2. 肝再生誘導のスイッチとそれを妨げる因子

Induction of liver regeneration, and its inhibitory factors

1. 京都大学医学部附属病院探索医療センター
2. 鹿児島大学大学院医歯学総合研究科消化器疾患・生活習慣病学

井戸　章雄[1]・宇都　浩文[2]
Akio Ido（准教授）　*Hirofumi Uto*（講師）
桶谷　真[2]・坪内　博仁[1,2]
Makoto Oketani（講師）　*Hirohito Tsubouchi*（教授）

Summary

肝臓は，切除や炎症，壊死などでその容量が不足したときに再生が誘導され，一定量を回復したところで終了する。肝再生を促進する増殖因子にはHGF，TGF-α，HB-EGFがある。肝細胞がこれらの増殖因子刺激に反応するためには，TNF-αやIL-6によって静止期(G_0期)にある細胞がG_1期に移行するプライミングステップが必須である。このステップには補体(C3a，C5a)も重要な役割を果たしている。一方，肝再生を抑制する因子としてはTGF-βおよびactivin Aがあり，特にTGF-βは，劇症肝炎における肝再生不全の一因となっていることが示唆されている。細胞外マトリックスは増殖因子の貯蔵や活性の制御に関与しており，肝再生早期の細胞外マトリックスの分解は接着分子を介して肝再生の開始シグナルとして作用している。また，脂肪肝では肝再生が障害されており，そのメカニズムの解明が進められている。

Surgery Frontier 14(2)：15-19，2007

Key Words　肝再生，増殖因子，サイトカイン，細胞外マトリックス，脂肪肝

はじめに

肝臓は古くから再生能力の高い臓器として知られており，正常の肝臓であれば，70％の肝切除を行った場合でも，ヒトでは2～6ヵ月程度，マウスでは2～3週間程度で元の大きさに復する[1]。肝臓の再生は通常の状態では起こらず，切除や炎症，壊死などでその容量が不足したときに誘導され，一定量を回復したところで終了する。一方，劇症肝炎などのように大量の肝臓を喪失すると，逆に肝再生は阻害され，致死的である。このように，肝再生は，それを誘導する因子および抑制する因子によって制御されている。本稿では肝再生を誘導または抑制する因子について述べる。

肝再生を制御する増殖因子，サイトカイン

成熟した肝臓では肝細胞数万個に1個ぐらいの割合で核分裂像が認められるに過ぎないが，肝細胞壊死や部分肝切除によって肝細胞数が減少すると，残存する肝細胞が急速に増殖を開始する。肝再生機序の概要を図1に示す。

TNF-αやIL-6は，通常，炎症性

◆メモランダム◆

HGF(肝細胞増殖因子)

hepatocyte growth factor (HGF)は，劇症肝炎患者の血漿から単離された，肝再生を強力に促進する増殖因子である。HGFは肝再生促進作用のみならず，抗アポトーシス(抗肝炎)作用も有しているため，現在，劇症肝炎を対象として，その組換え蛋白の第Ⅰ相臨床試験/第Ⅱ相臨床試験が医師主導治験として進められている。この試験においてHGFの安全性が確認されれば，肝不全治療薬としての臨床応用が期待される。

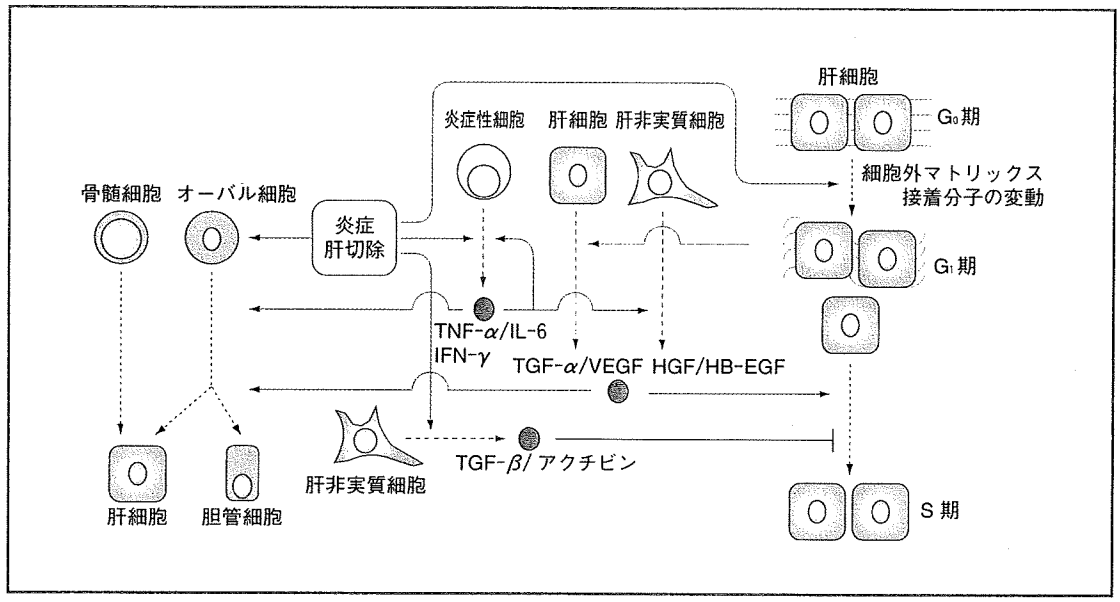

図1　肝再生機序の概要

サイトカインとして作用し，肝細胞に対する増殖促進作用はないが，これらのサイトカインが肝再生において重要な役割を果たしていることは，ノックアウトマウスを用いた解析から明らかにされている[2)3)]。すなわち，TNF-αやIL-6は，静止期(G_0期)にある成熟肝細胞を増殖因子に感受性の高いG_1期に移行させる(図2)。このプライミングとよばれるステップは可逆性であるが，HGF，TGF-α，HB-EGFといった増殖因子刺激によってG_1/Sチェックポイントを超えてS期に移行するためには必須である[4)5)]。また，自然免疫や液性免疫による細胞傷害の重要なエフェクター蛋白である補体も組織の再生に深くかかわっており，C3aおよびC5aがそのノックアウトマウスを用いた解析から，肝再生のプライミングに重要な役割を果たしていることが最近報告された[6)]。

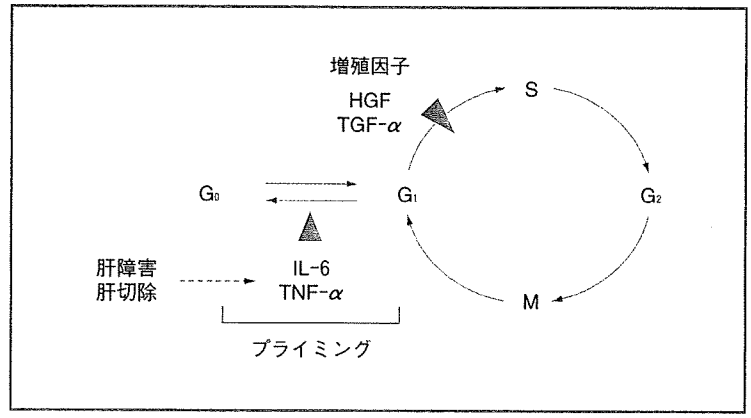

図2　肝再生のプライミングステップとサイトカイン

肝再生そのものに促進的に作用する増殖因子には，tumor necrosis factor (TNF)-α，Cinterleukin (IL)-6，hepatocyte growth factor (HGF)，transforming growth factor (TGF)-α，heparin binding epidermal growth factor-like growth factor (HB-EGF)，vascular endothelial growth factor (VEGF)が知られている。HGFは成熟肝細胞の増殖を強力に促進する増殖因子で，肝臓の発生にも重要な役割を果たしている。肝障害においては，

クッパー細胞，星細胞，血管内皮細胞などでHGFが産生される。HGFおよびその受容体であるc-metのノックアウトマウスでは，肝障害に伴う肝細胞死が著しく増加し，肝再生も強く阻害される[7]。TGF-αは肝細胞で産生されるが，HGFが肝細胞におけるTGF-αの産生を促進することも報告されている[8]。一方，HB-EGFは，部分肝切除後の再生肝内においてHGFやTGF-αに先行してクッパー細胞や血管内皮細胞で産生される[9]。HGFはその特異的受容体c-metを介してシグナルを伝達し，TGF-αとHB-EGFはepidermal growth factor (EGF)受容体(EGFR)を介してそのシグナルを伝達する。HGFは肝細胞増殖を促進する最も強力な因子で，EGFRを介するTGF-αやHB-EGFと協調して肝再生を促進し，その効果は相加的である(図3)[10]。VEGFはラット部分肝切除後に門脈周囲の肝細胞で発現増強し，肝再生を促進する[11]。

肝再生を抑制する因子としてtransforming growth factor (TGF)-β，activin Aが知られている。TGF-βは多様な作用をもつサイトカインであるが，他の多くの細胞に対するのと同様，肝細胞に対しても増殖を抑制する方向に作用し，増殖因子刺激による肝細胞増殖を有意に阻害し(図4)[10]，逆に，抗TGF-β抗体は胆道閉塞下の部分肝切除モデルにおける肝再生を促進する[12]。さらに，肝細胞特異的なTGF-βⅡ型受容体(tgfbr 2)ノックアウトマウスでは，部分肝切除で誘導される肝細胞増殖が増強する[13]。一方，劇症肝炎患者では血清TGF-βレベルが著しく上昇し，また，肝組織中のTGF-β1 mRNA発現も増強していることから，TGF-βは劇症肝炎の肝再生不全の一因となっている可能性が考えられる[14]。Activin Aは静止期の肝細胞で産生され，肝細胞の増殖をオートクリンに抑制しているが，肝障害や肝切除時には

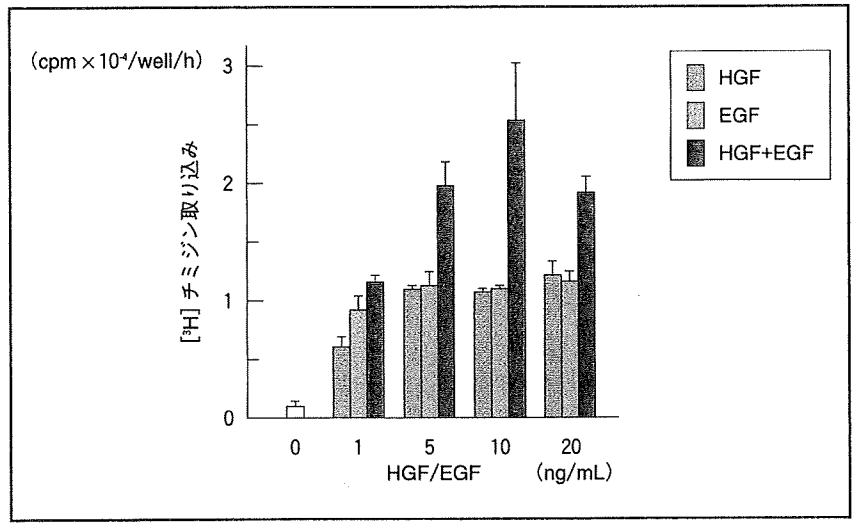

図3　初代培養ラット肝細胞の増殖に及ぼすHGFおよびEGFの相加作用
(文献10より引用)

