesting that HBV genotype A was associated with a higher rate of chronic HBV infection.[6] However, this study involved a total of 65 patients only and confounding factors such as age at infection, gender, mode of transmission, and coinfection with other hepatitis virus or human immunodeficiency virus were not analyzed.

HBV genotypes may contribute to the wide range in prevalence of HBV infection in different parts of the world through differences in rates of replication and abilities to evade immune clearance, but studies comparing

Abbreviations: HBV, hepatitis B virus, HBeAg, hepatitis B e antigen.

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Table 1. Geographic Distribution of HBV Genotypes and Serotypes

Genotypes	Serotypes	Distribution
A	adw2, ayw1	NW Europe, N America, Central Africa
B	adw2, ayw1	SE Asia, China, Japan
C	ayr, adrq+, adrq−, adw2	SE Asia, China, Japan
D	ayw2, ayw3	S Europe, Middle East, India
E	ayw4	Africa
F	adw4q−	American natives, Polynesia, Central and South America
G	adw2	United States, France

the replication capacity and immune response of the various HBV genotypes have not been performed. However, many studies have shown a strong relationship between HBV genotypes and mutations in the precore and core promoter regions that abolish or diminish the production of hepatitis B e antigen (HBeAg).[7-10] Thus, the most common precore mutation, a G to A substitution at nucleotide 1896 ($G_{1896}A$), which creates a premature stop codon (eW28X) is found in association with HBV genotypes B, C, and D but not genotype A. This accounts for the preponderance of HBeAg-negative chronic hepatitis B in Southern Europe and Asia. The basis for the genotype-dependent selection of the precore $G_{1896}A$ mutation is related to the need to maintain base pairing of the stem-loop structure of the pregenome encapsidation sequence (ϵ).[11,12] HBV genotypes B, C, and D frequently have a T at nucleotide 1858, which is directly opposite nucleotide 1896 in the stem of ϵ, whereas HBV genotype A usually has a C at nucleotide 1858, which forms a more stable bond with the wild type (G) rather than the variant sequence (A).[13]

In the last issue of HEPATOLOGY, Kato et al. explored the mechanism by which HBV genotype G, which has 2 stop codons in the precore region, maintains HBeAg production.[14] They found that all 4 patients with HBV genotype G, who were HBeAg positive, were also coinfected with HBV genotype A. In one patient who seroconverted from HBeAg to hepatitis B e antibody, a shift from predominant HBV genotype A to predominant HBV genotype G was shown. Based on partial sequencing of a few clones, the investigators suggested that there is evidence of recombination between the two genotypes, but more studies are needed to confirm these findings. It would also be important to determine if patients infected with one HBV genotype can be superinfected with other genotypes and if infection with multiple HBV genotypes results in more severe liver disease.

Several studies reported a correlation between HBV genotype and HBeAg clearance. These studies, all of Asian patients, found that the prevalence of HBeAg was higher in patients with genotype C compared to those with genotype B suggesting that HBeAg clearance occurred at higher rates among patients with genotype B.[8,10,15] One study of 466 Japanese patients found that HBeAg was present in 53% of genotype C versus 16% of genotype B patients.[10] This difference was maintained after matching for gender, age, and liver disease in the two groups. In a recent study of 269 Chinese patients, we found that spontaneous HBeAg seroconversion occurred approximately one decade earlier among patients with HBV genotype B.[16] We also showed that patients with genotype B were more likely to have a sustained biochemical remission after spontaneous HBeAg seroconversion.

A correlation between HBV genotype and liver disease has also been found in several studies from Asia. One study in Japan found that liver dysfunction (defined as abnormal aminotransferase levels) was observed less frequently in hepatitis B carriers with adw serotype (mainly genotype B) compared to those with adr serotype (mainly genotype C).[17] Another study found that hepatitis B surface antigen carriers with genotype B had lower histologic activity scores.[8] Two other studies involving a total of 490 Chinese patients with chronic HBV infection found that genotype C was more prevalent in patients with cirrhosis.[15,18] It is possible that a longer duration of high levels of HBV replication may contribute to more active liver disease and, in turn, a higher rate of progression to cirrhosis among patients with HBV genotype C. The relationship between HBV genotypes and hepatocellular carcinoma is inconclusive. One study found that HBV genotype B is associated with development of hepatocellular carcinoma at an earlier age,[18] but this finding was not confirmed by other studies.[15,19,20] The relationship between HBV genotypes and liver disease in other ethnic populations has not been examined.

HBV genotype has also been related to response to interferon therapy. One study of 64 German patients found that the rate of interferon-induced HBeAg seroconversion was higher among patients with genotype A than in those with genotype D (37% vs. 6%).[21] Another report involving 58 patients in Taiwan found that the rate of HBeAg loss was significantly higher in patients with genotype B compared to those with genotype C (41% vs. 15%).[22] A third study in 35 HBeAg-negative patients found that patients infected with HBV genotype A responded better than those with genotype D/E (70% vs. 40%).[23] The correlation between HBV genotype and response to other antiviral therapy (such as lamivudine) remains to be determined. One study based on 26 patients reported that patients with adw serotype were more likely to develop resistance to lamivudine than those with

ayw serotype but the correlation between serotype and response was not mentioned.[24]

In summary, there is growing evidence that HBV genotypes may influence HBeAg seroconversion rates, mutational patterns in the precore and core promoter regions, and the severity of liver disease. In addition, different HBV genotypes predominate in various parts of the world. Thus, the heterogeneity in disease manifestations and response to antiviral treatment among patients with chronic hepatitis B in different parts of the world may, at least in part, be attributed to differences in HBV genotypes. Further studies are needed to confirm these observations to determine if HBV genotyping should be included in the clinical evaluation of patients with chronic HBV infection and if treatment should be tailored accordingly.

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Hepatitis B Virus Genotype B Is Associated With Earlier HBeAg Seroconversion Compared With Hepatitis B Virus Genotype C

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Background & Aims: **Recent studies suggest that hepatitis B virus (HBV) genotype B is associated with less active liver disease than HBV genotype C. The aim of our study was to determine if HBV genotype B is associated with higher rates of spontaneous hepatitis B e antigen (HBeAg) seroconversion compared with genotype C.** ***Methods:*** **A retrospective study using stored sera from 332 Chinese patients with chronic HBV infection followed for a mean of 48 months (range, 12–98) were tested for HBV genotype using a line-probe assay.** ***Results:*** **HBV DNA was detected in 273 patients, 122 had HBV genotype B and 147 genotype C. Patients with genotype B had a significantly lower prevalence of HBeAg at presentation and significantly higher rates of spontaneous HBeAg seroconversion during follow-up. HBV genotype B patients who were HBeAg positive were significantly younger, and spontaneous HBeAg seroconversion occurred approximately 1 decade earlier compared with HBV genotype C patients. Multivariate analyses identified high alanine aminotransferase (baseline and follow-up), age >30 years, and genotype B as independent factors associated with spontaneous HBeAg seroconversion.** ***Conclusions:*** **HBV genotype B is associated with earlier HBeAg seroconversion than genotype C. This finding may explain the less active/progressive liver disease in patients with genotype B.**

Hepatitis B virus (HBV) is traditionally classified into 4 serotypes (adr, adw, ayr, and ayw) based on antigenic determinants of the hepatitis B surface antigen (HBsAg). More recently, HBV is classified into 7 genotypes (A–G) based on an intergroup divergence of 8% or more in the complete nucleotide sequence.[1–3] Several recent studies suggested that HBV genotypes may be related to the rate of recovery from acute HBV infection and the progression of liver disease during chronic HBV infection.[4–11] One study in Japan[4] found that liver dysfunction (defined as abnormal aminotransferase levels) was observed less frequently in hepatitis B carriers with adw serotype (mainly genotype B) compared with those with adr serotype (mainly genotype C). Another study[6] found that HBsAg carriers with genotype B had lower histologic activity scores than those with genotype C. A third study in Taiwan[7] reported that genotype B was less prevalent than genotype C among patients with cirrhosis. These data suggest that HBV genotype B is associated with less active and less advanced liver disease.

One explanation for the less aggressive liver disease associated with HBV genotype B may be related to a shorter duration of high levels of HBV replication. Two studies[4,5] found that HBsAg carriers with adw serotype were less often hepatitis B e antigen (HBeAg) positive than those with adr serotype, and carriers with adw serotype had slightly higher HBeAg seroconversion rate during follow-up.[5] More recent studies found that patients with genotype B were less often HBeAg positive than those with genotype C.[6,8] These data suggest that HBV genotype B is associated with higher rates of spontaneous HBeAg seroconversion compared with HBV genotype C. However, 3 of the 4 studies cited previously were conducted in Japan, and most of the findings were based on point prevalence.

The aim of our study was to determine if HBV genotype B is associated with higher rates of spontaneous HBeAg seroconversion compared with genotype C. We analyzed point prevalence as well as follow-up data of a cohort of 269 Chinese patients with chronic HBV infection followed for 1–8 years.

Patients and Methods

Patients

This is a retrospective study using stored sera from Chinese patients with chronic HBV infection seen in the Hepatitis Clinic, Queen Mary Hospital, Hong Kong, from 1984 to 1992. The clinical database was reviewed to identify all patients who had been followed for more than 1 year and had HBeAg/HBe antibody (anti-HBe) checked on at least 2 occasions during the first year with stable HBeAg status.

Abbreviations used in this paper: anti-HBe, hepatitis B e antibody; PCR, polymerase chain reaction; ULN, upper limit of normal.

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Patients were seen every 3 to 6 months or more often if clinically indicated. At each visit, liver biochemistry and HBV serology including HBsAg, HBeAg, and anti-HBe were checked. Serum was collected and stored for HBV DNA testing. Patients were censored when antiviral therapy was initiated.

