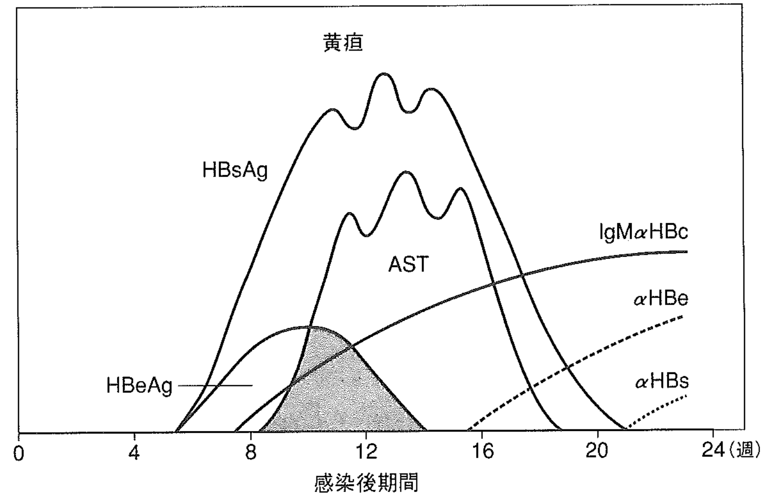
図1　急性B型肝炎の経過



HBsAg：B型肝炎表面抗原，HBeAg：B型肝炎e抗原，
IgMαHBc：B型肝炎コア抗原に対するIgM抗体，
AST：アスパラギン酸アミノトランスフェラーゼ，αHBe：B型肝炎e抗原に対する抗体，
αHBs：B型肝炎表面抗原に対する抗体

炎の急性増悪時にも弱陽性を示すことがあるが，その場合は IgG 型 HBc 抗体が 200 倍希釈でも高力価であり，両者の鑑別が可能である．

血中 HBV－DNA の検出はウイルス増殖の最も鋭敏な指標であり，血清トランスアミナーゼ値ともよく相関する．血清トランスアミナーゼ値は現在の肝炎の程度を知る最も有用なマーカーとなる．多峰性に変動を繰り返す場合は慢性化に注意が必要である．

B型急性肝炎では感染の鎮静化とともに陰性化する．しかし，肝細胞内には，一過性感染後も HBV－DNA が検出され，肝炎鎮静後も肝内からは完全にウイルスが消失していないことを当院では報告している[3]．

劇症化率はB型急性肝炎全体で約 2％ と推定される．しかし，プロトロンビン時間が 40％ 未満のいわゆる重症型の急性肝炎では約 30％ が，肝性脳症Ⅱ度以上を合併し劇症肝炎に進展する[4]．劇症化の予測には，厚生労働省の研究班による予知式[5]と，与芝らの予知式[6]がよく用いられる（表2）．双方の式で算出される劇症化確率はしばしば異なるため，複数の式を用いて予後を判断するべきである．

表2 急性肝炎重症型の劇症化予知式

与芝の式

λ = logit（P）= − 0.89 + 1.74 × 成因 + 0.056 × T. Bil（mg / dl）− 0.014 × ChE（IU / L）

成因：HAV または HBV 初感染　1，その他　2

研究班の式

λ = logit（P）= − 2.7469 + 0.0914 ×（年齢）+ 0.1255 × T. Bil（mg / dl）− 0.1534 × PT（%）

劇症化確率（p）= 1 / (1 + $e^{-\lambda}$)

B型急性肝炎の治療

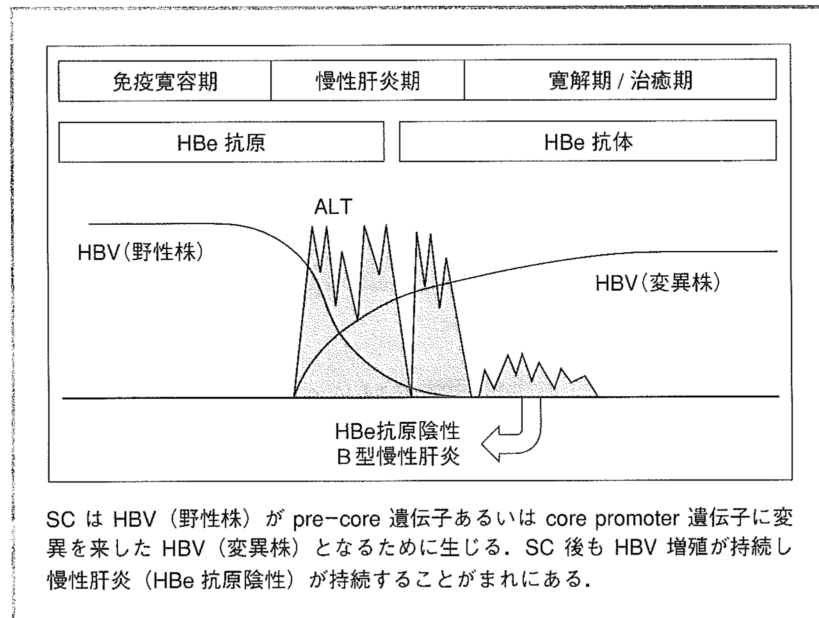
多くの場合3ヵ月以内に肝炎は鎮静化するので，入院による安静と食事療法の対処療法のみで根治可能である．食事は高タンパク，高カロリーが基本であるが，栄養過多による脂肪肝に注意が必要である．食欲低下を認める場合はビタミン剤を添加した補液が行われる．当院では全身倦怠感や食欲不振などの自覚症状の消失，血清トランスアミナーゼ値 100 IU / L 未満，血清総ビリルビン値 2.0 mg / dl 未満を退院の目処としている．

B型急性肝炎の中でも，重症化，劇症化を来す症例を数％で認める．血清総ビリルビン値やプロトロンビン時間などの肝予備能に注意しながら，劇症化の予知式[5)6)]などによる予測を行い，早めの対策が救命につながる．抗ウイルス薬のラミブジンをB型急性肝炎に使用し有効との報告[7)]もあるが，急性感染では血中のウイルスは早期に消失するため，ラミブジンがこの消失動態に影響するかは，まだ明らかなエビデンスはない．重症化，劇症化時の詳しい治療は劇症肝炎の治療の項に譲る．

B型慢性肝炎急性増悪の診断

B型慢性肝炎や血清トランスアミナーゼ正常の HB キャリアの患者が，急激な血清トランスアミナーゼ値の上昇と，時に肝機能の低下や全身倦怠感を引き起す．これを急性増悪と呼ぶ．その際に HBe 抗原陽性から HBe 抗体陽性へのセロコンバージョンを起すことがある（図2）[2)]．自然経過でのセロコンバージョンは年率 10 ～ 15％ と言われ，インターフェロン（IFN）などの抗ウイルス療法後にみられるこ

図2 HBe抗原からHBe抗体へのセロコンバージョン（SC）



SC は HBV（野性株）が pre–core 遺伝子あるいは core promoter 遺伝子に変異を来した HBV（変異株）となるために生じる．SC 後も HBV 増殖が持続し慢性肝炎（HBe 抗原陰性）が持続することがまれにある．

ともある．しかし，中には重症化，劇症化を来し，肝不全となる症例を認める．近年，HBe 抗原陰性でも HBV－DNA 陽性のB型慢性肝炎患者の存在が確認され，これらは HBe 抗原が産生されない pre-core 変異株や core promoter 変異株によることが報告されている[8]．HB キャリアやB型慢性肝炎からの急性増悪症例で肝不全となり死亡した症例の中には，HBV－DNA が 7.6 log copies / m*l* を超える高ウイルス症例のみならず，今述べた HBe 抗原陰性 HBe 抗体陽性の低ウイルス症例も認められる．したがって，抗原抗体系の血清マーカーのみにとらわれず，血清総ビリルビン値やプロトロンビン時間などの肝予備能を見ながら，重症化や劇症化を防ぐ必要がある．

B型慢性肝炎急性増悪の治療

急性増悪時の治療目標は ALT の正常化と重症化の防止である．基本的にはB型慢性肝炎の治療すなわち，IFN もしくはラミブジンなどの抗ウイルス薬の使用が中心となる．HB キャリアからの急性増悪例では予後の悪い亜急性型の劇症肝炎に進展する可能性が高く，肝移植も考慮しながらの診療が必要である．HBe 抗体陽性 HB キャリアに，他疾患でステロイドや免疫抑制剤を使用すると，肝炎が増悪する

