	odds ratio (95%CI)	p
年齢(<60歳/≧60歳)	0.453 (0.191~1.073)	0.0719
肝線維化(F1/F2~4)	0.356 (0.152~0.833)	0.0173
HCVコア蛋白量	0.454 (0.196~1.055)	0.0663
ISDR(変異数<2/≧2)	5.125 (1.590~16.520)	0.0062

表2 ペグインターフェロン/リバビリン併用療法(1b)のSVRに寄与する因子(多変量ロジステック解析)(n=140)

また、ISDRは治療開始前のHCV RNA量と相関しているが、多変量解析ではRNA量はISDRに勝る因子ではなかった。さらに投与前のウイルス量を1,000KIU以上と未満に分けて検討しても、同等のウイルス量群のなかでも、変異型の効果が高いことが判明し上記を裏付けているものと思われた[7,8]。

しかし、ISDR変異数が同じであっても治療反応性が異なる症例が存在することは事実であり、特にISDR変異数0ないしは1の症例でも、SVRとなる症例とそうでない症例が存在する。そこでわれわれは、ISDR変異数0ないしは1の症例で、他のウイルス学的条件と臨床的条件がそろった症例で、IFN反応性が良好な症例とそうでない症例について、HCV全ゲノムの相違を検討した。その結果、両者に相違がみられたのは、コア領域とNS2領域のアミノ酸であり、統計学に有意な相違が見られたのはコア領域の70番目のアミノ酸のみであった。さらにこの領域に注目してretrospectiveに、IFN治療効果とこのアミノ酸変異との関連を検討すると、ISDRが0ないしは1の難治が予測される症例であっても、このアミノ酸が、HCVのプロトタイプのHCV-Jにみられるアルギニン(R)であれば高率にSVRが期待できるものの、グルタミン(Q)に変異していると、IFN治療反応性がきわめて悪く、主治医の判断で治療中断した症例や、治療完遂してもSVRにならない症例が多数を占めていた。また、最近Akutaらは、コア領域の70番目と91番目のアミノ酸に変異がみられるとインターフェロン治療効果が劣り、特に50歳以上の女性ではその傾向が顕著であることを報告している[9]。コア蛋白は、ゲノムと結合しヌクレオカプシドを形成するほか、細胞内シグナル伝達や、ウイルス粒子の形成にも関与すると考えられており、今後の研究が待たれるところである。

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ウイルス性肝炎のプライマリケア

総論

慢性ウイルス性肝炎の診断と節目検診

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Key Words

肝炎ウイルス検診
節目検診
節目外検診
肝炎診療協議会
肝疾患診療連携拠点病院

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はじめに

B型肝炎，C型肝炎に代表されるウイルス性肝炎は，わが国の慢性肝炎の大部分を占める．しかも，年余にわたるウイルスの持続感染の結果，自覚症状がないまま慢性肝炎や肝硬変，さらには肝細胞癌へと進展する症例もあることから，ウイルス肝炎検診が急務とされた．そこで，わが国では，平成14年から肝炎ウイルス検診（節目検診，節目外検診）が施行され，当初目標の5年が経過した．平成19年度は，過去5年間に検診を受診しなかったものを拾い上げる目的で事業が継続された．

肝炎ウイルス検診とウイルス肝炎の診断

肝炎ウイルス検診は，平成14（2002）年4月1日から老人保健法に基づき，全国の市町村が実施している，基本健康診査（いわゆる住民検診）に追加するかたちで開始された．このうち「節目検診」は，40～70歳までの5歳刻み，すなわち40，45，50，55，60，65，70歳のすべての受診者を対象に検診を行い，5年間ですべての検診者のウイルス感染を診断しようとするものである．一方，「節目外検診」は，過去に肝機能異常を指摘されたことのある者，広範な外科的処置を受けたことがある者，妊娠分娩時に多量に出血をした者などの希望者を対象としている．検診では，B型肝炎はHBs抗原検査で診断し，C型肝炎はまずHCV抗体によるスクリーニングを行い，抗体価に応じてHCVコア抗原（コア蛋白），HCVRNAを測定し，ウイルス血症の有無の判定までを行うものとされている．

1．HBs抗原陽性の場合

HBs抗原陽性の場合は，原則としてB型肝炎ウイルス（HBV）キャリアである．日本肝臓学会の「慢性肝炎の治療ガイド2008」によれば，病歴（特に家族歴，飲酒歴）を聴取し，一般肝機能検査，末梢血検査，プロトロンビン時間を測定し，肝炎の有無，肝病態の進展度や活動性を把握し，HBVDNA量やHBe抗原，HBe抗体の測定によりウイルス学的状態を把握するよう記述されている．また，腹部エコー検査と腫瘍マーカー（AFP等）の測定により肝癌の合併の有無を診断することが重要である（図1）[1]．

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B 型肝炎ウイルスキャリアの多くは非活動性の「無症候性キャリア」で，1 割程度のみが，慢性肝炎，肝硬変，肝細胞癌に進展する(図 2)[1]．また，若年者では自然経過で，HBe 抗原から HBe 抗体へのセロコンバージョンが起こり，これを契機に肝炎の沈静化が起こることがしばしばあり，たとえ肝機能異常がみられても，ただちに治療対象とはならないことがある．したがって，B 型肝炎の診療にあたっては，現在の肝病態の進展度と活動性を把握し，これに応じた治療・経過観察方針を立てることが重要である．

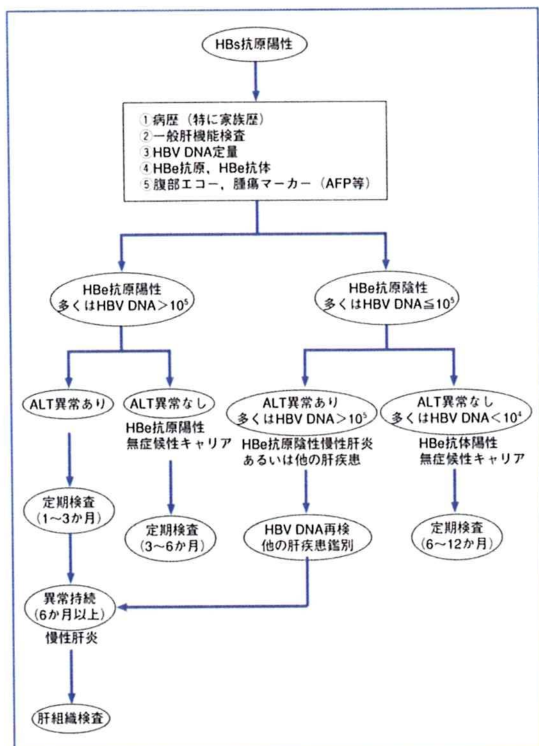


図 1　B 型肝炎の診断

2. HCV 陽性の場合

肝炎ウイルス検診では，はじめに HCV 抗体によるスクリーニングを行い，高力価陽性の場合は「現在 HCV に感染している可能性が極めて高い」として医療機関への受診勧奨を行う．また，中力価ないしは低力価の場合は，HCV コア抗原（コア蛋白）検査，さらには HCVRNA 検査を行い，ウイルス血症の有無を判定し，HCVRNA が陽性の場合のみ，受診勧奨を行うこととされている（図 3）．これは HCV 抗体が感染防御抗体ではないため，HCV 抗体低力価陽性者のなかには，感染既往者も含まれるためである．したがって，肝炎ウイルス検診によって HCV 感染が判定された場合は，この手順によって診断がなされており，医療機関などで HCV 抗体陽性を指摘された場合とは，異なることに注意が必要である．ただ単に「HCV 抗体陽性を指摘され

