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Ⅳ．研究成果の刊行物・別刷

今日の消化器疾患治療指針

第3版

編集

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慢性肝炎の治療(C型)

treatment of chronic hepatitis C

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【概念】

C型肝炎ウイルス(HCV)は，感染すると自然に排除される可能性は低く，その多くが持続感染する．宿主の免疫応答は，多くの場合不完全でHCVは排除されることなく，壊死炎症反応が持続する．この結果が慢性肝炎であり，壊死炎症の継続の結果，線維化は進展し肝硬変へと移行する．特に，肝線維化の程度と肝発癌率は密接に関連しており，肝線維化の進展に伴い発癌率が急速に増加することから，慢性肝炎の段階での診断と適切な治療がきわめて重要である．

治療方針

治療の第1の目標はHCVの排除である．現在，HCVを排除することができるのはインターフェロン(IFN)を用いる抗ウイルス療法のみである．かつてのIFN単独療法では全体の1/3の患者でウイルス排除が可能であるにすぎなかったが，リバビリン(RBV)を併用することで格段に治療効果が高まっている．また，IFNにポリエチレングリコール(Peg)を結合させたPeg-IFNは週1回の投与で持続的な効果を発揮し，治療効果・認容性に優れ副作用も軽減されている．Peg-IFN + RBV併用療法はきわめて強力であるが，その一方で副作用が多く，なかには重篤なものも起こりうることから慎重な適応判断が望まれる．一方，ウイルス排除が困難な場合やIFNが無効の場合は，肝炎を沈静化し肝発癌抑止を目指した治療を行うことが必要である．

治療法

❶インターフェロン療法

a) 初回治療：IFNをウイルス排除の目的で用いる場合には，予測される治療成績・副作用を考慮し，最も治療効果が得られる治療法を第1選択とし，年齢・性別・肝線維化の程度・合併症などの感染者(宿主)の条件を加味して決定する．治療成績を規定するウイルス側の因子としては，HCVのgenotype，ウイルス量が知られており，genotype 1b(セログループ1)では，さらにHCVの遺伝子変異(ISDR，コア領域70番，91番のアミノ酸置換)などが知られている．すなわちセログループ2型は1型よりIFN感受性が高く，ウイルス量は少ないと治療効果が高い．また，ISDR(インターフェロン感受性領域)内のアミノ酸変異数は多いほどIFN感受性が高く，コア領域70番・91番のアミノ酸置換があるとIFN感受性が劣る．

このような背景のもと，厚生労働省の研究班により，初回治療のガイドライン(表11-12)が示されている．ウイルス量は，現在一般的に用いられているreal time PCR法で5.0 log IU/mL以上は高ウイルス量とされ，genotype 1かつ高ウイルス量症例は最も難治であり，最も強力なPeg-IFNα-2b + RBVないしはPeg-IFNα-2b + RBV併用の48週間投与が推奨されるが，この治療法のウイルス排除率は40～50%程度にすぎない．一方，genotype 2では，この治療法を24週間行えば80～90%の高い治療効果が得られるほか，低ウイルス量症例では，RBV併用療法とIFN単独療法ではいずれも80～90%の高いSVR率で，差を認めないため，初回治療ではIFN単独療法が推奨される．また，治療効果を高めるために，治療開始12週後にHCV-RNA量が治療開始前の1/100以下に低下するがHCV-RNAが陽性(real time PCR法)で，36週までに陰性化した例では72週間投与が可能である．

表11-12　C型慢性肝炎に対する初回治療ガイドライン

	genotype 1	genotype 2
高ウイルス量 1 Meq/mL 5.0 log IU/mL 300 fmol/L 以上	Peg-IFNα-2b + リバビリン (48～72週間) Peg-IFNα-2a + リバビリン (48～72週間)	Peg-IFNα-2b + リバビリン(24週間)
低ウイルス量 1 Meq/mL 5.0 log IU/mL 300 fmol/L 未満	IFN(24週間) Peg-IFNα-2a(24～48週間)	IFN(8～24週間) Peg-IFNα-2a(24～48週間)

C型慢性肝炎の治療(ガイドラインの補足)

1. genotype1，高ウイルス症例へのPeg-IFN + リバビリン併用療法の投与期間延長(72週間投与)の基準：投与開始12週後にHCV-RNA量が前値の1/100以下に低下するがHCV-RNAが陽性(real time PCR法)で，36週までに陰性化した症例では，プラス24週(トータル72週間)の投与期間を延長する．
2. genotype1，高ウイルス症例へのPeg-IFN + リバビリン併用療法で，投与開始36週後にHCV-RNAが陽性(real time PCR法)でもALT値が正常化例は，48週まで継続治療を行い，治療終了後の長期ALT値正常化維持を目指す．
3. Peg-IFN + リバビリン非適応例・無反応例に対するIFN単独長期療法は，最初の2週間は通常量の連日または週3回間欠投与とし，最大8週間でHCV-RNAが陰性化しない症例は通常量の半分量を長期投与する．

〔平成20年度厚生労働科学研究費肝炎等克服緊急対策研究事業(肝炎分野)「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」より転載〕

処方例

〔体重60 kgの場合〕
Peg-IFNα-2b(ペグイントロン皮下注)
80 μg　週1回　皮下注
リバビリン(レベトールカプセル200 mg)　3C(カプセル)　分2　朝1C・夕2C　連日

ペグイントロンは通常1.5 μg/kgを週1回皮下投与する．投与量の目安は体重35～45 kgでは60 μg，46～60 kgで80 μg，61～75 kgで100 μg，76～90 kgで120 μg，91～120 kgでは150 μgである．レベトールは60 kg以下では3C(朝1C・夕2C)，61 kgを超え80 kg以下では4C(朝2C・夕2C)，80 kgを超えるものでは5C(朝2C・夕3C)である．