表 3　B型肝炎治療の新しいステージ分類

HB stage	0	I	II	III	IV	V
HBsAg	＋	＋	＋	＋	＋	−**
HBeAg	＋	＋	＋	−	−	−
HBV−DNA (copies/ml)	不問	$10^{7.6}\leqq$	$10^{7.6}>$	$10^{5}\leqq$	$10^{5}>$	不問
ALT	持続正常	持続正常以外	持続正常以外	不問	不問	不問
年齢	不問	若年／高年* (Ia／Ib)	若年／高年* (IIa／IIb)	不問	不問	不問
発癌リスク	極めて小	小／大	小／極めて大	極めて大	極めて小	極めて小
治療	不要	F_2以上 IFN／IFN＋ラミブジン	IFN／ラミブジン	ラミブジン	不要	不要

*：若年　男性 30 歳未満，女性 35 歳未満　　高年　男性 30 歳以上，女性 35 歳以上

**：HBsAg（＋）の時期が確認されていること

略語：巻末の略語集参照

ことがあり，注意が必要である．一般にウイルス量の増加が高度な症例では，肝炎も重症化する例が多いので，早期より積極的にラミブジンを使用するべきである．いずれにしても，急性感染同様に，劇症化の予知式[5)6)]などを用いて，早めの治療が必要である．

最も重要なのは，治療開始のタイミングである．特にラミブジンの開始時期にはさまざまな見解がある．プロトロンビン時間が 40％ をきるような重症例での早期使用は当然であるが，HB キャリアからの急性増悪と慢性肝炎からの急性増悪では異なった基準を設けるべきとの意見もある[9)]．すなわち，ラミブジンの有効性の検討結果から，HB キャリアからの急性増悪例ではプロトロンビン時間が 60％ 未満，慢性肝炎からの急性増悪例では総ビリルビン値が 5 mg／dl 以上で速やかにラミブジンを投与すべきと考えられる．

ラミブジン長期投与例においてみられる耐性株出現による breakthrough hepatitis が原因の急性増悪例では他の抗ウイルス薬の併用が試みられる．我が国では 2004 年 12 月より，アデホビルが保険適用となり，ラミブジン投与中に HBV の持続的な再増殖を伴う肝機能異常が確認された症例で，ラミブジンとの併用治療が可能となった．また，entecavir など，他の抗ウイルス薬の開発も進んでおり，今後

の臨床への応用が期待される．

当院の加藤ら[10]は年齢やウイルス量によって，B型肝炎治療のステージ分類（表3）を作成し，治療法の選択に使用している．HBs抗原，HBe抗原，HBV－DNA，ALT，年齢によってステージングを行い，発がんリスクを考慮して，ラミブジン，IFNなどの治療の適否を示している．しかし，これらは新たな抗ウイルス薬が使用可能になるまでの暫定的な治療選択であり，今後，選択肢が広がる可能性が十分考えられる．

おわりに

B型急性肝炎では，その多くが一過性感染で，無治療にて軽快する．まれに重症化や劇症化を引き起す．B型慢性肝炎急性増悪では，セロコンバージョンなど，一過性の悪化で改善する場合もあるが，一部に重症化や劇症化を起す症例を認める．どちらも治療の最大の目的は重症化や劇症化を未然に防ぎ，肝不全を引き起さないことである．そして，不幸にして，肝不全となったときには，肝移植を含めた集学的治療を行うことが重要である．今後，新たな抗ウイルス薬の開発により，B型肝炎に対する治療法は大きく変化する可能性が考えられる．

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加藤道夫

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B 型慢性肝炎の病態をどう把握し，治療方針を立てるか？

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はじめに●

B 型肝炎ウイルス(HBV)キャリアはHBe 抗原陽性無症候性キャリアから慢性肝炎，肝硬変，肝細胞癌あるいは臨床的治癒とされているHBe 抗体陽性無症候性キャリアまでさまざまな病態が存在する．そして，その経過もさまざまであるが，大別すると肝硬変，肝細胞癌に進行する群と臨床的治癒の状態に落ち着く群に2分される．これらHBV キャリアのそれぞれが現在どの病期にいるのか，発癌リスクはどの程度であるのか，積極的な治療の必要性はあるのか，そしてあるならどのような治療を選択すべきかという問いに対処するため，われわれはHBV キャリアのステージ分類を提唱した[1]．

HBV キャリアのステージ分類●

1995 年 11 月以降に当院を初診したHBV キャリア 207 例を対象にHBV キャリアを 8 ステージに分類した(表1)．対象の性別は男性 138 例，女性 69 例で，平均年齢はそれぞれ 44.3±13.4 歳，42.8±15.6 歳であった．

HB ステージ 0：HBs 抗原陽性，HBe 抗原陽性，ALT 正常値持続のいわゆる無症候性キャリアの状態．

HB ステージ I：HBs 抗原陽性，HBe 抗原陽性，ALT 異常値(持続正常以外)でHBV-DNA 量が $10^{7.6}$copies/mL 以上の高ウイルス群．若年例(男性：30 歳未満，女性：35 歳未満)をステージ Ia，高年例(男性：30 歳以上，女性：35 歳以上)をステージ Ib とする．

HB ステージ II：HBs 抗原陽性，HBe 抗原陽性，ALT 異常値(持続正常以外)でHBV-DNA 量が $10^{7.6}$copies/mL 未満の低ウイルス群．若年例をステージ IIa，高年例をステージ IIb とする．

HB ステージ III：HBs 抗原陽性，HBe 抗原陰性，HBV-DNA10^5copies/mL 以上のプレコア変異株の増殖が持続していると考えられる群である．

HB ステージ IV：HBs 抗原陽性，HBe 抗原陰性，HBV-DNA10^5copies/mL 未満のいわゆる臨床的治癒の状態である．

HB ステージ V：HB キャリア(HBs 抗原陽性の時期が確認されている例)でHBs 抗原が消失した状態である．

各ステージの例数，性別，平均年齢，ALT 値，血小板数および発癌率は表2に示す．HBe 抗原

表1　HBV キャリアのステージ分類

HB ステージ	0	I	II	III	IV	V
HBsAg	＋	＋	＋	＋	＋	−**
HBeAg	＋	＋	＋	−	−	−
HBV-DNA (copies/mL)	不問	$10^{7.6} \leqq$	$10^{7.6} >$	$10^5 \leqq$	$10^5 >$	不問
ALT	持続正常	持続正常以外	持続正常以外	不問	不問	不問
年齢	不問	若年/高年* (Ia/Ib)	若年/高年* (IIa/IIb)	不問	不問	不問
発癌リスク	きわめて小	小/大	小/きわめて大	きわめて大	きわめて小	きわめて小

*若年：男性 30 歳未満，女性 35 歳未満
高年：男性 30 歳以上，女性 35 歳以上
**HBsAg(＋)の時期が確認されていること

- 臨床的治癒コースはステージ Ia から IIa となり，速やかにステージ IV に移行する.
- 病態進展コースはステージ Ia から Ib，IIb と進行し，III までは到達するが IV には至らない.
- ステージ III とステージ IV は時間的経過の差ではなくて，病態の異なる集団である.

表2　各 HB ステージの背景因子と発癌率

HB ステージ	0	Ia	Ib	IIa	IIb	III	IV
例数(%)	9 (4.3)	23 (11.1)	44 (21.3)	10 (4.8)	31 (15.0)	49 (23.7)	41 (19.8)
性別 (男性/女性)	3/6	16/7	32/12	4/6	24/7	38/11 **	21/20 **
年齢(歳)	34.4±9.1	25.5±3.4	44.8±11.0	24.0±2.5	48.5±9.8	53.1±9.7 **	45.6±15.7 **
ALT (IU/L)	17.7±4.4	129.0±101.4	193.6±204.2	105.6±80.3	130.5±194.2	117.2±112.3 ***	41.0±39.7 ***
血小板数 ($\times 10^4$)	20.4±4.2	20.1±3.6	16.5±6.2	18.1±4.3	15.4±7.9	14.4±5.9 ***	19.3±7.5 ***
初診時発癌 (−/+)	9/0	23/0	44/0	9/1	24/6	39/10	35/6
初診後発癌例	0	0	3	0	4	9	1
発癌率(%)	0	0	6.8	0	16.7	23.1 *	2.9 *