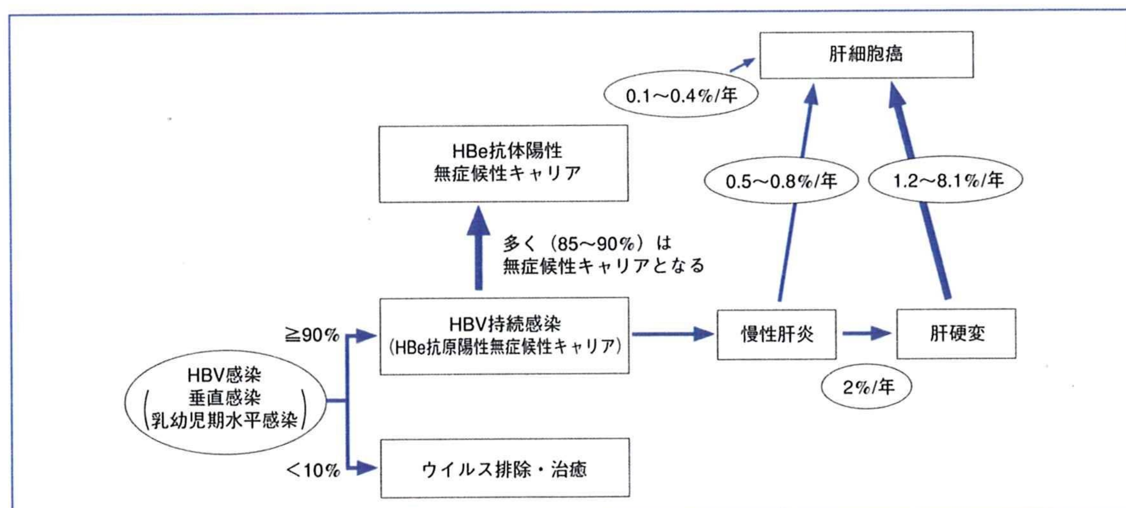
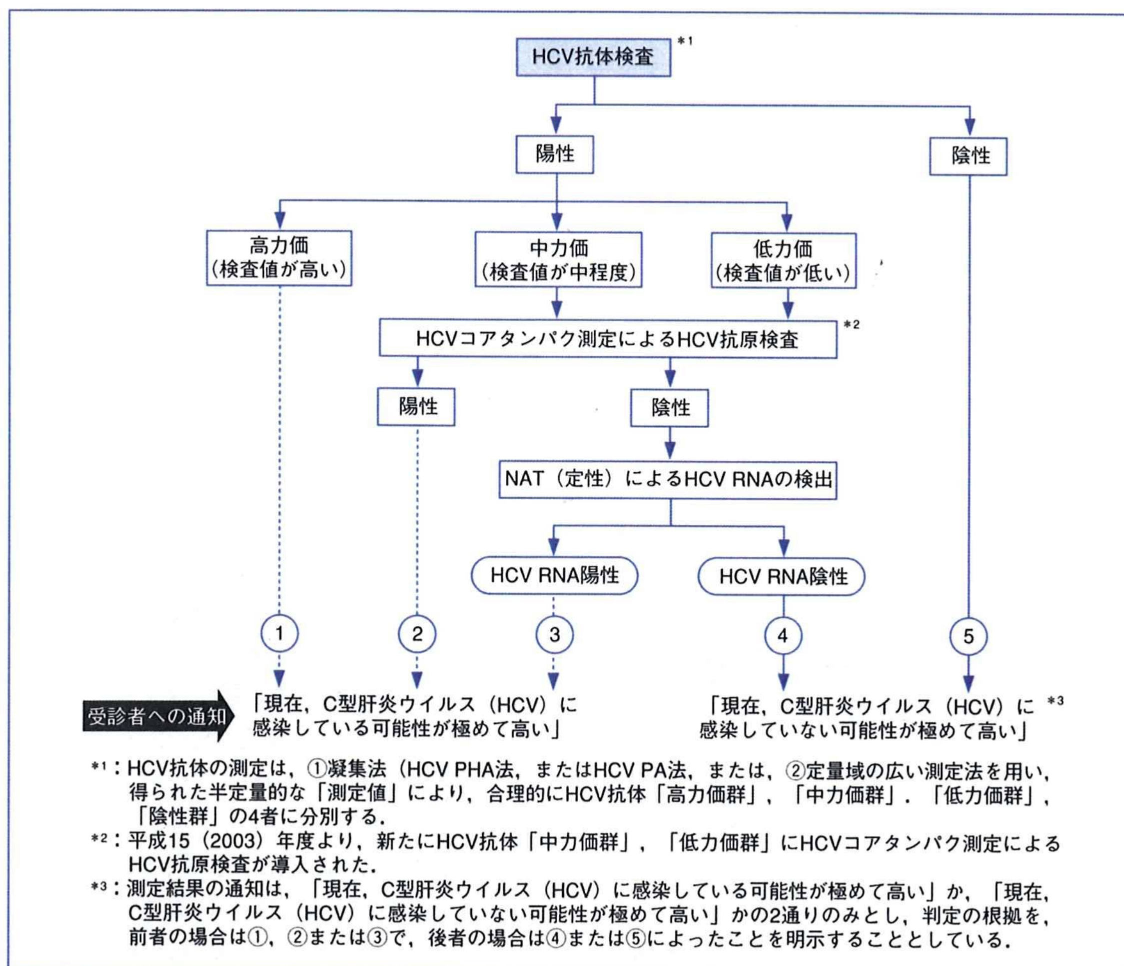


図 2　B 型肝炎の自然経過

た」場合には，HCV コア抗原検査ないしは HCVRNA 検査（2008 年 2 月現在，コマーシャルベースの検査ではリアルタイム PCR 法〈Taqman PCR 法〉が最も検出感度が高い）を行い，真のウイルス血症の有無を判定する必要がある．

C 型肝炎ウイルス（HCV）感染が診断された場合，まず肝炎の有無についての検査を行う．すなわち慢性肝炎であるか，無症候性キャリアの状態であるかを診断するのであるが，たとえ AST，ALT が施設内基準値であっても，ALT が 30 IU/mL 以上の場合は，わずかな肝炎が存在する可能性がある[2, 3]ほか，たまたま検査時に基準値以下であった可能性などを考慮する必要がある．また，最近では ALT の基準値を 30 IU/mL 以下とする意見が多く，たとえ血清 ALT 値が基準値以下であっても抗ウイルス療法の適応となることがある．C 型肝炎の自然経過は，感染すると 70 ～ 80%が慢性肝炎と移行し，平均 10 年，20 年，30 年の経過で慢性肝炎・肝硬変・肝細胞癌へと進展する（図 4）[1]．この間の肝線維化の程度と血小板数は，C 型肝炎では高い相関が認められる[4]ため，肝線維化の程度を肝生検なしで把握するためには，血小板数の測定は極めて重要である[5]．また，B 型肝炎と同様，肝病変の進展度と肝細胞癌の合併の診断には，腹部超音波検査や AFP をはじめと



*1：HCV抗体の測定は，①凝集法（HCV PHA法，またはHCV PA法，または，②定量域の広い測定法を用い，得られた半定量的な「測定値」により，合理的にHCV抗体「高力価群」，「中力価群」．「低力価群」，「陰性群」の4者に分別する．
*2：平成15（2003）年度より，新たにHCV抗体「中力価群」，「低力価群」にHCVコアタンパク測定によるHCV抗原検査が導入された．
*3：測定結果の通知は，「現在，C型肝炎ウイルス（HCV）に感染している可能性が極めて高い」か，「現在，C型肝炎ウイルス（HCV）に感染していない可能性が極めて高い」かの2通りのみとし，判定の根拠を，前者の場合は①，②または③で，後者の場合は④または⑤によったことを明示することとしている．

図 3　検診における C 型肝炎ウイルス検査

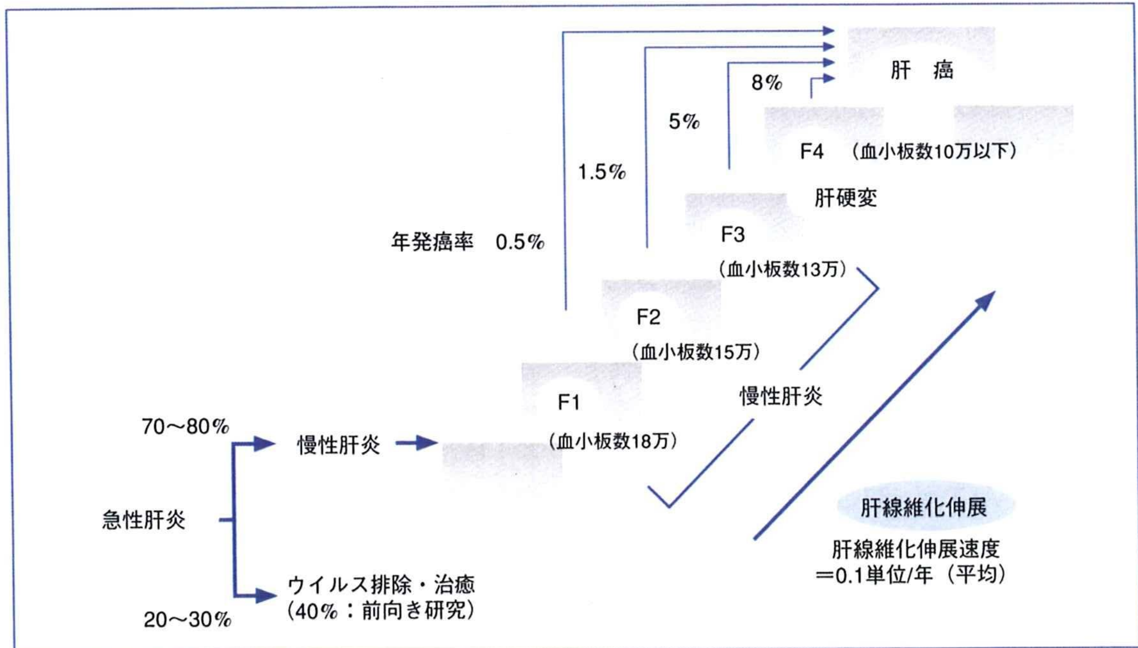


図 4　C 型肝炎の自然経過

した腫瘍マーカーの測定が重要であることはいうまでもない.

一方，C 型慢性肝炎の治療には，抗ウイルス療法と肝庇護療法があるが，根本的な治療には抗ウイルス療法，すなわちインターフェロン治療を検討することが好ましい．このためには，治療方針決定のために，HCV のウイルス型 (serogroup，genotype) の測定とウイルス量の測定は非必須であるが，さらに詳細な治療効果予測には HCV の NS5A に存在する ISDR (Interferon sensitivity determining region)[6] やコア蛋白変異の測定[7] が有用である.

肝炎ウイルス検診の実施状況と成果

厚生労働省によれば，平成 14 〜 18 年度までの 5 年間の肝炎ウイルス検診受診者は約 628 万人で，受診対象者の 26.4%であった (**表 1**)．このうち，「現在 C 型肝炎ウイルスに感染している可能性が極めて高い」と判定されたのは 0.9%の約 55,000 人で，HBs 抗原陽性は 1.1%の 70,000 人であった．一方節目外検診では，それぞれ 45,000 人，31,000 人発見されていることから，この 5 年間で HCV，HBV 感染者がそれぞれ約 10 万人発見されたことになる (**表 2**)．この数字は，それなりに意味のあることではあるが，受診対象者の 73%が検診を受けていないことから，まだ多くの未診断者が存在することになる．さらに，この検診は，老人保健法に基づく検診であり，国民健康保険加入者に限られることを考えると，これ以外の各種健康保険組合保険加入者への検診の普及を図ることが重要である.

