文　献

1) Thenappan A, Li Y, Shetty K et al : New therapeutics targeting colon cancer stem cells. Curr Colorectal Cancer Rep 5 : 209, 2009
2) Howe JR, Roth S, Ringold JC et al : Mutations in the SMAD4/DPC4 gene in juvenile polyposis. Science 280 : 1086–1088, 1998
3) Miyaki M, Kuroki T : Role of Smad4 (DPC4) inactivation in human cancer. Biochem Biophys Res Commun 306 : 799–804, 2003
4) Waite KA, Eng C : From developmental disorder to heritable cancer: it's all in the BMP/TGF-beta family. Nat Rev Genet 4 : 763–773, 2003
5) Yang X, Li C, Herrera PL et al : Generation of Smad4/Dpc4 conditional knockout mice. Genesis 32 : 80–81, 2002
6) Elliott RL, Blobe GC : Role of transforming growth factor Beta in human cancer. J Clin Oncol 23 : 2078–2093, 2005
7) Hruban RH, Adsay NV : Molecular classification of neoplasms of the pancreas. Hum Pathol 40 : 612–623, 2009
8) Hahn SA, Schutte M, Hoque AT et al : DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271 : 350–353, 1996
9) Hahn SA, Seymour AB, Hoque AT et al : Allelotype of pancreatic adenocarcinoma using xenograft enrichment. Cancer Res 55 : 4670–4675, 1995
10) McCarthy DM, Hruban RH, Argani P et al : Role of the DPC4 tumor suppressor gene in adenocarcinoma of the ampulla of Vater: analysis of 140 cases. Mod Pathol 16 : 272–278, 2003
11) Bartsch D, Hahn SA, Danichevski KD et al : Mutations of the DPC4/Smad4 gene in neuroendocrine pancreatic tumors. Oncogene 18 : 2367–2371, 1999
12) Wilentz RE, Iacobuzio-Donahue CA, Argani P et al : Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res 60 : 2002–2006, 2000
13) Duda DG, Sunamura M, Lefter LP et al : Restoration of SMAD4 by gene therapy reverses the invasive phenotype in pancreatic adenocarcinoma cells. Oncogene 22 : 6857–6864, 2003
14) Friess H, Yamanaka Y, Buchler M et al : Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 105 : 1846–1856, 1993
15) Biankin AV, Morey AL, Lee CS et al : DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol 20 : 4531–4542, 2002
16) Zhao S, Venkatasubbarao K, Lazor JW et al : Inhibition of STAT3 Tyr705 phosphorylation by Smad4 suppresses transforming growth factor beta-mediated invasion and metastasis in pancreatic cancer cells. Cancer Res 68 : 4221–4228, 2008
17) Bardeesy N, Cheng KH, Berger JH et al : Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev 20 : 3130–3146, 2006
18) Ijichi H, Chytil A, Gorska AE et al : Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. Genes Dev 20 : 3147–3160, 2006
19) Izeradjene K, Combs C, Best M et al : Kras (G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. Cancer Cell 11 : 229–243, 2007
20) Kojima K, Vickers SM, Adsay NV et al : Inactivation of Smad4 accelerates Kras (G12D)-mediated pancreatic neoplasia. Cancer Res 67 : 8121–8130, 2007

*　　*　　*

IL28B

坂本直哉*,** 中川美奈*,** 渡辺 守**

索引用語：インターフェロンλ，SNP（遺伝子多型）

1 はじめに

わが国におけるC型慢性肝炎の抗ウイルス療法は，1992年に認可されたインターフェロン(IFN)がその中心的役割を果たしてきた．現在，ペグインターフェロン(PEG-IFN)・リバビリン(RBV)併用療法が日本および世界の標準治療となっており，難治例であるゲノタイプ1型高ウイルス量症例のSVR率は40～50％である[1,2]．しかし，長期にわたる治療期間や副作用の問題，また副作用などによる薬剤の中止減量が治療効果低下につながることから，治療導入に際してはその効果予測，合併症リスクなど治療前の十分な評価が必要である．またIFNに対する反応性には人種間の差があり遺伝的な治療感受性決定因子が示唆されている[3]．一方2000年にヒトゲノムの配列が決定されて以来，近年の遺伝子情報処理技術の飛躍的な進歩により，さまざまな疾患発症の原因遺伝子の報告が盛んになされている．このような状況で，2009年に初めてC型肝炎の自然経過・治療効果に関連する遺伝子として，IL28Bの遺伝子多型が報告された．

2 C型肝炎の自然経過・治療効果とゲノムワイド解析

田中および筆者らは，PEG-IFN・RBV療法の有効性に関連するヒト遺伝子多型を同定するためゲノムワイド関連解析を実施した．すなわち，同療法が有効であった患者と無効(null-response)であった患者に対して，ヒト遺伝子の約90万カ所の一塩基置換(single nucleotide polymorphism, SNP)をDNA microarray (Affimetrix Human SNP Array)を用いて検出し，個々のSNPの表現型と治療効果の関連性を検討した．その結果，19番染色体上のIL28B遺伝子周辺に治療無効に強く関連する一連の有意なSNPを同定した[4]．すなわち，IL28B遺伝子座の代表的なSNPであるrs8099917 (TT/TG/GG)のminor allele (TG/GG)を持つ患者群は，持たない(TT)患者群に較べ強い有意差をもってPEG-IFN・RBV療法が無効(null

Naoya SAKAMOTO et al: IL28B
*東京医科歯科大学・分子肝炎制御学講座［〒113–8519 東京都文京区湯島 1–5–45］
**同 消化器内科

表1　IL28B SNP（rs12979860）と急性C型肝炎のウイルス消失率
（n=1,008，文献10より改変）

ゲノタイプ	血中ウイルス消失率	P値（対C/C）
T/T	23.4%	4×10^{-7}
C/T	29.5%	4×10^{-11}
C/C	53%	−

response）であった（$p=2.68 \times 10^{-32}$）．さらに年齢，性別，感線維化，治療歴，ウイルス量，ALT，血小板データとともに多変量解析を行った結果，IL28B SNPが治療無効を最も強く予測する独立した因子として同定された（$p<0.001$）．