* $p<0.05$，** $p<0.01$，*** $p<0.001$

陰性期のステージ III とステージ IV を比較すると，平均年齢はステージ IV が有意(p＜0.01)に若年齢であり，性別は女性は有意(p＜0.01)にステージ III 例で少数であった．また，ALT 値はステージ IV が有意(p＜0.001)に低値であった．ステージ III とステージ IV はステージ III からステージ IV へと移行するという時間的経過の差ではなくて，病態の異なる集団と考えられる．HBV キャリアの大多数が歩む臨床的治癒の状態へのコースはステージ Ia からステージ IIa となり，その後短期間ステージ III を経由した後速やかにステージ IV に移行するものと考えられる．そしてステージ IV が長期間続いた後 HBs 抗原が消失し，ステージ V となる．一方，肝硬変進展・肝癌発癌ハイリスク群はステージ Ia からステージ Ib，ステージ IIb と進行し，HBe 抗原が陰性化してステージ III までは到達するが HBV の増殖は持続し，ステージ IV に至ることはない(図1)．臨床的治癒コースの各ステージにおける初診時の血小板数と発癌リスクは，ステージ 0，Ia，IIa および IV でそれぞれ 20.4 万，0%，20.1 万，0%，18.1 万，0% および 19.3 万，2.9% とほとんど変化を認めないが，肝硬変進展・肝癌発癌ハイリスクコースにあたるステージ Ib，IIb および III ではそれぞれ 16.5 万，6.8%，15.4 万，16.7% および 14.4 万，23.1% とステージの移行に従っての血小板数の低下と発癌率の増加が認められ，ステージ Ib，IIb および III のキャリアに対する抗ウイルス治療の必要性が強く示唆される．

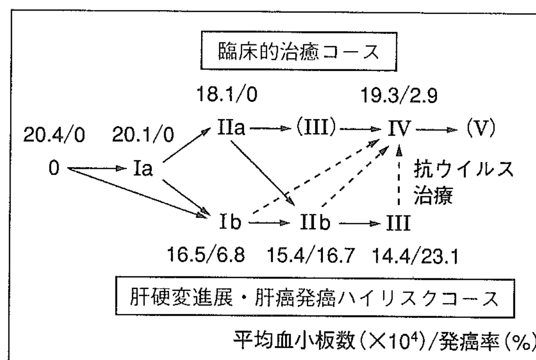

図1　HBV キャリアの経過(臨床的治癒コースと肝硬変進展・肝癌発癌ハイリスクコース)

- ステージ Ib，IIb および III は肝硬変進展・肝癌発癌のハイリスクコースである．
- ステージ III では CP，Pre C 両領域ともに変異型が有意に高率である．
- ステージ IV では両領域で野生型の残存率が高く，ウイルス量の減少による HBe 抗原消失と考えられる．

肝癌発癌例，肝予備能低下例と HB ステージ分類●

各ステージ別の発癌率はステージ 0 0%，ステージ Ia 0%，ステージ Ib 6.8%(3/44)，ステージ IIa 0%，ステージ IIb 16.7%(4/24)，ステージ III 23.1%(9/39)，ステージ IV 2.9%(1/35) であった．ステージ Ib，ステージ IIb およびステージ III は B 型肝癌発癌のハイリスク群で積極的に抗ウイルス治療を行う必要がある．また全発癌例(初診時有発癌例を含む)における性別および発癌確認時の年齢，ALT 値についてみると，性差は，男性 24.6%(138 例中 34 例)，女性 10.1%(69 例中 7 例)と男性で有意に発癌率が高率($p < 0.02$)であった．発癌例の年齢分布は 50 歳代が 55.0% と最も多く，60 歳代，40 歳代がそれぞれ 17.5%，15.0% で 40 歳未満は 25 歳と 35 歳の 2 例のみであった．また，発癌確認時の ALT 値は 30IU/L 未満が 6 例(15.0%)，40IU/L 未満 12 例(30.0%) および 50IU/L 未満 19 例(47.5%)と ALT 低値例が約半数を占めた．

また，初診時血小板数 10 万未満例を肝予備能低下例とすると，各ステージ別の肝予備能低下例の割合はステージ 0 0%，ステージ Ia 4.3%(1/23)，ステージ Ib 13.6%(6/44)，ステージ IIa 0%，ステージ IIb 25.8%(8/31)，ステージ III 26.5%(13/49)，ステージ IV 7.3%(3/41) であった．発癌例と同様にステージ Ib，ステージ IIb およびステージ III において肝予備能低下例が高率に認められた．

プレコア，コアプロモーター変異と HB ステージ分類●

対象 207 例中 111 例においてプレコア(PreC) およびコアプロモーター(CP)変異について検討した．各ステージにおける野生型の割合は PreC 領域，CP 領域でそれぞれ，ステージ 0 100%，75.0%，ステージ Ia 66.7%，33.3%，ステージ Ib 65.2%，34.8%，ステージ IIa 42.9%，14.3%，ステージ IIb 53.3%，13.3%，ステージ III 3.7%，7.4%，ステージ IV 37.5%，31.8% であった．HBe 抗原陰性期でもステージ III とステージ IV では様相が異なり，PreC 領域では野生型と変異型がステージ III ではそれぞれ 1 例，15 例であるが，ステージ IV では 9 例，12 例とステージ III で変異型が有意($p < 0.05$)に高率であった．また，CP 領域でも野生型と変異型を比較すると，ステージ III ではそれぞれ 2 例，25 例であるが，ステージ IV では 7 例，13 例とステージ III で PreC 領域と同様，変異型が有意($p<0.05$)に高率であった．PreC 領域と CP 領域のいずれかが野生株である率はステージ III ではわずか 11.1%(3/27) であったが，ステージ IV では 52.0%(13/25) と過半数を占めた．臨床的治癒期と考えられるステージ IV では両領域で野生型の残存率が高く，ウイルス量の減少によって HBe 抗原が消失した例が多いことを示す成績と考えられる．

HBV genotype と病態との関連●

HBV は分子進化学の発展により A 型から H 型までの 8 種の genotype に分類されている．Orito らのわが国における genotype 分布の解析[2]によると，沖縄と東北地方には genotype B が多く，それ以外の地域では genotype C が大半を占めており，わが国全体の比率としては genotype B が 12.2%，genotype C が 84.7% であった．genotype B は genotype C に比し予後良好と考えられており，PreC 領域と CP 領域の変異の有無についての検討でも，変異型は genotype B の 16% に比し genotype C では 58% と genotype C で有意に高率と報告されている[3]．当院で無作為に抽

- ステージⅠbでは若年齢を過ぎてもHBV-DNA量高値が持続し，抗ウイルス薬治療が必要である．
- ステージⅡb全例とステージⅢのALT異常男性例は抗ウイルス治療の絶対適応である．
- ステージⅢの発癌数は全ステージ中最大で，ALTの正異に関係なく発癌例がみられる．

出したB型慢性肝疾患60例中56例(93.3%)はgenotype Cであり，その他はgenotype A，B，FおよびB+Cが1例ずつであった．大阪でもB型慢性肝疾患の大半はgenotype Cであり，前述のPreC，CP変異とステージ分類との関係もgenotype Cのキャリアにおいての成績と考えられるが，genotype Bのキャリアでは変異型が有意に低値とのことで，HBe抗原陰性期でのステージⅢの比率がきわめて低率ではないかと推察される．

HBステージ分類と抗ウイルス治療の必要性●

ステージⅠaはステージ0の無症候性キャリアが肝炎期に移行した状態のすべてのHBキャリアが通過する高ウイルスのステージであり，発癌リスクがきわめてまれで通常は抗ウイルス治療の必要はない．しかし，組織学的に線維化ステージがF2以上に進行している例は早期に肝硬変に進展する可能性があり，抗ウイルス治療の適応と考えられる．ALT値が高値を持続する例は通常HBV-DNA量が減少しステージⅡaとなるが，ステージⅡaからは若年発症のB型肝癌例があり，ALT値持続高値例は抗ウイルス治療の適応となる．Ⅰa，Ⅱaとも薬剤としては若年で免疫応答が良好であるのでインターフェロン(IFN)が第一選択となると考える．ステージⅠbは若年齢を過ぎてもHBV-DNA量の高値が持続する群で，発癌リスクはステージⅡbよりは低頻度であるがリスク大で抗ウイルス治療の必要がある．Suzukiら[4]は多変量解析によって，高ウイルス群であることがYMDD変異株出現に最も寄与する因子であることを報告しており，ラミブジン(ゼフィックス®)単独での治療効果の持続は困難で，エンテカビルなどの抗ウイルス効果の強い薬剤あるいは併用治療が適応になると考えられる．ステージⅡbは発癌リスクがきわめて大で抗ウイルス治療の絶対適応である．薬剤はラミブジンなどの核酸アナログ単独あるいはIFN，HBワクチンとの併用の選択が考えられる．ステージⅢの発癌数は全ステージ中最大でALT値の正異に関係なく発癌例がみられる．受診キャリア中の頻度も最大で，全例に対して治療が必要かどうかは今後の検討課題と考えられるが，少なくともALT値異常の特に男性例は絶対適応であろう．薬剤は高年例が大半を占め，ラミブジンの治療効果が良好でYMDD変異株の出現も低率であるため，現在のところラミブジンが第一選択であり，YMDD変異株出現例にはアデホビル(ヘプセラ®)などの他の核酸アナログの併用あるいは切り替えで対応できると考えられる．ステージⅣはいわゆる臨床的治癒といわれる病態で，抗ウイルス治療の最終目標である．まれに発癌例を認めるが，治療の対象にはならない．ステージⅤも非B非C肝癌におけるオカルトB型肝癌の問題も残るが抗ウイルス治療の対象にはならないと考えられる．