表 1　肝炎ウイルス検診受診率 (C 型肝炎ウイルス検診，節目検診)

年度	受診対象者	受診者数	受診率 (%)
平成 14 年度	4,331,521	1,298,746	30.0
平成 15 年度	4,676,852	1,375,583	29.4
平成 16 年度	5,061,690	1,271,320	25.1
平成 17 年度	4,848,053	1,196,457	24.7
平成 18 年度	4,904,926	1,138,005	23.2
計	23,823,042	6,280,111	26.4

表 2 肝炎ウイルス検診実績

C 型肝炎ウイルス

	受診者（人）			「現在 C 型肝炎ウイルスに感染している可能性が極めて高い」と判定された者（人）			感染者率（%）		
	節目検診	節目外検診	計	節目検診	節目外検診	計	節目検診	節目外検診	計
平成 14 年度	1,298,746	624,734	1,923,480	14,672	16,721	31,393	1.1	2.7	1.6
平成 15 年度	1,375,583	454,687	1,830,270	13,324	10,167	23,491	1.0	2.2	1.3
平成 16 年度	1,271,320	347,431	1,618,751	10,385	6,446	16,831	0.8	1.9	1.0
平成 17 年度	1,196,457	331,356	1,527,813	8,909	5,067	13,976	0.7	1.5	0.9
平成 18 年度	1,138,005	596,190	1,734,195	7,453	6,806	14,259	0.7	1.1	0.8
計	6,280,111	2,354,398	8,634,509	54,743	45,207	99,950	0.9	1.9	1.2

B 型肝炎ウイルス

	受診者（人）			HBs 抗原検査において「陽性」と判定された者（人）			感染者率（%）		
	節目検診	節目外検診	計	節目検診	節目外検診	計	節目検診	節目外検診	計
平成 14 年度	1,291,195	631,918	1,923,113	15,239	9,191	24,430	1.2	1.5	1.3
平成 15 年度	1,382,663	466,462	1,849,125	15,842	6,678	22,520	1.1	1.4	1.2
平成 16 年度	1,279,704	356,230	1,635,934	13,950	4,804	18,754	1.1	1.3	1.1
平成 17 年度	1,205,423	341,400	1,546,823	12,735	4,395	17,130	1.1	1.3	1.1
平成 18 年度	1,145,291	604,301	1,749,592	11,742	6,407	18,149	1.0	1.1	1.0
計	6,304,276	2,400,311	8,704,587	69,508	31,475	100,983	1.1	1.3	1.2

厚生労働省ホームページ（http://www.mhlw.go.jp/houdou/2007/10/h1003-1.html）より改変

また，検診により見出された肝炎ウイルス感染者に対するフォローアップ体制は，一部の地方を除いていまだ確立されておらず，発見された肝炎ウイルス感染者の医療機関受診率の向上を図るとともに，経過観察，治療を系統的に行なえるような仕組みを作り上げることが今後の課題である[8]．

今後の肝炎対策事業と展望

平成 14 年から行なわれた，ウイルス肝炎検診は「C 型肝炎等緊急総合対策」の一環として行われ，一定の成果をあげてきた．しかし，上述のような残された課題に対し，平成 17 年に「C 型肝炎対策等に関する専門家会議」が設置され，「C 型肝炎対策等の一層の推進について」とする報告書がとりまとめられた．これに基づき，全国 C 型肝炎診療懇話会が開催され，この報告書として「都道府県における肝炎検査後肝疾患診療体制に対するガイドライン」が制定された．これによれば，各都道府県に医師会・肝炎に対する専門医，関係市町村や保健所の関係者によって構成される「肝炎診療協議会」を設置することが決定し，都道府県の実情に応じて，

①要診療者に対する保健指導
②かかりつけ医と専門医療機関の連携
③高度専門的ないし集学的な治療を提供可能な医療機関の確保
④受診状況や治療状況等の把握
⑤医療機関情報の収集と提供
⑥人材の育成

等について必要な検討を行うとともに，関

係者との連絡・調整を図ることが期待されている．

また，肝疾患診療においては，かかりつけ医と肝疾患に関する専門医療機関との連携，さらに高度先進的医療に対応する新たに設置される「肝疾患診療連携拠点病院」との間で連携を図ることが求められている（図3）．このなかでは，肝疾患専門医療機関では，

①専門的な知識を持つ医師による診断と治療方針の決定

②インターフェロンなどの抗ウイルス療法

③肝がんの高危険群の同定と早期診断

が可能である必要があり，2次医療圏に1か所以上の施設を選定することになっている．また，肝疾患診療連携拠点病院は，

①肝疾患に係る一般的な医療情報の提供

②都道府県内の専門医療機関に関する情報の収集や紹介

③医療従事者や地域住民を対象とした研修会や講演会の開催や肝疾患に関する相談支援

④肝疾患に関する専門医療期間との協議の場の設定

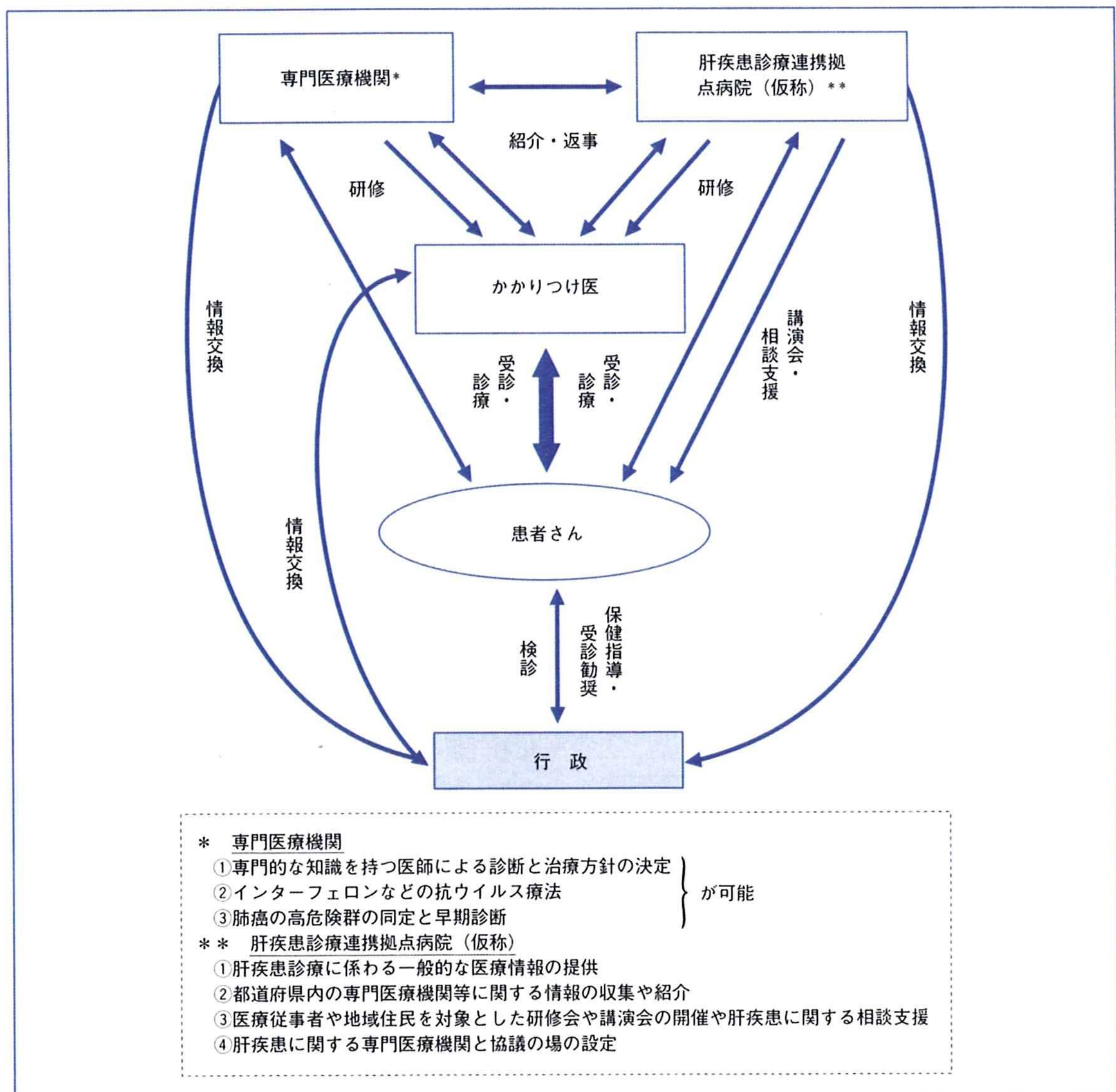


図5　都道府県における肝疾患診療ネットワーク（イメージ図）

を行うこととし，各都道府県のなかで肝疾患の診療ネットワークの中心的な役割を現在果たしているか，将来果たすことが期待される病院として，原則，都道府県に1か所選定することとされている．

以上のような活動が，平成20年度から本格的に開始するが，これらのネットワークが実際に稼動することが今後の肝炎対策の鍵を握るものと考えられる．

Y-PERSとY-PERSネットワーク

筆者らは，平成18年からYamanashi-PEG-interferon ribavirin study（Y-PERS）という組織を立ち上げてきた．これは，PEG-IFN＋Ribavirin治療を筆者らの大学病院と肝疾患専門医療機関と連携して行うことを意図して設立したもので，治療を通じて最適な治療方法と成績の検討，新たな知識の共有を目指している．さらに地域連携を図るために，地域のかかりつけ医の機能をもつ開業医・診療所の先生方との連携を図るY-PERSネットワークを設立した．ここでは，治療成績の検討会や講演会の開催などを随時行っており，まさに，今後，国が求めている肝炎対策を先取りしたものと考えられる[9]．