Serial alanine aminotransferase (ALT) values during the follow-up period were categorized into 3 patterns: (1) persistently normal ALT, (2) persistent or intermittent elevation in ALT levels that remain $\leq 5 \times$ upper limit of normal (ULN, 45 IU/L), and (3) fluctuations in ALT levels with peak values $>5 \times$ ULN.

Definition of Outcomes

HBeAg loss was defined as disappearance of serum HBeAg in a patient who was previously HBeAg positive. HBeAg seroconversion was defined as disappearance of HBeAg accompanied with the development of anti-HBe.

For purposes of analysis of sustained HBeAg loss or HBeAg seroconversion, only patients with at least 6 months follow-up were included. Sustained HBeAg loss or HBeAg seroconversion was defined as maintenance of this phenomenon throughout the remaining follow-up period with HBeAg/anti-HBe retested at least twice per year. Sustained normalization of ALT was defined as persistently normal ALT values from the period 6 months after HBeAg seroconversion until the last visit, with a minimum of twice yearly ALT tests. This definition differentiates elevated ALT values around the time of HBeAg seroconversion versus persistent or intermittent elevation after HBeAg seroconversion.

Hepatitis B Serology

Hepatitis B serologic markers HBsAg, HbeAg, and anti-HBe were tested using commercially available enzyme-linked immunosorbent assay kits from Abbott Laboratories (North Chicago, IL).

HBV Genotyping

Residual sera stored at -70°C were retrieved. The first available serum sample from each patient was tested for HBV genotype.

HBV genotypes were determined by line-probe assay (Inno-Lipa HBV genotyping assay; Innogenetics Inc., Ghent, Belgium).[12,13] In brief, HBV DNA was extracted under standard procedure.[14] HBV DNA was amplified by nested polymerase chain reaction (PCR) using biotinylated primers in the *S* gene provided by the manufacturer (Innogenetics). The reaction was performed in a Personal Cycler (Biometra Inc., Tampa, FL) The first-round PCR contained 40 cycles at 94°C for 30 seconds, 45°C for 30 seconds, and 72°C for 30 seconds, with a 10-minute extension step at 72°C at the end. The second-round PCR contained 35 cycles with the same steps as in the first round. All necessary precautions to prevent cross-contamination were observed, and negative controls were included at each step. Five microliters of the second-round PCR products were analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide and visualized with an ultraviolet transilluminator. The lower limit of detection of this PCR assay was 400 copies/mL. For samples with detectable HBV DNA after nested PCR reaction, 10 μL of amplified products were applied to strips precoated with specific oligonucleotide probes. After hybridization, washing, and chromogenic reaction, HBV genotypes were determined based on the pattern of reactive bands.

To validate the results of line-probe assay, all samples with unclassified genotypes and randomly selected samples from each genotype were sent for bidirectional automated sequencing. Sequencing was performed at the DNA sequencing core facility, the University of Michigan Medical Center, using the standard protocol for the Applied Biosystems DNA sequencer 377 (Perkin Elmer Corp., Foster City, CA) using second-round PCR primers. Sequencing results were compared with published sequences to ascertain HBV genotypes.[1,3]

Detection of Precore Stop Codon Variant ($G_{1896}A$)

To determine if the presence of precore stop codon variant is related to the rate of HBeAg seroconversion, the first available serum samples from HBeAg-positive patients were tested by a line-probe assay[13] (Inno-Lipa HBV precore assay, Innogenetics Inc.) designed to detect the G to A change at nucleotide 1896 ($G_{1896}A$), which results in a premature stop codon at position 28 in the precore region. Except for the primers, the procedure was similar to that described previously for HBV genotyping. To validate the results of line-probe assay, samples with indeterminate precore sequence and randomly selected samples with wild-type precore sequence and $G_{1896}A$ mutation were sent for bidirectional automated sequencing.

Statistical Analyses

Results were expressed as mean ± standard error. Data were entered into an Excel (Microsoft, Redmond, WA) database and analyzed using SPSS version 9.0 software package (SPSS Inc., Chicago, IL). Statistical analyses were performed by using χ^2 and Fisher exact test for categoric variables. Independent student *t* tests were used for continuous variables. Rates of spontaneous HBeAg seroconversion and HBeAg loss were estimated using Kaplan-Meier method and compared using log-rank test. Multivariate analyses using stepwise Cox regression were performed to identify independent factors associated with spontaneous HBeAg seroconversion. Results were considered statistically significant at $P < 0.05$.

Results

Baseline Characteristics

A total of 332 patients were studied. The study population included 199 (60%) men and 133 (40%) women; mean age was 30 ± 1 years (range, 2–75 years). All the patients were ethnically Chinese. The mean du-

ration of follow-up was 48 months (range, 12–98 months). At presentation, 172 (52%) patients were HBeAg (+) and anti-HBe (−), whereas 160 (48%) patients were HBeAg (−) and anti-HBe (+). The median serum ALT value was 34 IU/L (range, 6–1,700 IU/L).

HBV Genotypes

Serum HBV DNA was detected in 98% (168/172) HBeAg (+) and in 66% (105/160) HBeAg (−) patients after nested PCR reaction. The overall HBV DNA detection rate was 82% (273/332). Four HBV genotypes were found: 2 genotype A (0.7%), 122 genotype B (44.7%), 147 genotype C (53.9%), and 2 genotype D (0.7%). Only 5 (2%) samples cannot be typed by the Inno-Lipa assay. Direct sequencing of these 5 samples showed that 2 belonged to genotype B, 2 to genotype C, and 1 to genotype D. Thirty-two randomly selected samples that were typed by Inno-Lipa assay including 2 genotype A, 12 genotype B, 17 genotype C, and 1 genotype D were directly sequenced; the results were completely concordant.

Prevalence of HBeAg in Patients With HBV Genotype B and C

The baseline characteristics of the 269 patients infected with HBV genotype B and C are listed in Table 1. Genotype B was associated with a less marked male preponderance and lower baseline ALT values compared with genotype C, but these differences were not significant. At presentation, patients infected with genotype B had a significantly lower prevalence of HBeAg compared with those with genotype C (53% vs. 69%, $P < 0.01$).

Table 1. Baseline Characteristics of Patients Infected With HBV Genotype B and C

	Genotype B (n = 122) (%)	Genotype C (n = 147) (%)	P value
Mean age (*yr*)	28 ± 1	30 ± 1	NS
Gender (M:F)	73:49	103:44	NS
Mean ALT (*IU/L*)	83 ± 15	123 ± 17	NS
No. (%) HBeAg (+) anti-HBe (−)	65 (53)	102 (69)	<0.01
Initial ALT in HBeAg (+) patients			NS
Normal	38 (59)	44 (43)	
1-2 × ULN	11 (17)	19 (19)	
2-5 × ULN	10 (15)	23 (22)	
>5 × ULN	6 (9)	16 (16)	
Initial ALT in HBeAg (−) patients			NS
Normal	42 (74)	29 (64)	
1-2 × ULN	8 (14)	8 (18)	
2-5 × ULN	6 (10)	4 (9)	
5 × ULN	1 (2)	4 (9)	

NOTE. ULN = 45 IU/L.

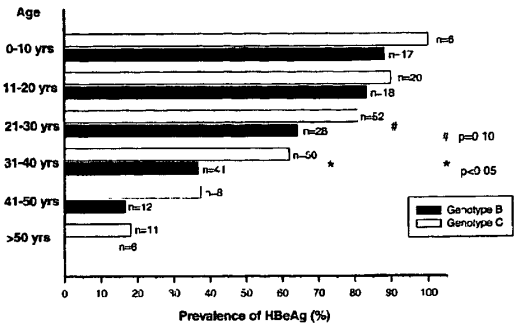

Figure 1. Age-specific prevalence of HBeAg among patients infected with HBV genotype B and C.

The age-specific prevalence of HBeAg in patients with genotype B and C are depicted in Figure 1. In both groups, there was a significant decrease in prevalence of HBeAg with age. However, the peak decline in prevalence of HBeAg occurred 1 decade earlier in patients with genotype B, resulting in a significantly lower prevalence of HBeAg by the fourth decade (37% vs. 62% in patients with genotype C, $P < 0.05$).

Accordingly, HBeAg (+) genotype B patients were significantly younger than HBeAg (+) genotype C patients (mean age 21±1 vs. 26±1 years, $P < 0.005$) (Table 2).

Rates of Spontaneous HBeAg Loss and HBeAg Seroconversion in Patients With HBV Genotypes B and C

One hundred sixty-seven HBeAg (+) patients were followed for 45±2 months (range, 12–96). Eighty-seven (52%) of these patients had liver biopsies. Patients with genotype B were less likely to have significant liver disease (chronic active hepatitis ± cirrhosis) than those with genotype C ($P < 0.05$) (Table 2). Compared with genotype C patients, genotype B patients were more likely to have the $G_{1896}A$ precore stop codon variant detectable in the initial sample ($P < 0.005$). The concordance between direct sequencing and Inno-Lipa HBV precore assay was >97%. During the follow-up period, approximately 40% of patients with each genotype developed flares in ALT levels (>5 × ULN), but a significantly higher percent of genotype B patients maintained persistently normal ALT levels (37% vs. 18% among genotype C patients, $P < 0.05$) (Table 2).