処方例

Peg-IFNα-2a(ペガシス皮下注)
180 μg　週1回　皮下注

b) 再治療：初回IFN無効例の再治療にあたっては，初回治療の無効の要因を検討することが重要である．すなわち，初回治療時にHCVがいったん陰性化し再燃した例や，治療期間や薬剤投与量が不十分であった症例では再治療によりウイルス排除が可能であり，最も強力なPeg-IFN + RBV治療を行うことが基本である．RBV併用療法を施行する場合には治療効果に寄与する因子(年齢，性別，肝疾患進行度，

表 11-13　C 型慢性肝炎に対する再治療ガイドライン

C 型慢性肝炎に対するインターフェロン(IFN)の再治療は初回治療での無効の要因を検討し，治癒目的の治療か，進展予防(発癌予防)を目指した ALT 値と AFP 値の正常化，あるいは安定化のための治療法を選択すべきである

1. 初回 IFN 無効例への再投与は IFN ＋リバビリン併用療法が，治療の基本である
2. リバビリン併用療法の非適応例あるいはリバビリン併用療法で無反応例では，IFN の長期投与が望ましい．なお，IFNα製剤(Peg 製剤を除く)は，在宅自己注射が可能
3. IFN 非適応例および IFN で ALT 値，AFP 値の改善が得られない症例は肝庇護剤(SNMC，UDCA)，瀉血療法を単独あるいは組み合わせて治療する
4. 進展予防(発癌予防)を目指した治療の ALT 目標値は Stage 1 (F1)では，持続的に基準値の 1.5 倍以下にコントロールする．Stage 2〜3 (F2〜F3)では，極力正常値 ALT ≦ 30 IU/L にコントロールする．
5. リバビリン併用療法を行う場合には治療効果に寄与する因子である，年齢，性別，肝疾患進行度，HCV ウイルスの遺伝子変異(コア領域 70，91 の置換，ISDR 変異)，real time PCR 法によるウイルス量などを参考にし，治療法を選択することが望ましい．

〔平成 20 年度厚生労働科学研究費肝炎等克服緊急対策研究事業(肝炎分野)「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」より転載〕

ISDR やコア領域の HCV の遺伝子変異)などを考慮し治療法を選択することが必要である(表 11-13)．この際，注意を要するのは，Peg-IFNα-2a + RBV 療法は，Peg-IFNα-2b + RBV 療法で無効ないしは再燃例には適応がないことである．一方，RBV 併用療法の非推奨例や無反応例では IFN を単独で 2 週間連日ないしは間欠投与し，8 週以内に HCV が陰性化した場合は完全治癒をめざし 2 年間の長期投与を行うが，8 週時点で HCV が陰性化しない場合には肝発癌予防を目的とし，IFN 単独少量長期療法により肝機能(ALT)の正常化をはかることが必要である．注意を要するのは，Peg-IFNα-2a + RBV 療法は，Peg-IFNα-2b + RBV 療法で無効ないしは再燃例には適応がないことである．

c) 肝発癌抑止を目的とした IFN 療法：HCV の排除が困難な場合でも，IFN 投与によって，肝機能(ALT)の正常化をはかり，肝発癌抑止効果が期待できる．これは「発癌抑制を目指した血清 ALT 正常 C 型肝炎症例の抗ウイルス治療ガイドライン」(表 11-14)にまとめられているが，これによれば血小板数が肝線維化の程度と相関することから，血小板数 15 万/μL 以下の肝線維化進展例では，たとえ ALT が 30 IU/L 以下であっても，肝発癌抑止のためには抗ウイルス療法を考慮することが明記されている．また，最近は IFNα製剤 α-2b 製剤では自己注射も可能となり，治療法の選択肢は広がっている．しかし，IFN は副作用も多いことから慎重な判断が求められる．

処方例

IFNα(HLBI)(スミフェロン DS)　600 万単位　週 3 回　筋注または皮下注

❷肝庇護療法：IFN の治療効果が高まったとはいえ，1/3 の患者ではウイルス排除は困難である．また，高齢者・肝線維化進展例や合併症のために IFN 投与が困難な症例も存在し，IFN 治療によっても ALT が正常化しない例も存在する．この場合，一般に用いられるのは，ウルソデオキシコール酸(UDCA)あるいはグルチルリチン製剤による肝庇護療法である．これらには抗ウイルス効果はみられないものの大きな副作用がなく，一定の効果が認められる．

処方例

強力ネオミノファーゲンシー　40〜60 mL　週 2〜3 回　静注
かつ/または
ウルソデオキシコール酸(ウルソ錠 100 mg)　6 錠　分 3　毎食後

❸瀉血療法：C 型慢性肝炎では肝に過剰な鉄が沈着し，鉄蓄積と肝線維化の程度が相関することや，肝臓の鉄濃度と肝機能成績が相関することから，瀉血により肝の除鉄を行うことの有用性が示されており，特に鉄制限食を併用することが重要と考えられている．IFN が無効な症例には選択肢の 1 つとして，2006 年から保険適用されている．実際には週 1 回 200 g の瀉血を Hb 11.0 g/dL 未満ないしは血清フェリチン値 10 ng/mL 未満となるまで，最長 16 週間繰り返し，その後は Hb 10.5〜10.9 g/dL，血清フェリチン値 10 ng/mL を維持するよう 4 週間ごとに 200〜400 g の瀉血を 1〜3 か月間繰り返す．