おわりに

わが国のHCV感染者は推定200万人，HBV感染者は推定140万人いるといわれている．しかしその多くが，いまだ感染者であることを診断されておらず，たとえ検診で感染が判明しても医療機関を受診していない現状がある．また，正しい知識と技術が提供されていない患者も多く存在することも事実である．したがって，これからはかかりつけ医や肝疾患専門医療機関，肝疾患診療連携拠点病院とが連携を図りつつ，系統的な経過観察，治療を行える仕組みづくりが必要である．

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Targeting Lipid Metabolism in the Treatment of Hepatitis C Virus Infection

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Recently, microdomains of organelle membranes rich in sphingomyelin and cholesterol (called "lipid rafts") have been considered to act as a scaffold for the hepatitis C virus (HCV) replication complex. Using the HCV cell culture system, we investigated the effect of myriocin, a sphingomyelin synthesis inhibitor, on HCV replication. We also investigated the combined effect of myriocin with interferon (IFN) and myriocin with simvastatin. Myriocin suppressed replication of both a genotype 1b subgenomic HCV replicon (Huh7/Rep-Feo) and genotype 2a infectious HCV (JFH-1 HCV) in a dose-dependent manner (for subgenomic HCV-1b, maximum of 79% at 1000 nmol/L; for genomic HCV-2a, maximum of 40% at 1000 nmol/L). Combination treatment with myriocin and IFN or myriocin and simvastatin attenuated HCV RNA replication synergistically in Huh7/Rep-Feo cells. Our data demonstrate that the sphingomyelin synthesis inhibitor strongly suppresses replication of both the subgenomic HCV-1b replicon and the JFH-1 strain of genotype 2a infectious HCV, indicating that lipid metabolism could be a novel target for HCV therapy.

Hepatitis C virus (HCV) is a major etiologic agent of liver diseases, affecting 170 million people worldwide [1]. Fifty-five percent to 85% of acute infections become persistent [2], and at least 20% of patients with chronic HCV infection progress to cirrhosis within 20 years [3]. With therapeutic advances, including the recent combination of pegylated interferon (IFN) plus ribavirin, half of patients can achieve a sustained virologic response [4]. However, the remaining half cannot clear the virus, demonstrating a strong need for HCV-specific therapies.

Positive-strand RNA viruses replicate intracellularly on certain membrane structures, including the endoplasmic reticulum [5], the Golgi apparatus [6], endosomes, and lysosomes [7]. During replication, RNA viruses form distinct replication complexes made of several membrane compartments and viral proteins [8]. In HCV, the membranous web (consisting of vesicles in a membranous matrix) has been described in the cellular matrix of HCV replicon–harboring cells [9, 10]. This membranous web is considered to be the HCV replication complex, consisting of viral and host proteins.

Recent studies suggest that the HCV replication complexes are formed on lipid rafts (which are detergent-insoluble microdomains of intracellular vesicular membranes rich in cholesterol and sphingolipid) [11–13]. It has been reported that viral nonstructural proteins and both positive- and negative-sense HCV RNAs were localized distinctively in a fraction of lipid rafts when subgenomic HCV replicon cells were subjected to membrane flotation analysis [12]. On the other hand, recent studies have demonstrated that agents related to lipid metabolism affect the replication of genotype 1 HCV. Leu et al. [14] reported that polyunsaturated fatty acids exerted strong anti-HCV activity on a subgenomic HCV-1b replicon. Moreover, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), which prevent cholesterol synthesis, have been shown to suppress replication of ge-

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nomic and subgenomic HCV-1b replicons [15, 16]. Even though the precise mechanism has not been defined, these agents may attenuate HCV replication through the destruction of lipid rafts, according to their pharmacological actions. If this is the mechanism, sphingomyelin, the remaining and essential component of lipid rafts, might play a role in HCV replication. With this in view, recent studies have demonstrated that a sphingomyelin synthesis inhibitor attenuated the replication of a subgenomic HCV-1b replicon in cultured cells [17] and the replication of genomic HCV-1 in a chimeric mouse model [18]. However, investigation of anti-HCV activity in these agents has been limited to genotype 1 HCV, and the combined effect of these agents has not been determined. If they do not target the HCV structure itself but exert their antiviral activity through destruction of the host's lipid raft, it would be plausible to speculate that they might be effective irrespective of the viral isolate, and the combined effect of these agents might be additive or synergistic.

In the present study, we investigated the role played by the sphingomyelin synthesis pathway and the mevalonate pathway in HCV replication, using a subgenomic HCV-1b replicon and the particle-producing cell culture HCV 2a model of JFH-1 HCV [19].

MATERIALS AND METHODS

Cell culture and HCV replicon. The human hepatoma cell lines Huh7 and Huh7.5.1 [20] were maintained in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal calf serum at 37°C in 5% CO_2. The subgenomic HCV replicon used was derived from Rep-Feo (genotype 1b) [21, 22], and a full-length genomic HCV RNA was derived from genotype 2a JFH-1 HCV [19]. Subgenomic or genomic HCV RNA was synthesized from replicon cDNA–harboring plasmids (pRep-Feo and pJFH-1) by means of T7 polymerase (RiboMax Large Scale RNA Production System; Promega) and transfected into these cells. For the subgenomic replicon, cell lines stably expressing the replicon were established (Huh7/Rep-Feo) in the presence of 500 μg/mL G418.

Reporter plasmids and luciferase assay. pISRE-TA-Rluc expressing the *Renilla* luciferase reporter gene under control of the IFN-stimulated response element (ISRE) was constructed by replacing the firefly luciferase gene with the *Renilla* luciferase gene of pISRE-TA-Luc, purchased from Invitrogen. Luciferase activity was quantified using the Bright-Glo or Dual-Luciferase assay system (both from Promega) and a luminometer (AB-2250; ATTO). Assays were performed in triplicate, and the results were expressed as mean ± SD percentages of the control values. QuantiLum recombinant luciferase (Promega) was used as the positive control for the analysis.

Reagents. The reagents used included myriocin (Biomol), IFN-α 2b (Santa Cruz Biotechnology), phytosphingosine hydrochloride (Sigma), 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CyD; Sigma), and simvastatin (Cosmobio).

Northern blotting. Total cellular RNA was extracted from cells by means of Isogen (Wako). The RNA was separated by denaturing agarose-formaldehyde gel electrophoresis and transferred to a membrane from a NorthernMax kit (Ambion). The membrane was hybridized with a digoxigenin-labeled probe that was specific for the nonstructural replicon sequence. The signals were detected in a chemiluminescence reaction by using a digoxigenin detection kit (Roche) and were visualized by using an LAS-1000 imaging system (Fuji Film).

Western blotting. Ten micrograms of total cell lysate was separated using NuPAGE 4%–12% Bis-Tris gel (Invitrogen) and was blotted onto an Immobilon polyvinylidene difluoride membrane (Roche). The membrane was incubated with an anti-core monoclonal antibody (MAb; Affinity Bioreagents), an anti-NS3 MAb (Virogen), an anti-NS5A MAb (gift from Burckstummer, Robert Koch Institute), or a anti–β-catenin MAb (Sigma). Detection was done in a chemiluminescence reaction (ECL; Amersham).

Dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium (MTS) assays. To evaluate cytotoxicity, MTS assays were performed using a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega), in accordance with the manufacturer's instructions.

Thin-layer chromatography (TLC). The lipid fraction of cells treated with myriocin was extracted using the method of Bligh and Dyer [23], and total lipids from the cells treated with myriocin were extracted with 3 mL of chloroform. The extracts were spotted onto silica gel TLC plates (Merck) and were chromatographed with chloroform-methanol-water (65:25:4 [vol/vol/vol]). The plate was visualized with a molybdenum spray.

Real-time reverse-transcription polymerase chain reaction (RT-PCR). TaqMan RT-PCR targeting the 5' untranslated region was used for the quantitation of intracellular genomic JFH-1 HCV RNA. The sequences of the sense and antisense primers and the TaqMan probe were 5'-TGCGGAACCGGTGAGTACA-3', 5'-CTTAAGGTTTAGGATTCGTGCTCAT-3', and 5'-(FAM)CACCCTATCAGGCAGTACCACAAGGCC(TAMRA)-3', respectively. The method has been described elsewhere [24].

Short interfering RNA (siRNA) analysis. The sequence encoding the LCB1 subunit of serine palmitoyltransferase (SPT) was selected as the target for siRNA (sense, 5'-AACAACAUCGUUUCAGGUCCUTT-3'; antisense, 5'-AGGGCCUGAAACGAUGUUGTT-3'). siRNA targeting enhanced green fluorescent protein (GFP) was used as the negative control (sense, 5'-CUUACGCUGAGUACUUCGATT-3'; antisense, 5'-UCGAAGUACUCAGCGUAATT-3'). (Underlined letters indicate deoxyribonucleotides.)