おわりに●

B型肝癌発癌抑止のためには，HBVキャリアがどの病期にいるかを診断することが肝要である．われわれが提唱したこのHBステージ分類はその診断に有用と考える．治療適応例には早期に適切な抗ウイルス治療を開始し，発癌例を1名でも減少させたいと考えている．

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Infection of Human Hepatocyte Chimeric Mouse With Genetically Engineered Hepatitis B Virus

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Studies of hepatitis B virus (HBV) mutants have been hampered by the lack of a small animal model with long-term infection of cloned HBV. Using a mouse model in which liver cells were highly replaced with human hepatocytes that survived over a long time with mature human hepatocyte function, we performed transmission experiments of HBV. Human serum containing HBV and the virus produced in HepG2 cell lines that transiently or stably transfected with 1.4 genome length HBV DNA were inoculated. Genetically modified e-antigen–negative mutant strain also was produced and inoculated into the mouse model. A high-level (≈10^{10} copies/mL) viremia was observed in mice inoculated with HBV-positive human serum samples. The level of viremia tended to be high in mice with a continuously high human hepatocyte replacement index. High levels and long-lasting viremia also were observed in mice injected with the *in vitro* generated HBV. The viremia continued up to 22 weeks until death or killing. Passage experiments showed that the serum of these mice contained infectious HBV. Genetically engineered hepatitis B e antigen–negative mutant clone also was shown to be infectious. Lamivudine effectively reduced the level of viremia in these infected mice. In conclusion, this mouse model of HBV infection is a useful tool for the study of HBV virology and evaluation of anti-HBV drugs. Our results indicate that HBeAg is dispensable for active viral production and transmission. (HEPATOLOGY 2005;42:1046-1054.)

Hepatitis B virus (HBV) is a small enveloped DNA virus and causes chronic infection of the liver that often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1-4] The lack of a practical small animal model has impeded the study of the biology of this virus and the development of effective antiviral therapies. Chimpanzee is the only natural host that allows active replication of HBV.[5-7] Although this animal is a valuable model for the study of hepatitis viruses,[8] the practical use of chimpanzees is severely limited both ethically and economically.

Several small animal models of HBV infection have been reported. The HBV transgenic mouse is a very useful model for the study of virology and evaluation of antiviral drugs.[9-12] However, the liver cells of this model are not permissive for HBV infection; therefore, studying virus–cell interactions such as receptor binding and entry is not possible. The HBV-trimera mouse is another useful mouse model.[13] In this model, *ex vivo* HBV-infected human liver fragments are implanted into lethally irradiated mice after SCID mouse bone marrow transplantation. Approximately 80% of the mice develop viremia 2 to 3 weeks after infection. However, the rate of positivity subsequently decreases to less than 20% 6 weeks after infection. The level viremia is approximately 10^5copies/mL. More recently, HBV-containing human serum samples were used to infect human hepatocyte repopulated mice.[14] A high-level viremia (4.5 and 10 × 10^8copy/mL) and HBs antigenemia are observed 8 weeks after injection. This mouse model is promising because HBV replicates in natural host cells, human hepatocytes. However,

Abbreviations: HBV, hepatitis B virus; WHV, woodchuck hepatitis virus; HSA: human serum albumin; PCR, polymerase chain reaction; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; DMEM, Dulbecco's modified Eagle's medium.

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Table 1. Table Inoculum Used for Infection Experiments

Inoculum	Source	Transfection	HBs Antigen	HBe Antigen	HBV DNA (LGE/mL)
Serum 1	HBV carrier 1	–	+	130	10.8
Serum 2	HBV carrier 2	–	+	150	8.7
CA59	pCAG-HB-wt	Stable	6.3 ± 2.8	15 ± 7	8.0 ± 0.2
CM3	pTRE-HB-wt	Transient	3.9 ± 1.5	105 ± 7	8.3 ± 0.4
Fresh CM3	pTRE-HB-wt	Transient	ND	ND	8.0
e-Negative	pTRE-HB (PC)	Transient	2.9 ± 0.2	0.5 ± 0.3	8.1 ± 0.3
Mouse CM3	CM3-infected mouse	–	ND	ND	ND

NOTE. Fifty microliters of each serum or cell culture supernatant was used for injection except for fresh CM3 (500-1,000 μL) and mouse CM3 (5 μL). Data are mean ± SD.

Abbreviations: LGE, log genome equivalent; ND, not determined.

long-term high-level viremia has not been reported so far in this model, probably because of technical difficulties in maintaining large quantities of human hepatocytes in these mice.

Long-term HBV viremia was reported after subcutaneous transplantation of immortalized human hepatocytes in RAG-2–deficient mice after transfection of circularized full-length HBV genome.[15] Viremia of up to 3×10^8 copy/mL was still observed in these mice at least 5 months after transplantation. This long-term viremia model should be useful for *in vivo* HBV studies. However, the production and selection of HBV-secreting immortalized human hepatocytes takes a long time, and the level of viremia in the transplanted animal depends on the volume of live immortalized cells in mice. The mode of viremia might be different from natural infection because the pregenome RNA is transcribed from integrated HBV. Whether the produced HBV re-infects implanted immortalized human hepatocytes has not been confirmed.

A useful woodchuck hepatitis virus (WHV) infection model was established by Petersen et al.[16] They showed high-level replacement of uPA/Rag-2 knockout mice liver with woodchuck hepatocytes and development of high-level (1×10^{11} virion/mL) WHV viremia. Dandri et al.[17] transplanted *Tupaia* hepatocyte into uPA/RAG-2 mice and showed up to 8.2×10^7 genome equivalent/mL viremia. This model is useful because viremia continued up to 29 weeks. However, probably because of different host cells, the replication levels of HBV are lower than those of woolly monkey HBV. Using SCID mouse homozygous for Alb-uPA transgene, the group of Mercer and colleagues[18] were the first group to report high-level replacement of mouse liver with human hepatocytes and successful infection of these mice with hepatitis C virus. Recently, we also created a human hepatocyte chimeric mouse in which the hepatocytes were highly replaced by implanted human liver cells.[19] The repopulation index calculated from serum human serum albumin (HSA) concentrations exceeded 70% in 32% of the transplanted mice, and these animals survived up to 80 days after transplantation with high replacement index. Using this chimeric mouse, we performed transmission experiments of HBV. Using serum samples obtained from patients with chronic HBV infection, high-level viremia (approximately 10^{10} copies/mL) was observed up to 22 weeks in mice inoculated with HBV-positive human serum samples. We also performed infection study using *in vitro*–generated HBV. Infectious HBV was produced in HepG2 cell lines by transfecting with 1.4 genome length HBV DNA. Because mice injected with this *in vitro*–produced virus developed viremia, we further performed passage study. In addition, we introduced point mutations in HBV genome to create an HBe antigen–negative variant. The mice inoculated with this HBe antigen–negative variant developed viremia. Lamivudine effectively suppressed replication of HBV in mice inoculated with human serum samples and wild-type *in vitro*–created HBV. This model is a useful tool for the study of the nature of HBV mutants and development of anti-viral drugs.

Materials and Methods

Human Serum Samples. Serum samples were obtained from four HBV carriers after obtaining written informed consent. Inocula for mice were obtained from two patients who tested positive for HBs and HBe antigens with slightly elevated levels of serum alanine aminotransferase and high-level viremia (Table 1). Serum samples for extraction and cloning of HBV were obtained from the remaining two patients who were positive for hepatitis B e antigen (HBeAg) and had high-level HBV DNA (6.9×10^9 and 9.8×10^{10} copies/mL by real-time polymerase chain reaction [PCR], respectively). All of these HBV belonged to genotype C.