【合併症・偶発症】

IFN にはさまざまな副作用がみられる．Peg-IFN の副作用も基本的には IFN と同様に注意深い経過観察が必要である．頻度の高い副作用としては，治療開始早期に出現するものとして発熱・頭痛などのインフルエンザ症状，食思不振・嘔気・味覚障害・心窩部痛・便通異常などの消化器症状，皮疹・掻痒感などの皮膚症状が，治療 2〜3 か月後に出現するものとして，脱毛や抑うつ症状などがある．また，血球成分の減少も知られ，最大の効果発揮と副作用の軽減のためには，血球検査の結果によって投与量を調整することも必要である．特に RBV 併用では溶血性貧血が問題となる．一方，稀には甲状腺機能異常，眼底出血，耐糖能異常，間質性肺炎，脳出血，心筋症などの重篤な副作用も報告されている．特に 65 歳以上で，高血圧，糖尿病を合併しているものでは脳出血の危険性が高まるとされ注意が必要である．

【患者説明のポイント】

IFN 療法にはさまざまな副作用が存在することから，起こりうる副作用と対処法について十分な説明が必要である．特に，普段と異なる症状が出現した場合には主治医に連絡・受診するように説明することが重要である．また RBV には催奇形性が存在することから，投与中と投与終了 6 か月間は避妊の必要を十分説明しておく必要が

表 11-14　発癌抑制を目指した血清 ALT 正常 C 型肝炎例への抗ウイルス治療ガイドライン

ALT 値	血小板数	
	≧ 15 × 10⁴/μL	＜ 15 × 10⁴/μL
≦ 30 IU/L	2〜4 か月ごとに血清 ALT 値フォロー ALT 異常を呈した時点で完治の可能性，発癌リスクを評価し，抗ウイルス療法を考慮	線維化進展例がかなり存在する 可能なら肝生検を施行し F2A2 以上の例に抗ウイルス療法を考慮 肝生検非施行例は 2〜4 か月ごとに血清 ALT 値を測定し，異常を示した時点で抗ウイルス療法を考慮
31〜40 IU/L	65 歳以下は抗ウイルス療法の考慮	慢性肝炎治療に準じる*

*遺伝子型，ウイルス量，年齢などを考慮し，通常の C 型慢性肝炎治療に準じて，治療法を選択する．

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ある．

【医療スタッフへの指示】

IFNの投与中にみられる副作用に抑うつ症状がある．多くは医師の診察時に診断できるが，注射の際の表情の変化や言動により医療スタッフが気づくことがある．この際には医師に連絡するよう指導する．

院内感染予防対策

prevention of hospital-acquired hepatitis B and C viral infection

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【概念】

肝炎ウイルスによる院内感染は，①輸血や血液製剤などによる患者への感染のほか，②病室内や血液透析などの医療行為を介して患者間または患者・医療従事者間で発生することがある．これら肝炎ウイルスによる院内感染は集団発生したり，重篤な肝炎を発症することがあるので，医療機関では院内感染に対する特別な対策が必要である．医療従事者は針刺し事故など観血的処置に伴い受傷することが多く対応を要する．

【頻度】

輸血後肝炎の頻度は0.1%以下であり，1999年の核酸増幅検査(NAT)の導入により血液製剤の安全性はさらに高まった．しかし，感染初期のウインドウ期間の献血液による感染は，きわめて低頻度であるものの完璧な阻止は困難である．ウインドウ期間は，HBs抗原が約59日，HBV-NATが約34日，HCV抗体が約84日，HCV-NATが約23日である．

HBV汚染血液の針事故による肝炎発生の頻度は全体で6～30%である．汚染源がHBe抗原陽性の場合は陰性に比しリスクが高いとされ，肝炎発生率はHBe抗原陽性血液では22～31%，HBe抗原陰性では1～6%で，汚染源のウイルス量(HBV-DNA)が多いほど感染のリスクが高くなる．特に注意すべきは，全体の2～3%は重症化・劇症化することがあり，これらはHBe抗体陽性の汚染源，すなわちHBV precore変異またはcore promoter変異株による感染で発症することが多い．

一方，HCV汚染血液の針刺し事故による肝炎発生の頻度は約1.8%であり，HBVに比し低率であるが，発症すると無治療の場合慢性化する頻度が高い．

【予防策】

医療行為の際，手袋などの標準予防策をとることが基本である．血液・体液を大量に浴びる可能性が高い場合は，必要に応じてマスク，ゴーグル，フェイスシールド，ガウンなどを着用する．針刺し事故の予防の3原則は，①針のリキャップの禁止，②適切な耐貫通性の廃棄容器の管理，③安全・防御装置のついた器材の導入である．

一方，透析ユニットや手術室などの観血的処置が行われる処置室では，患者間の感染を防止するために，①医療器材や薬剤は共用としない，②ディスポーザブル器材を使用する，③適切な消毒と滅菌を行う．④医療者が媒介しない対策(手洗いほか)など基本的な院内感染防止対策を徹底する．内視鏡の洗浄については，日本消化器内視鏡学会から指針が示されているので，これを参考にして適切に実施する．

【針刺し事故後の手順】

1) 事故直後，可及的速やかに，受傷部を流水と石けんで洗浄する．

2) 直ちに受診する．この際，汚染源が特定できる場合は，その症例のHBs抗原，HCV抗体，HIV抗体，RPR，TPLA(必要があればHTLV-1抗体)の有無を検索する．これらが未検査の場合は，患者(あるいは代理人)の了解を得たうえでこれらの検査を至急実施する．針の太さや傷の深さ，注入された血液量，患者血中のウイルス量に大きく左右されるため，詳しく問診する．