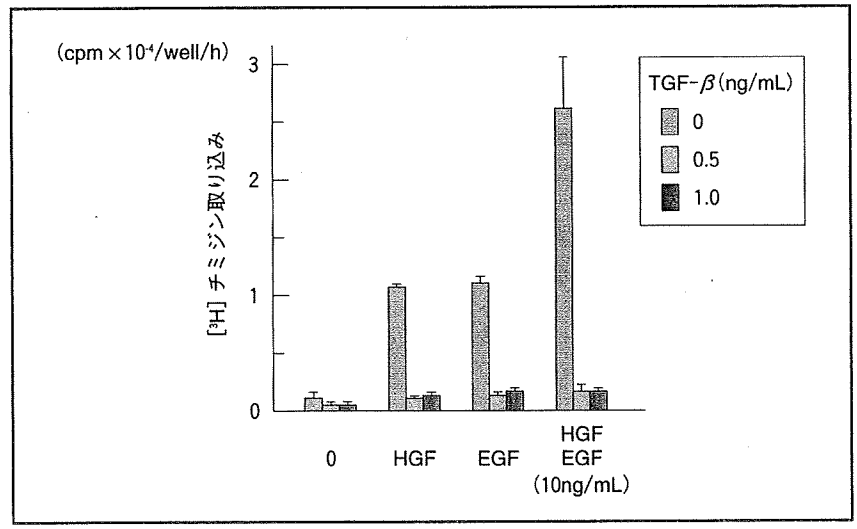

図4　HGFまたはEGFに誘導される肝細胞増殖に対するTGF-βの抑制作用
TGF-βは，HGF，EGFで誘導された初代培養ラット肝細胞のDNA合成活性を抑制する
(文献10より引用)

その発現が減少する。

近年，成熟肝細胞の増殖による肝再生が不可能なまでに肝実質が障害された場合には，肝細胞と胆管上皮細胞の二方向に分化する幹細胞様の細胞や前駆細胞が肝再生に関与することが明らかとなってきた。近年，肝幹(前駆)細胞と考えられるオーバル細胞の増殖および肝細胞分化はHGFで促進されること[15]，またオーバル細胞では成熟肝細胞に比してTGF-βによる増殖抑制が誘導されないことが示された[16]。一方，HGF以外にもfibroblast growth factor (FGF)，オンコスタチンM (oncostatin M ： OSM)などもES細胞や肝芽細胞の肝細胞分化に重要な因子であることが報告されている[17][18]。このように種々の増殖因子が，単に細胞増殖だけではなく，器官形成すなわち組織のリモデリングに重要な役割を果たしていると考えられる。

肝再生における細胞外マトリックスの意義

肝再生における細胞外マトリックスの役割は大きい。細胞外マトリックスはHGF，HB-EGF，TGF-βなどの増殖因子を結合することにより，増殖因子の貯蔵および活性の制御に関与している。HGFはプロテオグリカンと高い親和性を有し，非活性型のプロHGFは，Ⅰ型コラーゲン，Ⅲ型コラーゲン，Ⅴ型コラーゲン，Ⅵ型コラーゲンとも結合する。HB-EGFもプロテオグリカンと親和性を有し，HB-EGFとヘパラン硫酸プロテオグリカンとの結合は，HB-EGFのシグナル伝達を増強させる。また，肝芽細胞の肝細胞分化をさら進めるには，HGFやオンコスタチンMに加えてラミニンといった細胞外マトリックスからのシグナルが必要とされている[19]。一方，肝再生の早期には，セリンプロテアーゼとマトリックスプロテアーゼ(MMP)が活性化され，細胞外マトリックスの分解が開始される。MMP産生はIL-1やTNFなどのサイトカインだけでなく，basic FGF，TGF-βなどの増殖因子によっても誘導される。最近，MMP 9ノックアウトマウスでは部分肝切除後のHGF，TNF-α，VEGF発現が低下し，肝再生が遅延することが報告された[20]。このような肝再生早期の細胞外マトリックスの分解は，接着分子を介して初期遺伝子群の発現や細胞周期の移行など，肝再生の開始シグナルとして作用している可能性がある。

脂肪肝と肝再生

脂肪肝が肝切除術や肝移植術後の合併症および死亡率を高めることは，よく知られた事実である[21]-[23]。最近，非アルコール性脂肪肝(non-alcoholic fatty liver disease ： NAFLD)モデルであるZucker fa/faラットやob/obマウスでは部分肝切除後の肝再生が障害されることが報告され，脂肪化が肝細胞の増殖やアポトーシスなどに関する複数の細胞内シグナル伝達系に影響を及ぼしていることが示された[24][25]。一方，高脂肪食で誘導したNAFLDモデルマウスではinhibitor of nuclear factor-κBα(IκBα)の発現が増強しており，nuclear factor (NF)-κBの活性化とサイクリンD 1およびBcl-xLの誘導が阻害されることで肝再生が障害される[26]。この高脂肪食によるNAFLDマウスとZucker fa/faラットではレプチンレベルが上昇しているが，レプチンを欠損しているob/obマウスでは，肝再生の阻害がレプチン依存性であることも報告された[27]。

おわりに

肝再生の制御機構に関する研究は，主に実験動物において部分肝切除や肝障害モデルを用いて解析が進められてきた。これらは臨床的に肝再生不全が問題となる肝切除術や肝移植術後の肝不全や劇症肝炎の病態を忠実に反映するものではないが，肝再生に影響を及ぼすさまざまな因子について重要な情報を与えてくれている。今後，肝再生の制御機構および肝再生不全の病態が明らかとなり，肝再生を誘導する新たな治療法開発につながることを期待したい。

文　献

1) Michalopoulos GK, DeFrances MC ： Liver regeneration. Science 276 ： 60-66, 1997
2) Yamada Y, Kirillova I, Peschon JJ, et al ： Initiation of liver growth by tumor necrosis factor ： deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci USA 94 ： 1441-1446, 1997
3) Cressman DE, Greenbaum LE, DeAngelis RA, et al ： Liver failure and defective hepatocyte regeneration

in interleukin-6-deficient mice. Science 274 : 1379-1383, 1996
4) Fausto N : Liver regeneration. J Hepatol 32 : 19-31, 2000
5) Fausto N, Campbell JS : The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 120 : 117-130, 2003
6) Strey CW, Markiewski M, Mastellos D, et al : The proinflammatory mediates C 3 a and C 5 a are essential for liver regeneration. J Exp Med 198 : 913-923, 2003
7) Huh CG, Factor VM, Sanchez A, et al : Hepatocyte growth factor/c-met signaling pathways is required for efficient liver regeneration and repair. Proc Natl Acad Sci USA 101 : 4477-4482, 2004
8) Tomiya T, Ogata I, Yamaoka M, et al : The mitogenic activity of hepatocyte growth factor on rat hepatocytes is dependent upon endogenous transforming growth factor-alpha. Am J Pathol 157 : 1693-1701, 2000.
9) Kiso S, Kawata S, Tamura S, et al : Role of heparin-binding EGF-like growth factor as a hepatotrophic factor in rat liver regeneration after partial hepatectomy. Hepatology 22 : 1584-1590, 1995
10) Moriuchi A, Hirono S, Ido A, et al : Additive and inhibitory effects of simultaneous treatment with growth factors on DNA synthesis through MAPK pathway and G_1 cyclins in rat hepatocytes. Biochem Biophys Res Commun 280 : 368-373, 2001
11) Taniguchi E, Sakisaka S, Matsuo K, et al : Expression and role of vascular endothelial growth factor in liver regeneration after partial hepatectomy in rats. J Histochem Cytochem 49 : 121-130, 2001
12) Deneme MA, Ok E, Akcan A, et al : Single dose of anti-transforming growth factor-beta 1 monoclonal antibocy enhances liver regeneration after partial hepatectomy in biliary-obstructed rats. J Surg Res 136 : 280-287, 2006
13) Romero-Gallo J, Sozmen EG, Chytil A, et al : Inactivation of TGF-beta signaling in hepatocytes results in an increased proliferative response after partial hepatectomy. Oncogene 24 : 3028-3041, 2005
14) Miwa Y, Harrison PM, Farzaneh F, et al : Plasma levels and hepatic mRNA expression of transforming groth factor-beta 1 in patients with fulminant hepatic failure. J Hepatol 27 : 780-788, 1997
15) Hasuike S, Ido A, Uto H, et al : Hepatocyte growth factor accelerates the proliferation and differentiation of hepatic oval cells in a 2-acetylaminofluorene/partial hepatectomy model in rats. J Gastroenterol Hepatol 20 : 1753-1761, 2005
16) Nguyen LN, Furuya MH, Wolfraim LA, et al : Transforming growth factor-beta differentially regulates oval cell and hepatocytes proliferation. Hepatology 45 : 31-41, 2007
17) Hamazaki T, Iiboshi Y, Oka M, et al : Hepatic maturation in differentiating embryonic stem cells *in vivo*. FEBS Lett 497 : 15-19, 2001
18) Kamiya A, Kinoshita T, Miyajima A : Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways. FEBS Lett 492 : 90-94, 2001
19) Kamiya A, Kojima N, Kinoshita T, et al : Maturation of fetal hepatocytes *in vitro* by extracellular matrices and oncostatin M : induction of tryptophan oxigenase. Hepatology 35 : 1351-1359, 2002
20) Olle EW, Ren X, McClintock SD, et al : Matrix metalloproteinase-9 is an important factor in hepatic regeneration after partial hepatectomy in mice. Hepatology 44 : 540-549, 2006
21) Todo S, Demetris A, Teperman L, et al : Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation 47 : 903-905, 1989
22) D'Alessandro A, Kalayoglu M, Sollinger H, et al : The predictive value of donor liver biopsies for the development of primary nonfunction after orthotopic liver transplantation. Transplantation 51 : 157-163, 1991
23) Behrn KE, Tsiotos GG, De Souza NF, et al : Hepatic steatosis as a potential risk factor for major hepatic resection. J Gastrointest Surg 2 : 292-298, 1998
24) Selzner M, Clavien PA : Failure of regeneration of the steatotic rat liver : disruption at two different levels in the regeneration pathway. Hepatology 31 : 35-42, 2000
25) Yang SQ, Lin HZ, Mandal AK, et al : Disrupted signaling and inhibited regeneration in obese mice with fatty livers : implications for nonalcoholic fatty liver disease pathophysiology. Hepatology 34 : 694-706, 2001
26) DeAngelis RA, Markiewski MM, Taub R, et al : A high-fat diet impairs liver regeneration in C 57 BL/6 mice through overexpression of the NF-κB Inhibitor, IκBα. Hepatology 42 : 1148-1157, 2005
27) Leclercq IA, Field J, Farrell GC : Leptin-specific mechanisms for impaired liver regeneration in ob/ob mice after toxic injury. Gastroenterology 124 : 1451-1464, 2003

Original Article

Intervirology

Intervirology 2010;53:66–69
DOI: 10.1159/000252787

Published online: January 5, 2010

HCV Genetic Elements Determining the Early Response to Peginterferon and Ribavirin Therapy

Nobuyuki Enomoto Shinya Maekawa

First Department of Medicine, Faculty of Medicine, University of Yamanashi, Chuo, Japan

Key Words
Full open reading frame analysis · Hepatitis C virus · Peginterferon/ribavirin therapy

Abstract
The aim of this study was to search hepatitis C virus (HCV) genetic elements determining the early response to peginterferon/ribavirin therapy using HCV genome-wide analysis. From a total of 88 chronic hepatitis C patients with HCV-1b treated with peginterferon/ribavirin, the whole HCV amino acid sequence was determined and analyzed according to the viral response during the treatment. Mutations in NS5A-ISDR (interferon sensitivity-determining region) are associated with rapid viral response at week 4, and the core arginine70glutamine (R70Q) mutation is associated with no early viral response at week 12, revealing that core 70 and NS5A are the most important factors determining the virological kinetics during peginterferon and ribavirin therapy.