A total of 48 patients lost HBeAg, of whom 42 developed anti-HBe. Despite similar duration of follow-up, spontaneous HBeAg loss (40% vs. 22%, $P < 0.05$) and HBeAg seroconversion (37% vs. 18%, $P < 0.01$)

Table 2. Comparisons Between HBeAg (+) Genotype B and C Patients

	Genotype B (n = 65) (%)	Genotype C (n = 102) (%)	*P* value
Mean age (yr)	21 ± 1	26 ± 1	<0.005
Gender (M:F)	41:24	73:29	NS
Mean initial ALT (*IU/L*)	111 ± 26	145 ± 24	NS
Mean follow-up (*mo*)	47 ± 3	44 ± 2	NS
Histology			<0.05
Nonspecific changes/chronic persistent hepatitis	13/26 (50)	17/61 (28)	
Chronic active hepatitis	9/26 (35)	32/61 (52)	
Cirrhosis	4/26 (15)	12/61 (20)	
Precore region (codon 28)			<0.005
Wild-type sequence only	47 (72)	93 (91)	
Presence of $G_{1896}A$ variant	18 (28)	9 (9)	
ALT levels during follow-up			<0.05
Persistently normal	24 (37)	19 (18)	
≤5 × ULN	15 (23)	41 (41)	
Peak >5 × ULN	26 (40)	42 (41)	
No. with HBeAg loss	26 (40)	22 (22)	<0.05
Mean age at HBeAg loss (yr)	27 ± 2	34 ± 2	<0.05
No. with HBeAg seroconversion	24 (37)	18 (18)	<0.01
Mean age at HBeAg seroconversion (*yr*)	27 ± 2	35 ± 2	<0.05
HBeAg seroconversion in relation to initial ALT			
Normal	7/38 (18)	3/44 (7)	NS
Abnormal	17/27 (63)	15/58 (26)	<0.005
HBeAg seroconversion in relation to ALT levels during follow-up			
Persistently normal	1/24 (4)	1/19 (5)	NS
≤5 × ULN	6/15 (40)	9/41 (22)	NS
Peak >5 × ULN	17/26 (65)	8/42 (19)	<0.001

NOTE. ULN = 45 IU/L.

occurred more often in patients with genotype B. This difference was predominantly noted in patients who had elevated ALT level at presentation (63% vs. 26%, $P < 0.005$) and those who had flares in ALT during follow-up (65% vs. 19%, $P < 0.001$). The rates of spontaneous HBeAg seroconversion were similarly low in genotype B and C patients who had normal ALT levels (Table 2). By using Kaplan-Meier analyses, the cumulative rates of HBeAg loss at 1, 3, and 5 years were 8%, 30%, and 50% in genotype B patients and 0%, 23%, and 38% in genotype C patients ($P < 0.05$). The cumulative rates of spontaneous HBeAg seroconversion at 1, 3, and 5 years were 6%, 24%, and 46% in genotype B patients and 0%, 18%, and 34% in genotype C patients ($P < 0.05$) (Figure 2).

Predictive Factors for Spontaneous HBeAg Seroconversion

Factors that may be associated with spontaneous HBeAg seroconversion including gender, age, initial ALT level, ALT levels during follow-up, liver histology, presence of $G_{1896}A$ precore stop codon variant, and HBV genotype were analyzed. After univariate analyses, age >30 years, HBV genotype B, initial abnormal ALT, fluctuating ALT with peak >5 × ULN, and presence of $G_{1896}A$ variant significantly contributed to spontaneous HBeAg seroconversion. These factors were further analyzed by multivariate stepwise Cox regression model. All factors except the presence of $G_{1896}A$ variant were independently associated with spontaneous HBeAg seroconversion (Table

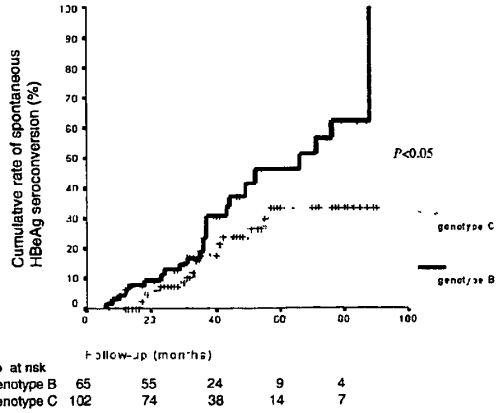

Figure 2. Cumulative rates of spontaneous HBeAg seroconversion in patients infected with HBV genotype B and C.

Table 3. Independent Factors Associated With Spontaneous HBeAg Seroconversion

Factor	No. of patients	Odds ratio	95% CI	*P* value
All patients				
ALT level during follow-up				
Fluctuating ALT, peak >5 × ULN	68	6.37	1.31–31.06	<0.05
Persistent or intermittent increase, ≤ 5 × ULN	56	4.34		
Persistently normal	43	1		
Initial ALT				
Abnormal	85	3.31	1.51–7.28	<0.01
Normal	82	1		
Genotype				
B	65	2.32	1.23–4.35	<0.01
C	102	1		
Age				
>30 yr	53	1.94	1.02–3.69	<0.05
≤30 yr	114	1		
Genotype B patients				
ALT level during follow-up				
Fluctuating ALT, peak >5 × ULN	26	11.79	1.39–99.81	<0.05
Persistent or intermittent increase, ≤5 × ULN	15	6.24		
Persistently normal	24	1		
Initial ALT				
Abnormal	27	2.51	0.98–6.42	0.056
Normal	38	1		
Genotype C patients				
Age				
>30 yr	36	3.03	1.19–7.71	<0.05
≤30 yr	66	1		

CI, confidence interval.

3). ALT level (at baseline and during follow-up) was the most important predictor of spontaneous HBeAg seroconversion, particularly for genotype B patients (Figure 3). As a group, the likelihood of spontaneous HBeAg seroconversion among genotype B patients was 2.3 times that of genotype C patients.

Biochemical and Virologic Remission After HBeAg Seroconversion

Of the 33 patients who had been followed for at least 6 months (range, 7–53 months) after spontaneous HBeAg seroconversion, 11 of 17 (65%) genotype B and

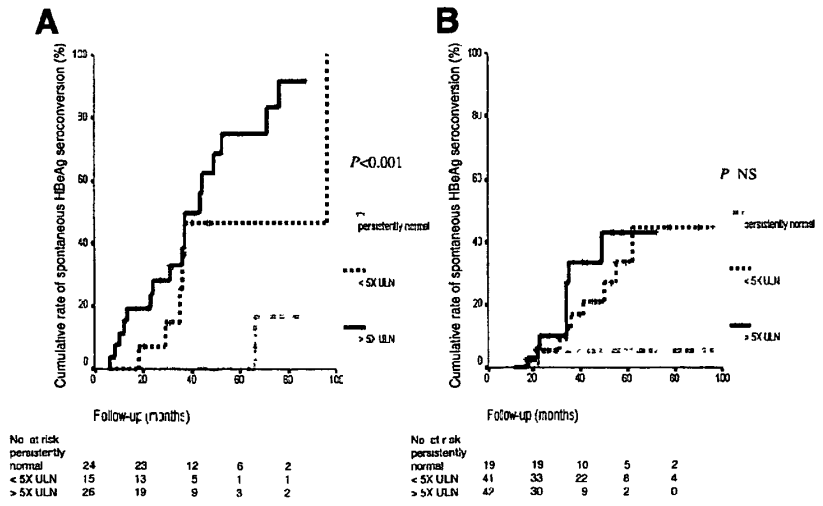

Figure 3. Cumulative rates of spontaneous HBeAg seroconversion in relation to ALT levels during follow-up in patients infected with (*A*) HBV genotype B and (*B*) C.

Table 4. Outcome of HBV Genotype B and C Patients After HBeAg Seroconversion

	Genotype B (n = 24) (%)	Genotype C (n = 18) (%)	*P* value
No. with follow-up >6 months after HBeAg seroconversion	17	16	
Mean follow-up after HBeAg seroconversion (mo)	26 ± 4	31 ± 4	NS
No. with sustained HBeAg seroconversion	11 (65)	8 (50)	NS
No. with sustained normalization of ALT	13 (76)	6 (38)	<0.05
No. with HBsAg loss	1 (6)	0 (0)	NS
No. with undetectable HBV DNA by PCR	5 (29)	3 (19)	NS

8 of 16 (50%) genotype C patients had sustained HBeAg seroconversion. Compared with genotype C patients, genotype B patients were significantly more likely to have sustained normalization in ALT levels (76% vs. 38%, $P < 0.05$) (Table 4).

Discussion

Our study showed that HBV genotype B is associated with earlier HBeAg seroconversion than HBV genotype C among Chinese patients with chronic HBV infection. We found that patients with HBV genotype B had a significantly lower prevalence of HBeAg at presentation and a significantly higher rate of spontaneous HBeAg seroconversion during follow-up. We also showed that HBV genotype B patients who were HBeAg positive were significantly younger, and spontaneous HBeAg seroconversion occurred approximately 1 decade earlier compared with HBV genotype C patients. Our findings corroborate that of previous reports from Japan.[4,5,8,11] Our results are also in accord with a recent report that patients with HBV genotype B had a higher rate of HBeAg loss during interferon therapy compared with those with genotype C.[15]

The mechanism(s) responsible for the difference in rate of spontaneous HBeAg seroconversion between HBV genotype B and C is not clear. One study[11] found that the mean serum HBV DNA levels were higher among genotype C patients compared with genotype B patients, but this finding may be related to a higher prevalence of HBeAg among the genotype C patients. Another study reported that the precore stop codon variant ($G_{1896}A$), which abrogates HBeAg production, is more commonly found in patients with HBV genotype B than those with genotype C.[6] However, this finding was not confirmed in another study.[8] Our study revealed that genotype B patients were more likely to have the $G_{1896}A$ variant at presentation, and univariate analysis showed that presence of this variant was associated with a higher rate of spontaneous HBeAg seroconversion. However, presence of the $G_{1896}A$ variant was not an independent predictor of spontaneous HBeAg seroconversion, suggesting that factors other than selection of the precore stop codon mutation may be more important in HBeAg seroconversion. Alternatively, relevance of the $G_{1896}A$ variant may be related to timing of the initial sample because 67% of the initial samples were collected more than 2 years before HBeAg seroconversion.