3) 院内の取り決め事項に従い，感染症対策委員会などに報告する

4) 労災申請を行う．

5) BまたはC型急性肝炎を発症した場合は，治療を行うとともに，5類感染症として7日以内に最寄りの保健所に届ける．

表11-15　汚染源がHBs抗原陽性の場合の対応

受傷者のHBs抗体	ワクチン接種歴	対応	経過観察
陰性または10 mIU/mL未満	なし	・48時間以内にHBIG投与および ・HBワクチン投与(直後，4週間後，20週間後)	毎月，6か月後までHBs抗体，AST，ALTを測定
	あり(抗体ができなかった)	・48時間以内にHBIG投与 ・ウイルス量が多い場合は1か月後にHBIGの追加投与を考慮	
	あり(抗体はできたが経過とともに抗体価が低下した)	・米国疾病予防管理センターガイドラインでは処置不要としているが，HBワクチン1回投与を考慮(ブースター効果)	経過観察不要 要定期健診
10 mIU/mL以上		・感染防御抗体があるため，薬物投与は不要	経過観察不要 要定期健診
不明(48時間以内に判明しない)	なし	・48時間以内にHBIG投与および ・HBワクチン投与(直後，4週間後，20週間後)	HBs抗体価判明後対応を決定
	あり	・48時間以内にHBIG投与を考慮および ・HBワクチン1回投与を考慮	

【肝炎発症の予防対策】

❶**汚染源が不明の針刺し事故**：直ちに受傷者のHBs抗原，HBs抗体，HCV抗体，HIV抗体，RPR，TPLA，AST，ALTを測定する．必要に応じてHTLV-1抗体を追加する．これらの検査は原則として毎月，6か月後まで施行する．HBs抗体が陰性(10 mIU/mL未満)でHBワクチン未接種の場合は早急にHBワクチンの接種を1クール行うことが必要である．

処方例

ビームゲン　0.5 mL　筋注(あるいは皮下注)　直後，4週間後，20週間後

❷**汚染源がHBs抗原陽性の場合**：直ちに受傷者のHBs抗原，HBs抗体，AST，ALTの測定を行い，表11-15にしたがい対応する．

処方例

1) HBIG：ヘブスブリン-IH 1,000単位＋生理食塩水100 mL 点滴注射　受傷後48時間以内

2) HBワクチン：ビームゲン　0.5 mL 筋注(あるいは皮下注)　直後，4週間後，20週間後

Amino Acid Substitution in Hepatitis C Virus Core Region and Genetic Variation Near the Interleukin 28B Gene Predict Viral Response to Telaprevir with Peginterferon and Ribavirin

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Genetic variation near the IL28B gene and substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG IFN/ribavirin is not clear. The aims of this study were to investigate the predictive factors of sustained virological response to a 12-week or 24-week regimen of triple therapy in 72 of 81 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 61% and 89%, respectively. Especially, the sustained virological response was achieved by 45% and 67% in the 12- and 24-week regimens, respectively. Multivariate analysis identified rs8099917 near the IL28B gene (genotype TT) and substitution at aa 70 (Arg70) as significant determinants of sustained virological response. Prediction of response to therapy based on a combination of these factors had high sensitivity, specificity, and positive and negative predictive values. The efficacy of triple therapy was high in the patients with genotype TT, who accomplished sustained virological response (84%), irrespective of substitution of core aa 70. In the patients having genotype non-TT, those of Arg70 gained high sustained virological response (50%), and sustained virological response (12%) was the worst in patients who possessed both genotype non-TT and Gln70(His70). *Conclusion:* This study identified genetic variation near the IL28B gene and aa substitution of the core region as predictors of sustained virological response to a triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV genotype 1b. (HEPATOLOGY 2010;52:421-429)

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).[1,2] At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 KIU/mL) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.[3] Such background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads.[4,5] Recently, a new strategy was introduced in the treatment of chronic HCV infection by

Abbreviations: aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGTP, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; PEG-IFN, pegylated interferon; PPV, positive predictive value

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means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection.[6] Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.[7,8] Specifically, HCV RNA is suppressed below the limits of detection in the blood in almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin.[9] However, treatment-resistant patients who do not achieve sustained virological response by the triple therapy have been reported.[9-11] The underlying mechanism of the response to the treatment is still not clear.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy,[12-14] and also affect clinical outcome, including hepatocarcinogenesis.[15,16] Furthermore, a recent report showed that aa substitutions in the core region can also be used before therapy to predict very early dynamics (within 48 hours) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin.[17] However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict sustained virological response to triple therapy.

Recent reports showed that genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19 is a host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,[18-21] and also affect clinical outcome, including spontaneous clearance of HCV.[22] However, it is not clear at this stage whether genetic variation near the IL28B gene can be used before therapy to predict sustained virological response to triple therapy.

The present study included 81 patients with HCV-1b and high viral loads who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict sustained virological response, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near the IL28B gene).

Patients and Methods

Study Population. Between May 2008 and September 2009, 81 patients infected with HCV were recruited for this study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki and was approved by the Institutional Review Board. Each patient gave informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy (telaprevir [MP-424], PEG-IFN, and ribavirin) (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

All of 81 patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C. (2) HCV-1 confirmed by sequence analysis. (3) HCV RNA levels of $\geq$5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20-65 years. (6) Body weight $\geq$35 kg and $\leq$120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of $\leq$50 mL/minute at baseline, diabetes requiring treatment or fasting glucose level of $\geq$110 mg/dL, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of $\geq$12 g/dL, neutrophil count $\geq$1500/mm^3, and platelet count of $\geq$100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. Furthermore, 72 of 81 patients were followed for at least 24 weeks after the completion of triple therapy. The treatment efficacy was evaluated by HCV-RNA negative at the end of treatment (end-of-treatment response) and 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 mg or 500 mg