このIL28Bの関連を示す同様の報告は，独立した3つの研究グループからほぼ同時に報告され，人種を越えた遺伝要因であることが証明された[5,6]．さらにGeらは，白人，黒人，ヒスパニックの患者群で検討した結果，IFN治療感受性に関連するIL28Bのmajor alleleの頻度はアジアで80～90%と最も多く，続いて白人およびヒスパニックが70～80%，そして黒人は30～50%と低値であり，この頻度がPEG-IFN・RBV療法の著効率と正に相関していることが示されている[6]．また，ThomasらはIL-28B SNPがC型急性肝炎の自然治癒率に関連することを報告しており，IL28 major typeの症例は，minor alleleを持つ症例に較べ自然治癒率が約3倍高率であった（表1）．

3　IL28Bとインターフェロンλファミリー

IL28BはIFNλ3と呼ばれ，IL28A（λ2）およびIL29（λ1）と類似の構造を持つファミリー蛋白である．IFNλは2003年にIFN関連蛋白の共通構造に基づいた蛋白データベース検索により同定された新しいクラスのIFNであり，生体における詳細な機能はいまだ不明である[7,8]．IFNλの3つのisoformは共通のクラスIIサイトカインレセプター（IL28R）とIL10Rβ複合体に結合したあと，IFNα同様細胞内でSTAT1/2をリン酸化しIFN誘導遺伝子の発現を誘導する[9]．つまり，IFNλはIFNα・βとレセプター下流のシグナルを共有しているが，シグナル活性化誘導能はIFNα，βに較べて弱い．一方，PEG-IFN・RBV療法抵抗性であるIL28B minor alleleを有する患者ではリンパ球のIL28B発現が有意に低く，IL28B産生不十分な遺伝子型と考えられる[4]．したがって，IFNλを大量投与，または産生誘導する治療が治療不応例に対する新たな方策となる可能性が十分ある．事実，現在PEG化されたIL29製剤の臨床試験が欧州で進行しており，IFN-α製剤に較べ副作用が少なく有効性が期待されている．

4　IL28Bゲノタイピング法

IL28B遺伝子座にはIFN治療効果に強く関連する複数のSNPが存在するが，これらはすべてが強い連鎖不平衡を伴っていおりハプロタイプを形成している．したがって，最も関連の強い特定の一つのSNP（タグSNP）を調べれば残りのSNPを推測することができる．このタグSNPとしては，現在のところrs12979860[6]，またはrs8099917[4,5]の2箇所が*de-facto* standardとして使用され，それぞれ固有の核酸がmajor alleleおよびminor allele

表2　ゲノタイピングに用いられるIL28B SNPs

NCBI reference ID	Allele		報告者
	major	minor	
rs12979860	C	T	Ge, et al. [6]
rs8099917	T	G	Tanaka, et al. [4] , Suppiah, et al. [5]

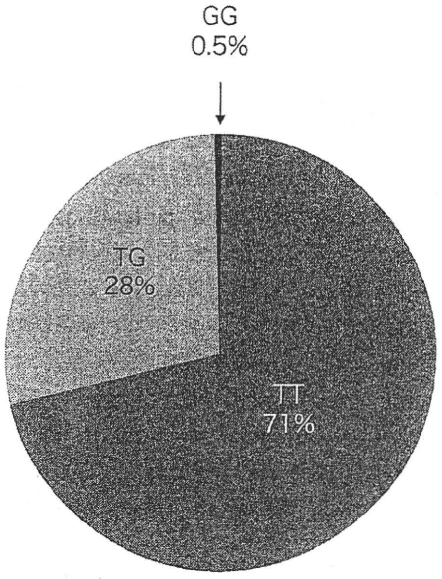

図1　C方慢性肝炎患者におけるIL28B SNP (rs8099917)の頻度

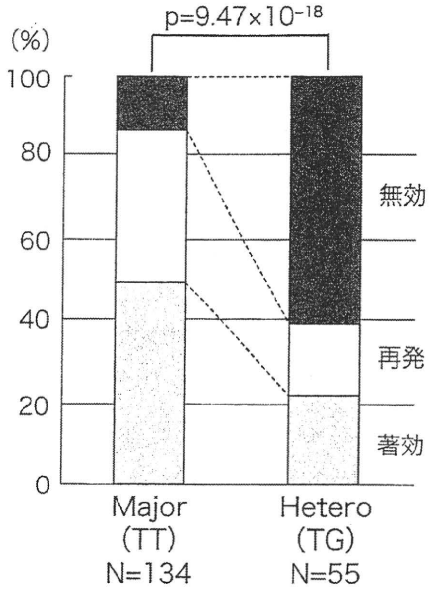

図2　C方慢性肝炎患者，ゲノタイプ1b，高ウイルス量症例におけるIL28B SNP (rs8099917)とPEG-IFN/RBV治療効果

を代表している(表2)．SNPの検出法にはいくつかの方法があるが，少数の特定のSNPを検出するにはシーケンス法，Taqmanアッセイなどが一般的に用いられる．

ゲノタイピングを含む遺伝子検査では生涯変化しない個人の重要な遺伝学的情報が扱われるため，検査実施時に，個人の遺伝学的情報の保護，検査に用いた生体試料の取り扱い，検査前後の遺伝カウンセリングなどを被検者に十分に説明し，インフォームド・コンセントを取った上で行わなければならない．また厚労省の「遺伝子検査に関するガイドライン」に則して，しかるべき施設で倫理委員会の承認を得たうえで施行しなければいけない．

5　IL28B多型とPEG-IFN・RBV治療効果

2004年12月より本学および関連施設で行ったPEG-IFN/Ribavirin療法の最終効果判定可能であったGenotype1,213例を対象にIL28B SNP rs8099917の解析を行い，ウイルス，宿主因子と併せ治療効果との関連を検討した．IL28Bゲノタイプの頻度はTT：73.1%，TG：25.8 %，GG：1.0 % であり，minor allele (G)頻度は0.139であった(図1)．IL28B SNPは治療効果と強く関連しており，SVRはTT群49%に比し，TG + GG群では22%と有意に低率であり($p=9.47\times10^{-18}$)，その61%がnull response (無効)であった．TG，GG群