Analysis of HBV Markers. Hepatitis B surface antigen (HBsAg) and HBeAg were measured by commercially available ELISA (Abbott Japan, Osaka, Japan). For quantitative analysis of HBV DNA, 100 μL serum samples or culture supernatants were used. DNA was extracted from these samples by SMITEST (Genome

Science Laboratories, Tokyo, Japan) and was dissolved in 20 μL H_2O. One microliter DNA solution was amplified by Light Cycler (Roche Diagnostics, Japan, Tokyo) for quantitation of HBV. The primers used for amplification were 5′-TTTGGGCATGGACATTGAC-3′ and 5′-GGTGAACAATGTTCCGGAGAC-3′. The amplification condition included initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 5 seconds, extension at 72°C for 6 seconds. The lower detection limit of this assay is 300 copies. Nested PCR was used to detect a small amount of HBV DNA with the outer primers X1F1 (5′-CGCGGGACGTCCTTTGTCTA-3′) and X2R1 (5′-GTTCACGGTGGTCTCCATGC-3′) and inner primers X1F2 (5′-TACGTCCCGTCGGCGCT-GAA-3′ and X2R2 (5′-CAGAGGTGAAGCGAAGT-GCA-3′). The amplification condition included 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes after 2 minutes of initial denaturation at 94°C followed by 7 minutes of final extension using Gene Taq (Wako Pure Chemicals, Tokyo, Japan) with anti-Taq high (TOYOBO Co., Osaka, Japan) according to the instructions provided by TOYOBO.

Cloning of HBV DNA and Plasmid Construction. Full-length HBV DNA was amplified using these HBV DNA samples by the method of Gunther et al.[20] and cloned into pBluescript SK+ (Stratagene, La Jolla, CA). HBV DNA, 1.4 genome length, obtained from one of these two patients was cloned into pcDNA3 (Invitrogen, San Diego, CA) after replacement of CMV promoter with CAG to yield pCAG-HB-wt. Similarly, 1.4 genome length HBV DNA from the other patient was cloned into a plasmid vector pTRE2 (BD Biosciences, Franklin Lakes, NJ) and designated pTRE-HB-wt. A modified plasmid pTRE-HB-PC was generated by introducing a G-to-A point mutation to nucleotide 1896 to create precore stop codon (TTG to TAG). The substitution was introduced by a commercially available site directed mutagenesis kit (QuickChange Site-Directed Mutagenesis Kit, Stratagene). Nucleotide sequences of the HBV cloned into plasmids pCAG-HB-wt and pTRE-HB-wt were deposited into the GenBank database under accession numbers AB206817 and AB206816, respectively.

Transfection of HepG2 Cell Lines With 1.4 Genome Length HBV DNA and Endogenous Polymerase Reaction Analysis. HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum at 37°C and under 5% CO_2. The cells were seeded to semi-confluence in 6-well tissue culture plates. For transient transfection experiments, two plasmids; pTRE-HB-wt and pTRE-HB-PC, were used. Two micrograms of each plasmid was transfected using Fugene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) according to the instructions provided by the supplier. Three to five days after transfection, the culture supernatant was collected for infection of mice and quantitative analysis of HBV DNA by real-time PCR. Alternatively, calcium phosphate precipitation was performed to prepare fresh supernatant for large-dose administration experiments. Concentrated supernatants were prepared by using Microsep 10K spin filter, according to the instructions provided by the manufacturer (Pall Life Sciences., Ann Arbor, MI). The HBV particles produced in the supernatants were immunoprecipitated with protein A sepharose and mouse anti-HBs monoclonal antibody 2Z824Z (Institute of Immunology, Tokyo, Japan) and subjected to endogenous polymerase reaction[21] and Southern blot analysis after sodium dodecyl sulfate/proteinase K digestion followed by phenol extraction and ethanol precipitation. The DNA was electrophoresed in a 1% agarose gel and transferred onto a nylon membrane. The transferred DNA was detected with full-length HBV DNA probe synthesized with the PCR DIG probe synthesis kit and the DIG Nucleic Acid Detection kit and CSPD, ready-to-use (Roche Diagnostics) in the Fluor-S Max MultiImager (BIO-RAD Laboratories, Hercules, CA).

For the production of stably transfected cell lines, HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. Cells were seeded into 90-mm-diameter culture dishes. Twenty micrograms of the plasmid pCAG-HB-wt was transfected by calcium precipitation. Twenty-four hours after transfection, the cells were split and cultured in G418 selection DMEM (1 mg/mL). One hundred fifty colonies were isolated and amplified for identification of virus-producing cell lines. Clones positive for both HBs and HBe antigens were selected and further analyzed for production of HBV particles. Finally, one of five cell lines that produced more than 10^5 copy/mL HBV DNA in supernatant were selected and used for further experiments. This cell line produced stable levels of HBV DNA for more than 12 months (data not shown).

Analysis of HBV Produced in the Supernatant of Transfected HepG2 Cell Lines by Sucrose Density Gradient. Five milliliters HBV-positive serum (10^8 copy/mL) or 100 mL cell culture supernatant (10^7 copy/mL) was layered on a 20% (wt/wt) sucrose gradient, and centrifuged at 24,000 rpm for 1 hour at 4°C with a Beckman SW28 rotor Beckman Coulter, Fullerton, CA). The precipitate was resuspended with 500 μL phosphate-buffered saline. These HBV samples were layered on a linear 20% to 50% (wt/wt) sucrose gradient. Centrifugation was carried out at 24,000 rpm for 21 hours at 4°C with a Beckman SW40 rotor. The gradients were frac-