Table 1. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1

Demographic data	
Number of patients	81
Sex (M/F)	44 / 37
Age (years)*	55 (23-65)
History of blood transfusion	24 (29.6%)
Family history of liver disease	13 (16.0%)
Body mass index (kg/m^2)*	22.5 (13.2-32.4)
Laboratory data*	
HCV genotype (1a/ 1b)	1/80
Level of viremia (log IU/mL)	6.7 (5.1-7.6)
Serum aspartate aminotranferase (IU/L)	34 (15-137)
Serum alanine aminotransferase (IU/L)	42 (12-175)
Serum albumin (g/dL)	3.9 (3.2-4,6)
Gamma-glutamyl transpeptidase (IU/L)	36 (9-229)
Leukocyte count (/mm^3)	4,800 (2,800-8,100)
Hemoglobin (g/dL)	14.3 (11.7-16.8)
Platelet count ($\times$ 10^4/mm^3)	17.1 (9.1-33.8)
Alpha-fetoprotein (μ g/L)	4 (2-39)
Total cholesterol (mg/dL)	180 (110-276)
Fasting plasma glucose (mg/dL)	92 (64-125)
Treatment	
PEG-IFNα -2b dose (μ g/kg)*	1.5 (1.3-2.0)
Ribavirin dose (mg/kg)*	11.7 (7.2-18.4)
Telaprevir dose (1,500 / 2,250 mg/day)	10/71
Treatment regimen (T12PR12 group / T12PR24 group)	20/61
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine / glutamine [histidine] /ND)	47/33/1
Core aa 91 (leucine / methionine / ND)	43/37/1
ISDR of NS5A (wild-type / non wild-type / ND)	76/4/1
Genetic variation near IL28B gene	
rs8099917 genotype (TT / TG / GG / ND)	42/30/2/7
rs 12979860 genotype (CC / CT / TT /ND)	42/32/2/5
Past history of IFN therapy	
Treatment-naive / Relapsers to previous treatment / nonresponders to previous treatment	27/33/21

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values. ND, not determined.

three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose 1.5 μg/kg (range: 1.3-2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200-600 mg twice a day after breakfast and dinner (daily dose: 600-1000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm,^{3} or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1000/mm^3, 500/mm^3 or 50,000/mm,^{3} respectively. When hemoglobin decreased to <10 g/dL, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg and 1000 mg to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dL. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 81 patients at the commencement of treatment. They included 44 males and 37 females, ages 23 to 65 years (median, 55 years).

Measurement of HCV RNA. The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2-7.8 log IU/mL, and the undetectable samples were defined as negative.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b. In the present study, aa substitutions of the core region and NS5A-ISDR (IFN-sensitivity determining region) of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by polymerase chain reaction (PCR) using the following primers: (1) Nucleotide sequences of the core region: The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134-153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096-1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234-253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934-953) primers. (2) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5′-ATG CCC ATG CCA GGT TCC AG-3′, nucleotides: 6662-6681) and ISDR2 (antisense, 5′-AGC TCC GCC AAG GCA GAA GA-3′, nucleotides: 7350-7369) primers, and the second-round PCR with ISDR3 (sense, 5′-ACC GGA TGT GGC AGT GCT CA-3′, nucleotides: 6824-6843) and ISDR4 (antisense, 5′-GTA ATC CGG GCG TGC CCA TA-3′, nucleotides: 7189-7208) primers. ([1,2]; nested PCR.) All samples were initially denatured at 95°C for 2 minutes. The 35 cycles of

amplification were set as follows: denaturation for 30 seconds at 95°C, annealing of primers for 30 seconds at 55°C, and extension for 1 minute at 72°C with an additional 7 minutes for extension. Then 1 μL of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo).

With the use of HCV-J (Access. No. D90208) as a reference,[23] the sequence of 1-191 aa in the core protein of HCV 1b was determined and then compared with the consensus sequence constructed on 81 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91).[12] The sequence of 2209-2248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al.[24] was determined and the numbers of aa substitutions in ISDR were defined as wildtype (0, 1) or nonwildtype (≥2).

Genetic Variation Near the IL28B Gene. Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described.[25,26]

In this study, genetic variations near the IL28B gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome,[18-22] were investigated.

Statistical Analysis. Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to sustained virological response. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All P values less than 0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with sustained virological response included the following variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the IL28B gene, and aa substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using SPSS (Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy

Results

Virological Response to Therapy. Sustained virological response was achieved by 44 of 72 (61.1%) patients. In all, 64 of 72 (88.9%) patients were considered end-of-treatment response. According to treatment regimen, sustained virological response were achieved by 45.0% (9 of 20 patients) and 67.3% (35 of 52 patients), in the T12PR12 group and the T12PR24 group, respectively. Of eight patients who could not achieve end-of-treatment response, six (75.0%) patients resulted in reelevation of viral loads regardless of HCV-RNA temporary negative, and the other two patients (25.0%) did not achieve HCV-RNA negative during treatment.

Especially in the T12PR24 group, according to the past history of treatment, sustained virological response were achieved by 76.4% (13 of 17 patients), 86.4% (19 of 22 patients), and 23.1% (3 of 13 patients), in treatment-naive, relapsers to previous treatment, and nonresponders to previous treatment, respectively.