ではTT群に比し治療早期抗ウイルス効果(RVR, EVR)はいずれも低率であった. ISDR変異2以上の症例ではIL28B多型にかかわらず治療高感受性であったが(SVR TT：71％, TG＋GG：75％), ISDR変異0,1症例ではIL28B変異群でSVRは有意に低率であった(46％ vs. 18％, $p<0.001$). また, IL28B変異とコア70/91変異は連鎖しており, TG＋GG治療抵抗群でコア変異が有意に高頻度であった($p<0.01$). 以上の結果より, genotype 1かつISDR変異2以上の症例ではIL28B多型の如何に関わらず良好な治療効果が得られる一方, ISDR変異1以下, IL28B minor allele症例では高率にnull responseとなる. 現在のところnull responderに対しては有効な再治療法がなく, 今後登場するSTAT-C薬を含んだ新規治療法の早期導入が待たれる.

6 おわりに

C型慢性肝炎の治療にあたっては, IL28Bを含めた詳細な宿主およびウイルス因子解析による治療前効果予測により, 抗ウイルス療法導入症例を慎重に見極めることが治療の質の向上に重要である. 今後新規薬剤の登場とともに上述の宿主遺伝子情報を検討し, 根治の見込める患者を高い的中率で選別し, 治療導入を強く勧め, また治療抵抗性と判定された患者はSTAT-C併用療法, あるいはPEG-IFN-λ療法に振り替えるなど, 治療のオーダーメイド化の展開が期待される.

謝　辞

名古屋市立大学　田中靖人先生

国立国際医療センター　溝上雅史先生

お茶の水リバーカンファレンス共同研究協力施設

文　献

1) Fried MW, Shiffman ML, Reddy KR et al : Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. New England Journal of Medicine 347 : 975–982, 2002
2) Zeuzem S, Feinman SV, Rasenack J et al : Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 343 : 1666–1672, 2000
3) Rosen HR, Gretch DR : Hepatitis C virus: current understanding and prospects for future therapies. Mol Med Today 5 : 393–399, 1999
4) Tanaka Y, Sakamoto N, Nakagawa M et al : Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41 : 1105–1109, 2009
5) Suppiah V, Moldovan M, Ahlenstiel G et al : IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41 : 1100–1104, 2009
6) Ge D, Fellay J, Thompson AJ et al : Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461 : 399–401, 2009
7) Sheppard P, Kindsvogel W, Xu W et al : IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 4 : 63–68, 2003
8) Kotenko SV, Gallagher G, Baurin VV et al : IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 4 : 69–77, 2003
9) O' Brien TR : Interferon-alfa, interferon-lambda and hepatitis C. Nat Genet 41 : 1048–1050, 2009
10) Thomas DL, Thio CL, Martin MP et al : Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461 : 798–801, 2009

*　　*　　*

IL-6と肝炎・肝発癌

植山真由美* 中川美奈*
坂本直哉* 渡辺守*

索引用語：IL-6，肝炎，肝細胞癌，男女差

1 はじめに

多機能性サイトカインとして知られるインターロイキン6(IL-6)は，局所炎症反応や組織障害に反応してマクロファージ・リンパ球・線維芽細胞・血管内皮細胞など多岐にわたる細胞より産生され，局所炎症性細胞に対する作用をはじめとして脳神経・内分泌系，免疫系，造血系細胞に対して幅広く作用する[1]．肝臓においてはクッパー細胞より産生され，CRP・ハプトグロビンなどの急性期蛋白を誘導する生体防御機構に関与する[2]．さらに血管内皮細胞増殖因子(vascular endothelial growth factor; VEGF)の産生を誘導することにより血管新生を促進し肝組織の再生・修復に寄与している(図1)．

肝疾患においてIL-6の重要性を示唆する報告はこれまでなされており，健常人に比べて慢性肝炎，肝硬変，肝細胞癌と病態が進行するにつれて血清IL-6値が高くなるという報告や[3,4]，肝癌マウスのIL-6発現に雌雄で相違があり，肝発癌の性差にIL-6が関与している可能性が示唆されている[5]．本稿ではIL-6と肝炎・肝癌との関連について，肝癌発症率の性差にも着目して概説する．

2 肝臓におけるIL-6発現

肝臓におけるIL-6の発現にはToll様受容体(Toll-like receptor; TLR)が関与している．TLRは細胞表面またはエンドゾームに局在する膜貫通型タンパク質で，TLRを発現している細胞が病原体の侵入を認識するとアダプター分子であるMyd88(Myeloid differentiation factor; 骨髄分化因子)を経由してシグナル伝達経路が活性化し炎症性サイトカインやⅠ型インターフェロン産生が誘導される[6]．肝細胞にウイルス感染もしくは細胞障害性サイトカインが作用して肝細胞が壊死やアポトーシスをきたすと肝壊死物質がクッパー細胞表面上に発現したTLRに結合し，Myd88依存性に転写因子であるNF-κB(nuclear factor-kappa B)を核に移行させIL-6の転写制御が行われる[7]．B細胞リンパ腫細胞株であるRaji細胞にHCVを感染させると細胞表面

Mayumi UEYAMA et al: Interleukin-6 and hepatitis, hepatocellular carcinoma
*東京医科歯科大学大学院医歯学総合研究科消化器病態学［〒113-8510 東京都文京区湯島1-5-45］