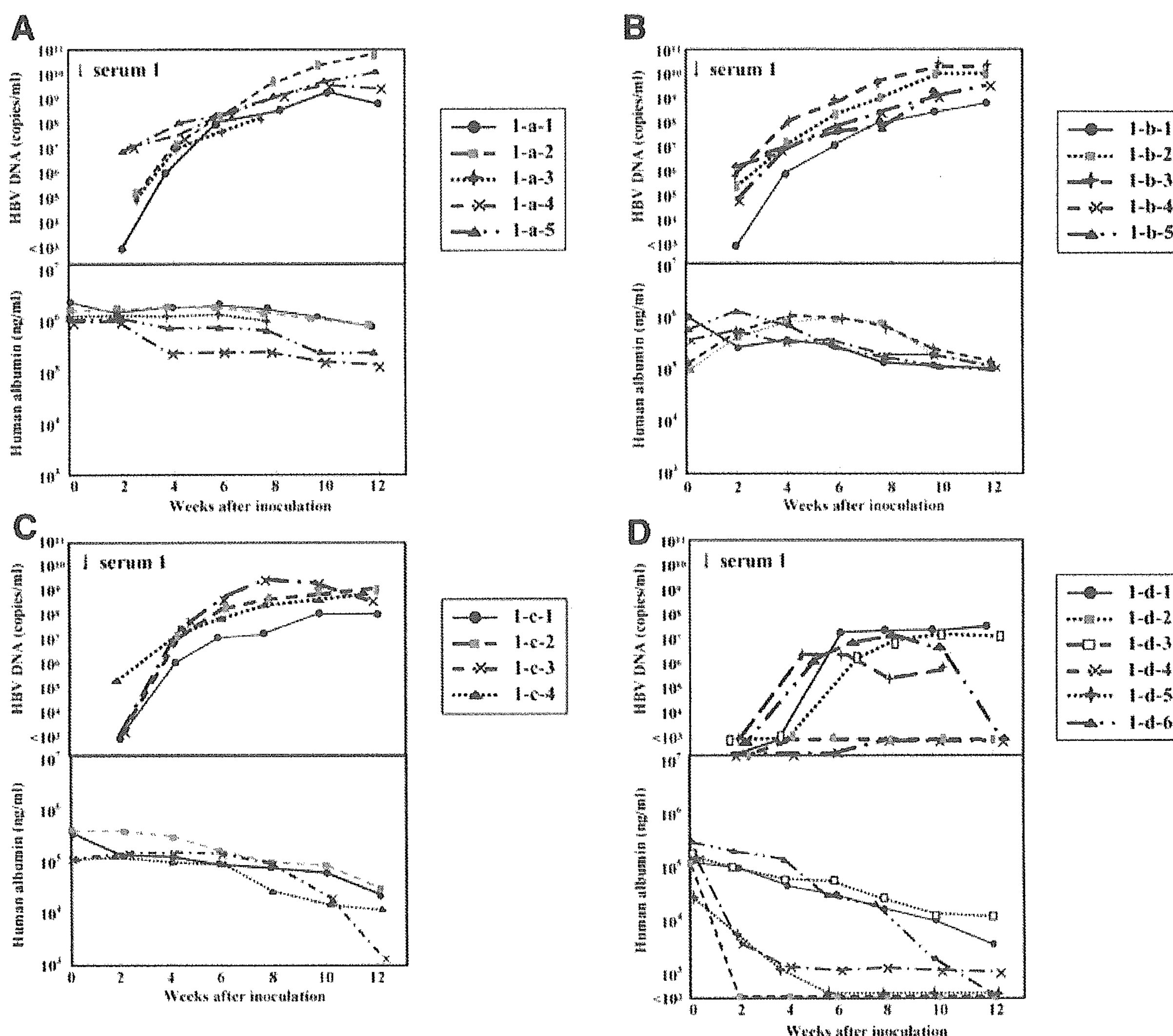

Fig. 1. Time course studies in 20 mice inoculated with human serum samples positive for hepatitis B virus (HBV). Fifty microliters serum samples (serum 1, Table 1) was intravenously injected into each mouse. The upper half of each panel shows HBV DNA, and the lower panel shows concentrations of human serum albumin (HSA). Mice were divided according to the levels of HSA; (A) initial HSA > 1,000,000 ng/mL with only slight decline (n = 5); (B) initial HSA > 500,000 ng/mL, with slight decline (n = 5); (C) initial HSA > 200,000 ng/mL, but declined to less than 100,000 ng/mL during observation (n = 4); (D) initial HSA =100,000 ng/mL, but diminished to less than 30,000 ng/mL (n = 6).

tionated into 500-μL samples, and the density of each fraction was calculated from the weight and volume. Each fraction was diluted 10-fold and tested for HBV DNA by real-time PCR.

Generation of Human Hepatocyte Chimeric Mice and Analysis of Serum Samples. Generation of the $uPA^{+/+}/SCID^{+/+}$ mice and transplantation of human hepatocytes were performed as described previously by our group.[19] Animal protocols were performed in accordance with the guidelines of the local committee for animal experiments. Infection, extraction of serum samples, and sacrifice were performed under ether anesthesia. HSA was measured with a Human Albumin ELISA Quantitation kit (Bethyl Laboratories Inc., Montgomery, TX) according to the instructions provided by the manufacturer. Serum samples obtained from mice were aliquoted and stored in liquid nitrogen until use.

Histochemical Analysis of Mouse Liver. The liver specimens of infected mice were fixed with 10% buffered-paraformaldehyde and embedded in paraffin blocks for histological examination. The liver sections were stained with hematoxylin-eosin or subjected to immunohistochemical staining by using an antibody against hepatitis B core antigen (HBc-Ag) (DAKO Diagnostika, Hamburg, Germany) or HSA (Bethyl Laboratories Inc.). Endogenous peroxidase activity was blocked with 0.3% H_2O_2

and methanol. Immunoreactive materials were visualized by using a streptavidin-biotin staining kit (Histofine SAB-PO kit; Nichirei, Tokyo) and diaminobenzidine.

Results

Human Hepatocyte Chimeric Mice Develop High-Level and Long-Term Viremia After Inoculation of Serum Samples Obtained From Carriers. Twenty chimeric mice were inoculated with 50 μL serum 1 (Table 1). We used mice that had relatively low-level HSA because we had previously found that mice with low-level replacement are susceptible to HBV (Chayama K and Tateno C, unpublished results). The HSA of these mice was 300,000 ng/mL (median, range, 40,000-3,090,000, Fig. 1A). All 20 mice tested positive for HBV DNA by nested PCR 2 to 4 weeks after inoculation. Eighteen of 20 mice developed quantitatively measurable viremia, but two mice showed very low-level viremia that was detectable only by nested PCR. Mice with persistently high-level HSA tended to show high virus titer (Fig. 1A-C). The maximum level of viremia was 9.5×10^{10} copy/mL. The viremia reached a plateau 4 to 6 weeks after infection. In contrast, mice with a rapid decrease in HSA or persistently low-level HSA showed low virus titer (Fig. 1D). We also performed infection experiments using serum 2 (Table 1). Of the five mice inoculated with this serum, all developed quantitatively measurable viremia 2 to 4 weeks after inoculation (Fig. 2). The level of viremia reached 1×10^7 to 1×10^9 copies/mL. The level of viremia also tended to be high in mice with high HSA levels.

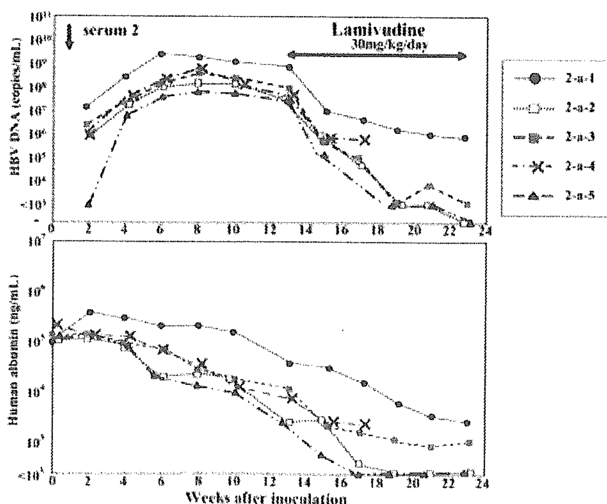

Fig. 2. Time course studies in five mice inoculated with human serum samples positive for hepatitis B virus (HBV). Fifty microliters serum samples (serum 2, Table 1) was intravenously injected into each mouse. The upper panel shows HBV DNA, and the lower panel shows concentrations of human serum albumin. The effects of lamivudine are shown in the upper panels.

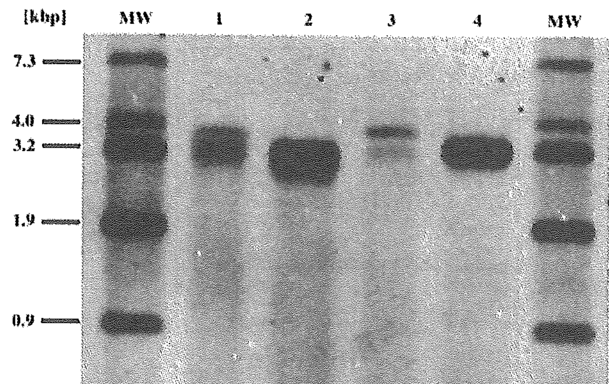

Fig. 3. Formation of fully repaired, relaxed circular hepatitis B virus (HBV) DNA after endogenous polymerase reaction. HBV particles produced into supernatant were immunoprecipitated with a monoclonal antibody against HBs antigen and subjected to Southern blot analysis before (lanes 1 and 2) and after (lanes 3 and 4) endogenous polymerase reaction. The undigested (lanes 1 and 3) and unique *SmaI*-digested DNA was electrophresed in a 1% agarese gel and detected by Southern blot hybridization.

HBV Generated in HepG2 Cell Lines Are Infectious to Human Hepatocyte Chimeric Mice. HBV markers and endogenous polymerase experiments with Southern blot analysis of HBV produced by transiently or stably transfected HepG2 cell lines are shown in Table 1 and Fig. 3. The results indicated that these cell lines produced the expected HBV antigens and HBV DNA into the supernatant. Using virus particles produced by transient transfection of plasmid pTRE-HB-wt, we performed endogenous polymerase chain reaction experiments. Formation of fully double-stranded, relaxed circular DNA was observed after the reaction (Fig. 3). Sucrose density gradient analysis of HBV produced by stably transfected cell line (CA59, Table 1) showed that the produced viruses were sedimented to similar fractions of HBV obtained from the serum of the HBV carrier (Fig. 4), suggesting that HBV particles similar to those in serum are produced in these cell lines.