Sustained Virological Response According to Amino Acid Substitutions in Core and NS5A Regions. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions (74.4%) showed sustained virological response than that of patients who showed Gln70(His70) (41.4%) (Fig. 1, $P = 0.007$). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 (65.0%) and Met91 (56.3%) (Fig. 1). Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype

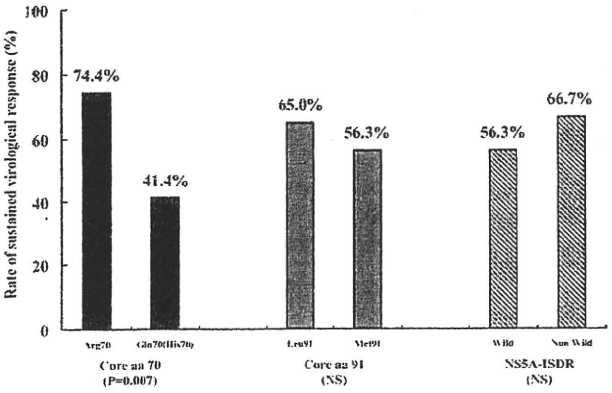

Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ($P = 0.007$). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91. Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype and nonwildtype.

(56.3%) and nonwildtype (66.7%) (Fig. 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

Sustained Virological Response According to Genetic Variation Near the IL28B Gene. According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients) in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Fig. 2, $P < 0.001$) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients), in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Fig. 2, $P < 0.001$) (Table 2).

Predictive Factors Associated with Sustained Virological Response. Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12, $P = 0.007$), genetic variation in rs8099917 (genotype TT; OR 13.6, $P < 0.001$), and rs12979860 (genotype CC; OR 10.8, $P < 0.001$). Two factors were identified by multivariate analysis as independent parameters that significantly influenced sustained virological response (rs8099917 genotype TT; OR 10.6, $P < 0.001$; and Arg70; OR 3.69, $P = 0.040$) (Table 3).

Assessment of Amino Acid Substitutions in Core Region and Genetic Variation Near the IL28B Gene as Predictors of Sustained Virological Response. The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near the IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The nonsustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of nonsustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70 the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

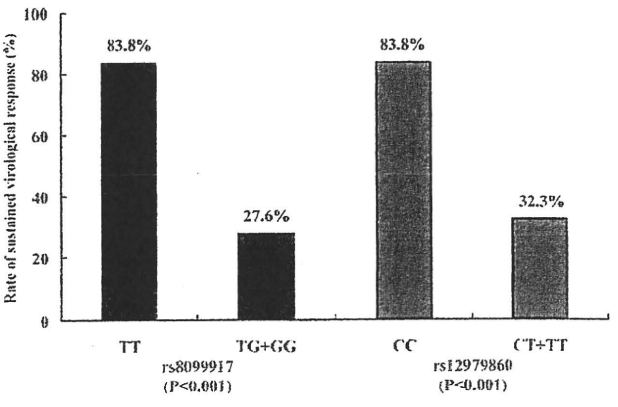

Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ($P < 0.001$ or $P < 0.001$, respectively).

Table 2. According to Genetic Variation Near the IL28B Gene, Background at Commencement of Triple Therapy and Treatment Efficacy

	rs8099917 genotype			rs12979860 genotype		
	TT (n = 42)	TG+GG (n = 32)	TT vs. TG+GG P	CC (n = 42)	CT+TT (n = 34)	CC vs. CT+TT P
Demographic data						
Sex (M/F)	22 / 20	18 / 14	NS	22 / 20	19 / 15	NS
Age (years)*	54 (23-65)	56 (36-65)		54 (23-65)	55 (36-65)	NS
History of blood transfusion	15 (35.7%)	9 (28.3%)	NS	15 (35.7%)	9 (26.5%)	NS
Family history of liver disease	6 (14.3%)	6 (18.8%)	NS	6 (14.3%)	6 (17.6%)	NS
Body mass index (kg/m^2)*	22.1 (13.2-32.4)	22.4 (18.7-26.5)	NS	22.1 (13.2-32.4)	22.3 (18,7-26.5)	NS
Laboratory data*						
HCV genotype (1a / 1b)	0 / 42	1 / 31	NS	0 / 42	1 / 33	NS
Level of viremia (log IU/mL)	6.9 (5.4-7.5)	6.6 (5.1-7.4)	NS	6.9 (5.4-7.5)	6.5 (5.1-7.4)	NS
Serum aspartate aminotransferase (IU/L)	38 (15-118)	31 (20-137)	0.036	38 (15-118)	31 (20-137)	0.031
Serum alanine aminotransferase (IU/L)	50 (12-175)	36 (17-136)	0.029	50 (12-175)	35 (17-136)	0.014
Serum albumin (g/dL)	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS
Gamma-glutamyl transpeptidase (IU/L)	29 (9-194)	53 (9-154)	0.008	29 (9-194)	53 (9-229)	0.004
Leukocyte count (/mm^3)	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS
Hemoglobin (g/dL)	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS
Platelet count ($\times 10^4$/mm^3)	16.8 (9.9-33.8)	17.1 (9.1-24.8)	NS	16.8 (9.9-33.8)	17.8 (9.1-28.8)	NS
Alpha-fetoprotein (μ g/L)	4 (2-39)	5 (2-38)	NS	4 (2-39)	5 (2-38)	NS
Total cholesterol (mg/dL)	184 (112-276)	178 (110-263)	NS	184 (112-276)	178 (110-263)	NS
Fasting plasma glucose (mg/dL)	97 (80-125)	90 (66-111)	0.038	97 (80-125)	91 (66-111)	0.030
Treatment regimen						
T12PR12 group / T12PR24 group	12 / 30	7 / 25	NS	12 / 30	7 / 27	NS
Ammo acid substitutions in the HCV genotype 1b						
Core aa 70 (arginine / glutamine [histidine])	30 / 12	13 / 18	0.016	30 / 12	13 / 20	0.009
Core aa 91 (leucine / methionine)	25 / 17	13 / 18	NS	25 / 17	14 / 19	NS
ISDR of NS5A (wild-type / non wild-type)	39 / 3	30 / 1	NS	39 / 3	32 / 1	NS
Past history of IFN therapy						
Treatment-naive / Relapsers to previous treatment / Nonresponders to previous treatment	16 / 24 / 2	7 / 6 / 19	<0.001	16 / 24 / 2	8 / 7 / 19	<0,001
Treatment efficacy**						
End-of-treatment response (%)	35 (94.6%)	23 (79.3%)	NS	35 (94.6%)	25 (80.6%)	NS
Sustained virological response (%)	31 (83.8%)	8 (27.6%)	<0.001	31 (83.8%)	10 (32.3%)	<0.001

Data are number and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.
**Treatment efficacy according to rs8099917 genotype was evaluated in 66 patients, and that according to rs12979860 genotype was evaluated in 68 patients.