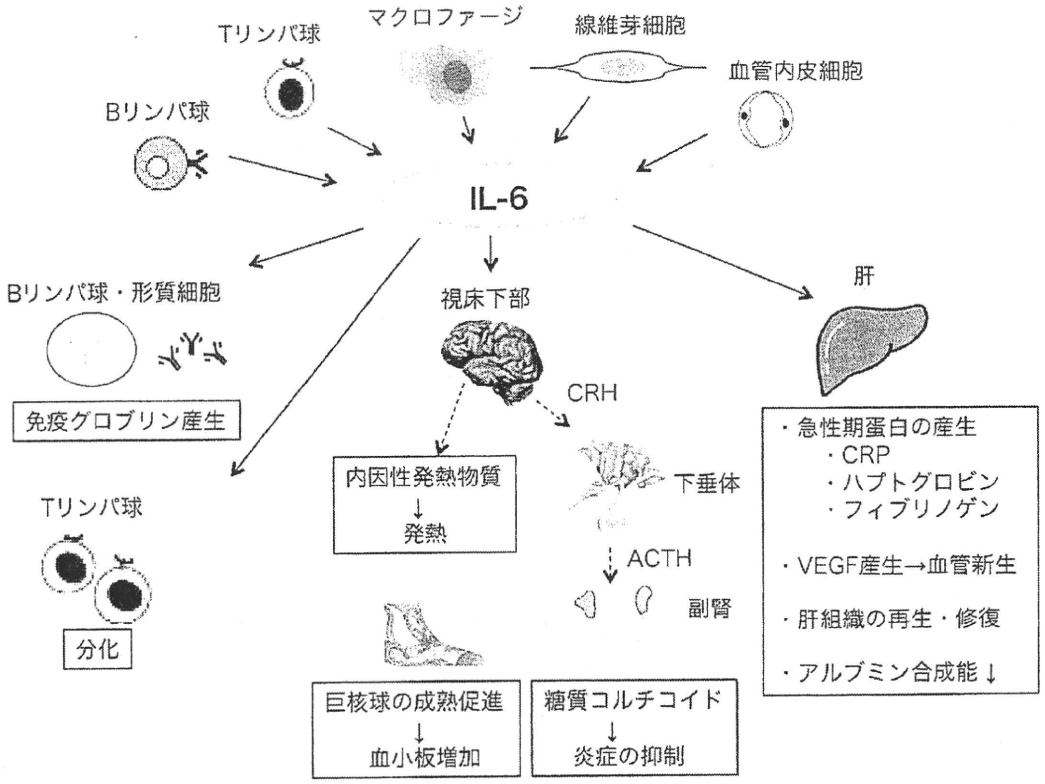

図1　IL-6の発現と全身の作用機序(文献1より引用，一部改訂)

IL-6はリンパ球・単球/マクロファージ・線維芽細胞・血管内皮細胞などから産生される．抗体産生やリンパ球分化への誘導，視床下部からのCRH放出促進などの生体防御反応をはじめ，肝臓では急性期炎症蛋白合成などに関与している．
CRH：副腎皮質刺激ホルモン放出ホルモン　ACTH：副腎皮質刺激ホルモン

にTLR4の発現が誘導され上清中IL-6濃度が上昇することが示されており[8]，IL-6が肝細胞表面上の受容体に結合すると，JAK-STAT経路を介してc-Myc，VEGF，Bcl-xLといった細胞増殖，血管新生促進，抗アポトーシス作用のある遺伝子の発現を調節し[9]，肝炎の遷延ならびに肝細胞癌への進展に関与すると考えられている(図2)．

3　IL-6と肝炎

血清中IL-6は健常人と比してウイルス性肝炎[10]・アルコール性肝炎[11]・NASH[12]などの慢性肝炎や急性肝炎・劇症肝炎で高値となる[13]ことが知られ，肝炎の回復と相関してIL-6値も正常化することから肝障害の程度を評価する指標として有用であると考えられている[13]．C型慢性肝炎に対するインターフェロン(interferon; IFN)治療経過とIL-6の推移については，投与前血清IL-6値がHAI scoreならびにHCV-RNA量と相関しSVR症例では治療後IL-6も低下すること[14]やIFN投与後の体温と血清IL-6値が相関すること[15]，Peg-interferon (Peg-IFN) α2a/Ribavirin (RBV) 併用療法開始後2週目で高値となるが治療終了時には治療前レベルと同等になる[16]という報告がある．

われわれの検証ではPeg-IFN/RBV併用療法を導入したC型慢性肝炎患者173名の治療前血清IL-6値を測定すると，non SVR症例ではSVR症例に比較しIL-6が高い症例の占める

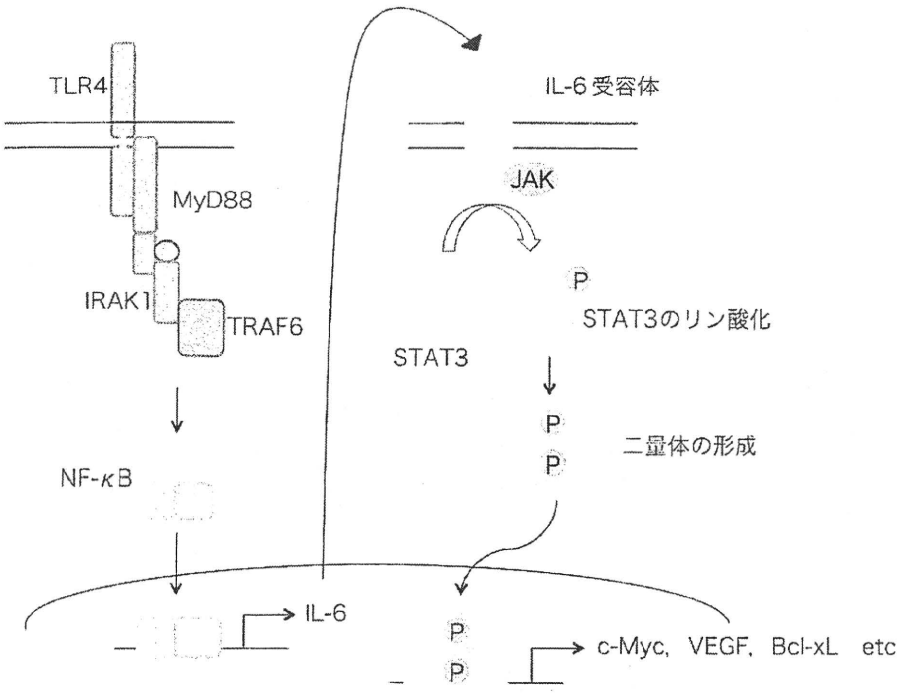

図2　Myd88依存性サイトカイン産生ならびにIL-6のシグナル伝達

TLRにリガンドが結合するとアダプターであるMyd88がTLRと結合し，IRAK1を活性化する．IRAK1はTRAF6と相互作用して転写因子のNF-κBを核に移行させ炎症性サイトカインの発現を誘導する．IL-6は細胞膜上に発現した受容体に結合するとJAK-STAT経路が活性化され，STAT3が二量体を形成後核内に移行し多くの遺伝子転写を制御する．