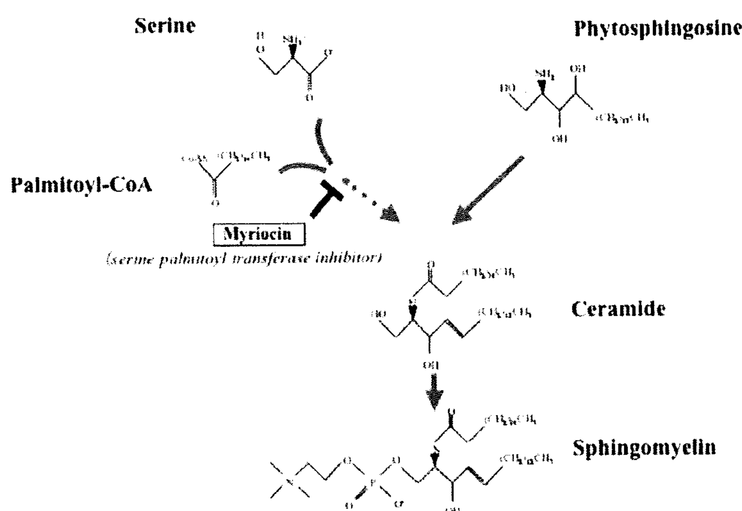


Figure 1. The sphingomyelin synthesis pathway. Serine palmitoyltransferase catalyzes the first committed step of sphingomyelin biosynthesis from serine and palmitoyl-coenzyme A (CoA). Myriocin inhibits the catalyzing activity of serine palmitoyltransferase. Phytosphingosine is known to work as a precursor of ceramide in both mammalian and fungal cells.

Statistical analyses. Statistical analyses were performed using Student's *t* test; statistically significant differences were defined as those for which $P < .05$.

RESULTS

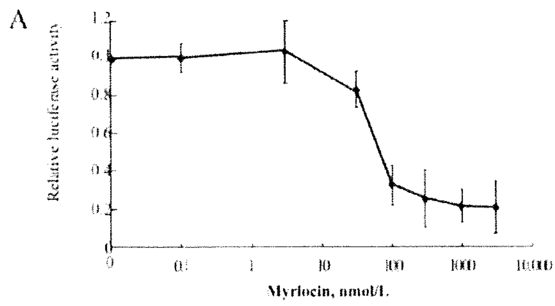
Specific suppression of the replication of a subgenomic HCV-1b replicon by an inhibitor of sphingomyelin synthesis. To clarify the role played by the sphingomyelin synthesis pathway in HCV replication, we added myriocin, a specific inhibitor of SPT that catalyzes the first committed step of sphingomyelin biosynthesis (figure 1), to the medium of Huh7/Rep-Feo cells. The luciferase activity, reflecting replication of the subgenomic HCV-1b replicon, dropped to 37% and 21% of the control at myriocin concentrations of 100 and 1000 nmol/L, respectively (figure 2*A*, *upper panel*), but myriocin did not cause toxicity to the cultured cells (figure 2*A*, *lower panel*). The result indicates that the decrease in HCV replication is due to a specific suppressive effect of myriocin and not to the cytotoxicity of myriocin. Northern hybridization analysis also demonstrated a substantial reduction of the subgenomic HCV replicon RNA in Huh7/Rep-Feo cells treated with myriocin in a dose-dependent manner (figure 2*B*). Similarly, Western blot analysis demonstrated a decrease in HCV NS5A after treatment with myriocin (figure 2C).

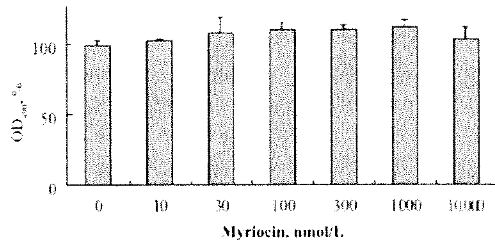
No enhancement of ISRE promoter activity after myriocin treatment. To determine whether the effect of myriocin in suppressing the subgenomic HCV replicon was associated with the activation of IFN-stimulated genes, the ISRE–*Renilla* luciferase plasmid was transfected into Huh7/Rep-Feo cells, and these cells were cultured with various concentrations of myriocin. As a positive control for the enhancement of ISRE reporter activity, the ISRE–*Renilla* luciferase–transfected cells were cultured with IFN. Myriocin had no significant effect on ISRE promoter activity, whereas IFN significantly up-regulated ISRE activity (figure 2*D*, *upper panel*). In contrast, firefly luciferase activity in the Huh7/Rep-Feo cells, reflecting HCV replication, was inhibited by both IFN and myriocin in a dose-dependent manner (figure 2*D*, *lower panel*). These results demonstrate that the action of myriocin on HCV replication is independent of the IFN pathway.

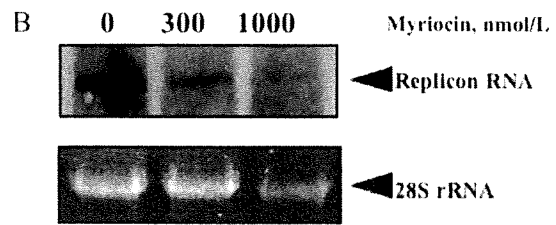
Decrease in the sphingomyelin content of Huh7 cells after myriocin treatment. To clarify whether myriocin really inhibits the biosynthesis of sphingomyelin in Huh7 cells, we treated Huh7 cells with 100 nmol/L myriocin and analyzed the change in the cellular phospholipid composition by TLC. As demonstrated in figure 2*E*, the cellular sphingomyelin content decreased after myriocin treatment, but no significant change was observed in other cellular phospholipids.

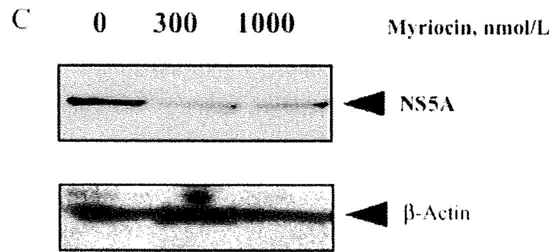
Restoration of HCV replication by addition of phytosphingosine. To confirm that suppression of HCV RNA replication was due to depletion of sphingomyelin, we incubated replicon cells with phytosphingosine, a precursor of ceramide in mammalian and fungal cells, in the presence of myriocin. Treatment with phytosphingosine restored HCV replication in a dose-dependent manner (figure 2*F*, *upper panel*). On the other hand, phytosphingosine by itself did not have any effect on HCV replication (figure 2*F*, *lower panel*). This result indicates that inhibition of HCV replication was the direct result of depletion of sphingomyelin.

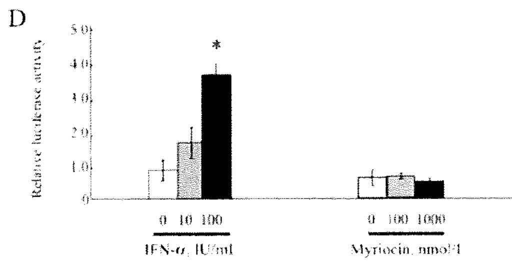
Suppression of HCV replication by knocking down SPT with siRNA. Next, we determined whether inhibition of SPT expression suppresses HCV replication by knocking down SPT with siRNA. As demonstrated in the upper panel of


A
Relative luciferase activity
Myriocin, nmol/L

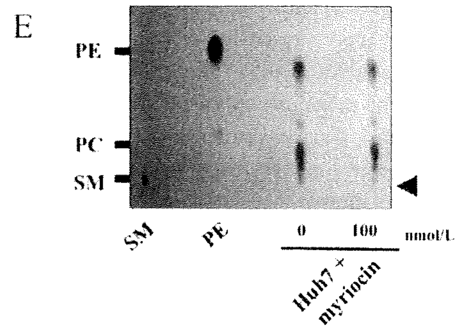

OD, %
100
50
0
0
10
30
100
300
1000
10000
Myriocin, nmol/L

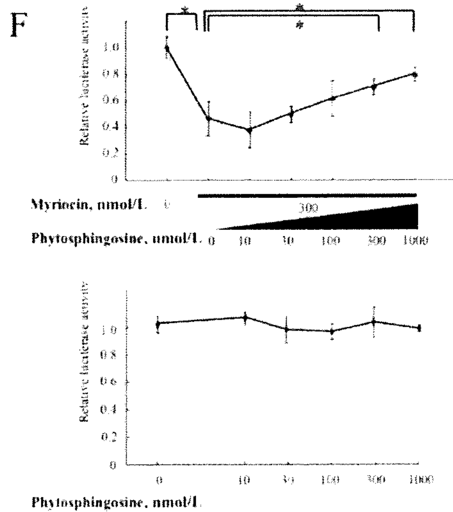

B
0
300
1000
Myriocin, nmol/L
Replicon RNA
28S rRNA

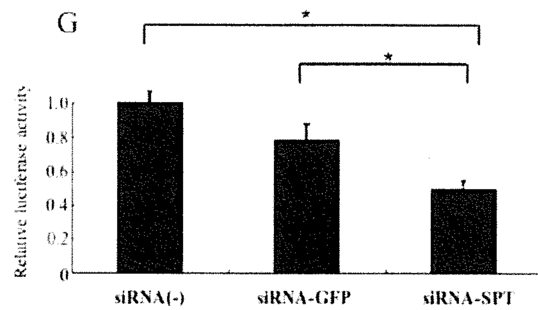

C
0
300
1000
Myriocin, nmol/L
NS5A
β-Actin


D
Relative luciferase activity
0 10 100
IFN-α, IU/mL
0 100 1000
Myriocin, nmol/L

Relative luciferase activity
0 10 100
IFN-α, IU/mL
0 100 1000
Myriocin, nmol/L


E
PE
PC
SM
SM
PE
0
100
nmol/L
Huh7 + myriocin


F
Relative luciferase activity
Myriocin, nmol/L
300
Phytosphingosine, nmol/L
0
10
30
100
300
1000
Relative luciferase activity
Phytosphingosine, nmol/L


G
Relative luciferase activity
siRNA(-)
siRNA-GFP
siRNA-SPT

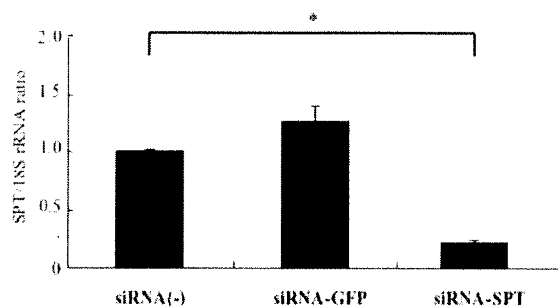

SPT/18S rRNA ratio
siRNA(-)
siRNA-GFP
siRNA-SPT

figure 2G, HCV replication was suppressed significantly by siRNA targeting SPT compared with no siRNA or siRNA targeting GFP (negative control). We confirmed with real-time PCR that the siRNA targeting SPT significantly decreased expression of SPT mRNA (figure 2G, *lower panel*). This result indicates that the SPT enzyme plays an important role in HCV replication.