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver diseases, and worldwide 170 million people are infected with HCV. With the introduction of the recent combination therapy of pegylated-interferon (PEG-IFN) and ribavirin (RBV), half of patients can eradicate the virus (sustained virological response, SVR). The SVR rate of HCV to the PEG-IFN/RBV therapy is dependent on HCV genotypes, and the viral kinetics during the treatment strongly affect the final viral clearance [1, 2]. It is generally considered that HCV structures affect the treatment response since the SVR rate to PEG-IFN/RBV therapy depends upon viral genotypes as described above. However, comprehensive analysis of the contribution of HCV structures to different responses has not yet been conducted. In the present study, in order to clarify the relationship between HCV sequences and viral responses, we have determined the complete HCV open reading frame sequences obtained from pretreatment patients' serum, and investigated their response by searching for HCV genetic elements determining the early response to PEG-IFN/RBV therapy using HCV genome-wide analysis.

Methods

A total of 88 chronic hepatitis C patients with HCV-1b treated with PEG-IFN/RBV were studied. From pretreatment sera, the whole HCV deduced amino acid sequence (3,010 amino acids) was determined in each patient by direct RT-PCR.

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0066$26.00/0

Accessible online at:
www.karger.com/int

Nobuyuki Enomoto, MD
First Department of Medicine, Faculty of Medicine
University of Yamanashi
1110 Shimokato, Chuo, Yamanashi 409-3898 (Japan)
Tel. +81 55 273 9584, Fax +81 55 273 6748, E-Mail enomoto@yamanashi.ac.jp

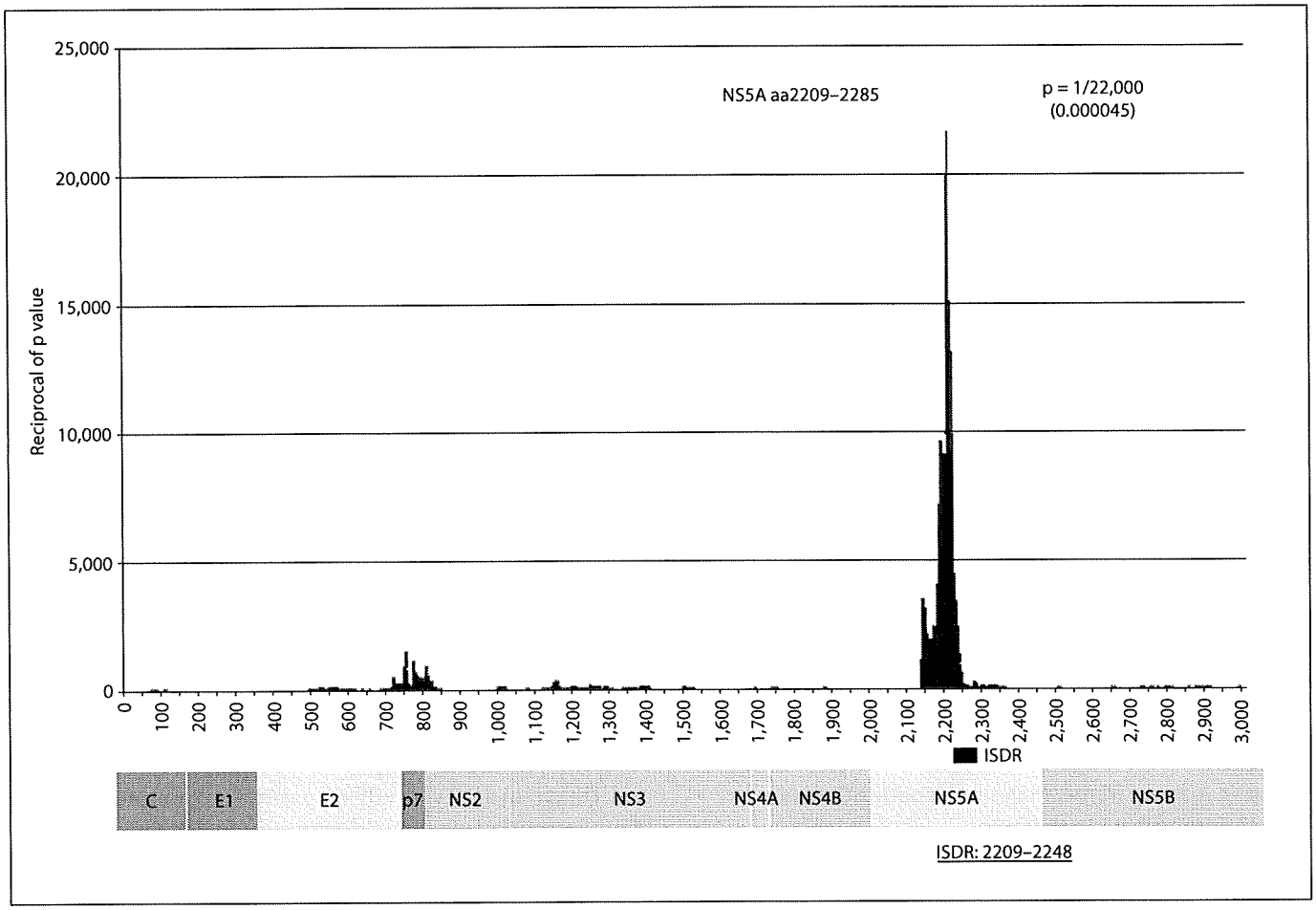

Fig. 1. Reciprocal of p value for sliding window analysis with 77 amino acid width for RVR versus others.

Amino acid usage of each of the 3,010 positions was compared according to the different virological response in order to identify the single amino acid differences determining the virological response. In addition, sliding window analyses were carried out in order to identify the amino acid region associated with the virological response. The number of the amino acid changes in the fixed stretch of the sequence (window: 2–100 amino acids) were compared according to the virological response, scanning the whole HCV amino acid sequence by sliding this window one by one.

Results

Of 88 patients studied, 9 showed rapid viral response (RVR; HCV-RNA undetectable at week 4) and 71 showed early viral response (EVR; over 2-log drop of HCV-RNA at week 12). The other 17 patients showed no EVR, indicating these patients are highly resistant to the treatment.

Mutations in the region overlapping NS5A-ISDR (interferon sensitivity-determining region, aa2209–2248) are associated with the good response to PEG-IFN/RBV therapy as shown in sliding window analysis comparing RVR patients at week 4 and others (fig. 1). In contrast, the core R(arginine)70Q (glutamine) mutation is associated with a poor response resulting in no EVR at week 12 by single amino difference analysis comparing non-EVR patients and the others (fig. 2).

Discussion

In the present study, using a sliding window analysis comparing all HCV amino acids, the amino acid region located in ISDR was extracted as the most significant region discriminating the RVR and non-RVR patients. By

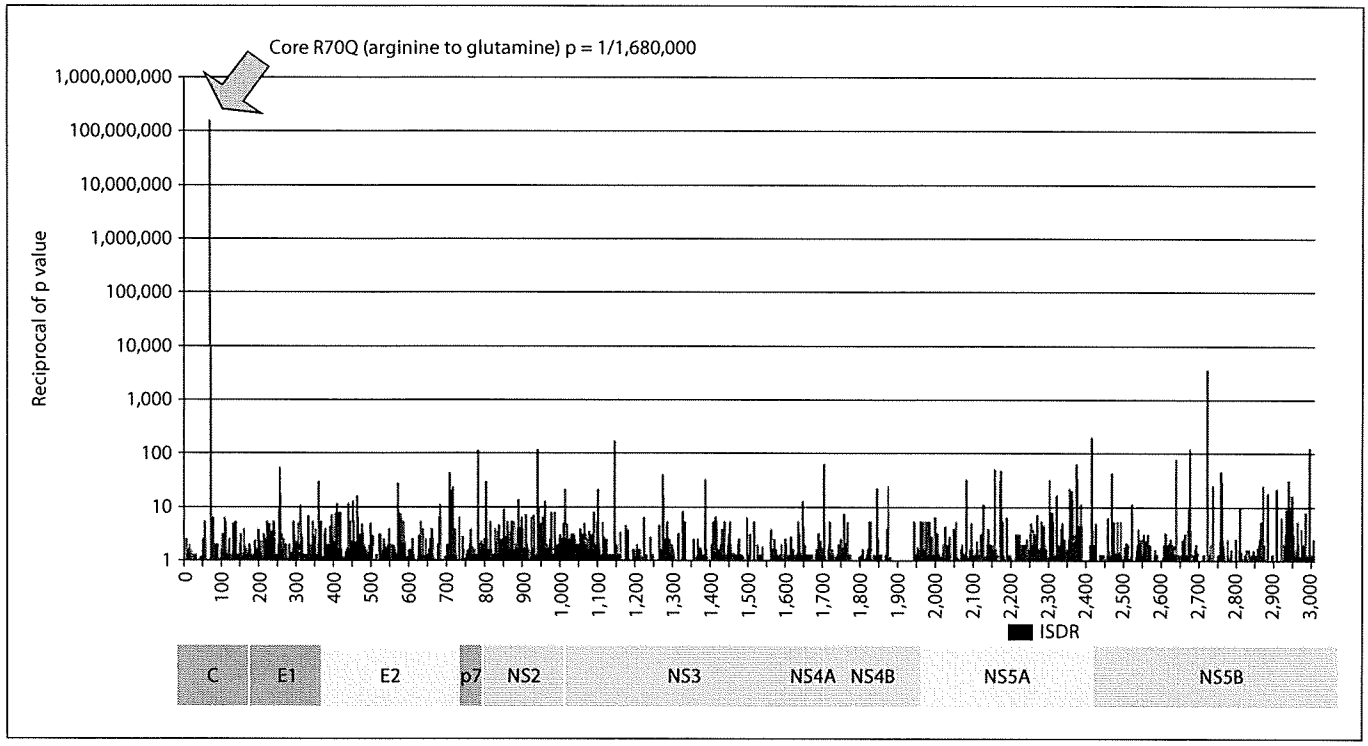

Fig. 2. Reciprocal of p value for single amino acid difference along the whole HCV sequence for non-EVR versus others.

comparing amino acids between the non-EVR patients and the others, remarkable differences were clustered in a single amino acid polymorphism in the core 70. Recent studies have proven that the initial viral response at week 4 and week 12 of the PEG-IFN/RBV therapy could be a useful predictor of the final outcome, indicating that the present findings are important for predicting treatment outcome and individualizing the treatment regimen for each patient as well as understanding the mechanism of diverse response to PEG-IFN/RBV therapy.