In accordance with previous reports,[16–18] we found that ALT levels (baseline and during the course of follow-up) were the most important factor in predicting spontaneous HBeAg seroconversion. The rates of spontaneous HBeAg seroconversion were uniformly low in patients with HBV genotype B and C who had normal ALT levels. The rates of spontaneous HBeAg seroconversion were significantly higher among patients who had abnormal baseline ALT levels and those who had ALT flares during follow-up (>5 × ULN). However, the association between high ALT levels and increased rate of HBeAg seroconversion was predominantly seen in patients with HBV genotype B. Our results indicate that ineffective ALT flares representing abortive immune clearance are more common in patients with HBV genotype C and may account for the more active liver disease and higher prevalence of cirrhosis as shown in some studies.[6–8,10] Because liver biopsies were performed in only 52% of the patients, we were not able to determine if baseline liver histology was predictive of subsequent HBeAg seroconversion.

We confirmed that age and presumably duration of infection is an important predictive factor of spontaneous HBeAg seroconversion.[18,19] In addition, we found that spontaneous HBeAg seroconversion occurred 1 decade earlier in patients with HBV genotype B. Thus, patients with genotype B are subjected to a shorter duration of high levels of HBV replication.

Our study also showed that compared with genotype C patients, HBV genotype B patients who had spontaneous HBeAg seroconversion were significantly more likely to have a sustained biochemical remission. In addition, genotype B patients had a trend toward a higher rate of sustained HBeAg seroconversion than genotype C patients.

In summary, our results showed that HBV genotype B is associated with earlier HBeAg seroconversion compared with HBV genotype C among Chinese patients with chronic HBV infection. A shorter exposure to high levels of HBV replication, less abortive ALT flares before HBeAg seroconversion, and more sustained biochemical remission after HBeAg seroconversion may contribute to the lower prevalence of cirrhosis in patients with HBV genotype B. The association between spontaneous HBeAg seroconversion rate and HBV genotype suggests that HBV genotype may also play a role in determining response to antiviral therapy. Our results suggest that heterogeneity in disease manifestations, natural course, and response to antiviral treatment among patients with chronic hepatitis B in different parts of the world may, at least in part, be attributed to differences in HBV genotypes. Further studies involving other patient populations and other HBV genotypes are needed to confirm our observations and to determine if HBV genotyping should be included in the clinical evaluation of patients with chronic HBV infection and if treatment should be tailored accordingly.

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Editorial

Clinical utility in quantifying serum HBV DNA levels using PCR assays

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See Article, pages 543–546

The evaluation of patients with hepatitis B virus (HBV) infection has evolved from serological to molecular diagnostic assays. Advances in molecular biology techniques in the early 1980s led to the development of hybridization assays for serum HBV DNA with detection limits of 10^6–10^7 copies/ml. The introduction of polymerase chain reaction (PCR) assays in the late 1980s decreased the limit of HBV DNA detection to 10^2–10^3 copies/ml. The availability of molecular diagnostic assays has improved our understanding of the pathogenesis and natural history of HBV infection and facilitated the monitoring of response to treatment. They also generated new questions and dilemmas. Patients with undetectable serum HBV DNA using hybridization assays were previously thought to have non-replicative infection. These patients were deemed not to require treatment and hepatic inflammation if present was attributed to other causes of liver disease.

Using hybridization assays, patients who have recovered from acute HBV infection invariably have undetectable HBV DNA in serum. However, recent studies using more sensitive PCR assays found that low levels of HBV DNA, generally $<10^3$ copies/ml [1,2], may persist for many years after recovery from acute HBV infection [3]. Studies using PCR assays also found that the vast majority of patients with chronic HBV infection including those who are hepatitis B e antigen (HBeAg) negative, hepatitis B e antibody (anti-HBe) positive have detectable HBV DNA in serum [1,2,4–9]. These findings raise a number of clinically important questions. First, what level of serum HBV DNA is associated with progressive liver disease? Second, at what level of serum HBV DNA is treatment indicated? Third, to what level should serum HBV DNA be reduced during treatment in order to ensure sustained virologic response, HBeAg seroconversion, and remission of liver disease?

The National Institutes of Health (NIH) Workshop on 'Management of Hepatitis B' held in September 2000 attempted to address some of these questions [10]. Consensus definition and diagnostic criteria for clinical terms relating to HBV infection were discussed. An arbitrary serum HBV DNA level of 10^5 copies/ml was proposed to differentiate chronic hepatitis B from inactive carrier state. This level was chosen to include serum HBV DNA levels that would be detected by all commercial as well as in house non-PCR based assays. Studies in the 1980s and early 1990s before PCR assays became popular demonstrated that most patients who developed spontaneous or treatment induced HBeAg seroconversion with undetectable serum HBV DNA had normal aminotransferase (ALT) levels, reduced histologic activity, decreased risks of hepatic decompensation, and improved survival. The majority of these patients would have detectable HBV DNA if they had been tested by PCR assays. Thus, it seems that low serum HBV DNA levels may not necessarily be pathogenic and may not require treatment. However, the selection of 10^5 copies/ml as a cut-off value for differentiating chronic hepatitis B (HBeAg positive as well as HBeAg negative) from inactive carrier state has not been validated. The threshold HBV DNA level that is associated with progressive liver disease is not known and may be dependent on host factors such as immune response, viral factors such as HBV genotype and mutations in the core promoter and precore regions, and environmental factors such as alcohol consumption. In addition, many patients with chronic HBV infection have fluctuating HBV DNA levels. Finally, assays for HBV DNA quantification are not well standardized.

It is generally accepted that HBV is not a cytopathic virus and that liver disease associated with HBV infection is mainly immune-mediated. Evidence that HBV is not directly cytopathic is most obvious in children and adolescents with perinatally acquired HBV infection, in whom HBeAg and high serum HBV DNA levels are present, but

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ALT levels and liver histology are usually normal. Indeed, several studies found that HBeAg positive patients with normal ALT have higher serum HBV DNA levels than those with elevated ALT [4,9,11]. Nevertheless, presence of HBV is needed for ongoing necroinflammation.

Several studies have attempted to examine the range of serum HBV DNA levels in HBeAg positive as well as in HBeAg negative patients and to determine if there is a relation between serum HBV DNA levels and liver disease. Serum HBV DNA levels generally exceeded 10^5 copies/ml among patients with HBeAg positive chronic hepatitis and may be as high as 10^{10} copies/ml [1,2,4–8], but the correlation between serum HBV DNA levels and histologic activity was poor [7]. Serum HBV DNA levels tended to be lower (10^4–10^8 copies/ml) among patients with HBeAg negative chronic hepatitis B (HBeAg negative, anti-HBe positive, elevated ALT) [4,5] and may at times be undetectable in non-PCR based assays [9,12]. One study found that HBeAg negative patients with high serum HBV DNA levels ($>10^7$ copies/ml) were more likely to have increased histologic activity and fibrosis scores but only six patients had high HBV DNA levels in that study [7]. Reports on the relation between precore and core promoter variants and serum HBV DNA levels and liver disease are inconclusive [4,7,9,13]. The inability to demonstrate a correlation between serum HBV DNA levels and liver disease is in part related to the fact that HBV induced liver damage is predominantly immune-mediated. However, many of the earlier studies have also been hampered by the lack of sensitivity and/or standardization of the HBV DNA assays used, and the absence of serial serum samples and/or liver histology.

Recent availability of commercial quantitative PCR assays and establishment of international standards for HBV DNA assays have enabled some of the questions regarding HBV DNA levels and liver disease to be re-examined. In this issue of the Journal, Martinot-Peignoux et al. [14] determined serum HBV DNA levels of inactive HBsAg carriers (HBeAg negative, anti-HBe positive, with persistently normal ALT levels) using a commercially available PCR assay (Cobas Amplicor HBV Monitor™), which has a detection limit of 200 copies/ml. They found that 71 (84%) of 85 inactive carriers had detectable HBV DNA, but only two (2%) had levels $>10^5$ copies/ml. Annual testing of 38 patients followed for 1–6 years found that serum HBV DNA levels remained stable in most patients, with median values ranging from 10^3 to 10^4 copies/ml. Only one (3%) patient had HBV DNA level $>10^5$ copies/ml. The authors concluded that their results support the NIH recommendation of 10^5 copies/ml as a cut-off HBV DNA level for differentiating inactive carriers from those with chronic hepatitis B. However, this study did not include patients with chronic hepatitis B. Thus, the reliability of an HBV DNA level of 10^5 copies/ml in differentiating inactive carriers from patients with chronic hepatitis B cannot be established. Indeed, a recent report from Greece found that 13% of 134 patients with HBeAg negative chronic hepatitis had serum HBV DNA levels less than 10^5 copies/ml indicating that a cut-off value of 10^5 copies/ml would lead to misclassification of these patients and possibly denial of treatment [15]. In this study, all 68 inactive carriers but only 11% of patients with HBeAg negative chronic hepatitis B had serum HBV DNA levels $<3\times10^4$ copies/ml. The authors concluded that a cut-off HBV DNA level of 3×10^4 copies/ml is more appropriate for differentiating inactive carriers from patients with HBeAg negative chronic hepatitis B but this study was based on serum HBV DNA levels taken at a single time point. Clearly, more studies on a larger number of patients followed over a longer period of time are needed to validate this cut-off value.

Establishment of a threshold serum HBV DNA level that is associated with liver injury can be important for predicting prognosis and for defining indications for treatment. However, given the variable nature of chronic HBV infection, it is possible that this threshold level may be different for each individual and may vary with time depending on the host immune status and other exogenous factors. Thus, the main objective of defining such a threshold level, if it exists, is to identify individuals with very low risk of progressive liver disease, in whom current treatment offers little or no benefit, and who may require less frequent monitoring. Nevertheless, repeat testing is necessary even for inactive carriers as serum HBV DNA levels may fluctuate with time. In addition, decision on treatment should not be based simply on serum HBV DNA level. Other factors such as histologic activity, likelihood of response and other comorbid conditions should be considered.

With the recognition that nosocomial transmission of HBV infection can occur from HBeAg negative health care workers, who have chronic HBV infection [16], some countries such as England, have attempted to establish guidelines on restrictions on practice based on serum HBV DNA levels [17]. This policy must be carefully reviewed because of the wide fluctuations in serum HBV DNA levels particularly in patients with HBeAg negative chronic hepatitis B.