Inhibition of the replication of a subgenomic HCV-1b replicon by an HMG-CoA reductase inhibitor (simvastatin). HMG-CoA reductase inhibitors have been reported to suppress replication of subgenomic and genomic HCV-1b replicons [15, 16]. Because cholesterol is another important component of lipid rafts, it may be speculated that depletion of cholesterol by HMG-CoA reductase inhibitors disrupts the lipid raft, affecting the ability of the HCV replicon to replicate in Huh7 cells. To confirm the effect of HMG-CoA reductase inhibitors on the subgenomic HCV-1b replicon, we examined the effect of simvastatin by means of Huh7/Rep-Feo cells. Cultures of Huh7/Rep-Feo cells with simvastatin at concentrations of 0–100 μmol/L showed a dose-dependent reduction of the subgenomic HCV-1b replicon (figure 3, *upper panel*). The MTS assay showed that treatment with simvastatin had no toxic effect on Huh7/Rep-Feo cells in the dose range used (figure 3, *lower panel*). These results demonstrated that simvastatin specifically suppressed replication of a subgenomic HCV-1b replicon. However, because recent studies showed that statins suppress HCV replication through inhibition of geranylgeranylation of certain proteins rather than inhibition of cholesterol synthesis [15], we also examined the effect on HCV replication of 2-HP-β-CyD, an agent known to deplete cholesterol directly from membranes. As demonstrated in figure 4A, 2-HP-β-CyD also suppressed HCV replication without cytotoxicity. To confirm that 2-HP-β-CyD did not inhibit firefly luciferase activity nonspecifically rather than by suppressing HCV RNA, we incubated recombinant firefly luciferase with various concentrations of 2-HP-β-CyD in the culture medium, and the medium was subjected to luciferase analysis. As demonstrated in figure 4B, 2-HP-β-CyD did not affect luciferase activity. These results indicate that cholesterol itself plays an important role in HCV replication.

Synergistic inhibitory effects of myriocin with IFN, simvastatin with IFN, and myriocin with simvastatin. We carried out the following assay to determine whether myriocin and IFN have a synergistic inhibitory effect on HCV replication. Huh7/Rep-Feo cells were treated with combinations of myriocin and IFN at various concentrations. The relative dose-inhibition curves of IFN were plotted for each fixed concentration of myriocin (0, 30, 100, and 300 nmol/L). As demonstrated in the upper panel of figure 5A, the curves shifted to the left with increasing concentrations of myriocin, demonstrating the synergy of the 2 drugs against the subgenomic HCV-1b replicon. Isobologram analysis also confirmed the synergy (figure 5A, *lower panel*). To determine whether this synergistic effect was associated with upregulation of the IFN-stimulated gene responses, we investigated the combined effect of myriocin and IFN on ISRE activity. As demonstrated in figure 5B (*upper panel, right*), myriocin did not enhance the ISRE–*Renilla* luciferase activity induced by IFN, but

Figure 2. Specific inhibition of the replication of a subgenomic hepatitis C virus (HCV) genotype 1b replicon by myriocin. *A*, Inhibition of HCV replicon replication by myriocin. By use of Huh7/Rep-Feo cells expressing a selectable chimeric luciferase reporter Feo gene, the intracellular replication level of an HCV replicon was quantified on the basis of luciferase activity [22, 25]. Huh7/Rep-Feo cells were cultured with various concentrations of myriocin. After 96 h of treatment, the luciferase assay was performed, as described in Materials and Methods (*upper panel*). In the dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium (MTS) assay, Huh7/Rep-Feo cells were cultured with various concentrations of myriocin for 96 h (*lower panel*). Data are means ± SDs of triplicates from 2 independent experiments. *B*, Northern hybridization. Huh7/Rep-Feo cells were cultured with various concentrations of myriocin and harvested at 96 h after administration. Ten micrograms of total cellular RNA was electrophoresed in each lane. The membrane containing the HCV replicon RNA was hybridized using a digoxigenin-labeled probe specific for the replicon sequence (*upper panel*), and 28S human ribosomal RNA (rRNA) was used as an internal control (*lower panel*). Lane 1, no myriocin; lane 2, 300 nmol/L myriocin; lane 3, 1000 nmol/L myriocin. *C*, Western blotting. Ten micrograms of total cellular protein was electrophoresed in each lane. Anti-NS5A monoclonal antibody was used as the primary antibody to detect HCV proteins (*upper panel*), and β-actin was used as an internal control (*lower panel*). Lane 1, no myriocin; lane 2, 300 nmol/L myriocin; and lane 3, 1000 nmol/L myriocin. *D*, No enhancement of interferon (IFN)–stimulated response element (ISRE) promoter activity by myriocin. To investigate whether the effect of myriocin was associated with the activation of IFN-stimulated genes, the ISRE–*Renilla* luciferase plasmid was transfected into Huh7/Rep-Feo cells in the presence of myriocin. The upper panel demonstrates the ISRE–*Renilla* luciferase activity at 48 h after transfection. The lower panel demonstrates the firefly luciferase activity of the Huh7/Rep-Feo cells, reflecting HCV replication. Data are means ± SDs of triplicates from 2 independent experiments. $^*P < .05$. *E*, Decrease in the sphingomyelin (SM) content of Huh7 cells after myriocin treatment. The change in the cellular phospholipid content was analyzed by thin-layer chromatography. Huh7 cells were cultured alone or with 100 nmol/L myriocin for 96 h. PC, phosphatidylcholine; PE, phosphatidylethanolamine. *F*, Restoration of the HCV replication that was suppressed by myriocin after the addition of phytosphingosine. Huh7/Rep-Feo cells were cultured with myriocin alone or with various concentrations of phytosphingosine. The luciferase assay was performed after 72 h of treatment (*upper panel*). Huh7/Rep-Feo cells were also cultured with phytosphingosine alone as indicated for 72 h (*lower panel*). Data are means ± SDs of triplicates from 2 independent experiments. $^*P < .05$. *G*, Suppression of HCV replication by knocking down of serine palmitoyltransferase (SPT) with short interfering RNA (siRNA). Huh7/Rep-Feo cells were transfected with 10 nmol/L siRNA oligonucleotides targeting the LCB1 subunit of SPT or control siRNA targeting green fluorescent protein (GFP). The luciferase activity of the HCV replicon was measured 72 h after transfection (*upper panel*). SPT mRNA expression at 72 h after siRNA transfection was analyzed by real-time polymerase chain reaction. The SPT mRNA level was measured relative to 18S rRNA (*lower panel*). Values are shown as ratios to negative control levels and as the means ± SDs of triplicates from 2 independent experiments. siRNA(−), no siRNA. $^*P < .05$

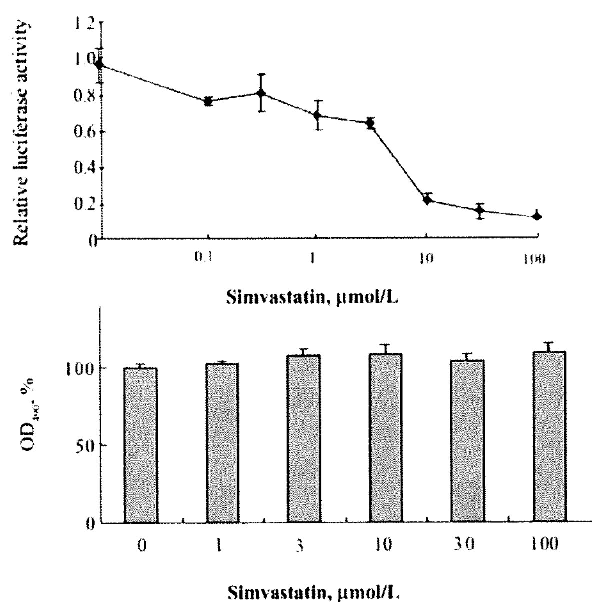


Figure 3. Inhibition of replication of a subgenomic hepatitis C virus genotype 1b replicon by simvastatin. Huh7/Rep-Feo cells were cultured with various concentrations of simvastatin, and the luciferase assay was performed after 48 h of treatment *(upper panel)*. The dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium assay was performed after Huh7/Rep-Feo cells were cultured with various concentrations of simvastatin for 48 h *(lower panel)*. Data are means ± SDs of triplicates from 2 independent experiments.

it significantly enhanced IFN-induced suppression of the firefly luciferase activity reflecting HCV replication *(lower panel, right)*. This demonstrated that the synergistic effect was not caused by up-regulation of the IFN-stimulated genes. We also assessed the synergy of simvastatin with IFN and of myriocin with simvastatin. In each case, the 2 drugs showed synergistic effects at the concentrations indicated (figure 5C and 5D). In all cases, the MTS reduction values at the drug concentrations used in this assay did not show any significant decrease (data not shown). These results indicate that the synergistic effects on HCV replication of IFN with myriocin, IFN with simvastatin, and myriocin with simvastatin were exerted through their pharmacological effects and were not due to the augmentation of cytotoxicity.