ISDR was first identified as the region significantly related to SVR in the era of IFN monotherapy in Japanese patients [3, 4]. 'Mutant type', meaning 4 or more mutations in the region, was associated with high SVR rate, while the rate was low in the 'intermediate type' (1–3 mutations) and wild type (no mutation). Though there were controversies as to the predictive value of ISDR, since studies in Europe and in North America did not necessarily reproduce evident correlation between ISDR and SVR, a recent meta-analysis proved its value by demonstrating a clear relationship all over the world, even in Western countries [5]. The present study reproduced the significance of ISDR in PEG-IFN/RBV therapy. Mutations in ISDR make HCV highly sensitive to IFN, leading to RVR. Current guidelines indicate that RVR patients with low viral load before treatment can be treated with 24 weeks instead of the standard 48 weeks of therapy. Since most ISDR mutant patients show low viral loads, these easy-to-treat patients in genotype 1b should be mainly infected with HCV with ISDR mutations, suggesting ISDR genotyping would identify the patients treatable with the abbreviated regimen.

On the other hand, in the present study, the polymorphism of core 70 was extracted as the most significant position to determine poor virological response in 12 weeks (non-EVR). The contribution of core region amino acid polymorphism in resistance to (PEG-)IFN/RBV therapy was previously reported by Akuta et al. [6], who first found that the polymorphisms in a combination of core 70 and 91 were closely related to the final outcome. The importance of core 70 polymorphism alone, however, was considered rather weak in their study for its smaller p value. Their end point was the final outcome of the treatment, which could be influenced by a variety of factors other than viral genetics, such as host factors (age, sex, fibrosis, body weight, etc.) and treatment (dose of

PEG-IFN/RBV). Further studies are needed to clarify the significance of the core mutations for final outcome of the treatment in the context of the HCV genome-wide analysis.

Different viral responses by polymorphisms in core 70 were also recently suggested in North American patients by Donlin et al. [7]. However, it was reported that the association with core 70 was weaker in their study. Very recently, the IL28B (interferon-lambda-3) gene polymorphism has been found to be closely associated with treatment response in patients in the United States, European Union and Japan by human genome-wide analysis [8–10]. The favorable IL28B genotype is found most frequently in Asian patients, second in European-Americans, and least in African-Americans, indicating that a well-known racial difference in treatment efficacy can be explained by the IL28B polymorphism. The interaction between viral and human genome polymorphisms should be studied further with regard to the treatment response.

Conclusion

HCV genome-wide analysis with a large number of patients successfully revealed that core 70 and NS5A are the most important factors determining the virological kinetics during PEG-IFN/RBV therapy. Viral genome-wide analysis is a promising tool for elucidating the unknown viral factors for different pathological pictures, such as disease progression.

Disclosure Statement

Supported in part by a Grant-in-Aid for Research on Hepatitis (grant ID H19-002) from the Ministry of Health, Labor, and Welfare, Japan. The authors report receiving grant support from Schering-Plough and Roche.

References

1 Neumann AU, Pianko S, Zeuzem S, Yoshida EM, Benhamou Y, Mishan M, et al: Positive and negative prediction of sustained virologic response at weeks 2 and 4 of treatment with albinterferon alfa-2b or peginterferon alfa-2a in treatment-naive patients with genotype 1, chronic hepatitis C. J Hepatol 2009; 51:21–28.

2 Lee SS, Ferenci P: Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. Antivir Ther 2008;13(suppl 1):9–16.

3 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al: Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b: sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.

4 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.

5 Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, et al: Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. Gut 2004;53:1345–1351.

6 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, et al: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.

7 Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, et al: Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol 2007; 81:8211–8224.

8 Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al: Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

9 Suppiah V, Moldovan M, Ahlenstiel G: *IL28B* is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nat Genet 2009;41:1100–1104.

10 Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al: Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nat Genet 2009; 41:1105–1109.

J Gastroenterol (2009) 44:1009–1015
DOI 10.1007/s00535-009-0126-7

REVIEW

Viral factors influencing the response to the combination therapy of peginterferon plus ribavirin in chronic hepatitis C

Shinya Maekawa · Nobuyuki Enomoto

Received: 13 August 2009 / Accepted: 13 August 2009 / Published online: 11 September 2009

Abstract Hepatitis C virus (HCV) is a single-stranded RNA virus known for its high genetic variability owing to the lack of a proofreading mechanism of its RNA dependent RNA polymerase. Until now, numerous studies have been undertaken to clarify the correlation between pretreatment HCV genetic variability and the therapeutic response. Even with the recent combination therapy of peginterferon plus ribavirin for chronic hepatitis C, viral response is variable, and only half of treated patients could clear the virus [sustained viral response (SVR)]. In this review, the contribution of viral genetic variability affecting the treatment outcome is discussed according to each HCV genomic region.

Keywords Hepatitis C virus · Peginterferon plus ribavirin therapy · Viral predictive factor

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver diseases worldwide; 180 million people, or some 3% of the world's population, are infected with HCV. Seventy percent of acute infections become persistent, and 50–75% of patients with chronic HCV infection progress to hepatocellular carcinoma. Though interferon-based therapy for HCV has been greatly advanced, half of patients still cannot eradicate the virus [sustained virological response (SVR)] even with the most recent combination therapy of peginterferon plus ribavirin [1].

HCV has a 9.5 kb single positive stranded RNA genome, and contains a single open reading frame flanked by 5′ and 3′ untranslated regions (UTR). HCV is classified as hepacivirus, a family of flaviridae. HCV is known for its high mutation rate owing to the lack of proofreading activity of its RNA dependent RNA polymerase (1.4×10^{-3} to 1.9×10^{-3} substitutions/nucleotide/year [2, 3]). In accord with this mechanism, HCV presents a high degree of genetic variability, and the resultant molecular polymorphisms of HCV are suspected as one of the major causes determining the treatment responses.

HCV genotypes

By phylogenetic analysis, HCV is classified into six major genotypes, and then further classified into subtypes in each genotype determined by their genetic distances [4]. Among all the viral factors investigated, viral genotypes are the most important, and a well-established predictive factor determining the treatment outcome. Geographically, genotypes 1–3 are associated with worldwide epidemic, while genotypes 4–6 are endemic. In comparison among major genotypes 1–3, a high SVR rate (~84%) was observed in patients with genotype 2 or 3, while a low SVR rate(~42%) was observed in genotype 1 [5–7]. Comparing between genotypes 2 and 3, genotype 2 could have more favorable outcomes [8, 9]. The study of genotype 4 was mainly from Egypt, and the SVR rate was reported to be intermediate (55–69%) [10, 11]. In genotypes 5 and 6, the SVR rate has been considered to be intermediate between the SVR of genotype 1 and genotypes 2–3, but studies

S. Maekawa · N. Enomoto (✉)
First Department of Internal Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan
e-mail: enomoto@yamanashi.ac.jp

S. Maekawa
e-mail: maekawa@yamanashi.ac.jp

focusing on the response of genotype 5 and 6 are limited because of their minor distribution [12].

Genomic regions and the treatment response

5′UTR

The 5′UTR of the HCV genome is 341 nucleotides long, and is the most conserved region throughout the HCV genome among different HCV genotypes. The 5′UTR together with the first 30 nucleotides of the core region acts as an internal ribosome entry site (IRES) regulating the cap-independent translation of HCV RNA to polyprotein. Secondary and tertiary conformation of IRES has the critical role in the initiation of polyprotein translation, and an IRES contains four highly structured domains (Domain I–IV). Since the structures play a pivotal role in HCV replication, changes in the conformation of an IRES, as well as changes in primary nucleotide sequence, result in a decrease of efficiency of protein translation. Therefore, it was suspected that IRES heterogeneity might correlate with the response to interferon-based therapy clinically. Several studies for interferon-based therapy, including peginterferon plus ribavirin, have been undertaken to date, however, its clinical value as a predictive factor for therapy is still in question, since most of these studies showed conflicting results of the relationship between 5′UTR variability and treatment response [13–18].

Core

The core protein is considered to form the viral nucleocapsid of HCV, and its mature form consists of a secondary structure made of a large folded multimer of ~24 monomers. It is 21 kDa in size, and is separated into two domains, an N-terminal two-thirds hydrophilic domain (D1, residues 1–117) and a C-terminal one-third hydrophobic domain (D2, residues 118–170), respectively. The D1 domain contains many positively charged amino acids, and is implicated to bind RNA. The D2 domain is required for proper folding of domain D1. The mature core protein shares high homology among HCV genotypes. The core protein has been reported to interact with a variety of cellular proteins and to influence numerous host cell functions, such as its proapoptotic or antiapoptotic actions [19], immunomodulatory roles [20], or oxidative stress [21]. Recently, much attention has been paid to its relationships with liver steatosis, insulin resistance and hepatocellular carcinoma [22, 23]. HCV core proteins of genotype 3a and 1b were reported to interfere with the insulin signaling pathway in different ways depending on genotype-specific mechanisms [24].

As its contribution to the clinical treatment response, Akuta et al. first reported that substitutions of the amino acid 70 and 91 in the core protein were significantly related to the final outcome in the 48 weeks of interferon plus ribavirin combination therapy in 50 Japanese patients infected with genotype 1b HCV [25]. In successive studies, they reported that substitutions in those core regions were related to the final outcome, viral kinetics, early viral response, and extended 72 weeks of therapy [26–29]. They also reported substitution of core protein was associated with elevated alpha-fetoprotein, and hepatocarcinogenesis [29, 30]. Correlation of substitutions in the core protein in the treatment of interferon-based therapy was also reported in several other studies [31–33].

E2

E2 is a type I transmembrane protein 70 kDa in size which assembles with E1 protein forming a heterodimer to become the mature viral envelope. It is a glycoprotein possessing several potential conserved glycosylation sites. Because the protein is essential for the virus's entry into hepatocytes, E2 interacts with potential HCV receptors, CD81, SR-BI [34] and occludin [35]. In E2, hypervariable region 1 (HVR1) was identified in the first 27 amino acids of the E2 ectodomain. HVR1 is known for its significant genomic variability and is suspected to be the target of antibodies. Its significant genetic variability could be induced by antibody selection.

PePHD

A region between amino acid residues of E2 659–670 is well-conserved, and is known as the phosphorylation site of PKR/eIF-2α phosphorylation homology domain (PePHD). The PePHD motif is similar to the phosphorylation sites of PKR and eIF2alpha. The PePHD has been shown to interact with PKR, one of the important antiviral proteins of the host cell, and inhibit antiviral action of PKR in vitro, suggesting a possible mechanism of HCV for countering the antiviral effects of interferon [36–38]. According to those observations, mutations in this PePHD were suspected to influence the clinical response to interferon-based therapy. However, the results of those studies are conflicting, and its clinical importance as the predictive value for treatment outcome has been controversial. Though some studies supported its significance [39–42], other recent studies could not find evident correlations [43–49].

NS5A

NS5A is phosphorylated on multiple serine and threonine residues, and forms two distinct molecules of basal

phosphorylated form (p56) and hyperphosphorylated form (p58), being 56 and 58 kDa in size, respectively. The protein has three distinct domains (domains I, II, and III) being separated by low complexity sequences (LCS I and II). The study of the X-ray crystal structure analysis of domain I suggested that the NS5A is a dimer, and it forms a large putative RNA binding groove. Recent genetic study has shown many residues in domain II are essential for RNA replication, while domain III is less conserved and might be dispensable. Though the true function of NS5A is still under investigation, the protein is considered as a component of the HCV replication complex, where it modulates HCV replication through interaction with other viral proteins. Among all HCV proteins, NS5A has been most extensively explored for its relationship to interferon-based therapy.