In the next step, we inoculated each chimeric mouse with 50 μL of the supernatants produced by transiently or stably transfected cell lines (Table 1). Three mice were inoculated with CM3 (Fig. 5A). Four weeks later, one of these three mice developed measurable viremia. It reached a high level (7.3×10^8 copy/mL) at week 14. A serum sample obtained from this mouse at week 6 was stored in liquid nitrogen and used in the subsequent passage experiments. The other two mice developed viremia, but its level was so low that HBV was only detectable by nested PCR. At week 13, these two mice (5-a-2, 5-a-3, Fig. 5A) were inoculated with serum 1, which induced high-level viremia in mice with high HSA levels (Fig. 1). These mice did not develop measurable viremia, suggesting that the

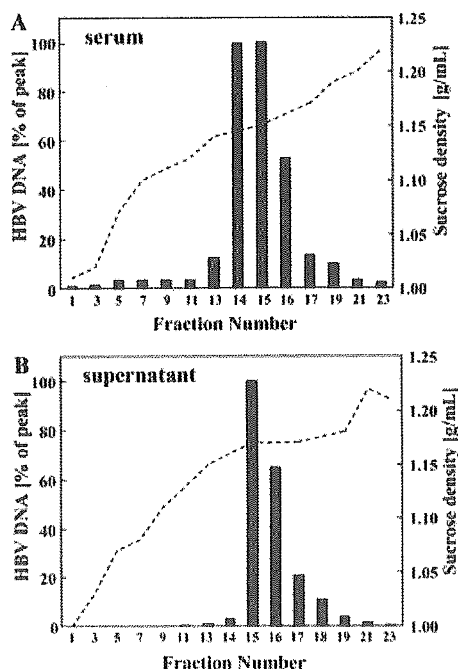

Fig. 4. Sucrose density gradient analysis of hepatitis B virus (HBV) produced into the supernatant of the transfected HepG2 cell line (CA59). Sucrose density is indicated by the dotted line. The amount of HBV DNA in each fraction was measured by real-time PCR.

low-level viremia in the latter two mice was due to low-levels of human hepatocyte replacement. Similarly, six mice were inoculated with supernatant CA59. One of these six mice developed quantitatively measurable viremia (peak, 2.6×10^9 copies/mL) (Fig. 5B). Two of the six mice developed viremia only detectable by nested PCR. For a more efficient reverse genetics infection procedure, we used mice with higher human albumin concentrations and inoculated each with 500 to 1,000 μL freshly prepared high-titer virus particles. This resulted in infection of all 10 mice (Fig. 5C). Thus, we established a highly effective infection procedure of reverse genetics of HBV.

Infection of Genetically Engineered Mutant Viruses. Four mice were inoculated with the supernatant of genetically engineered e-antigen–negative HBV generated in a pTRE-HBV-PC-transfected HepG2 cell line (e-negative, Table 1). Three of these four mice developed quantitatively measurable, but relatively low-level (less than 10^7 copies/mL) viremia, 2 to 6 weeks after inoculation (Fig. 6). Nucleotide sequence analysis of the precore region showed that the sequence obtained from the infected mice was completely in agreement with the transfected plasmid with precore stop codon at 1896.

Passage Experiment of HBV From a Mouse Infected by* In Vitro *Generated HBV to Naïve Chimeric Mice. Each of four naïve mice was injected with 5 μL serum samples obtained from a mouse that developed HBV viremia after inoculation of *in vitro* generated virus (CM3, Table 1). All four mice developed viremia at 2 to 6 weeks after inoculation (Fig. 7). One of the four mice that developed measurable viremia died at week 5 (7-a-3, Fig. 7). Another mouse (7-a-2) was weak and was sacrificed at week 13. The high-level viremia in the third mouse (7-a-1) increased further to 8.5×10^9 copies/mL. The remaining mice developed viremia detectable only by nested PCR (7-a-4).

Histochemical Analysis of the Liver of Mice Infected With HBV. Liver specimens from mice that became positive for HBV DNA after the inoculation of the described passage experiment were subjected to histological and immunohistochemical analyses. Multiple foci of replaced human hepatocytes were noted in hematoxylin-eosin–stained sections (Fig. 8A) that were positive for HSA (Fig. 8B). Such positive human hepatocytes were also positive for the HBV core antigen in serial sections (Fig. 8C).

Effect of Lamivudine Treatment in Mice Infected With HBV. Five mice that became positive for HBV DNA by inoculation with serum 2 (Fig. 2) were fed lamivudine (30 mg/kg/day)-containing food. A rapid reduction of HBV DNA level was observed in all 5 mice. Although two of five mice showed graft failure reflected by a decrease in HSA levels to the lower limits of the assay (2-a-2 and 2-a-5), the reduction of HBV DNA levels appeared before the decrease in HSA in these mice, suggesting that the decrease in HBV DNA was due to both the effect of lamivudine and the loss of virus replicating human hepatocytes (Fig. 2). Similarly, 1 mouse with high-level viremia as described in the above passage experiment (7-a-1, Fig. 7) showed a marked reduction of HBV DNA.

Discussion

The major finding of the current study was the successful establishment of a model of HBV infection with long-term and high-level HBV viremia in the human hepatocyte chimeric mouse. The level of viremia correlated with the degree of human hepatocyte replacement indicated by HSA levels. We also showed that HBV created *in vitro* using HepG2 cell lines are infectious to this mouse model. Thus, a combination of chimeric mouse and molecularly cloned virus enabled us to prepare a practical model for the study of HBV virology. Chimpanzee is also a useful model for the study of HBV virology. Injection of molecularly cloned HBV into the liver of chimpanzee induced HBV infection and hepatitis.[22] However, there might be some difference between hepatocytes of human and chimpanzee that could affect the nature of infection and the replication of this narrow host virus.

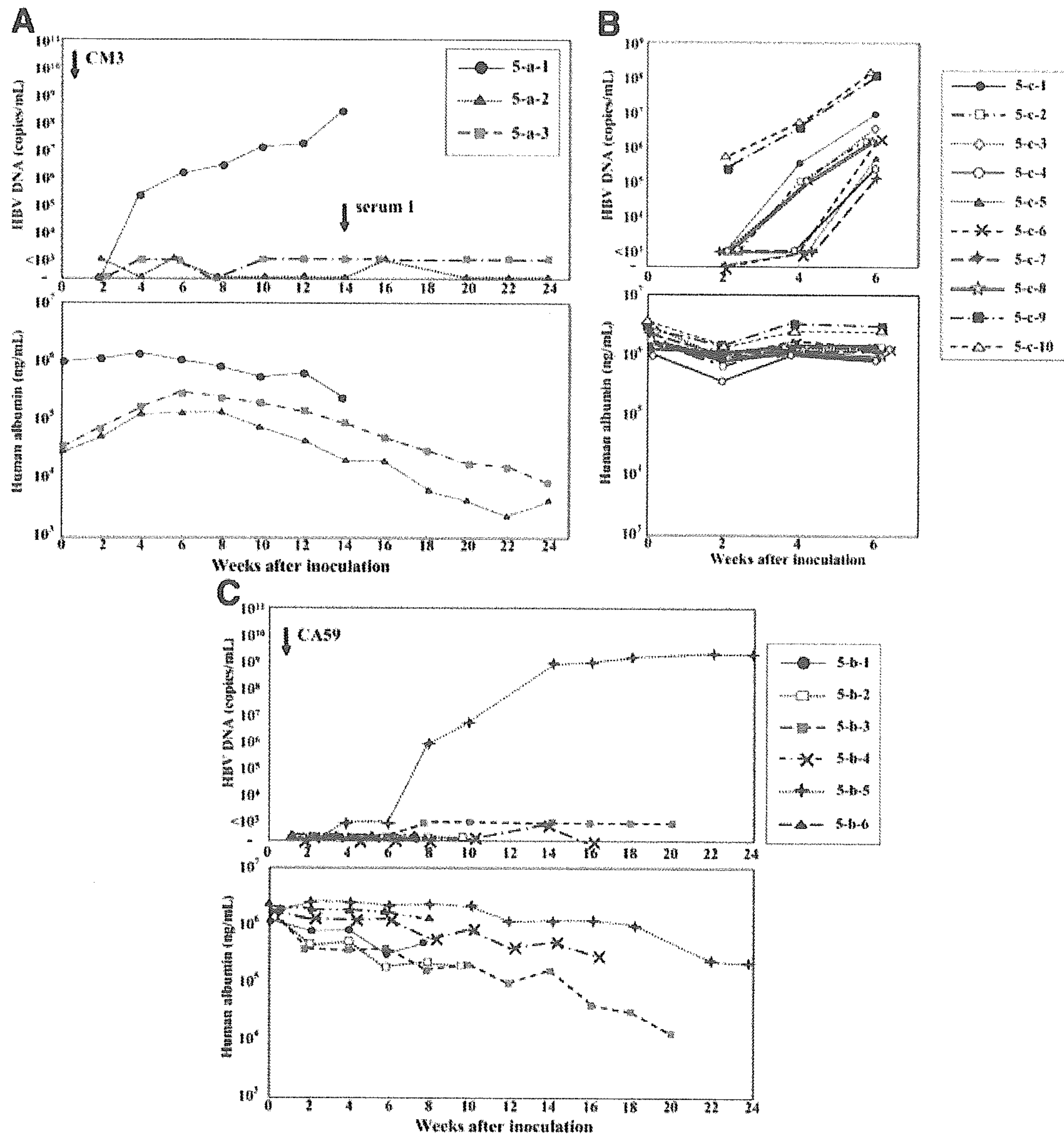

Fig. 5. (A) Time course studies in three mice inoculated with supernatants of HepG2 cell lines transfected with hepatitis B virus (HBV) DNA. Fifty microliters culture supernatant (CM3, Table 1) was inoculated intravenously into each mouse. Upper panel: HBV DNA; lower panel: concentrations of human serum albumin. (B) Time course studies in 6 mice inoculated with supernatants of HepG2 cell lines transfected with HBV DNA. Fifty microliters culture supernatant (CA59, Table 1) was inoculated intravenously into each mouse. (C) Time course studies in 10 mice inoculated with freshly prepared supernatants of HepG2 cell lines transfected with HBV DNA. Five hundred (mice 5-c-1 to 5-c-4) and 1,000 μL culture supernatants (5-c-5 to 5-c-8) and 500 μL concentrated (from 1.1×10^9 copies/mL to 1.3×10^{10} copy/mL by ultrafiltration) supernatant (5-c-9 and 5-c-10) were inoculated intravenously into each mouse.