IRAK1（IL-1 receptor associated kinase），TRAF（TNF receptor associated factor 6），STAT3（Signal Transducers and Activator of Transcription-3）

割合が有意に高かった(カットオフ値:5)．このことからIL-6がIFN治療抵抗性に関与している可能性が示唆された．また，男性，高齢，高ウイルス量，線維化の進展，ISDR変異少，core変異有と治療抵抗性を予測する患者背景の際に高値となる傾向を認めたことから，今後血清IL-6が治療効果予測因子となりうる可能性について引き続き検討中である．

IL-6が肝障害の進行や治療難治の程度と相関するという知見とは相反する報告もあり，肝損傷・肝切除後の再生ならびに肝機能維持において重要な因子であるとの報告もいくつかなされている[17-19]．肝切除後の肝細胞再生は1日以内に始まることが知られているが，TNF-αやIL-6が休止状態にあるG0 phaseにある肝細胞をG1周期へと移行させることが再生の契機となるという[17]．また，IL-6ノックアウトマウスを用いた研究では，IL-6-/-マウスはIL-6+/+マウスと比べて肝細胞のアポトーシスを高率にきたすものの，IL-6投与により肝障害の程度がIL-6+/+マウスと同等にまで回復することが示された[18]．また肝の一部を切除したIL-6-/-マウスでは黄疸・食欲不振を高率に認め死亡率が40％であったのに対し，IL-6+/+マウスならびにIL-6-/-マウスにIL-6を投与したマウス群の死亡率はそれぞれ10％，13.8％と有意に死亡率の低下を認めた[19]．以上のようにIL-6が肝再生や生存率に重要な役割を担っていることがノックアウト

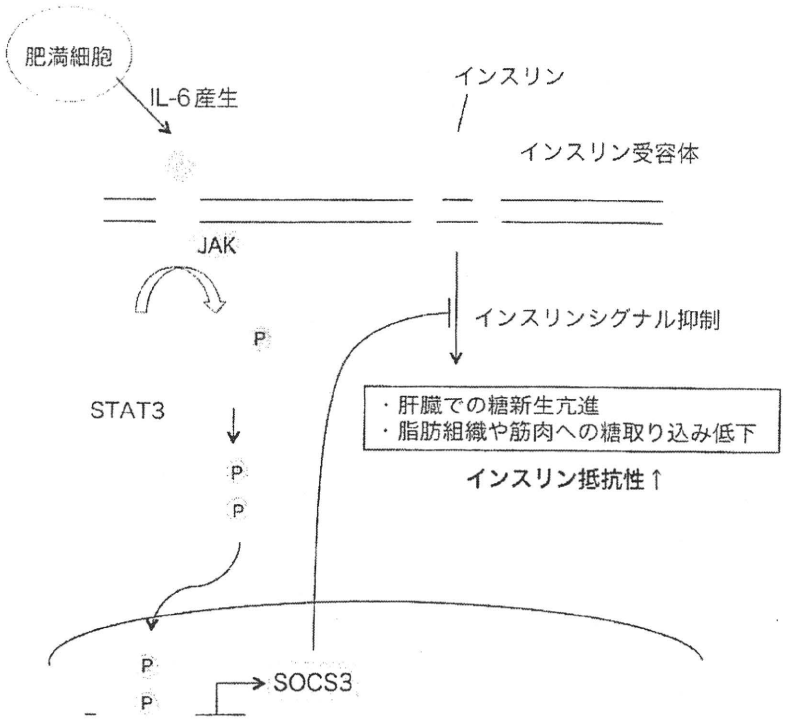

図3　IL-6シグナルとインスリン抵抗性の関係

過剰な脂肪蓄積により肥大化した脂肪細胞からIL-6が分泌される．IL-6が受容体に結合するとSTAT3が活性化されSOCS3の発現が増強する．これが肝臓，筋肉，脂肪組織でのインスリン作用を阻害しインスリン抵抗性を惹起する．

マウスの実験から得られているが，多機能性サイトカインとしてさまざまな働きをもつIL-6の多面性については今後解明すべき点が多いと思われる．

4　IL-6と脂質代謝・インスリン抵抗性

IL-6は血球系細胞などから分泌され，生体防御反応や免疫機能を制御するサイトカインである以外に脂肪組織から分泌される生理活性蛋白(アディポカイン)としての役割も担っており，非炎症時の血中IL-6の約30％が脂肪組織由来であるといわれている[20]．アディポカインは中枢神経系における食欲の制御や血圧調節，末梢組織での糖脂質代謝調節などの多様な作用を併せ持つが，血清中IL-6は肥満者[20]や2型糖尿病患者[21]で高値となることが報告されており，インスリン抵抗性の惹起因子と考えられている．その機序としてSOCS3 (suppressor of cytokine signaling-3) が注目されている．過剰な脂肪蓄積により肥大化した脂肪細胞からIL-6が分泌されIL-6が受容体に結合すると，STAT3 (Signal Transducers and Activator of Transcription-3) が活性化され，SOCS3の発現が増強，SOCS-3が肝臓，筋肉，脂肪組織でのインスリンシグナル伝達を阻害することでインスリン抵抗性が惹起されると報告されている[22] (図3)．また，こうして惹起されたインスリン抵抗性が肝硬変や肝発癌を促進することも報告されていることから[23]，IL-6は糖尿病と肝炎・肝発癌の病態解明の接点としても，今後重要な役割を担う可能性が示唆される．