ISDR and PKR-BD

The interferon sensitivity determining region (ISDR), located in the C-terminal half of NS5A, was originally identified as the 40 amino acid region (aa2209–2248) significantly related to the treatment outcome in the monotherapy of interferon-alpha in Japanese patients infected with genotype-1b HCV [50, 51]. The "mutant-type," having 4 or more mutations in the region, was associated with a high SVR rate (16/16: 100%), while the SVR rate was low in the "intermediate-type" [1–3 mutations: SVR rate 5/38 (13%)], or the "wild-type" [no mutation: SVR rate 30/30 (0%)]. Following studies from Japan were also concordant with the initial study [52–54]. However, controversy occurred as to the predictive value of ISDR since studies from Europe and North America did not necessarily report evident correlations between ISDR and treatment outcomes [55–60]. However, a recent meta-analysis study clearly confirmed its value, even in the Western countries [61]. Different results observed in North America and Europe might have been been caused partly by smaller rates of mutant-type patients in Western countries, and by the different treatment regimen in Japan compared to Western countries [62–66]. Though ISDR was found in the era of interferon monotherapy, its predictive value in the treatment outcome of the recent peginterferon plus ribavirin regimen has continued to be reported in most large cohort studies [26, 33, 67–69]. In searching for the biological ISDR function, Gale et al. reported that NS5A represses PKR through a direct interaction with the PKR binding domain (PKR-BD, aa2209–2274) and that the PKR-BD contains the ISDR [70]. Thus, they insisted that inactivation of PKR may be one mechanism by which HCV avoids the antiviral effects of interferon.

V3 domain and IRRDR

The V3 domain located in the C-terminal region of NS5A (aa2356–2379) was originally identified as a genomic region of genotype-1b HCV showing a marked heterogeneity between Japanese and American isolates [71]. A correlation of its mutations and the response to interferon-based therapy was first reported by Duverlie et al., and they reported that sequences of the V3 domain were highly conserved in resistant strains, but were highly variable in sensitive strains [72]. Most following studies also reported concordant results [46, 47, 68, 73, 74]. El-Shamy et al. reported a high degree of sequence variations in the V3 and the flanking pre-V3 regions (aa2334–2355) of NS5A, and they designated the region as the interferon/ribavirin resistance-determining region (IRRDR) (aa2334–2379). They reported that substitution number in the IRRDR was closely correlated with early virological response (EVR) by week 16 in 47 HCV-1b-infected patients treated with peginterferon plus ribavirin [75]. In their follow up study for the same group of patients, sequence variation in the IRRDR was also significantly related to the final outcome. The positive predictive values of IRRDR of 6 or more for SVR was 89% (16/18), whereas negative predictive values of IRRDR of 5 or less for non-SVR was 81% (22/27) [76].

Other region in NS5A

Pfeiffer et al. reported that two responsible mutations resided in the C-terminal region of NS5A: G404S and E442G were considered as mechanisms accounting for ribavirin resistance during HCV RNA replication, using HCV replicon-containing cell lines in the presence of increasing concentrations of ribavirin [77]. However, the clinical importance of such mutations and their relevance to ribavirin-related therapy is not evident.

NS5B

NS5B is 68 kDa proteins in size, and known as an RNA-dependent RNA polymerase. The enzyme synthesizes HCV–RNA using HCV–RNA as a template. NS5B is considered as one component of the HCV–RNA replication complex, and its activity as an RNA polymerase is modulated by NS3 and NS5A. Since this enzymatic activity is critical for HCV replication, the correlation between its mutations and treatment response has been explored, to date, in several studies.

Though the viral inhibitory mechanism of ribavirin in the treatment of HCV is unknown, its action as a mutagen is especially focused on the NS5B protein. During

ribavirin monotherapy, Young et al. reported that a specific mutation of NS5B amino acid 415 Phe-to-Tyr (F415Y) had emerged in five out of five patients infected with genotype-1a HCV [78]. To clarify the biological relevance of this mutation in ribavirin monotherapy, they introduced NS5B F415Y mutations into subgenomic HCV replicons, and reported that they observed different drug sensitivities in HCV replicons according to this NS5B polymorphism in a ribavirin dose-dependent manner. However, subsequent studies done in Japan and the UK could not find an evident relationship between specific selection of NS5B 415 mutations and the treatment of combination therapy of peginterferon and ribavirin. Sugihara et al. reported that they did not find specific mutations in NS5B 415 in the both serum obtained before and after therapy in 18 patients infected with genotype-1b HCV [79], and Ward et al. could not find evidence of a relationship of these mutations in the therapy of peginterferon and ribavirin in 15 patients infected with genotype-1a [80]. Hamano et al. explored genetic changes of genotype-1b HCV during the treatment of interferon-alpha and ribavirin, and reported that mutations at positions 300–358 of NS5B, including polymerase motif B–E, occurred more frequently in SVR patients or in end-of-treatment response patients when compared to null-response patients [81]. Mutation rate of NS5B in patients undergoing treatment with ribavirin monotherapy was also explored in patients treated with peginterferon/ribavirin therapy, since error catastrophe from an increase in mutation rate could be a possible mechanism of ribavirin in HCV infection [82]. Lutchman et al. reported that ribavirin was only associated with an early transient increase in the HCV mutation rate, but lethal mutagenesis and error catastrophe was unlikely to be the sole mechanism of ribavirin [83].

Conclusions

Viral genetic variability of HCV and its potential correlation to the interferon-based treatment response is briefly discussed here. Understanding the biological features of drug-resistant HCV, may helpl us to predict the treatment response in each patient in advance. Furthermore, though trials of HCV specific protease inhibitors are on-going, and are just about to be incorporated into the new standard therapy, understanding those biological features of HCV would further clarify and focus which patients will most benefit from being treated with the new treatment regimens. This viral genetic approach could be crucial even in the era of HCV protease inhibitors for achieving global eradication of HCV.

References

1. Zeuzem S. Interferon-based therapy for chronic hepatitis C: current and future perspectives. Nat Clin Pract Gastroenterol Hepatol. 2008;5(11):610–22.
2. Ogata N, Alter HJ, Miller RH, Purcell RH. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. Proc Natl Acad Sci USA. 1991;88(8):3392–6.
3. Okamoto H, Kojima M, Okada S, Yoshizawa H, Iizuka H, Tanaka T, et al. Genetic drift of hepatitis C virus during an 8.2-year infection in a chimpanzee: variability and stability. Virology. 1992;190(2):894–9.
4. Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005;42(4):962–73.
5. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347(13):975–82.
6. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140(5):346–55.
7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358(9286):958–65.
8. Dalgard O, Mangia A. Short-term therapy for patients with hepatitis C virus genotype 2 or 3 infection. Drugs. 2006;66(14):1807–15.
9. von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, et al. Peginterferon-alpha-2a (40 kDa) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology. 2005;129(2):522–7.
10. El-Zayadi AR, Attia M, Barakat EM, Badran HM, Hamdy H, El-Tawil A, et al. Response of hepatitis C genotype-4 naive patients to 24 weeks of Peg-interferon-alpha2b/ribavirin or induction-dose interferon-alpha2b/ribavirin/amantadine: a non-randomized controlled study. Am J Gastroenterol. 2005;100(11):2447–52.
11. Kamal SM, El Tawil AA, Nakano T, He Q, Rasenack J, Hakam SA, et al. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut. 2005;54(6):858–66.
12. Nguyen MH, Keeffe EB. Prevalence and treatment of hepatitis C virus genotypes 4, 5, and 6. Clin Gastroenterol Hepatol. 2005;3(10 Suppl 2):S97–101.
13. Araujo FM, Sonoda IV, Rodrigues NB, Teixeira R, Redondo RA, Oliveira GC. Genetic variability in the 5' UTR and NS5A regions of hepatitis C virus RNA isolated from non-responding and responding patients with chronic HCV genotype 1 infection. Mem Inst Oswaldo Cruz. 2008;103(6):611–4.
14. El Awady MK, Azzazy HM, Fahmy AM, Shawky SM, Badreldin NG, Yossef SS, et al. Positional effect of mutations in 5'UTR of hepatitis C virus 4a on patients' response to therapy. World J Gastroenterol. 2009;15(12):1480–6.
15. Katano Y, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, et al. Association with 5'-untranslated region and response to interferon in chronic hepatitis C. Hepatogastroenterology. 2007;54(75):854–7.
16. Soler M, Pellerin M, Malnou CE, Dhumeaux D, Kean KM, Pawlotsky JM. Quasi species heterogeneity and constraints on the