A key element in the evaluation of new treatment of chronic hepatitis B is to determine the degree of reduction in serum HBV DNA levels. The availability of quantitative PCR assays for HBV DNA permits more accurate assessment of dose–response effect and comparison of the potencies of various antiviral compounds. In addition, the ability to detect lower HBV DNA levels enables investigators and physicians to determine if continued treatment is associated with further decrease in virus levels as well as earlier detection of relapse or breakthrough infection. Thus, quantitative PCR assays have improved our ability to monitor treatment response, but several important questions have arisen. First, to what level should HBV DNA be reduced to ensure sustained virologic and clinical remission? The answer to this question is predicated on the availability of potent antiviral agents that when used singly or in combination will be

able to decrease serum HBV DNA to the desired level in the vast majority of patients. Such level has not been determined. Ideally, one would like to reduce serum HBV DNA to levels undetectable by the most sensitive PCR assays but current treatment rarely achieves this goal. Second, to what level should HBV DNA be reduced to achieve HBeAg seroconversion? To date, only one study had addressed this issue. In a study of 23 patients who received lamivudine therapy, six (50%) of 12 patients whose serum HBV DNA decreased to $<10^4$ copies/ml developed HBeAg seroconversion versus none of 11 whose serum HBV DNA remained $>10^4$ copies/ml [18]. This study suggested that there may be a threshold HBV DNA level associated with lamivudine induced HBeAg seroconversion. In an ongoing study of patients with spontaneous or interferon induced HBeAg seroconversion, we found that serum HBV DNA levels ranged from 10^4 to 10^8 copies/ml at the time when HBeAg first became undetectable (personal observations). Thus, further studies are needed to determine if HBeAg seroconversion is associated with a specific HBV DNA level. Third, how should treatment response of HBeAg negative chronic hepatitis B be defined? Patients with HBeAg negative chronic hepatitis B tend to have lower serum HBV DNA levels that may at times be undetectable using non-PCR based assays, and HBeAg loss or seroconversion cannot be used as an endpoint. Clearly, quantitative PCR assays for HBV DNA are very important in monitoring response of these patients, but it is not known if reduction in serum HBV DNA to levels undetectable by PCR assays is necessary or sufficient for sustained remission and if treatment can be withdrawn after serum HBV DNA become undetectable by PCR assay.

In summary, quantitative PCR assays for serum HBV DNA have provided many answers and also raised new questions. Studies similar to that of Martinot-Peignoux et al. should be repeated in more diverse patient populations to address the questions raised so results of these tests can be appropriately interpreted and used in the management of patients with chronic HBV infection.

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附錄四

Outcome of Liver Transplantation for Hepatitis B: Report of a Single Center's Experience

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Results of liver transplantation (LT) for hepatitis B have improved significantly with the use of hepatitis B immune globulin (HBIG) and/or lamivudine. The aim of this study is to review the long-term outcome of patients who underwent LT for hepatitis B. Records of 41 patients who underwent LT for hepatitis B and survived 3 months or longer post-LT were reviewed. Twenty patients were administered no immunoprophylaxis or short-term intramuscular HBIG, whereas 21 patients were administered high-dose intravenous (IV) HBIG. Median post-LT follow-up in these 2 groups was 76 months (range, 4 to 155 months) and 25 months (range, 4 to 68 months), respectively. Hepatitis B recurred in 15 (75%) and 4 patients (19%) who underwent LT in the pre-HBIG and post-HBIG eras, respectively. Cumulative rates of recurrent hepatitis B at 1 and 3 years post-LT in these 2 groups were 66% and 77% and 20% and 20%, respectively ($P < .001$). Recurrent hepatitis B in the post-HBIG era correlated with antibody to hepatitis B surface antigen titer less than 100 IU/L. Nine patients with recurrent hepatitis B were administered lamivudine for 13 to 49 months (median, 28 months); 6 patients continued to have stable or improved liver disease, whereas 3 patients developed virological breakthrough with slow deterioration of liver disease. Long-term IV HBIG is effective in preventing recurrent hepatitis B. The risk for recurrent hepatitis B is negligible after the first year post-LT. Among patients with no virological breakthrough, lamivudine can stabilize or improve liver disease for up to 4 years in patients with recurrent hepatitis B post-LT. (*Liver Transpl 2001;7:724-731.*)

Early results of liver transplantation (LT) for hepatitis B were poor, with a recurrent hepatitis B rate of 80% and 2-year mortality rate of 50%.[1-3] Long-term (>6 months) immunoprophylaxis with high-dose hepatitis B immune globulin (HBIG) has been effective in reducing the rates of recurrent hepatitis B, as well as graft and patient mortality, after LT.[4-7] Nevertheless, hepatitis B still recurs in some patients administered long-term HBIG therapy. Recurrence may be secondary to a high viral load pre-LT or immune escape mutations in the hepatitis B virus (HBV) S gene.[8,9] Overall recurrence rates with HBIG monotherapy vary from 15% to 50%, but may be as high as 80% in patients who are hepatitis B e antigen (HBeAg) or HBV DNA positive before LT.[4,10-15] Pharmacokinetic studies showed significant variability in antibody to hepatitis B surface antigen (anti-HBs) titers post-LT, particularly during the first 3 months.[16] Until now, the efficacy of HBIG beyond the first 2 to 3 years post-LT has not been reported.

In the last few years, lamivudine has been shown to be effective in decreasing recurrent hepatitis B post-LT.[17,18] However, the long-term efficacy of lamivudine monotherapy may be limited by the selection of drug-resistant mutants.[19] Recent studies showed that the combination of HBIG and lamivudine may be more effective and may reduce recurrent hepatitis B to less than 5%; however, the number of patients involved in these studies is small, and the duration of post-LT follow-up is limited.[20-23]

Lamivudine also has been shown to be effective in the treatment of patients with recurrent hepatitis B post-LT.[24] However, breakthrough infection caused by lamivudine-resistant mutants developed in 27% of patients after 1 year of treatment. Thus, the long-term benefits of lamivudine treatment in patients with recurrent hepatitis B post-LT remain to be determined.

The primary aim of this study is to review the long-term outcome of patients who underwent LT for hepatitis B. Secondary aims are to determine (1) the efficacy of indefinite high-dose HBIG in the prevention of recurrent hepatitis B, and (2) long-term safety and efficacy of lamivudine in the treatment of recurrent hepatitis B.

Patients and Methods

Patients

Between January 1984 and May 2000, a total of 930 patients underwent LT at the University of Michigan Medical Center

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Table 1. Characteristics of Patients at Transplant Listing

	Pre-HBIG Era (n = 20)	Post-HBIG Era (n = 21)	*P*
Age (yr)	38 ± 3	49 ± 2	< 01
Men/women	17/3	19/2	NS
White/black/Asian	18/2/0	20/1/0	NS
Cirrhosis/FHF/HCC	17/3/0	18/2/1	NS
1st/re-LT	19/1	17/4	NS
HCV coinfection	1/3	3/17	NS
HDV coinfection	0/12	2/10	NS
HBeAg	7/13	14/19	NS
HBV DNA	NA	13/17	

Abbreviations: NS, not significant; FHF, fulminant hepatic failure; HCC, hepatocellular carcinoma; NA, not available.

(Ann Arbor, MI). Fifty-nine patients (6 3%) underwent LT for HBV-related acute or chronic liver failure. All patients had detectable hepatitis B surface antigen (HBsAg) in serum at the time of LT. Medical records of 41 patients who survived more than 3 months post-LT were reviewed to assess hepatitis B recurrence and outcome (Table 1). For each patient, baseline demographic data, indications for LT, and results of sequential liver biochemistry tests, hepatitis B serological tests, and HBV DNA tests, as well as the use of antiviral therapy pre-LT and prophylactic immune/antiviral therapies post-LT, were recorded.

Outcome

Primary outcomes of this study are patient survival and rate of recurrent hepatitis B post-LT. Recurrent hepatitis B is defined as the detection of HBsAg more than 1 month post-LT. Breakthrough HBV infection during lamivudine therapy is defined as the persistent reappearance of HBV DNA in serum after its initial disappearance on at least 2 occasions, determined by non–polymerase chain reaction (PCR)-based assays.

Prophylactic Therapy

Patients were divided into 2 groups based on the use of immunoprophylaxis post-LT. During the pre-HBIG era (before January 1994), 20 patients were administered no or only short-term (<6 months) intramuscular HBIG (Abbott Laboratories, Abbott Park, IL). In the post-HBIG era (after January 1994), 21 patients were administered 10,000 IU of intravenous (IV) HBIG during the anhepatic phase, daily for the next 6 days, and monthly thereafter (Table 1). HBIG protocols were approved by the University of Michigan Institution Review Board. Four patients in the post-HBIG era were also administered lamivudine (100 mg/d) before LT (median, 9.5 months; range, 1 to 17 months). These 4 patients were administered the same dose of HBIG as listed. Three patients were HBeAg positive and had detectable HBV DNA before lamivudine treatment. All 3 patients had undetectable HBV DNA and cleared HBeAg before LT. No patient developed lamivudine resistance before LT.

Monitoring Post-LT

During the pre-HBIG era, patients were followed up monthly during the first year post-LT and every 3 to 6 months thereafter. During the post-HBIG era, patients were administered monthly HBIG infusions and follow-up care at the Transplant Ambulatory Care Unit. Hepatitis B serological assays were performed every 3 to 6 months during the pre-HBIG era. HBsAg and trough anti-HBs titers were checked before each infusion, and serum HBV DNA was tested every 4 months in the post-HBIG era.