Suppression of JFH-1 HCV replication by myriocin and simavastatin. The experiments described thus far were done using the subgenomic HCV-1b replicon system. Recently, Wakita et al. [19] established an infectious HCV model in cultured cells. This system, known as the JFH-1 system and based on genotype 2a HCV, secretes viral particles into the medium, and the medium is infectious for chimpanzees. This JFH-1 system completely mimics HCV infection in vivo and is considered more suitable for analyzing the effect of drugs. Therefore, we examined the effect of myriocin and simvastatin using the JFH-1 system. Huh7.5.1/JFH-1 HCV cells were cultured for 96 h with 1000 nmol/L myriocin, 10 µmol/L simvastatin, 1000 IU/mL IFN, and a combination of 1000 nmol/L myriocin and 10 µmol/L simvastatin. The intracellular JFH-1 HCV RNA titer was analyzed using real-time RT-PCR. As demonstrated in figure 6A, intracellular JFH-1 HCV RNA treated with myriocin or simvastatin decreased to 60% of control in 96 h, demonstrating that the inhibitory effect of myriocin and simvastatin on replication was not restricted to the subgenomic HCV-1b replicon. When both agents were used in combination, JFH-1 HCV RNA also

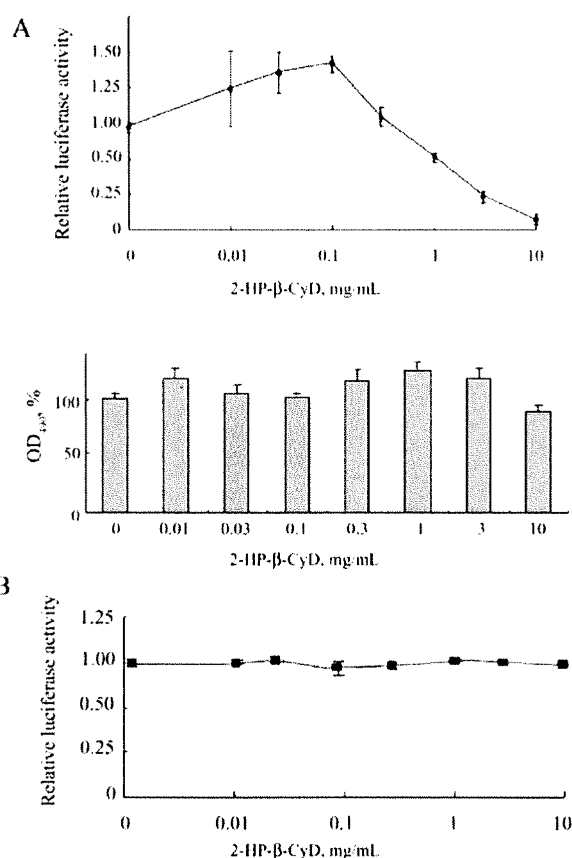


Figure 4. Inhibition of replication of a subgenomic hepatitis C virus genotype 1b replicon by 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CyD). *A,* Huh7/Rep-Feo cells cultured with various concentrations of 2-HP-β-CyD for 48 h. The luciferase assay was performed after 48 h of treatment *(upper panel)*. The dimethylthiazol carboxymethoxyphenyl sulfophenyl tetrazolium assay was performed after Huh7/Rep-Feo cells were cultured with various concentrations of 2-HP-β-CyD for 48 h *(lower panel)*. Data are means ± SDs of triplicates from 2 independent experiments. *B,* Recombinant firefly luciferase incubated with various concentrations of 2-HP-β-CyD in the culture medium at 37°C for 48 h. The medium was collected and subjected to luciferase analysis. Data are means ± SDs of triplicates from 2 independent experiments.

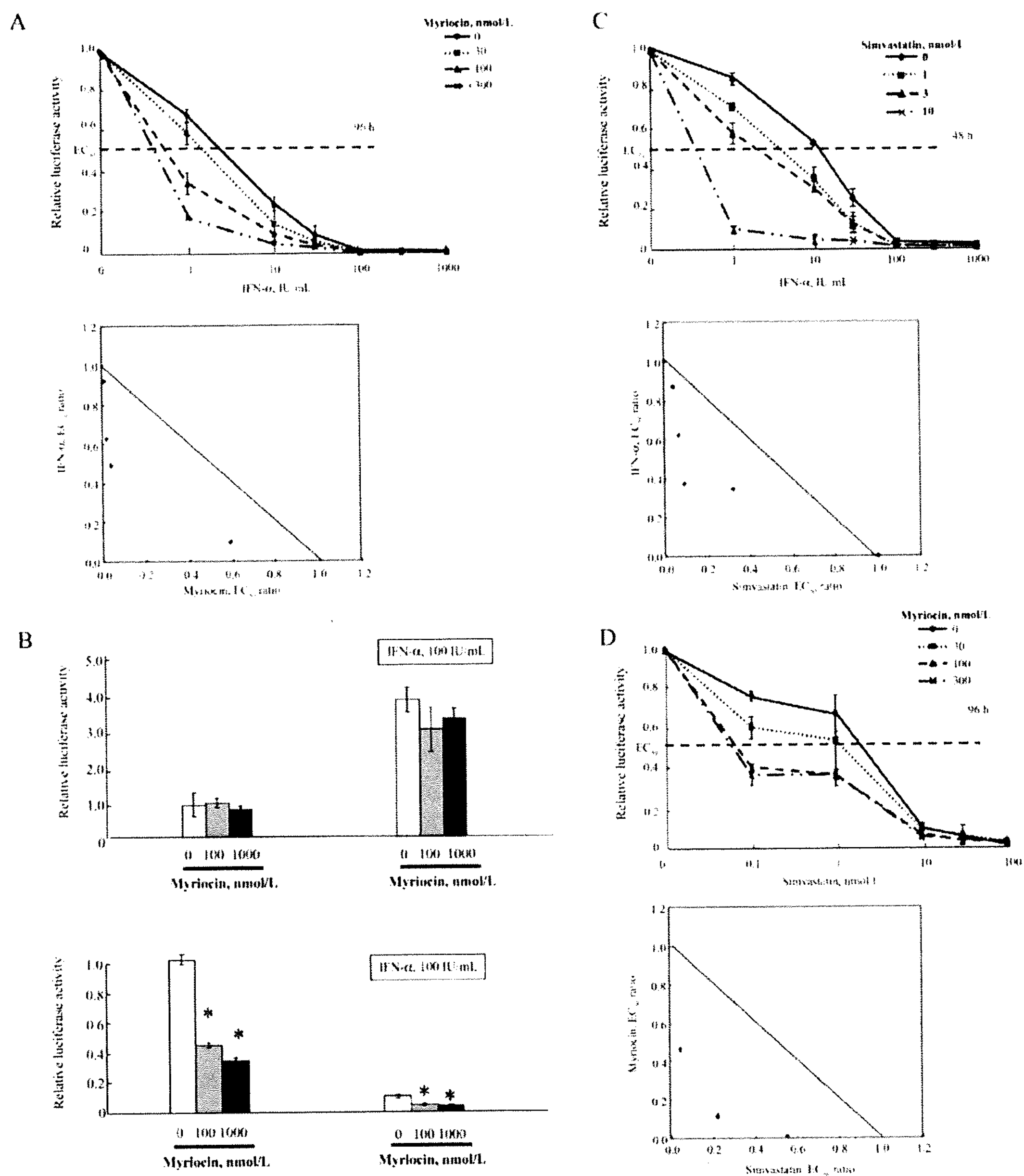


Figure 5. Synergistic inhibitory effects of myriocin with interferon (IFN), simvastatin with IFN, and myriocin with simvastatin. *A,* Synergistic inhibitory effect of myriocin with IFN on hepatitis C virus replication. Huh7/Rep-Feo cells were treated with combinations of myriocin and IFN at various concentrations. The upper panel shows the relative dose-inhibition curves of IFN plotted for each fixed concentration of myriocin (0, 30, 100, and 300 nmol/L). The lower panel shows the isobologram analysis for the combination of myriocin with IFN. *B,* IFN-stimulated response element (ISRE) promoter activity induced by a combination of myriocin with IFN. Huh7/Rep-Feo cells transfected with ISRE–*Renilla* luciferase were cultured with various concentrations of myriocin alone *(left)* or with 100 IU/mL IFN *(right)*. The upper panel demonstrates the ISRE–*Renilla* luciferase activity at 48 h after transfection. The lower panel demonstrates the firefly luciferase activity of the Huh7/Rep-Feo cells, reflecting hepatitis C virus (HCV). Data are means ± SDs of triplicates from 2 independent experiments. $^*P < .05$. *C,* Synergistic inhibitory effect of simvastatin with IFN on HCV replication. *D,* Synergistic inhibitory effect of simvastatin and myriocin on HCV replication.

decreased to almost 60% of the control at 48 and 96 h after treatment. However, no evident synergistic inhibitory effect was observed (figure 6*A*). To clarify the inhibitory effect of myriocin on JFH-1 HCV, we performed Western blot analysis for JFH-1 HCV proteins. As demonstrated in figure 6*B*, a substantial decrease in the core and NS3 proteins of JFH-1 HCV was observed 96 h after treatment with myriocin, confirming the RT-PCR results (figure 6*B*).