evolution of the 5′ noncoding region of hepatitis C virus (HCV): relationship with HCV resistance to interferon-alpha therapy. Virology. 2002;298(1):160–73.
17. Thelu MA, Drouet E, Hilleret MN, Zarski JP. Lack of clinical significance of variability in the internal ribosome entry site of hepatitis C virus. J Med Virol. 2004;72(3):396–405.
18. Yamamoto C, Enomoto N, Kurosaki M, Yu SH, Tazawa J, Izumi N, et al. Nucleotide sequence variations in the internal ribosome entry site of hepatitis C virus-1b: no association with efficacy of interferon therapy or serum HCV-RNA levels. Hepatology. 1997;26(6):1616–20.
19. Kountouras J, Zavos C, Chatzopoulos D. Apoptosis in hepatitis C. J Viral Hepat. 2003;10(5):335–42.
20. Hahn YS. Subversion of immune responses by hepatitis C virus: immunomodulatory strategies beyond evasion? Curr Opin Immunol. 2003;15(4):443–9.
21. Wang T, Weinman SA. Causes and consequences of mitochondrial reactive oxygen species generation in hepatitis C. J Gastroenterol Hepatol. 2006;21(Suppl 3):S34–7.
22. Mori Y, Moriishi K, Matsuura Y. Hepatitis C virus core protein: its coordinate roles with PA28gamma in metabolic abnormality and carcinogenicity in the liver. Int J Biochem Cell Biol. 2008;40(8):1437–42.
23. Serfaty L, Capeau J. Hepatitis C, insulin resistance and diabetes: clinical and pathogenic data. Liver Int. 2009;29(Suppl 2):13–25.
24. Pazienza V, Clement S, Pugnale P, Conzelman S, Foti M, Mangia A, et al. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. Hepatology. 2007;45(5):1164–71.
25. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, et al. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon–ribavirin combination therapy. Intervirology. 2005;48(6):372–80.
26. Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. J Med Virol. 2009;81(3):452–8.
27. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol. 2007;79(11):1686–95.
28. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol. 2007;46(3):403–10.
29. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology. 2007;46(5):1357–64.
30. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Substitution of amino acid 70 in the hepatitis C virus core region of genotype 1b is an important predictor of elevated alpha-fetoprotein in patients without hepatocellular carcinoma. J Med Virol. 2008;80(8):1354–62.
31. Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, et al. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol. 2007;81(15):8211–24.
32. Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, et al. Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol. 2009;81(4):640–9.
33. Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, et al. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol. 2009;11 [Epub ahead of print].
34. Grove J, Nielsen S, Zhong J, Bassendine MF, Drummer HE, Balfe P, et al. Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies. J Virol. 2008;82(24):12020–9.
35. Benedicto I, Molina-Jimenez F, Barreiro O, Maldonado-Rodriguez A, Prieto J, Moreno-Otero R, et al. Hepatitis C virus envelope components alter localization of hepatocyte tight junction-associated proteins and promote occludin retention in the endoplasmic reticulum. Hepatology. 2008;48(4):1044–53.
36. Pavio N, Taylor DR, Lai MM. Detection of a novel unglycosylated form of hepatitis C virus E2 envelope protein that is located in the cytosol and interacts with PKR. J Virol. 2002;76(3):1265–72.
37. Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science. 1999;285(5424):107–10.
38. Taylor DR, Tian B, Romano PR, Hinnebusch AG, Lai MM, Mathews MB. Hepatitis C virus envelope protein E2 does not inhibit PKR by simple competition with autophosphorylation sites in the RNA-binding domain. J Virol. 2001;75(3):1265–73.
39. Gupta R, Subramani M, Khaja MN, Madhavi C, Roy S, Habibullah CM, et al. Analysis of mutations within the 5′ untranslated region, interferon sensitivity region, and PePHD region as a function of response to interferon therapy in hepatitis C virus-infected patients in India. J Clin Microbiol. 2006;44(3):709–15.
40. Saito T, Ito T, Ishiko H, Yonaha M, Morikawa K, Miyokawa A, et al. Sequence analysis of PePHD within HCV E2 region and correlation with resistance of interferon therapy in Japanese patients infected with HCV genotypes 2a and 2b. Am J Gastroenterol. 2003;98(6):1377–83.
41. Sarrazin C, Bruckner M, Herrmann E, Ruster B, Bruch K, Roth WK, et al. Quasi species heterogeneity of the carboxy-terminal part of the E2 gene including the PePHD and sensitivity of hepatitis C virus 1b isolates to antiviral therapy. Virology. 2001;289(1):150–63.
42. Sarrazin C, Kornetzky I, Ruster B, Lee JH, Kronenberger B, Bruch K, et al. Mutations within the E2 and NS5A protein in patients infected with hepatitis C virus type 3a and correlation with treatment response. Hepatology. 2000;31(6):1360–70.
43. Gaudy C, Lambele M, Moreau A, Veillon P, Lunel F, Goudeau A. Mutations within the hepatitis C virus genotype 1b E2-PePHD domain do not correlate with treatment outcome. J Clin Microbiol. 2005;43(2):750–4.
44. Hung CH, Lee CM, Lu SN, Lee JF, Wang JH, Tung HD, et al. Mutations in the NS5A and E2-PePHD region of hepatitis C virus type 1b and correlation with the response to combination therapy with interferon and ribavirin. J Viral Hepat. 2003;10(2):87–94.
45. Munoz de Rueda P, Casado J, Paton R, Quintero D, Palacios A, Gila A, et al. Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. J Virol. 2008;82(13):6644–53.
46. Murphy MD, Rosen HR, Marousek GI, Chou S. Analysis of sequence configurations of the ISDR, PKR-binding domain, and V3 region as predictors of response to induction interferon-alpha and ribavirin therapy in chronic hepatitis C infection. Dig Dis Sci. 2002;47(6):1195–205.

47. Vuillermoz I, Khattab E, Sablon E, Ottevaere I, Durantel D, Vieux C, et al. Genetic variability of hepatitis C virus in chronically infected patients with viral breakthrough during interferon–ribavirin therapy. J Med Virol. 2004;74(1):41–53.
48. Yang SS, Lai MY, Chen DS, Chen GH, Kao JH. Mutations in the NS5A and E2-PePHD regions of hepatitis C virus genotype 1b and response to combination therapy of interferon plus ribavirin. Liver Int. 2003;23(6):426–33.
49. Yoon J, Lee JI, Baik SK, Lee KH, Sohn JH, Lee HW, et al. Predictive factors for interferon and ribavirin combination therapy in patients with chronic hepatitis C. World J Gastroenterol. 2007;13(46):6236–42.
50. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest. 1995;96(1):224–30.
51. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med. 1996;334(2):77–81.
52. Chayama K, Tsubota A, Kobayashi M, Okamoto K, Hashimoto M, Miyano Y, et al. Pretreatment virus load and multiple amino acid substitutions in the interferon sensitivity-determining region predict the outcome of interferon treatment in patients with chronic genotype 1b hepatitis C virus infection. Hepatology. 1997;25(3):745–9.
53. Komatsu H, Fujisawa T, Inui A, Miyagawa Y, Onoue M. Mutations in the nonstructural protein 5A gene and response to interferon therapy in young patients with chronic hepatitis C virus 1b infection. J Med Virol. 1997;53(4):361–5.
54. Kurosaki M, Enomoto N, Murakami T, Sakuma I, Asahina Y, Yamamoto C, et al. Analysis of genotypes and amino acid residues 2209–2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology. 1997;25(3):750–3.
55. Chung RT, Monto A, Dienstag JL, Kaplan LM. Mutations in the NS5A region do not predict interferon-responsiveness in American patients infected with genotype 1b hepatitis C virus. J Med Virol. 1999;58(4):353–8.
56. Hofgartner WT, Polyak SJ, Sullivan DG, Carithers RL Jr, Gretch DR. Mutations in the NS5A gene of hepatitis C virus in North American patients infected with HCV genotype 1a or 1b. J Med Virol. 1997;53(2):118–26.
57. Khorsi H, Castelain S, Wyseur A, Izopet J, Canva V, Rombout A, et al. Mutations of hepatitis C virus 1b NS5A 2209–2248 amino acid sequence do not predict the response to recombinant interferon-alfa therapy in French patients. J Hepatol. 1997;27(1):72–7.
58. Odeberg J, Yun Z, Sonnerborg A, Weiland O, Lundeberg J. Variation in the hepatitis C virus NS5a region in relation to hypervariable region 1 heterogeneity during interferon treatment. J Med Virol. 1998;56(1):33–8.
59. Squadrito G, Leone F, Sartori M, Nalpas B, Berthelot P, Raimondo G, et al. Mutations in the nonstructural 5A region of hepatitis C virus and response of chronic hepatitis C to interferon alfa. Gastroenterology. 1997;113(2):567–72.
60. Zeuzem S, Lee JH, Roth WK. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alpha. Hepatology. 1997;25(3):740–4.
61. Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, et al. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. Gut. 2004;53(9):1345–51.
62. Pawlotsky JM, Germanidis G, Neumann AU, Pellerin M, Frainais PO, Dhumeaux D. Interferon resistance of hepatitis C virus genotype 1b: relationship to nonstructural 5A gene quasi species mutations. J Virol. 1998;72(4):2795–805.
63. Saiz JC, Lopez-Labrador FX, Ampurdanes S, Dopazo J, Forns X, Sanchez-Tapias JM, et al. The prognostic relevance of the nonstructural 5A gene interferon sensitivity determining region is different in infections with genotype 1b and 3a isolates of hepatitis C virus. J Infect Dis. 1998;177(4):839–47.
64. Sarrazin C, Berg T, Lee JH, Ruster B, Kronenberger B, Roth WK, et al. Mutations in the protein kinase-binding domain of the NS5A protein in patients infected with hepatitis C virus type 1a are associated with treatment response. J Infect Dis. 2000; 181(2):432–41.
65. Sarrazin C, Berg T, Lee JH, Teuber G, Dietrich CF, Roth WK, et al. Improved correlation between multiple mutations within the NS5A region and virological response in European patients chronically infected with hepatitis C virus type 1b undergoing combination therapy. J Hepatol. 1999;30(6):1004–13.
66. Witherell GW, Beineke P. Statistical analysis of combined substitutions in nonstructural 5A region of hepatitis C virus and interferon response. J Med Virol. 2001;63(1):8–16.
67. Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology. 2008; 48(6):1753–60.
68. Veillon P, Payan C, Le Guillou-Guillemette H, Gaudy C, Lunel F. Quasi species evolution in NS5A region of hepatitis C virus genotype 1b during interferon or combined interferon–ribavirin therapy. World J Gastroenterol. 2007;13(8):1195–203.
69. Yen YH, Hung CH, Hu TH, Chen CH, Wu CM, Wang JH, et al. Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209–2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. Aliment Pharmacol Ther. 2008;27(1):72–9.
70. Gale MJ Jr, Korth MJ, Tang NM, Tan SL, Hopkins DA, Dever TE, et al. Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. Virology. 1997;230(2):217–27.
71. Inchauspe G, Zebedee S, Lee DH, Sugitani M, Nasoff M, Prince AM. Genomic structure of the human prototype strain H of hepatitis C virus: comparison with American and Japanese isolates. Proc Natl Acad Sci USA. 1991;88(22):10292–6.
72. Duverlie G, Khorsi H, Castelain S, Jaillon O, Izopet J, Lunel F, et al. Sequence analysis of the NS5A protein of European hepatitis C virus 1b isolates and relation to interferon sensitivity. J Gen Virol. 1998;79(Pt 6):1373–81.
73. Puig-Basagoiti F, Forns X, Furcic I, Ampurdanes S, Gimenez-Barcons M, Franco S, et al. Dynamics of hepatitis C virus NS5A quasi species during interferon and ribavirin therapy in responder and non-responder patients with genotype 1b chronic hepatitis C. J Gen Virol. 2005;86(Pt 4):1067–75.
74. Sarrazin C, Herrmann E, Bruch K, Zeuzem S. Hepatitis C virus nonstructural 5A protein and interferon resistance: a new model for testing the reliability of mutational analyses. J Virol. 2002;76(21):11079–90.
75. El-Shamy A, Sasayama M, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, et al. Prediction of efficient virological response to pegylated interferon/ribavirin combination therapy by NS5A sequences of hepatitis C virus and anti-NS5A antibodies in pretreatment sera. Microbiol Immunol. 2007;51(4):471–82.
76. El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology. 2008;48(1):38–47.

77. Pfeiffer JK, Kirkegaard K. Ribavirin resistance in hepatitis C virus replicon-containing cell lines conferred by changes in the cell line or mutations in the replicon RNA. J Virol. 2005;79(4):2346–55.
78. Young KC, Lindsay KL, Lee KJ, Liu WC, He JW, Milstein SL, et al. Identification of a ribavirin-resistant NS5B mutation of hepatitis C virus during ribavirin monotherapy. Hepatology. 2003;38(4):869–78.
79. Sugihara K, Orito E, Tanaka Y, Kato T, Lau JY, Ohno T, et al. Variations in the viral NS5B region in Japanese patients with chronic hepatitis C virus genotype 1b infection. No specific amino acid substitution was identified as determinants of treatment response to interferon/ribavirin combination therapy. Intervirology. 2006;49(6):319–26.
80. Ward CL, Dev A, Rigby S, Symonds WT, Patel K, Zekry A, et al. Interferon and ribavirin therapy does not select for resistance mutations in hepatitis C virus polymerase. J Viral Hepat. 2008 [Epub ahead of print].
81. Hamano K, Sakamoto N, Enomoto N, Izumi N, Asahina Y, Kurosaki M, et al. Mutations in the NS5B region of the hepatitis C virus genome correlate with clinical outcomes of interferon-alpha plus ribavirin combination therapy. J Gastroenterol Hepatol. 2005;20(9):1401–9.
82. Asahina Y, Izumi N, Enomoto N, Uchihara M, Kurosaki M, Onuki Y, et al. Mutagenic effects of ribavirin and response to interferon/ribavirin combination therapy in chronic hepatitis C. J Hepatol. 2005;43(4):623–9.
83. Lutchman G, Danehower S, Song BC, Liang TJ, Hoofnagle JH, Thomson M, et al. Mutation rate of the hepatitis C virus NS5B in patients undergoing treatment with ribavirin monotherapy. Gastroenterology. 2007;132(5):1757–66.