Hepatitis serological tests (HBsAg, HBeAg, anti-HBs, antibody to HBeAg, antibody to hepatitis C virus, and antibody to hepatitis D virus [HDV]) were performed using commercially available enzyme-linked immunoassays (Abbott Laboratories). Serum HBV DNA was determined by non–PCR-based assays, which included liquid hybridization (Abbott) and branched DNA assays (Bayer Corp, Norwood, MA). Serum anti-HBs titer was measured using an enzyme-linked assay (AUSAB; Abbott). Lamivudine-resistant HBV P gene mutations were examined by PCR and direct sequencing.[25]

Management of Patients With Recurrent Hepatitis B Post-LT

Before the availability of lamivudine, patients with recurrent hepatitis B did not undergo antiviral therapy. Re-LT was performed in selected patients with recurrent liver failure. After lamivudine became available in 1996, 9 patients with recurrent hepatitis B were treated with lamivudine (100 mg/d). Of these, 7 patients underwent LT in the pre-HBIG, and 2 patients, in the post-HBIG era. The latter 2 patients had not been administered lamivudine previously.

Statistical Analyses

Data were entered into an Excel (Microsoft Corp, Redmond, WA) database and analyzed using SPSS version 9.0 software package (SPSS Inc, Chicago, IL.). Statistical analyses were performed using Chi-squared and Fisher's exact tests for categorical variables. Paired and unpaired Student's *t*-tests were used for continuous variables. Time to recurrent hepatitis B was estimated using the Kaplan-Meier method and compared using the log-rank test. Results are considered statistically significant at $P < .05$.

Results

Baseline characteristics of the patients are listed in Table 1. Sex, ethnicity, indications for LT, frequency of HDV coinfection, HBeAg status at listing, and liver biochemistry test results at LT were similar between the 2 groups. However, patients who underwent LT in the pre-HBIG era were significantly younger ($P < .01$).

Most patients who underwent LT in the post-HBIG

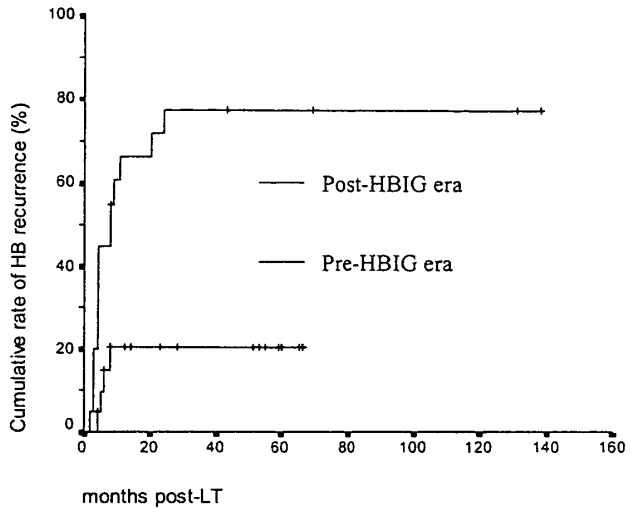

Figure 1. Kaplan-Meier curves showing rates of recurrent hepatitis B post-LT in patients who underwent LT in the pre-HBIG and post-HBIG eras.

Pre-HBIG No.	20	5	4	3	2	2	2
Post-HBIG No.	21	10	8	3			

era had features that predicted high risks for recurrent hepatitis B: 90% had cirrhosis, 74% were HBeAg positive, and 76% had detectable serum HBV DNA at listing; only 20% had HDV coinfection.

Median duration of post-LT follow-up in the pre-HBIG and post-HBIG eras was 76 months (range, 4 to 155 months) and 25 months (range, 4 to 68 months), respectively.

Recurrent Hepatitis B

In the pre-HBIG era, 15 patients (75%) developed recurrent hepatitis B: 13 patients experienced recurrence in the first year, and 2 patients, in the second year post-LT. In the post-HBIG era, only 4 patients (19%) developed recurrent hepatitis B, all 4 patients during the first year post-LT. Cumulative rates of recurrent hepatitis B after 1, 3, and 5 years post-LT were 66%, 77%, and 77% among patients who underwent LT in the pre-HBIG era and 20%, 20%, and 20% among patients who underwent LT in the post-HBIG era ($P < .001$; Fig. 1). None of the 4 patients administered combination prophylaxis of IV HBIG and lamivudine developed recurrent hepatitis B after a median of 17 months (range, 9 to 25 months) of post-LT follow-up. Thus, hepatitis B recurred in 4 of 17 patients (24%) administered HBIG monotherapy.

Factors Associated With Recurrent Hepatitis B in the Post-HBIG Era

Anti-HBs titer was the most important factor associated with recurrent hepatitis B in the post-HBIG era. Of the 4 patients with recurrent hepatitis B, 2 patients missed

Table 2. Outcome of Patients Post-LT

	Pre-HBIG Era (n = 20)	Post-HBIG Era (n = 21)	P
Median duration of post-LT follow-up (mo)	76	25	< 05
Recurrent hepatitis B	15 (75)	4 (19)	<.001
All-cause mortality	6	3	NS
Death related to recurrent hepatitis B	5	2	NS
No recurrent hepatitis B	5 (25)	17 (81)	<.001
All-cause mortality	3	3	NS

NOTE Values expressed as number of patients (percent) unless stated otherwise.
Abbreviation NS, not significant.

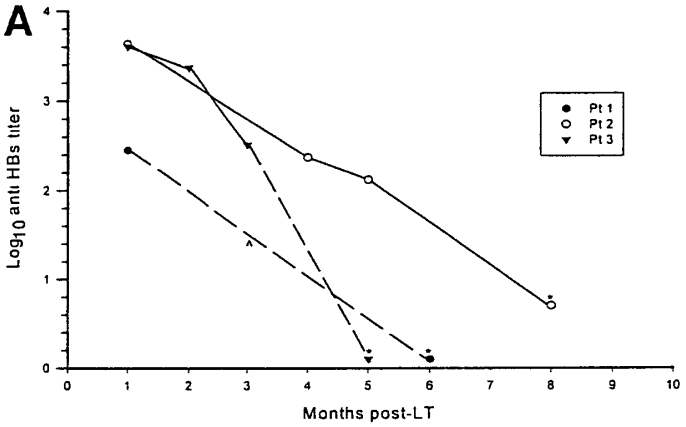

Figure 2. (A) Sequential anti-HBs titer (log_{10} values, IU/L) of the 4 patients who developed recurrent hepatitis B despite IV HBIG.

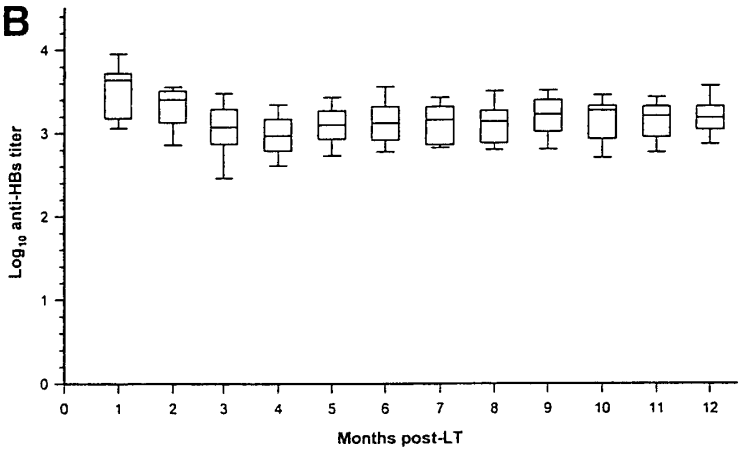

Figure 2. (B) Sequential anti-HBs titer (log_{10} values, IU/L) during the first year post-LT in the 17 patients administered IV HBIG who did not have recurrent hepatitis B. Box plots indicate 75th percentile, median, and 25th percentile values, the wickers indicate the highest and lowest values.

1 dose of HBIG during the first 3 months post-LT and hepatitis B recurred during months 4 and 6 post-LT. The other 2 patients were not able to maintain an anti-HBs titer greater than 100 IU/L after the first 4 months despite strict adherence to the HBIG protocol, and hepatitis B recurred during months 5 and 8 post-LT (Fig. 2A). In the remaining 17 patients with no recurrence, lowest trough anti-HBs titers during months 0 to 3, 4 to 6, and 7 to 12 post-LT were 217, 417, and 515 IU/L, respectively (Fig. 2B). Despite wide variation in anti-HBs titers during the first year, a stable anti-HBs titer greater than 500 IU/L was maintained in all uninfected patients after the first year post-LT.

In the post-HBIG era, data on HBeAg and HBV DNA at presentation were available for 19 patients. None of the 6 patients who were HBeAg and HBV DNA negative at presentation developed recurrent hepatitis B compared with 3 of 13 patients (23%) who were HBeAg and/or HBV DNA positive at presentation. HBeAg and HBV DNA results at presentation were not available in the fourth patient who developed recurrent hepatitis B.