Predicting Sustained Virological Response by Amino Acid Substitutions in Core Region in Combination with Genetic Variation Near the IL28B Gene. Sustained virological response by core aa 70 in combination with rs8099917 genotype is shown in Fig. 3. In patients with rs8099917 genotype TT, sustained virological response was not different between Arg70 (85.7%) and Gln70(His70) (77.8%). In contrast, in patients with rs8099917 genotype TG and GG, a significantly higher proportion of patients with Arg70 (50.0%) showed sustained virological response than that of patients with Gln70(His70) (11.8%) ($P = 0.038$).

Based on a strong power of substitution of core aa 70 and rs8099917 genotype in predicting sustained virological response (Table 3), how they increase the predictive value when they were combined was evaluated. The results are schematically depicted in Fig. 3. Together they demonstrate three points: (1) the efficacy of triple therapy was high in patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70; (2) in patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%); and (3) sustained virological response (11.8%) was the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

Discussion

Two previous studies (PROVE1 in the US, and PROVE2 in Europe) showed that the T12PR12 and T12PR24 group of telaprevir, PEG-IFN, and ribavirin could achieve sustained virological response rates of 35%-60% and 61%-69%, respectively.[10,11] In the

Table 3. Multivariate Analysis of Factors Associated with Sustained Virological Response of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1

Factor	Category	Odds Ratio (95% CI)	P
rs8099917 genotype	1: TG+GG	1	
	2: TT	10.6 (3.07-36.5)	<0.001
Substitution of aa 70	1: Gln70 (His70)	1	
	2: Arg70	3.69 (1.06-12.8)	0.040

Only variables that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown. 95% CI: 95% confidence interval.

present Japanese study, the sustained virological response rates were 45% and 67% in the T12PR12 and T12PR24 group, respectively, as in the two previous studies. There were differences at three points between the present study and two previous studies: (1) PEG-IFN in two previous studies was used at a fixed dose of PEG-IFNα-2a, but that of the present study was a body weight-adjusted dose of PEG-IFNα-2b; (2) The body mass index of our patients (median; 23 kg/m^2) was much lower than that of the participants of the previous study by McHutchison et al.[10] (median; >25 kg/m^2); and (3) The present study was performed based on Japanese patients infected with HCV-1b, except for only one patient with HCV-1a. Especially in PROVE-1, the viral breakthrough rate was higher in HCV-1a subjects compared to HCV-1b, and one of the reasons might be due to the low genetic barrier to the emergence of the R155K variant in HCV-1a.[10,27] Further studies of a larger number of patients matched for background, including genotype, race, body mass index, treatment regimen, and past history of IFN therapy are required to investigate the rate of the sustained virological response by triple therapy.

IL28A, IL28B, and IL29 (IFN-λ-2, IFN-λ-3, and IFN-λ-1, respectively) are novel IFNs identified recently.[28,29] They are similar to type 1 IFNs in terms of biological activities and mechanism of action, in contrast to their differences in structure and genetics.[30] The antiviral effects of IFN-λ against hepatitis B virus and HCV have been reported.[31] Furthermore, α and λ IFNs act synergistically against HCV.[32-34] Recent reports showed that genetic variation near the IL28B gene (rs8099917, rs12979860) are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,[18-21] and also affect clinical outcome, including spontaneous clearance of HCV.[22] At the 2009 meeting of the American Association for the Study of Liver Diseases, Thompson et al.[35] reported that genetic variation near the IL28B gene also affected the viral suppression in the first 2 to 4 weeks of PEG-IFN plus ribavirin, and this phenomenon probably explains much of the difference in treatment response rate. The present study is the first to report that genetic variation near the IL28B gene significantly also affect sustained virological response by triple therapy. These results should be interpreted with caution because races other than Japanese populations were not included. Any generalization of the results should await confirmation by studies of patients of other races to explore the relationship between genetic variation near the IL28B gene and the response to triple therapy.

The present study indicated that the use of the combination of aa substitution of the core region and genetic variation near the IL28B gene had high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response. The efficacy of triple therapy was high in the patients with TT, irrespective of substitution of core aa 70. In the patients having non-TT, those of Arg70 gained high sustained virological response, and sustained virological response was the worst in patients who possessed both non-TT, and Gln70(His70). Along with a high sustained virological response, combined PEG-IFN and ribavirin are accompanied by severe side effects and entail high

Table 4. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Sustained Virological Response, According to Substitution of Core aa 70 and Genetic Variation Near IL28B Gene

	% (Number)			
	Sensitivity	Specificity	PPV*	NPV**
(A) rs8099917 genotype TT	79.5 (31/39)	77.8 (21/27)	83.8 (31/37)	72.4 (21/29)
(B) Substitution at aa 70 of arginine (Arg70)	76.9 (30/39)	63.0 (17/27)	75.0 (30/40)	65.4 (17/26)
(A) and (B)	61.5 (24/39)	85.2 (23/27)	85.7 (24/28)	60.5 (23/38)
(A) and/or (B)	94.9 (37/39)	55.6 (15/27)	75.5 (37/49)	88.2 (15/17)

*PPV; Sustained virological response rates for patients with a combination of Arg70 or rs8099917 genotype TT (prediction of sustained virological response).