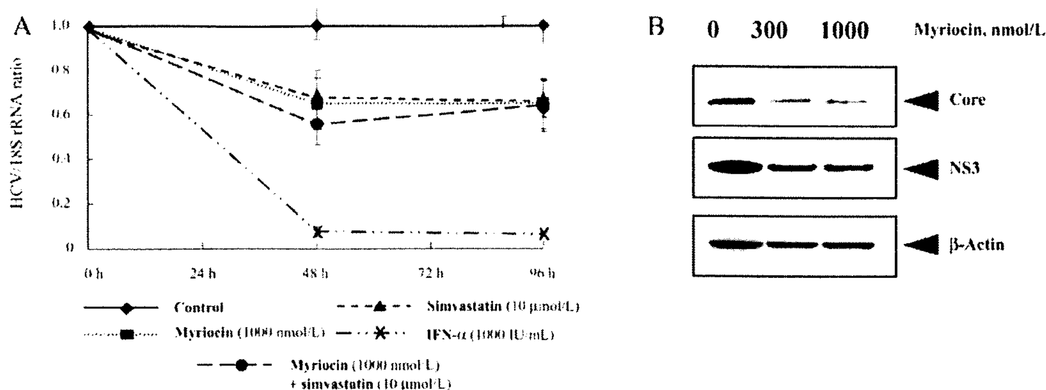


Figure 6. Suppression of JFH-1 hepatitis C virus (HCV) replication by myriocin and simvastatin. *A*, Cells containing JFH-1 HCV treated for 96 h with 1000 nmol/L myriocin, 10 μmol/L simvastatin, 1000 IU/mL IFN, or a combination of 1000 nmol/L myriocin and 10 μmol/L simvastatin. The cells were collected at 48 and 96 h, and the JFH-1 HCV RNA level relative to 18S rRNA was analyzed by real-time polymerase chain reaction. Values are shown as the ratios to negative control values (cells receiving no treatment) and as means ± SDs. *B*, Western blotting. Cells containing JFH-1 HCV were treated with 300 or 1000 nmol/L of myriocin and harvested at 96 h after administration. Ten micrograms of total cellular protein was electrophoresed in each lane. Anti-core monoclonal antibody (MAb) and anti-NS3 MAb were used as the primary antibodies to detect JFH-1 HCV proteins. β-Actin was detected as an internal control. Lane 1, no myriocin; lane 2, 300 nmol/L myriocin; and lane 3, 1000 nmol/L myriocin

DISCUSSION

In the present study, we demonstrated that the sphingomyelin synthesis inhibitor myriocin suppressed not only replication of a subgenomic HCV-1b replicon but also replication of the JFH-1 strain of infectious genotype 2a HCV. We also demonstrated that simvastatin suppressed replication of both a subgenomic HCV-1b replicon and JFH-1 HCV. When a subgenomic HCV-1b replicon was used, the anti-HCV activity of both myriocin and simvastatin was enhanced synergistically with IFN. Moreover, when myriocin and simvastatin were used together, their anti-HCV activity was enhanced synergistically.

What is the mechanism by which myriocin suppresses viral replication? Because myriocin is a specific inhibitor of SPT, which catalyzes the first committed step of sphingomyelin biosynthesis, we speculated that myriocin exerts its action by inhibiting production of downstream substrates, especially sphingomyelin. The findings that siRNA targeted against SPT decreased HCV replication and that HCV replication was restored by addition of phytosphingosine, a precursor of sphingomyelin, demonstrated that the effect was specific to SPT activity. Moreover, the fact that treatment of Huh7 cells with myriocin did not enhance the ISRE promoter activity indicated that the inhibitory effects of myriocin were independent of those of IFN. It is known that intracellular replication of most RNA viruses occurs on certain membrane structures—including the endoplasmic reticulum, the Golgi apparatus, endosomes, and lysosomes—by making replication complexes at these sites [5–7]. For HCV, it has been reported by several groups that in vitro replication activity is located in the membrane fractions of cultured cells [26–28]. In addition, newly synthesized HCV RNA and the nonstructural proteins in replicon cells were colocalized in detergent-resistant membrane structures, most likely lipid rafts [18]. Caveolin-2, a lipid raft protein, was also shown to colocalize with the nonstructural proteins [18]. According to these findings, the HCV replication complex machinery is considered to form on a lipid raft. Therefore, because sphingomyelin is the major component of the lipid raft, it is plausible to speculate that myriocin disrupted lipid raft formation and inhibited HCV replication.

Cholesterol is another major component of lipid rafts and might also be targeted for anti-HCV therapy. Because cholesterol is synthesized in the mevalonate pathway, an inhibitor of the pathway might act to disrupt lipid rafts. In accordance with this concept, statins, which are HMG-CoA reductase inhibitors, already have been reported to suppress the replication of genomic and subgenomic HCV-1b replicons [15, 16]. In the present study, we also confirmed that simvastatin suppressed replication of a subgenomic HCV-1b replicon without toxicity. Moreover, we showed for the first time that the suppressive effect was also observed in an infectious HCV-2a model of JFH-1 HCV. Meanwhile, recent studies found that the effect of statins was attributable to inhibition of geranylgeranylation rather than depletion of cholesterol, because addition of geranylgeraniol rescued HCV suppression induced by statins [15]. However, although geranylgeranylation might play a role in HCV regulation, the importance of cholesterol itself has not yet been determined. To clarify further the role played by cholesterol in HCV replication, we investigated the effect of 2-HP-β-CyD, which is known to deplete cholesterol directly from cells. As demonstrated in figure 4, specific suppression of HCV replication by 2-HP-β-CyD indicated the importance of cholesterol itself for HCV replication. It is unlikely that these agents suppressed replication of the subgenomic replicon through inhibi-

tion of encephalomyocarditis virus internal ribosome entry site (EMCV-IRES) activity, because they also significantly suppressed replication of a full-length genomic HCV (JFH-1 HCV) that does not include EMCV-IRES (figure 6*A*; data for 2-HP-β-CyD not shown).

Although we observed an inhibitory effect of myriocin and simvastatin on both the subgenomic HCV-1b replicon and JFH-1 HCV, there was a difference in efficacy between the 2 HCV systems; the subgenomic HCV-1b replicon was more sensitive to and was more strongly inhibited by either agent alone or in combination, compared with JFH-1 HCV. This result was unexpected, because we had speculated that these agents might be effective irrespective of the viral isolate if these agents targeted not the virus itself but rather host factors, such as lipid rafts. However, there are several differences between these 2 systems, and we cannot directly compare the results. In particular, the subgenomic HCV replicon lacks viral structural proteins and has only an HCV RNA intracellular replication step, whereas JFH-1 HCV includes all steps of the HCV life cycle. We do not know the precise target of the agents, and further studies are still needed.

Is it really possible to use these agents in clinical HCV treatment? Especially because statins have been used in the treatment of hyperlipidemia for many years worldwide with proven safety, it would be ideal if we could use statins as one therapeutic application for anti-HCV therapy. Most recently, O'Leary et al. [29] undertook a human pilot study and treated 10 patients with atorvastatin for 12 weeks; they reported that there was no statistically significant change in HCV RNA levels compared with pretreatment levels. The reason for the discrepancy between in vitro and in vivo findings is unknown. However, as also discussed by O'Leary et al., the most plausible explanation for this discrepancy is that the plasma concentrations of atorvastatin after a conventionally approved dose were unlikely to reach those found to be effective in cell culture medium. According to their calculations, to inhibit HCV RNA replication the plasma atorvastatin concentration should be 3 logs higher than that achieved by a conventional dose. However, even though it would be difficult to inhibit HCV RNA replication with statins alone, a clinical antiviral effect might be still achieved if statins were used in combination with IFN (or myriocin), because a synergistic effect was observed in our in vitro study. To determine the synergistic effect in vivo, however, further clinical trials are needed. On the other hand, although promising in vitro, myriocin has not yet been used for human clinical diseases, and its safety has not been established. However, in chimeric mice, the plasma myriocin concentration equivalent to culture medium effectively inhibited HCV RNA replication, and drug toxicity was not observed at this concentration [30]. This finding suggested the possibility that myriocin could be used in vivo, although further studies are needed.

In conclusion, we have demonstrated that inhibition of the sphingomyelin synthesis pathway and the mevalonate pathway both effectively suppressed HCV replication in vitro, indicating that lipid metabolism could be an important target for new anti-HCV therapies.

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The presence of steatosis and elevation of alanine aminotransferase levels are associated with fibrosis progression in chronic hepatitis C with non-response to interferon therapy☆

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***Background/Aims*: Interferon (IFN) therapy leads to regression of hepatic fibrosis in chronic hepatitis C patients who achieve a sustained virologic response (SVR), while the beneficial effect is limited in those who fail to do so. The aim of the present study was to define factors associated with progression of fibrosis in patients who do not achieve a SVR.**

***Methods*: Fibrosis staging scores were compared between paired liver biopsies before and after IFN in 97 chronic hepatitis C patients who failed therapy. The mean interval between biopsies was 5.9 years. Factors associated with progression of fibrosis were analyzed.**

***Results*: Fibrosis progressed in 23%, remained unchanged in 47% and regressed in 29%. Steatosis and a high average alanine aminotransferase (ALT) between biopsies were independent factors for progression of fibrosis with risk ratios of 5.53 and 4.48, respectively. Incidence and yearly rate of progression of fibrosis was 64% and 0.22 ± 0.29 fibrosis units per year in those with both risk factors compared to 8% and −0.04 ± 0.17 fibrosis units per year in those negative for both factors.**

***Conclusions*: Hepatic steatosis and elevated ALT levels are risk factors for progression of fibrosis in chronic hepatitis C patients who fail to achieve a SVR to IFN therapy and therefore may be therapeutic targets to halt the potentially progressive disease.**
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***Keywords*: Steatosis; ALT; Fibrosis**

1. Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. Mortality associated with HCV infection results from the development of liver cirrhosis and hepatocellular carcinoma, which now is the leading indication for liver transplantation [1]. Treatment with interferon (IFN), alone or in combination with ribavirin (RBV), can eradicate HCV infection in some patients, leading to sustained nor-

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