Treatment and Outcome of Patients With Recurrent Hepatitis B

Of the 15 patients who underwent LT in the pre-HBIG era who developed recurrent hepatitis B, 6 patients died 4 to 30 months (median, 14 months) post-LT (Table 2). Five patients died of causes related to recurrent hepatitis B: 3 patients of progressive liver failure 4, 9, and 12 months after the diagnosis of recurrent hepatitis B, and 2 patients of postoperative complications after

Table 3. Effect of Lamivudine Treatment in 9 Patients With Recurrent Hepatitis B Post-LT

Patient No	Time to Recurrence (mo post-LT)	Pre-Lamivudine Treatment								Last Visit					
		Interval Between Recurrence and Lamivudine Treatment (mo)	HBeAg	HBV-DNA	ALT (IU/L)	TB (mg/dL)	ALB (g/dL)	PT (s)	Histology	Duration of Treatment (mo)	HBeAg	HBV-DNA	ALT (IU/L)	TB (mg/dL)	ALB (g/dL)	PT (s)
1	24	12	(+)	(+)	145	0.9	3.4	13.2	Cirrhosis	48	(+)	(−)	68	1.2	3.9	10.6
2	11	96	(+)	(+)	56	0.4	3.3	10.5	Cirrhosis	14	(−)	(−)	57	0.9	3.5	10.5
3	4	69	(+)	(+)	148	2.7	3.5	12.3	Cirrhosis	49	(+)	(−)	77	1.1	3.6	10.3
4	3	119	(+)	(+)	83	4.5	2.0	13.4	Cirrhosis	23	(+)	(+)	91	0.8	4.2	9.7
5	8	131	(+)	(+)	294	12.5	2.6	11.9	Cirrhosis	13	(+)	(−)	53	3.9	3.4	9.9
6	3	104	(+)	(−)	36	0.6	3.6	12.9	CHB	49	(+)	(−)	61	1.0	3.5	11.0
7	3	107	(−)	(−)	41	1.4	2.7	13.8	Cirrhosis	48	(−)	(−)	36	0.8	3.7	10.7
8	8	4	(+)	(+)	54	0.6	3.0	12.2	CHB	20	(+)	(+)	48	0.5	3.1	11.5
9	4	2	(+)	(+)	73	0.9	2.4	10.0	CHB	28	(+)	(+)	120	1.1	3.5	10.3

Abbreviations: CHB, chronic hepatitis B; ALT, alanine aminotransferase; TB, total bilirubin; ALB, albumin; PT, prothrombin time.

re-LT for recurrent hepatitis B. The sixth patient died of sepsis 26 months after the diagnosis of recurrent hepatitis B with normal graft function at the time of death. One patient was lost to follow-up 121 months after LT; liver biochemistry test results were normal during the last follow-up. Of the remaining 8 patients, 1 patient had normal graft function and undetectable serum HBV DNA 129 months after the diagnosis of recurrent hepatitis B. The other 7 patients were administered lamivudine treatment.

Of the 4 patients who underwent LT in the post-HBIG era who developed recurrent hepatitis B, 1 patient died of progressive graft failure 9 months post-LT, and 1 patient died of sepsis 2 months after re-LT for recurrent hepatitis B. The other 2 patients were administered lamivudine treatment.

Nine patients were administered lamivudine therapy (Table 3). The median duration of treatment was 28 months (range, 13 to 49 months). Six patients had histological cirrhosis before the onset of treatment. The interval from diagnosis of recurrent hepatitis B to initiation of lamivudine treatment varied from 2 to 131 months (median, 96 months). Eight patients were HBeAg positive and 7 patients had detectable serum HBV DNA before treatment. All patients had undetectable serum HBV DNA and improvement in liver biochemistry results within 6 months of treatment. However, only 1 patient cleared HBeAg and no patient cleared HBsAg. Three patients developed virological breakthroughs after 11, 12, and 19 months of lamivudine therapy. Two patients (no. 8 and 9) were confirmed to have mutations involving the YMDD motif of the polymerase gene. All 3 patients had slow deterioration in liver disease; 1 patient was started on adefovir dipivoxil treatment (patient 9) and 1 patient was maintained on lamivudine therapy only because of noncompliance and chronic renal insufficiency (patient 4), whereas the third patient died of a cerebrovascular accident (patient 8). Six patients had persistently undetectable serum HBV DNA and continued improvement in liver disease (Fig. 3) 13 to 49 months (median, 48 months) after the initiation of lamivudine treatment.

Discussion

In this study, we reported the outcome of patients with hepatitis B up to 14 years post-LT. We found that hepatitis B recurred in 75% of patients administered no prophylaxis or short-term low-dose HBIG and 19% of those administered long-term high-dose HBIG. These data are similar to previously published reports[4,10-16] and support the use of high-dose IV HBIG in the pre-

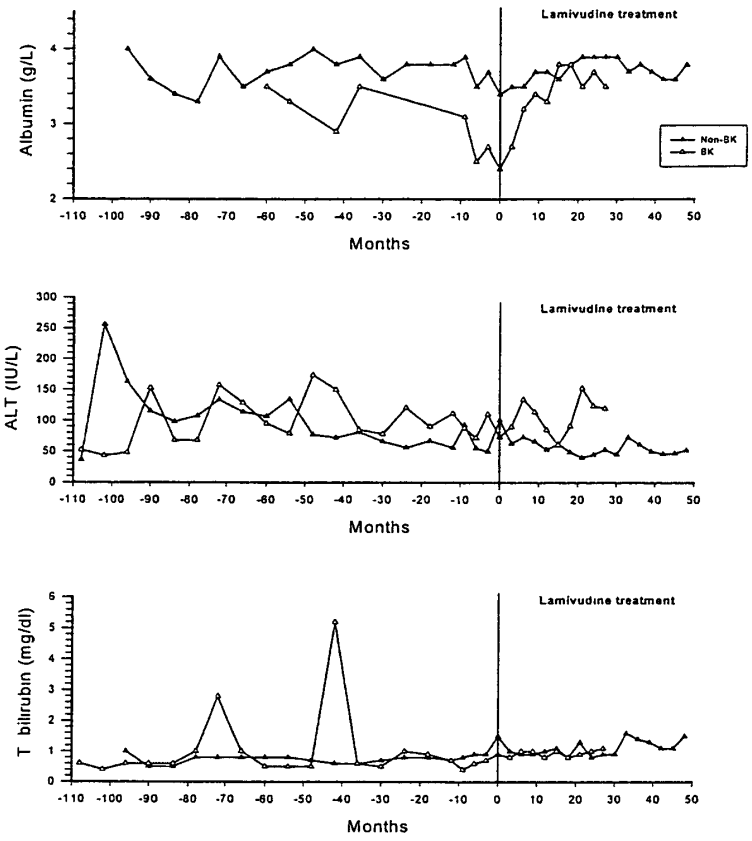

Figure 3. Serial values (median) of serum albumin, alanine aminotransferase, and total bilirubin in 9 patients administered lamivudine for recurrent hepatitis B. Biochemical values from the time recurrent hepatitis B was diagnosed were plotted to show changes before and after lamivudine treatment. (BK, virological breakthrough.)

vention of recurrent hepatitis B post-LT. Of the 4 patients who underwent LT in the post-HBIG era and developed recurrent hepatitis B, 3 of 3 patients tested were HBeAg positive and had detectable HBV DNA pre-LT. Two patients missed 1 dose of HBIG during the first 3 months post-LT, and 2 patients were unable to maintain anti-HBs titers greater than 100 IU/L despite strict adherence to a fixed-dose protocol. These data support the observations that (1) patients with a high viral load pre-LT have an increased risk for recurrent hepatitis B post-LT, (2) a fixed-dose regimen of HBIG administration may be insufficient in maintaining protective levels of anti-HBs in patients who are HBeAg and/or HBV DNA positive pre-LT, and (3) adequate anti-HBs titers must be maintained to prevent recurrent hepatitis B.[4,15,16] Our data suggest that close monitoring of anti-HBs titer with supplemental HBIG when titers decrease to less than a protective level or more aggressive prophylactic regimen is needed for patients with a high viral load pre-LT. In accordance with other reports,[20-23] none of our 4 patients administered combination prophylaxis with HBIG and lamivudine developed recurrent hepatitis B after a median follow-up of 17 months.

We found most (89%) hepatitis B recurrence during the first year post-LT, and all recurrence within the first 2 years. These data suggest that despite the persistence of HBV DNA in the liver or extrahepatic reservoirs,[14,26] neutralization of circulating virions and prevention of early graft infection when patients are heavily immunosuppressed may be the most important steps in the prevention of recurrent hepatitis B post-LT. Two of our patients who underwent LT in the pre-HBIG era had no evidence of reinfection up to 10 years after LT. Our findings suggest that prophylactic regimens may be tapered after the first 1 to 2 years post-LT because the risk for recurrent hepatitis B decreases. We are currently evaluating a tapering regimen in patients who have no evidence of recurrent hepatitis B after 1 year post-LT.

As in other studies,[1,27] we found that patients with recurrent hepatitis B post-LT had rapidly progressive liver disease; 37% developed graft failure within 1 year,

and 78% of deaths were related to recurrent hepatitis B. The dismal outcome of patients with recurrent hepatitis B improved after lamivudine became available. Of the 9 patients administered lamivudine treatment, none died of graft failure after a median treatment duration of 28 months, although 6 patients had cirrhosis and 4 patients had mild hepatic decompensation when treatment was initiated. In accordance with other reports,[24,28,29] all our patients had virological response, as well as clinical and biochemical improvement initially. Six patients with no breakthrough infection continued to have improved or stable liver disease up to 4 years after the initiation of lamivudine treatment. Of the 3 patients who developed virological breakthroughs, all had slow deterioration in liver disease. However, comorbid medical conditions and the addition of adefovir dipivoxil preclude us from determining the long-term effects of lamivudine-resistant HBV mutants in these patients.

In summary, our study showed that long-term high-dose IV HBIG is effective in preventing recurrent hepatitis B post-LT, especially in patients with a low viral load pre-LT or when anti-HBs titer was maintained at greater than 100 IU/L. We found that the risk for recurrent hepatitis B was very low after the first year post-LT. These data indicate that aggressive prophylaxis is needed during the initial post-LT period, especially in patients with a high viral load pre-LT, but the prophylactic regimen can be tapered with time. Our data showed that in patients with recurrent hepatitis B, lamivudine is effective in stabilizing or improving liver disease for up to 4 years, but the clinical benefits may be negated in patients with virological breakthrough infection.

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Does OKT3 Increase the Risk of Recurrent Hepatitis B in Patients Transplanted for Hepatitis B?

Levels of HBV-DNA and HbsAg after acute liver allograft rejection treatment by corticoids and OKT3. *Gonzalez RA, de la Mata M, de la Torre J, Mino G, Pera C, Pena J, Munoz E.* Clin Transplant 2000:14:208-211. (Reprinted with permission.)