BioSystems 99 (2010) 70–78

Contents lists available at ScienceDirect

BioSystems

journal homepage: www.elsevier.com/locate/biosystems

Reproducibility and usability of chronic virus infection model using agent-based simulation; comparing with a mathematical model

Jun Itakura[a,*], Masayuki Kurosaki[a], Yoshie Itakura[a], Sinya Maekawa[b], Yasuhiro Asahina[a], Namiki Izumi[a], Nobuyuki Enomoto[b]

[a] Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan
[b] First Department of Internal Medicine, Faculty of Medicine, University of Yamanashi, 1110, Shimogatou, Chuou-shi, Yamanashi 409-3898, Japan

ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form 27 August 2009
Accepted 6 September 2009

Keywords:
Agent-based model
Virus infectious disease

ABSTRACT

We created agent-based models that visually simulate conditions of chronic viral infections using two software. The results from two models were consistent, when they have same parameters during the actual simulation. The simulation results comprise a transient phase and an equilibrium phase, and unlike the mathematical model, virus count transit smoothly to the equilibrium phase without overshooting which correlates with actual biology in vivo of certain viruses. We investigated the effects caused by varying all the parameters included in concept; increasing virus lifespan, uninfected cell lifespan, uninfected cell regeneration rate, virus production count from infected cells, and infection rate had positive effects to the virus count during the equilibrium period, whereas increasing the latent period, the lifespan-shortening ratio for infected cells, and the cell cycle speed had negative effects. Virus count at the start did not influence the equilibrium conditions, but it influenced the infection development rate. The space size had no intrinsic effect on the equilibrium period, but virus count maximized when the virus moving speed was twice the space size. These agent-based simulation models reproducibly provide a visual representation of the disease, and enable a simulation that encompasses parameters those are difficult to account for in a mathematical model.

1. Introduction

All viruses need hosts as a basis for their life. When a virus enters the host body, it invades cells and uses both its own enzymes and those of the host cells to replicate. Host cells infected by viruses launch a self-defense system known as the innate immune system (See and Wark, 2008; Naniche, 2009), which inhibits viral replication and uses the human leukocyte antigen system and cytokines to elicit an immune response. Immune cells that have received signals from host cells activate other immune cells, neutralize viruses in the serum by means of antibodies, and prevent the virus from replicating and proliferating by destroying or curing host cells. Viral infection is a disorder based on the interactions between viruses and cells.

The power relationship between these agents changes along with the progression of the disease. In the very early stages of infection, as the host defense mechanisms are immature, the virus has the ability to overwhelm the host cells, actively replicate, and proliferate. Subsequently, as the capacity of the immune system improves, the speed of viral proliferation drops and the virus count reaches a peak. Infected host cells begin to be disrupted by the immune system or virus particles, and symptoms appear as a result. If the immune system is stronger than the virus, then the viral counts decline, and, in transient viral disorders, the virus is finally eliminated and the host recovers. In chronic viral disorders, however, the power relationship between the virus and host cells reaches equilibrium, and a long-term power balance is maintained with the virus count reaching a plateau.

Mathematical models have been proposed to study the dynamics of such viral disorders, and are regarded as being of value in understanding this phenomenon (Ho et al., 1995; Nowak et al., 1996; Neumann et al., 1998). However, these models are difficult to understand for clinicians, and their applicability is somewhat limited in everyday practice. In clinical research, measurements of viral dynamics in patients for short duration have been made for human

Abbreviations: HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

* Corresponding author. Tel.: +81 422 32 3111; fax: +81 422 32 9551.
E-mail address: jitakura@musashino.jrc.or.jp (J. Itakura).

0303-2647/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.biosystems.2009.09.001

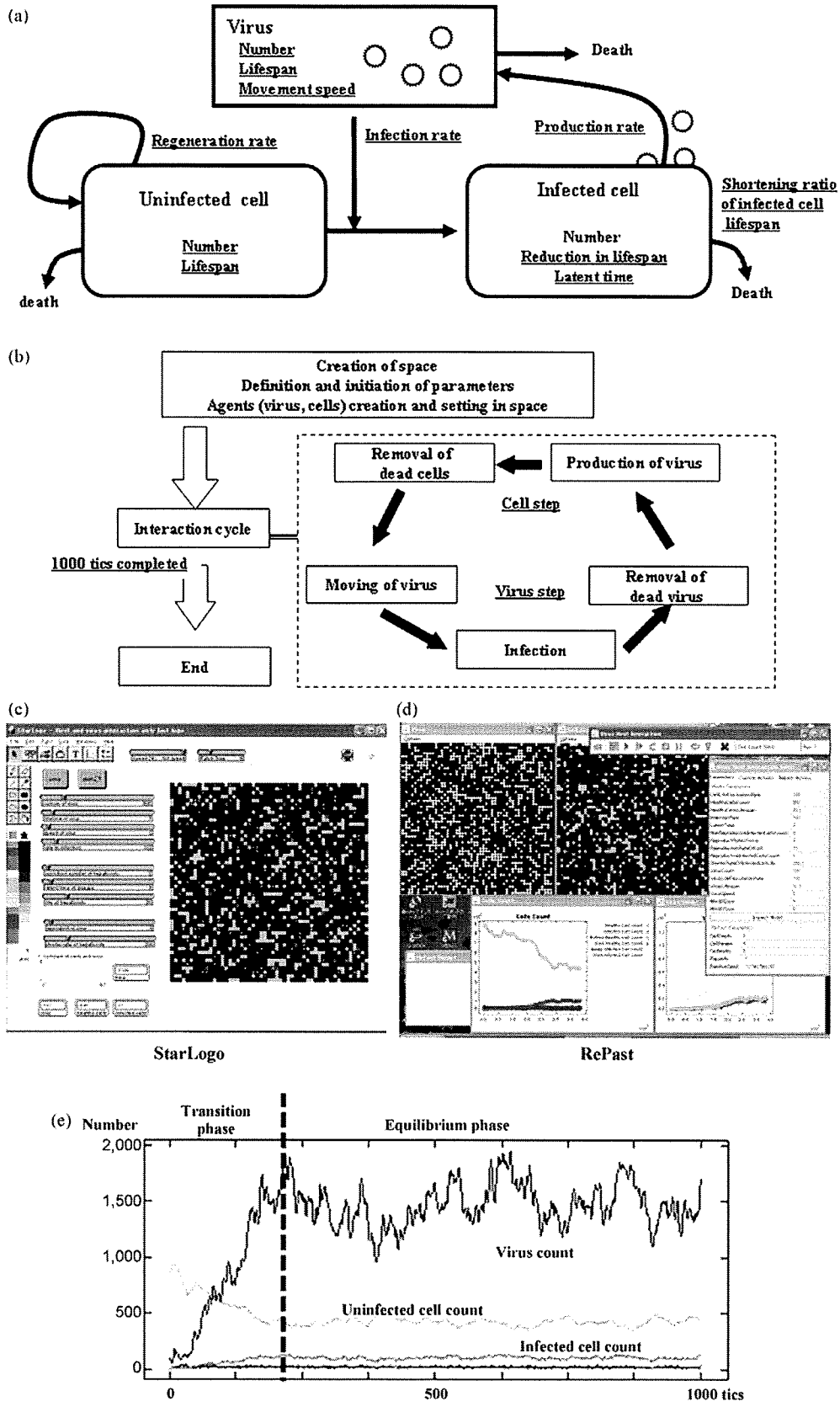

Fig. 1. Simulation design and an example of simulation results. (a) Model concept. Viruses, uninfected cells, and infected cells were treated as agents, and parameters were set for each of these and for interactions between agents (underlined). (b) Flowchart of the program. After preparing the simulation, we entered the interaction cycle, in which virus steps (such as movement) and cell steps were repeated. One cycle was counted as 1 tic, and the simulation concluded after 1000 tics. (c and d) Simulation screen using (c) StarLogo and (d) RePast. Yellow circles are viruses, green squares are uninfected cells, and orange and red indicate infected cells, with orange indicating the latent period. In StarLogo, all the agents are shown on the same screen, but in RePast, viruses and cells are shown in separate windows. (e) Example of a simulation chart in StarLogo. After the start of simulation the virus count and infected cell count increase while the uninfected cell count decreases, with equilibrium state reached after a certain number of tics.

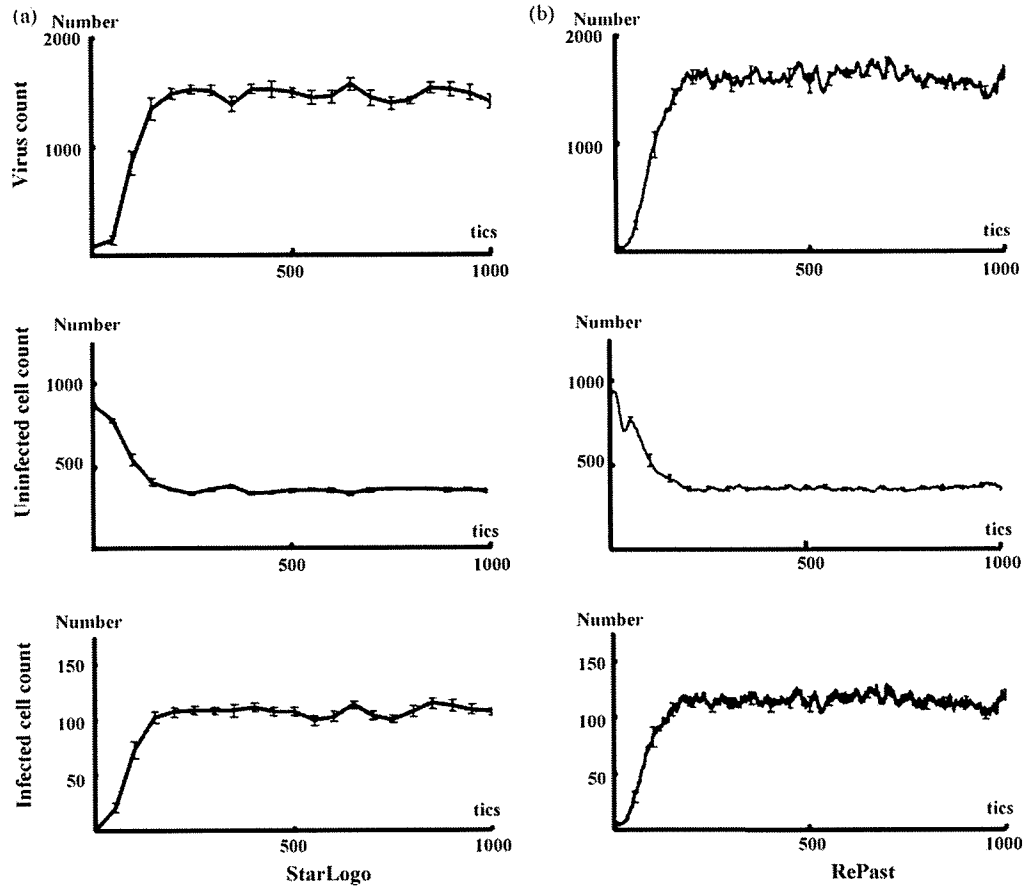

Fig. 2. Comparison of simulation results in (a) StarLogo and (b) RePast. The results were consistent when the parameters were made consistent. (Virus count [average ± SD]: StarLogo 1458.03 ± 173.1, RePast 1462.71 ± 178.8, $p = 0.94$. Uninfected cell count: 364.24 ± 30.4, 368.11 ± 33.4, $p = 0.83$. Infected cell count: 105.73 ± 13.0, 107.74 ± 13.0, $p = 0.24$. Unpaired Student's t-test.) Parameter values were set as follows: initial virus count, 100; uninfected cell count, 880; infected cell count, 0; virus speed of movement, 5 grids/tic; infection rate, 10%; uninfected cell regeneration rate, 1%; latent period, 3 tics; and virus reproduction rate, 5/cells/tic. The following parameter settings were taken from actual measurements: virus lifespan, 4.5 tics; uninfected cell lifespan, 49.8 tics; and infected cell lifespan, 6.7 tics.

immunodeficiency virus (HIV) (Ho et al., 1995), hepatitis B virus (HBV) (Nowak et al., 1996) and hepatitis C virus (HCV) (Neumann et al., 1998), and research is also underway on a range of models based on animal experiments and cell culture systems. As chronic viral disorders persist over long periods of time complete follow-up of viral dynamics is difficult. Furthermore, limitations of items that can be measured, such as the difficulty of measuring whole numbers of host cells, make it extremely difficult to investigate their consistency in mathematical models.