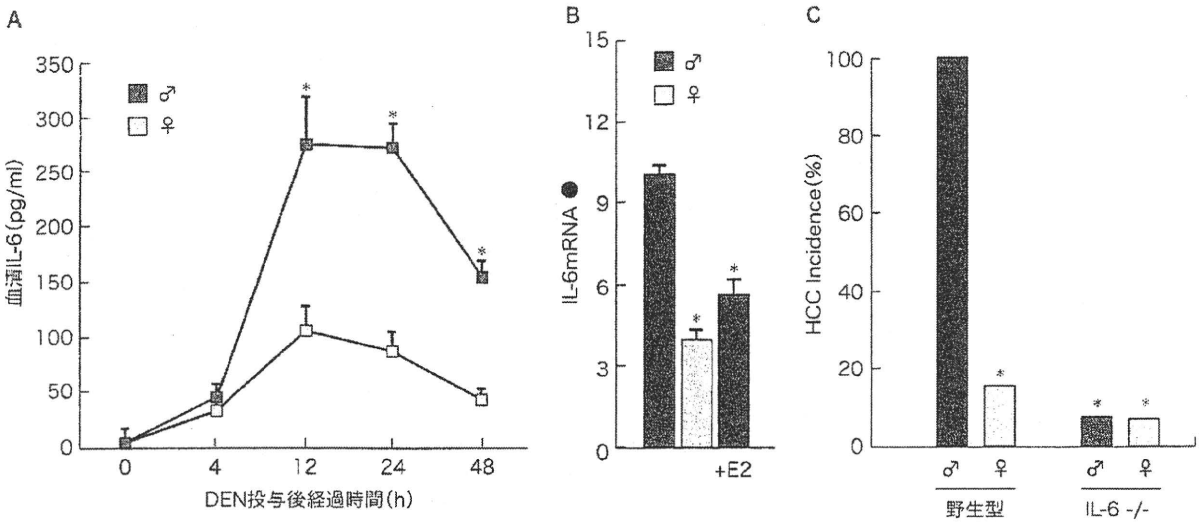

図4　DEN投与後の血清IL-6，肝臓IL-6mRNA，発癌率の性別による比較（文献5より引用，一部改変）

DEN投与後の血清IL-6値は雄では雌の約3倍(A)，肝発現するIL-6mRNAも雄は雌の2倍以上となり，雄にエストラジオールを投与すると雌の発現レベルまで低下した(B)．DEN投与にて雄マウスでは全例肝発癌を認めたのに対し，雌マウスの発癌率は約1割にまで減少し，IL-6-/-雄マウスでの発癌率は野生型雌レベル以下に低下した(C)．

(E2：エストラジオール　*p<0.005)

5　IL-6と肝発癌

全悪性腫瘍の約1～2割は感染や炎症が発生母地となり発症するが，代表的なものとして胃癌(ピロリ菌感染)，大腸癌(炎症性腸疾患)，膵癌(慢性膵炎)などがある．肝細胞癌においてもB型肝炎ウイルス(HBV)やC型肝炎ウイルス(HCV)といった肝炎ウイルスの関与が大きいが[9]，ウイルス性肝炎以外にアルコール性肝炎，非アルコール性脂肪性肝炎(non-alcoholic steatohepatitis; NASH)などからも発生し，これら脂肪性肝炎の病態にはインスリン抵抗性の関与が大きいと考えられている[24]．

肝細胞癌は国籍を問わず男性の有病率が女性よりも高いことが報告されており(男：女＝2：1～4：1)，好発年齢は女性と比べて男性の方が約5年早いことが知られている[25]．男女差が生じる理由としてはウイルス感染率，アルコール摂取量，喫煙率，肥満の差異に由来するとされるが，遺伝子や性ホルモンの関与も報告されている．IL-6と肝炎・肝細胞癌に関してもこれまで種々の報告があり，血清中のIL-6は腫瘍径と相関することや[4]，AFPとともに測定することで診断率が高くなることから肝細胞癌の有効な腫瘍マーカーとして今後有用となり得ると考えられている[26]．

性ホルモンと肝発癌の性差についてMyD88依存性IL-6産生がDEN(diethylnitrosamine)誘発HCC発生に関与していることがNauglerら[5]により明らかにされた．同筆者らはクッパー細胞によるIL-6産生がエストロゲンの介入により阻害されるため女性では肝発癌リスクが低下することを明らかにした．DEN投与後の血清IL-6値は，雄では雌の約3倍となり(図4-A)，肝臓に発現するIL-6mRNAも雄では雌の2倍以上であるのにエストロゲンを投与した雄では雌の発現レベルまで低下した(図4-B)．またDEN投与後に雄