Abstract

The aim of this work was to analyze whether the treatment of acute rejection of orthotopic liver transplants (OLT), either with corticoids or OKT3, has any effect on levels of hepatitis B virus (HBV)-DNA and HbsAg in individuals which were originally affected by cirrhosis or fulminant hepatic failure as a result of B virus. We have found that HBV-DNA is present in macrophages, B cells and both CD4+ and CD8+ T cells after OLT in all cases studied. Interestingly, the levels of HBV-DNA and HbsAg in the serum analyzed were increased extremely rapidly in the patients treated with OKT3 in an acute rejection episode. However, the serum levels of HBV-DNA and HbsAg found were lower when the patients were treated with steroids, and were not found in non-treated patients. As the serum levels of HBV-DNA increase, the process of liver reinfection could be accelerated; therefore, these results may help to understand how OKT3 and corticoids immunosuppressive therapy may accelerate the reinfection of OLT by HBV. In conclusion, our results suggest that special care must be taken in the use of OKT3 in the treatment of acute liver rejection episodes in chronic or fulminant HBV transplanted patients.

Comments

Reactivation of hepatitis B virus (HBV) replication and exacerbation of liver disease have been reported in patients with chronic hepatitis B administered immunosuppressive or cytotoxic therapy.[1-3] Reactivation of HBV replication may occur not only in patients with replicative HBV infection (hepatitis B e antigen positive and detectable serum HBV DNA by non–polymerase chain reaction [PCR] assay), but also in patients with low or nonreplicative infection (hepatitis B e antigen negative and undetectable serum HBV DNA by non-PCR assay) and those with resolved infection (hepatitis B surface antigen [HBsAg] negative, antibody to HBsAg positive, and antibody to hepatitis B core antigen positive). This is related to the persistence of HBV, albeit at low levels, in the liver and circulating blood and the presence of HBV DNA in extrahepatic reservoirs, especially peripheral-blood mononuclear cells (PBMCs).[4,5]

Among the immunosuppressive agents used after orthotopic liver transplantation (OLT), corticosteroids may have the most potent effect on HBV replication. In addition to indirect effects through immunosuppression, corticosteroids may directly augment HBV replication through a glucocorticoid-responsive element in the HBV genome.[6] Other antirejection drugs, such as cyclosporine and tacrolimus, were reported to have no direct stimulatory effect on HBV replication.[7,8]

The effects of other antirejection therapies, such as OKT3 (Orthoclone OKT3; Ortho Biotech Inc, Raritan, NJ, and muromonab CD3), on HBV replicarion have not been studied. OKT3, a monoclonal antibody against T cells, induces a rapid lymphocytopenia generally attributed to lymphocyte opsonization and phagocytosis.[9,10] It also modulates the expression of the T-cell receptor complex so that T cells become blinded to the antigens presented by the allograft.[9,10] Furthermore, T-cell homocytolysis,[11] as well as apoptosis,[12] have been proposed as possible mechanisms of action of OKT3. The use of OKT3 in patients who underwent OLT for hepatitis C has been associated with early and severe recurrence of hepatitis C.[13,14]

In the study by Gonzalez et al,[15] 11 patients who underwent OLT for hepatitis B and developed acute rejection were studied. All patients were administered low-dose hepatitis B immune globulin (HBIG) intramuscularly (IM) as prophylaxis against HBV reinfection. No patient was administered antiviral therapy. Rejection episodes were treated with 3 doses of methylprednisolone and/or an increased dosage of oral prednisone. Steroid-resistant rejection episodes were treated with OKT3 for 10 to 14 days. Three patients were administered corticosteroids and OKT3, 6 patients were administered corticosteroids only, and 2 patients were not treated. All 3 patients treated with OKT3 developed high serum HBV DNA levels, and 2 of these patients died of recurrent hepatitis B during the second and third years post-OLT. Serum HBV DNA was present at low levels in 3 of the 6 patients treated with corticosteroids alone, but not in the 2 patients who were not treated. The investigators suggested that OKT3 may lead to a rapid increase in serum HBV DNA levels in patients who undergo OLT for hepatitis B and cautioned that special care be exercised when OKT3 is administered to these patients. They speculated that the devastating effects of OKT3 may be related to the release of viral particles secondary to lysis of T cells, release of cytokines that may facilitate entry of HBV into

hepatocytes and other cells, or activation of HBV replication within PBMCs by mitogens. These hypotheses are interesting and need to be tested. Although the investigators showed increasing serum HBV DNA levels in the 3 patients administered OKT3, the findings were based on a small number of patients tested at very few time points, and details about these 3 patients in relation to the other patients, such as total dose and duration of corticosteroid therapy and viral replication status before OLT and just before the rejection episode, were not provided. Thus, more data are needed to determine whether the use of OKT3 is associated with a more rapid increase in serum HBV DNA levels in patients who undergo OLT for hepatitis B.

Deciphering the mechanisms by which OKT3 increases serum HBV DNA levels is more difficult. Using PCR assay, the investigators found HBV DNA present in $CD4^+$ and $CD8^+$ T cells, B cells, and macrophages even before the rejection episode. It is possible that OKT3-mediated lysis of T cells results in the release of HBV DNA into the circulation. Whether lymphokines induced by OKT3 facilitated the entry of HBV into hepatocytes or promoted HBV replication is less certain. Currently, very few data exist on the mechanisms of entry of HBV into hepatocytes and other cells. Some of the lymphokines induced by OKT3, such as interferon-γ and tumor necrosis factor, have been shown to downregulate HBV expression.[16,17] Some studies suggested that HBV can replicate in PBMCs, but these findings are controversial.[18,19] It is more likely that the effects of OKT3 on HBV replication are mediated through reduced immune-mediated virus clearance through lymphocyte depletion and antigenic modulation of the T-cell receptor complex.

The important clinical question is what to do when patients who undergo OLT for hepatitis B develop an episode of rejection. It is obvious that these patients should continue to be administered prophylaxis for HBV reinfection. Currently, the most effective therapies include long-term, high-dose, intravenous HBIG or combination therapy with HBIG and lamivudine.[20,21] Because of the expense associated with high-dose intravenous HBIG, many centers are evaluating the efficacy of lower doses, shorter duration, or IM administration of HBIG in combination with lamivudine. The short-term efficacies of these regimens are impressive, but longer follow-up of a larger number of patients is needed to determine the most cost-effective prophylactic regimen for patients who undergo OLT for hepatitis B. The efficacy of IM HBIG alone has not been well documented and may account for the high reinfection rate reported by Gonzalez et al.[15] Even in patients administered adequate prophylaxis, the occurrence of rejection episodes necessitating more aggressive immunosuppressive therapy may increase the risks for HBV reinfection. In vitro studies using the human hepatoblastoma cell line showed that the addition of antiviral therapy can inhibit the increase in HBV replication induced by prednisolone and azathioprine.[7] It seems reasonable to consider the addition of lamivudine in patients administered HBIG monotherapy and closely monitor the antibody to HBsAg titer to determine whether greater or additional doses of HBIG should be administered to patients administered IM HBIG with or without lamivudine when patients who underwent transplantation for hepatitis B develop steroid-resistant rejection episodes necessitating OKT3 therapy.

Although significant progress has been made in the prevention of recurrent hepatitis B after OLT, much remains to be learned. Adequate prophylaxis should be administered to all patients, and close monitoring is required, especially in patients with replicative infection pre-OLT or who require more intense immunosuppression post-OLT. The effects of more selective anti-rejection drugs, such as anti–interleukin-2 receptor antibody, on HBV replication should be evaluated.

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Detecting Alcoholic Relapse Posttransplant

Carbohydrate-deficient transferrin is not a useful marker for the detection of chronic alcohol abuse. *Schmitt VM, Stieber P, Jungst D, Blizer M, Wachtler M, Heberger S.* Eur J Clin Invest 1998; 28:615-621. (Reprinted with permission.)

Abstract

Background: **The role of carbohydrate-deficient transferrin (CDT) as a reliable marker for the detection of chronic alcohol abuse has been discussed controversially.**
Methods: **Therefore, we investigated CDT in the sera from 405 subjects with different alcohol intake. Besides healthy control subjects (n = 42), inpatients and outpatients in a department of gastroenterology (n = 325) and patients admitted to a department of otorhinolarynology (n = 38) were studied. A total of 213 patients suffered from various forms of liver diseases, and 89 patients had liver transplantation. CDT values were determined by a double-antibody radioimmunoassay.**
Results: **In the 241 alcohol-abstinent subjects, CDT levels ranged from 3 to 90 units L^{-1} (median = 12); the 92 moderate drinkers (20-60 g of alcohol per day) showed values from 3 to 40 units L^{-1} (median = 12), and the 72 subjects with chronic alcohol abuse (> 60 g per day) revealed CDT levels from 3 to 100 units L^{-1} (median = 16). The diagnostic specificity for alcohol abuse was 86.8% for men (sensitivity 36.9%) and 95% for women (sensitivity 0%).**
Conclusion: **Our data indicate that measurement of CDT does not reach clinical use in the detection of chronic alcohol abuse in an unselected population because of its insufficient specificity and sensitivity.**

Comments

Alcohol-related liver disease accounts for up to 50% of the patients who die of end-stage liver disease.[1] Patients with alcoholic cirrhosis have posttransplantation survival rates similar to those for patients who undergo transplantation for non–alcohol-related liver disease.[2-4] However, relapse has been a significant concern in this population. Most studies report relapse rates after transplantation of 0% to 30%.[5] These studies used various definitions of relapse. Campbell et al[6] reported a small minority of patients who returned to problematic drinking, leading to rehospitalization and, in some cases, death.

The search for a way to detect alcohol use after transplantation has included developing biochemical markers that reliably detect relapse. Abnormalities in transferrin levels, later named carbohydrate-deficient transferrin (CDT), were found in the cerebrospinal fluid of patients with alcohol cerebellar degeneration in 1976,[7] and later, in the serum of alcohol abusers.[8] Since then, CDT has been studied as a marker of excessive