A critical difference between the chimeric mouse model reported here and chimpanzee is that there is no immune system active for HBV in the mouse model. Although the chimpanzee model is known to cause hepatitis and is suitable for the study of HBV-induced hepatitis,[23] the mouse model is expected to be free from inflammation because these mice are SCID and do not have any human cytotoxic T lymphocytes. Actually, we observed no lymphocyte infiltration or focal necrosis of human hepatocytes in our mouse model. Recently, similar morphological changes in a similar model were reported by Meuleman et al.[24]; they also observed no alteration of liver architecture by HBV and hepatitis C virus infections. Interestingly, however, we observed a poor increase in the viral titer during the early phase of infection in some mice (Figs. 5A, 6). This might represent some innate anti-viral defense mechanism of liver cells themselves against viral infection. Further investigation is necessary to explore this issue.

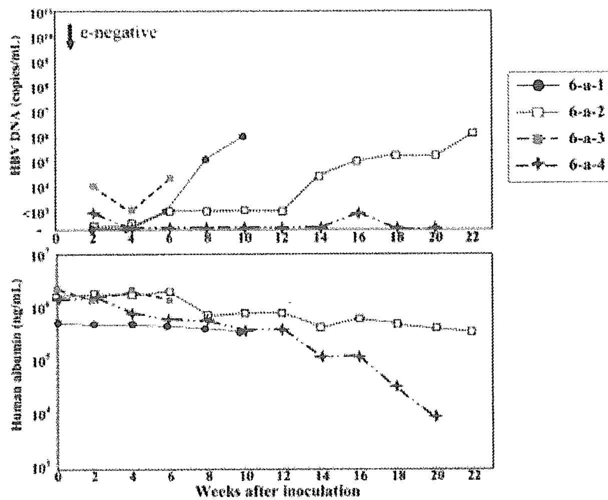

Fig. 6. Time course studies in four mice inoculated with supernatants of HepG2 cell lines transfected with e-antigen-negative (G1896A) hepatitis B virus (HBV) DNA. Fifty microliters culture supernatant (e-negative, Table 1) was inoculated intravenously into each mouse. The upper panel shows HBV DNA, and the lower panel shows the concentrations of human serum albumin.

A mouse model without any inflammation is an advantageous phenotype because it allows the study of HBV replication without any influence of immunological reaction. The model is also beneficial for studying the effects of drugs without any influence of fluctuation of the virus by immunological reaction. The HBV-infected mouse described here opens the way to create a long desired practical small animal model that overcomes economical and ethical problems associated with the chimpanzee model.

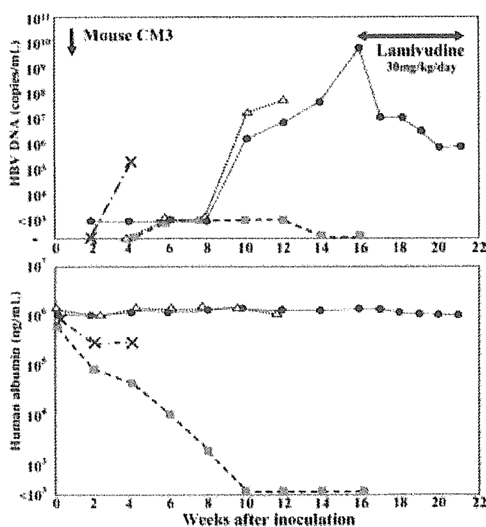

Fig. 7. Passage experiments in four mice. Five microliters mouse serum (mouse CM3, Table 1) was intravenously inoculated into each of four naïve mice. Upper panel: HBV DNA, lower panel: concentrations of human serum albumin.

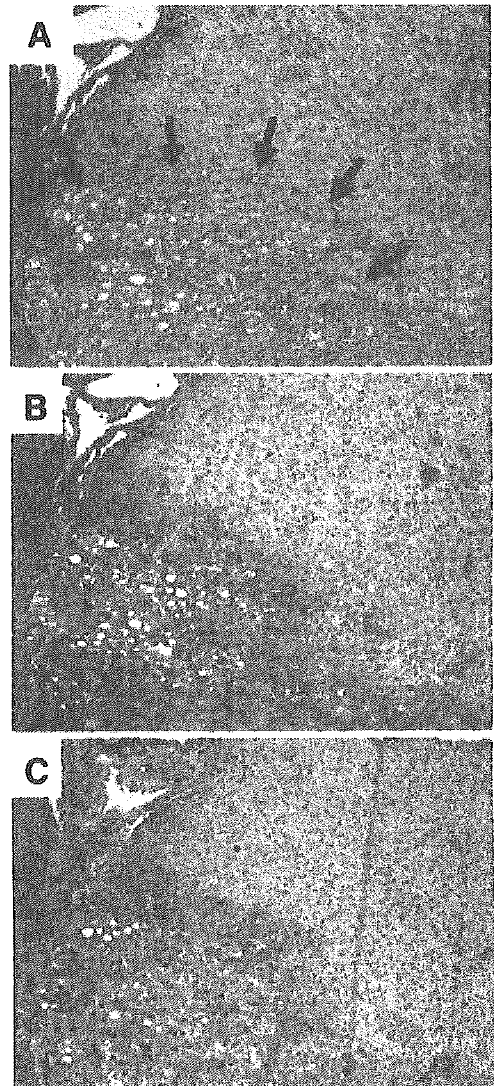

Fig. 8. Histochemical analysis of liver samples obtained from mice infected with hepatitis B virus generated in the 1.4 genome transfected HepG2 cell line. (A) Hematoxylin-eosin staining. Human hepatocytes are indicated by arrows. Immunohistochemical staining with anti-human serum albumin antibody (B) and anti-HBc-Ag antibody (C) (original magnification, ×40.

We showed in this study that reverse genetics of HBV can be achieved highly efficiently by using mice with high human albumin levels and inoculating mice with large amounts of freshly prepared virus particles (Fig. 5C). We further showed in this study that e-antigen is completely dispensable for infection and replication. The e-antigen–negative HBV-containing serum was previously used in chimpanzee and is known to induce more severe hepatitis.[25] However, it is difficult to exclude the possible presence of a small amount of e-antigen–producing virus that might help infection and

replication of HBe antigen-negative HBV strain. Our results clearly demonstrate that HBV can infect and replicate in the complete absence of e-antigen–producing species. However, the level of viremia was relatively low (less than 1×10^7 copies/mL) in these mice. Whether this is due to lack of e-antigen should be further confirmed in a larger number of mice with high replacement index.

Because the mice treated with lamivudine showed a reduction of viremia (Figs. 2, 7), our infected mouse is suitable for the study of new drugs. Lamivudine is a potent anti-HBV drug that reduces the virus and induces clinical remission and histological improvement.[26-28] Emergence of drug-resistant HBV mutants against this drug as well as other anti-viral drugs is a serious problem in the treatment of HBV,[29-31] as has been seen in the therapy of human immunodeficiency virus infection. Our model is especially useful for the study of the biology and drug susceptibility of such mutants, because almost all such drug resistances are based on only one or two point mutation(s).[29,31]

In conclusion, the mouse model presented in this study is very useful for the study of HBV biology and evaluating of anti-HBV drugs. Furthermore, many applications of this model are expected because we can easily create, manipulate, and modify the model compared with other models.

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