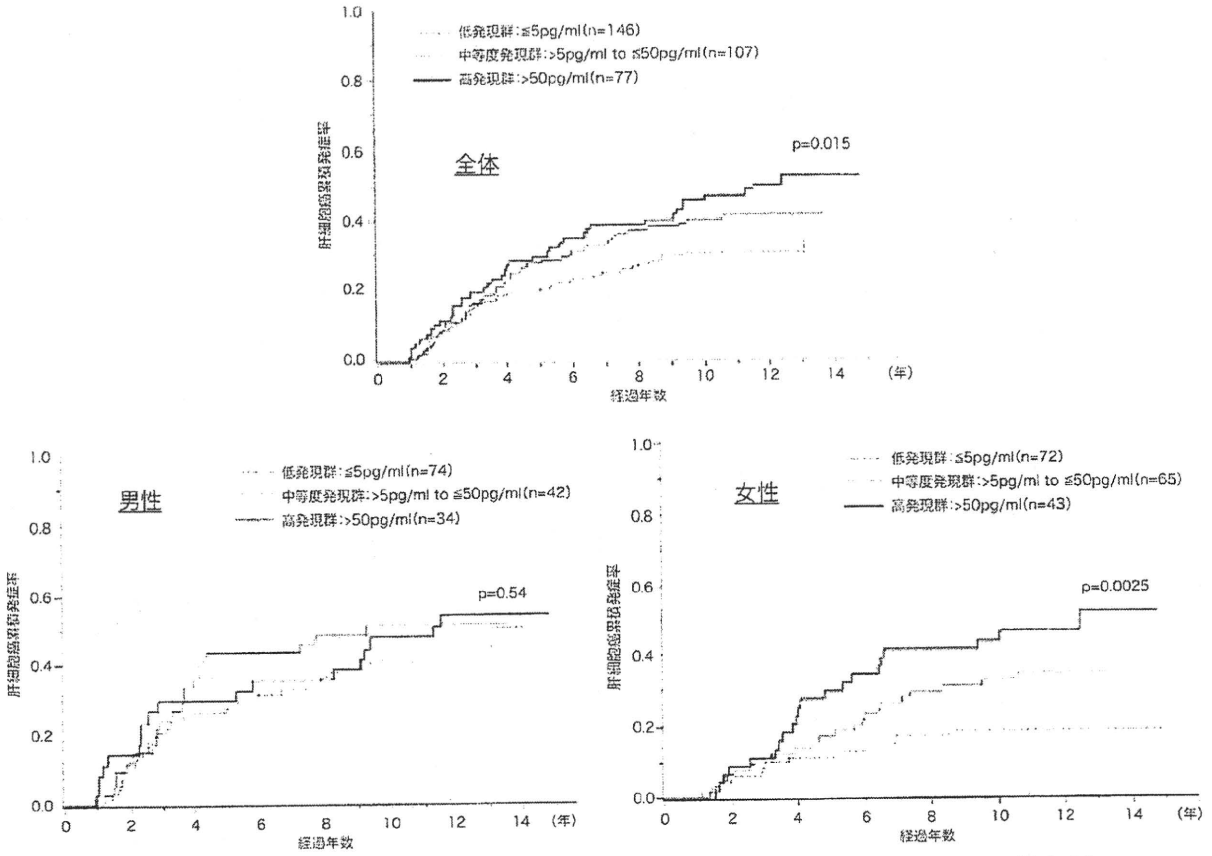

図5　血清IL-6値による肝細胞癌累積発症率の性別による比較（文献28より引用，一部改変）

血清IL-6値により低発現群(<5 pg/ml)，中等度発現群(5～50 pg/ml)，高発現群(50 pg/ml<)に分けて比較したところ，全体としてIL-6が高値になるにつれ発癌率が上昇し，男性よりも女性において有意な相関を認めた．

マウスでは全例肝発癌を認めたのに対し，雌マウスの発癌率は約1割にまで減少し，雄マウスでもIL-6をノックアウトすると発癌率は野生型雌レベル以下に低下したことがわかった(図4-C)．さらにMyd88をノックアウトした雄マウスにおいてもDEN投与後の肝発現IL-6mRNAならびに腫瘍数・腫瘍径は野生型マウスと比すると低下していることを示した．以上よりIL-6はクッパー細胞でTLRのMyd88依存性に発現制御がなされているが，エストロゲンはMyd88より上流でこの経路を阻害し，IL-6発現ならびにDEN誘発肝発癌を抑制していることが明らかとなった．

また，癌抑制遺伝子によるIL-6発現の調節ならびに肝発癌・生存率の関与を検討した報告がある．JiらはS100P(S100 calcium binding protein P)などの発現を制御する癌抑制遺伝子microRNA26a(miR26a)に着目し[27]，Microarrayにてmicro RNA26aの発現が低下するとNF-κBならびにIL-6が関与する遺伝子ネットワークが活性化することを見いだした．腫瘍内miR26a発現量は同一患者の正常組織より低下しているのに対しIL-6mRNAは対照的に腫瘍内において正常組織よりも発現量が高いことを示した．また腫瘍内miR26a発現量が低いと生存期間が短いことからmiR26a発現低下がIL-6発現増加をもたらし発癌・進展に関与すると示唆した．性別に関しては正常組織のmiR26a発現量は女性が男性よりも高いことから男性と比して発

癌率が低い要因であると示唆した．

Nakagawaら[28]は健常人における血清IL-6は男性が女性よりも高値となる傾向を示し，C型慢性肝炎患者の血清IL-6は健常人よりも高値となることを明らかにした．またIL-6と発癌率の関係について検討したところ全体的にはIL-6が高値になるにつれ発癌率が上昇する傾向を認めた．性別で比較すると女性において血清IL-6と発癌率の相関は明確であったのに対し男性では有意差を認めなかった．対象女性の多くが閉経後であったことからエストロゲンとIL-6の逆相関像は明確にはならなかったものの，多変量解析においてエストロゲンに比してIL-6が肝細胞癌発症の独立した危険因子であると考えられた(図5)．

6 おわりに

多機能性サイトカインとしてさまざまな働きをもつIL-6の多面性については今後解明すべき点が多いと思われるが，アディポカインとしての役割も併せもつIL-6はインスリン抵抗性の惹起因子でもあり，肝炎・肝発癌の病態解明に重要な因子であることは間違いない．IL-6を軸とした病態解明が新たな治療戦略へつながる可能性もあり，今後も引き続き検討を重ねてゆく必要があると思われる．