The recent ascend of dynamic-models owes much to advances in computers. Computer performance has improved markedly in recent years, not only in terms of their calculating capacity but also with regard to image displays, and models that offer a visual representation of viral disorders are now being reported (Gilbert and Bankes, 2002; Duca et al., 2007; Shapiro et al., 2008; Castiglione et al., 2007). One advantage of such visual models is that by providing a visual representation, they make understanding the disease status easy. Another benefit is that they enable parameters to be identified that are hidden as background noise in mathematical models. However, these models have some problems; it is difficult to prove the reproducibility of the simulation results derived from different languages or libraries, difficult to prove the validity of the model's concepts, and difficult to prove that the simulation results accurately reflect the reality. In this study, we created agent-based computer models that visually simulate the conditions of chronic viral infections using two software. The reproducibility of two agent-based computer models and the differences between agent-based models and the mathematical model were analyzed. This agent-based model enabled us to investigate how each parameter included in the concept affects the conditions of chronic viral infections.

2. Methods

2.1. Selection of Software

In this study, we used two different types of softwares: StarLogo version 2.0 (http://education.mit.edu/starlogo/) supplied by MIT Media Laboratory and Recursive Porous Agent Simulation Toolkit (RePast-3.0, http://repast.sourceforge.net/) supplied by the Argonne National Laboratory. StarLogo uses Logo, one of the simplest programming languages, and has a fixed graphical user interface. RePast is a library that uses Java, another programming language, which also has a fixed graphical user interface.

Logo is an assembly language, and StarLogo carries out processing sequentially. Java is an object-oriented language, and RePast has a faster processing speed than StarLogo. In addition, StarLogo has a number of stipulations to simplify simulations, such as parameters can only be set up to five decimal places and the simulation space is also fixed as 51 × 51 square grids. RePast, on the other hand, has fewer such restrictions. Thus, it offers a higher degree of freedom in program settings than StarLogo. Taking simulation space as an example, in spite of the restrictions imposed by the underlying operating system's image display system, any number of grids can be set and a hexagonal grid could also be chosen rather than a square one. However, users must stipulate and set all parameters themselves. This means that they must first declare the shape of the grid and the number of grids they will use to fill the simulation space. Java is also more difficult to learn than Logo, and debugging and correcting the program is also more difficult. Thus, it is difficult to judge whether or not the results agree with the planned simulation.

In effect, these two different types of softwares are polar opposites. It is simple to start a simulation in StarLogo, but producing results takes time and it is difficult to carry out more complex simulations. In RePast it is difficult to compose the program and judge whether or not the planned study has actually been achieved, but the

simulation itself takes only a short time to complete and there are lesser restrictions in the construction of a simulation model.

2.2. Concept for Modeling

We applied the basic virus–host interaction mathematical model to the agent-based simulation system with slight modifications. The mathematical model was used to describe the dynamics of HIV (Ho et al., 1995), HBV (Nowak et al., 1996), and HCV (Neumann et al., 1998) and the only agents involved were host cells and viruses, without the inclusion of immune cells. The effects of the immune system are expressed by varying parameters such as lifespan of host cells and viruses.

Fig. 1a illustrates the study concept. Viruses have the ability to infect healthy host cells (uninfected cells) and the infected cells produce new viruses. Both cells and viruses have definite lifespans, and the lifespan of infected cells is usually shorter than that of uninfected cells. Uninfected cells automatically regenerate within the space, whereas infected cells only arise due to infection of uninfected cells. Viruses also lack the ability to regenerate themselves and are only produced from infected cells.

2.3. Parameter Settings

In the present study, as the StarLogo settings are circumscribed, we limited the simulation space to 51 × 51 square grids. However, we made an exception here while investigating the effects of size of space on the simulation results. The numbers of viruses, uninfected cells, and infected cells could only be set before the start of the simulation. As described in the later, our simulation ran in cycles, with 1 cycle defined as 1 tic.

In mathematical simulation models, the death rate is required as a parameter. However, in our program we set lifespans for viruses and uninfected cells. These lifespans were not uniform, but were set to have a deviation of about 10%. The lifespan of cells was shortened by infection with ratio decided beforehand.

The infection ratio was meaningful only when an infected cell and a virus coincidentally occupied the same grid, and this was used to calculate the probability of the infection occurring in that situation. The virus production rate was set as the number of viruses produced by an infected cell during 1 tic. Infected cells could be set as a parameter indicating the latent period between the time of virus infection and the time of virus replication.

In order to emulate the tissue repair capacity, we set uninfected cell regeneration rate such that grids without any cells had a specified probability of producing uninfected cells on top of themselves. As a result, the more the cell count declined within a space the more regenerated uninfected cells were produced, whereas the number of regenerated cells declined as cell count increased.

The number of grids through which a virus could move in 1 tic was set as the speed of movement, and the direction of movement was set within a range of 90° toward the top of the simulation space. The program used a circulatory method of movement; when a virus arrived at the top of the space, it was translocated to the bottom, and moved again toward the top. Cells were fixed on the grid.

2.4. Simulation Flowchart

Fig. 1b shows a flowchart of the program. First, the simulation space was produced, after which each parameter was defined and the initial settings were made. Next the agents – viruses and uninfected and infected cells – were produced. The simulation cycle was as follows. Viruses moved to a new grid, and if an uninfected cell was present, this was infected with a probability based on the infection rate. The lifespan of the virus decreased, and viruses that had completed their lifespan and those that had caused an infection were removed from the space. Infected cells produced new viruses, the lifespans of both uninfected and infected cells decreased. Then, cells that had completed their lifespan were eliminated and a new cycle began. The program was set such that the simulation ended after this cycle had repeated 1000 times. This meant that one simulation was complete after 1000 tics.

2.5. Data Collection

The RePast model was programmed such that data for each tic was saved automatically as a text file at the end of the simulation. This text file could be opened by a database software. The StarLogo model was programmed to stop the simulation and collect data after every 50 tics.

2.6. Mathematical Model

In order to compare the results of this agent-based simulation, we used a viral infection mathematical model, which we improved as follows.

$$\frac{dT}{dt} = s[2601 - (T + I)] - dT - bVT \quad (1)$$

$$\frac{dI}{dt} = bVT - dI \quad (2)$$

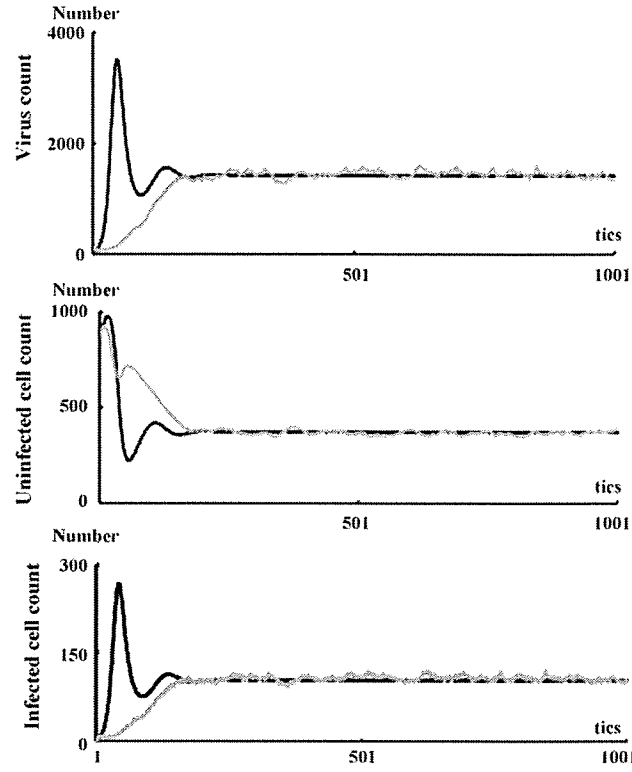

Fig. 3. Comparison of results of agent-based simulation and mathematical simulation. Both sets of results were consistent for the equilibrium phase, but differed in the shift in transition phase. Black line: mathematical model; grey line: results of simulation in RePast. Parameter values were set as follows: initial virus count, 100; uninfected cell count, 880; infected cell count, 0; virus speed of movement, 5 grids/tic; infection rate, 10%; uninfected cell regeneration rate, 1%; latent period, 3 tics; virus reproduction rate, 5/cells/tic; virus lifespan, 10 tics; uninfected cell lifespan, 50 tics; and cell lifespan-shortening ratio as a result of infection, 69%.

$$\frac{dV}{dt} = pI - cV \quad (3)$$

where, T is the uninfected cell count, I is the infected cell count, and V is the virus count. Uninfected cells are supplied to the space with a probability $s[2601 - (T+I)]$, as the number of grids in this agent-based simulation model was 2601 (51 × 51). The death rate of uninfected cells is d, the death rate of infected cells is δ, and the death rate of viruses is c. The infection rate is indicated by β. Viruses are released from infected cells at a probability p.

2.7. Statistical Analysis

Statistical analyses were performed by statistical tests using the program StatView 5.0 (SAS Institute Inc.). All tests of significance were two-tailed, with p values of <0.05 considered to be significant.

3. Results

3.1. Reproducibility of Chronic Viral Infection Disease Models Using Agent-based Simulation Methods

We constructed the chronic viral infection model with StarLogo library. Fig. 1c shows the simulation screen, and Fig. 1e shows one sample result. Immediately after the start of the simulation, the virus count temporarily dropped in accordance with the onset of an infection. Subsequently, the virus count started to increase with an increase in the infected cells and a decrease in the uninfected cells. After a certain number of tics (around 300 in this example), although the virus count, infected cell count, and uninfected cell count had some fluctuation, an equilibrium state was reached. We use the following descriptive terms in this paper: the transient phase is the period during which virus growth peaks, and the equilibrium phase is the period during which an equilibrium state is