**NPV; nonsustained virological response rates for patients with a combination of Gln70(His70) or rs8099917 genotype non-TT (prediction of nonsustained virological response).

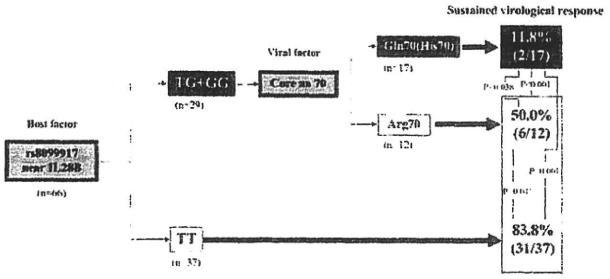

Fig. 3. Predicting sustained virological response by aa substitution in core region in combination with genetic variation near the IL28B gene. Efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70. In the patients having genotype TG and GG, those of Arg70 gained a high sustained virological response (50.0%), and sustained virological response (11.8%) were the worst in patients who possessed both genotypes TG and GG, and Gln70(His70).

costs. Hence, the patients who do not achieve sustained virological response need to be identified as early as possible, in order to free them of unnecessary side effects and high costs. The present study is the first to report that the combination of aa substitution of the core region and genetic variation near the IL28B gene are very useful as pretreatment predictors of sustained virological response by triple therapy, and further studies based on a larger number of patients are necessary to investigate the present results.

Other limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR),[36] were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation,[37] were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both *in vivo* and *in vitro* studies.[38,39] Thus, viral factors before and during triple therapy should be investigated in future studies and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, triple therapy with telaprevir, PEG-IFN, and ribavirin in Japanese patients infected with HCV-1 and high viral load achieved high sustained virological response rates. Furthermore, the aa substitution pattern of the core region and genetic variation near the IL28B gene seem to affect treatment efficacy. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of triple therapy and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

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Influence of Amino-Acid Polymorphism in the Core Protein on Progression of Liver Disease in Patients Infected With Hepatitis C Virus Genotype 1b

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The substitution of amino acid (aa) 70 of arginine for glutamine and/or that of aa91 of leucine for methionine in the core protein in patients infected with hepatitis C virus (HCV) genotype 1b is associated with a poor response to pegylated interferon and ribavirin. Factors influencing these substitutions were sought in 1,097 patients infected with HCV-1b who had not received antiviral treatment. HCV variants with Arg70 and Leu91 (wild-type) decreased, while those with Gln70 and/or Met91 (mutant types) increased with age ($P < 0.001$). Of the 1,097 patients, 464 (42.3%) were infected with the Gln70 variant and the remaining 633 patients with the Arg70 variant. The proportion of patients with the Gln70 variant increased with the severity of liver disease ($P < 0.001$), elevated γ-glutamyl transpeptidase (γ-GTP) levels ($P < 0.001$) and a decrease in platelet count ($P = 0.008$). In univariate analysis patients with hepatocellular carcinoma, elevated aspartate aminotransferase (AST ≥ 58 IU/L) and γ-GTP (≥61 IU/L), and decreased albumin levels (<3.9 g/dl) were more frequent in the patients with the Gln70 variant than the Arg70 variant ($P = 0.003$, 0.005, <0.001, and 0.031, respectively). In multivariate analysis HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 IU/L (1.647 [1.268–2.139]) increased the risk for the Gln70 variant. In conclusion, the substitution of amino aa70 of Arg for Gln in patients infected with HCV-1b increases with age, and it is associated with severe liver disease accompanied by elevated AST and γ-GTP levels, as well as the development of hepatocellular carinoma. ***J. Med. Virol. 82:41–48, 2010.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: cirrhosis; core protein; hepatitis C; hepatocellular carcinoma; interferon; ribavirin

INTRODUCTION

Worldwide, an estimated 170 million people are infected with hepatitis C virus (HCV) persistently [Cohen, 1999]. Decompensated cirrhosis and hepatocellular carcinoma (HCC) can develop in about 30% of patients infected with HCV [Alberti et al., 1999; Seeff, 2002]. HCV has six major genotypes and dozens of subgenotypes, and they have distinct geographic distributions and are associated with the progression of liver disease [Simmonds, 1995]. Host and virological factors can influence the severity of liver disease and the response to antiviral treatment. HCV infection in the childhood and women runs a milder course than that in adulthood and men, and the intake of alcohol accelerates the progression of liver disease [Poynard et al., 1997; Kenny-Walsh, 1999; Vogt et al., 1999; Wiese et al., 2000]. Genotypes 1 and 4 aggravate liver disease and decrease the response to antiviral treatment, in comparison with genotypes 2, 3, and 6 [Tsubota et al., 1994; Hui et al., 2003; Hadziyannis et al., 2004; Legrand-Abravanel et al., 2005; Yuen and Lai, 2006]. High levels of HCV RNA in the serum can induce severe liver disease and decrease treatment response [Tsubota et al., 1994].

In Japan, genotype 1b in a high viral load (>100 KIU/ml) accounts for >70% of HCV infection, and decreases the treatment response in patients with chronic hepatitis C [Kumada et al., 2006]. Even with pegylated interferon (PEG-IFN) combined with ribavirin, the sustained virological response for longer than 24 weeks after the withdrawal of treatment is achieved merely in

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