文 献

1) Naka T, Kishimoto T : Joint disease caused by defective gp130-mediated STAT signaling. Arthritis Res 4 : 154–156, 2002
2) Heinrich PC, Castell JV, Andus T : INTERLEUKIN-6 AND THE ACUTE PHASE RESPONSE. Biochemical J 265 : 621–636, 1990
3) Malaguarnera M, DiFazio I, Romeo MA et al : Elevation of interleukin 6 levels in patients with chronic hepatitis due to hepatitis C virus. J Gastroenterol 32 : 211–215, 1997
4) Malaguarnera M, DiFazio I, Laurino A et al : Role of the interleukin 6 in hepatocellular carcinoma. Bull Cancer 83 : 379–384, 1996
5) Naugler WE, Sakurai T, Kim S et al : Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317 : 121–124, 2007
6) Akira S, Takeda K : Toll-like receptor signalling. Nat Rev Immunol 4 : 499–511, 2004
7) Wands J : Hepatocellular carcinoma and sex. N Engl J Med 357 : 1974–1976, 2007
8) Machida K, Cheng KT, Sung VM et al : Hepatitis C virus induces toll-like receptor 4 expression, leading to enhanced production of beta interferon and interleukin-6. J Virol 80 : 866–874, 2006
9) Naugler WE, Karin M : The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14 : 109–119, 2008
10) Lee Y, Park US, Choi I et al : Human interleukin 6 gene is activated by hepatitis B virus-X protein in human hepatoma cells. Clin Cancer Res 4 : 1711–1717, 1998
11) Khoruts A, Stahnke L, McClain CJ et al : CIRCULATING TUMOR-NECROSIS-FACTOR, INTERLEUKIN-1 AND INTERLEUKIN-6 CONCENTRATIONS IN CHRONIC-ALCOHOLIC PATIENTS. Hepatology 13 : 267–276, 1991
12) van der Poorten D, Milner KL, Hui J et al : Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. Hepatology 48 : 449–457, 2008
13) Sun YQ, Tokushige K, Isono E et al : ELEVATED SERUM INTERLEUKIN-6 LEVELS IN PATIENTS WITH ACUTE HEPATITIS. J Clin Immunol 12 : 197–200, 1992
14) Malaguarnera M, DiFazio I, Laurino A et al : Serum interleukin 6 concentrations in chronic hepatitis C patients before and after interferon-alpha treatment. Int J Clin Pharmacol Ther 35 : 385–388, 1997
15) Cotler SJ, Reddy KR, McCone J et al : An analysis of acute changes in interleukin-6 levels after treatment of hepatitis C with consensus interferon. J Interferon Cytokine Res 21 : 1011–1019, 2001
16) Wan L, Kung YJ, Lin YJ et al : Th1 and Th2 cytokines are elevated in HCV-infected SVR(-) patients treated with interferon-alpha. Biochem Biophys Res Commun 379 : 855–860, 2009
17) Michalopoulos GK, DeFrances MC : Liver regeneration. Science 276 : 60–66, 1997

18) Kovalovich K, DeAngelis RA, Li W et al : Increased toxin-induced liver injury and fibrosis in interleukin-6-deficient mice. Hepatology 31 : 149–159, 2000
19) Cressman DE, Greenbaum LE, DeAngelis RA et al. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274 : 1379–1383, 1996
20) Mohamed-Ali V, Goodrick S, Rawesh A et al : Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 82 : 4196–4200, 1997
21) Pickup JC, Mattock MB, Chusney GD et al : NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40 : 1286–1292, 1997
22) Ogawa W, Kasuga M : CELL SIGNALING Fat Stress and Liver Resistance. Science 322 : 1483–1484, 2008
23) Siegel AB, Zhu AX : Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. Cancer 115 : 5651–5661, 2009
24) Sanyal AJ, Campbell-Sargent C, Mirshahi F et al : Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120 : 1183–1192, 2001
25) El-Serag HB, Rudolph L : Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology 132 : 2557–2576, 2007
26) Porta C, Amici M, Quaglini S et al : Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma. Ann Oncol 19 : 353–358, 2008
27) Ji JF, Shi J, Budhu A et al : MicroRNA Expression, Survival, and Response to Interferon in Liver Cancer. N Engl J Med 361 : 1437–1447, 2009
28) Nakagawa H, Maeda S, Yoshida H et al : Serum IL-6 levels and the risk for hepatocarcinogenesis in chronic hepatitis C patients: an analysis based on gender differences. Int J Cancer 125 : 2264–2269, 2009

* * *

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

Norio Akuta,[1] Fumitaka Suzuki,[1] Miharu Hirakawa,[1] Yusuke Kawamura,[1] Hiromi Yatsuji,[1] Hitomi Sezaki,[1] Yoshiyuki Suzuki,[1] Tetsuya Hosaka,[1] Masahiro Kobayashi,[1] Mariko Kobayashi,[2] Satoshi Saitoh,[1] Yasuji Arase,[1] Kenji Ikeda,[1] Kazuaki Chayama,[3] Yusuke Nakamura,[4] and Hiromitsu Kumada[1]

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

From the [1]Department of Hepatology, Toranomon Hospital, Tokyo, Japan; [2]Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan; [3]Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan; [4]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Received February 4, 2010; accepted March 17, 2010.

Address reprint requests to: Norio Akuta, M.D., Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, 105-0001, Japan. E-mail: akuta-gi@umin.ac.jp; fax: +81-000-000000.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.23690

Potential conflict of interest: Nothing to report.

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 aminotranlferase (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 (× 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

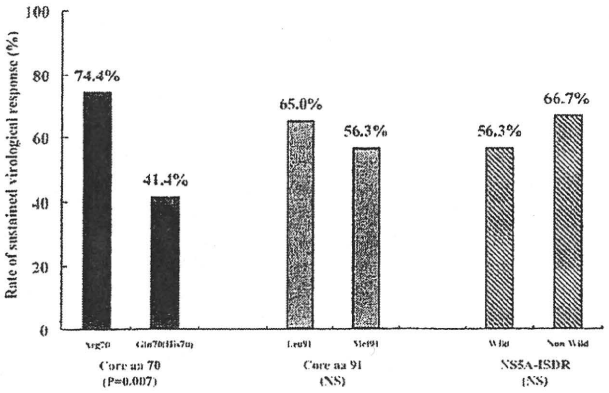

Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ($P = 0.007$). In contrast, according to the substitution of core aa 91, the sustained virological 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).

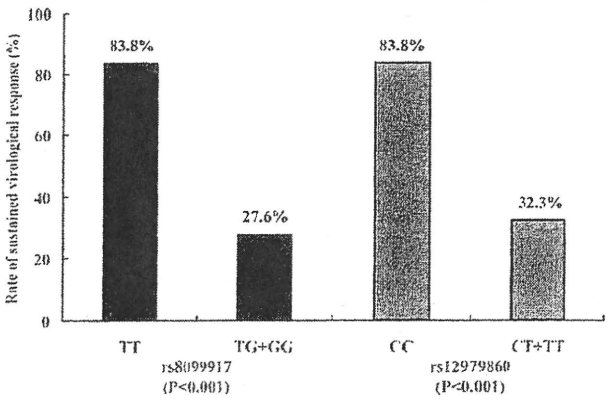

Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ($P < 0.001$ or $P < 0.001$, respectively).

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 (× 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).