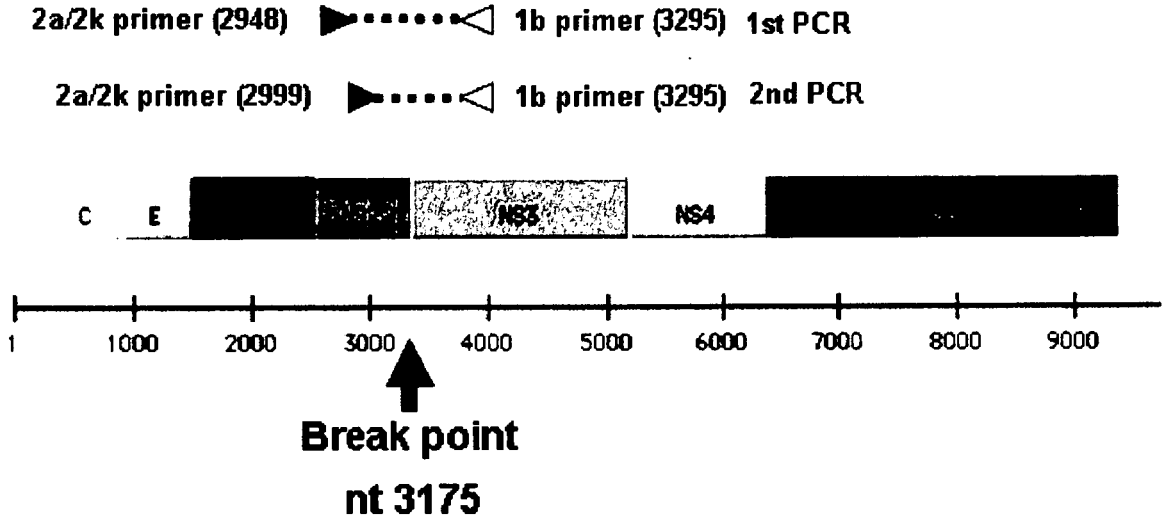
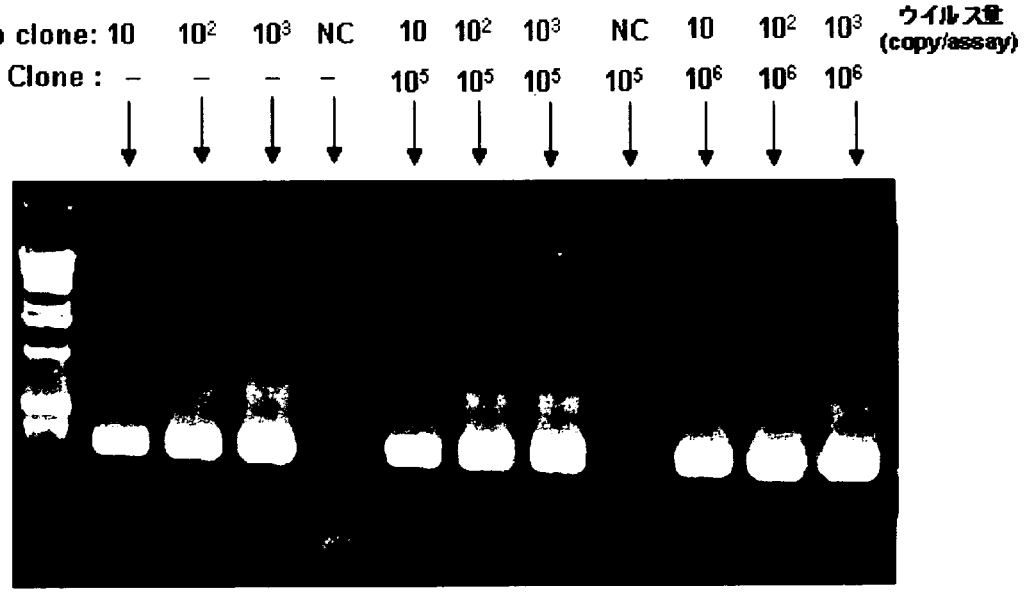
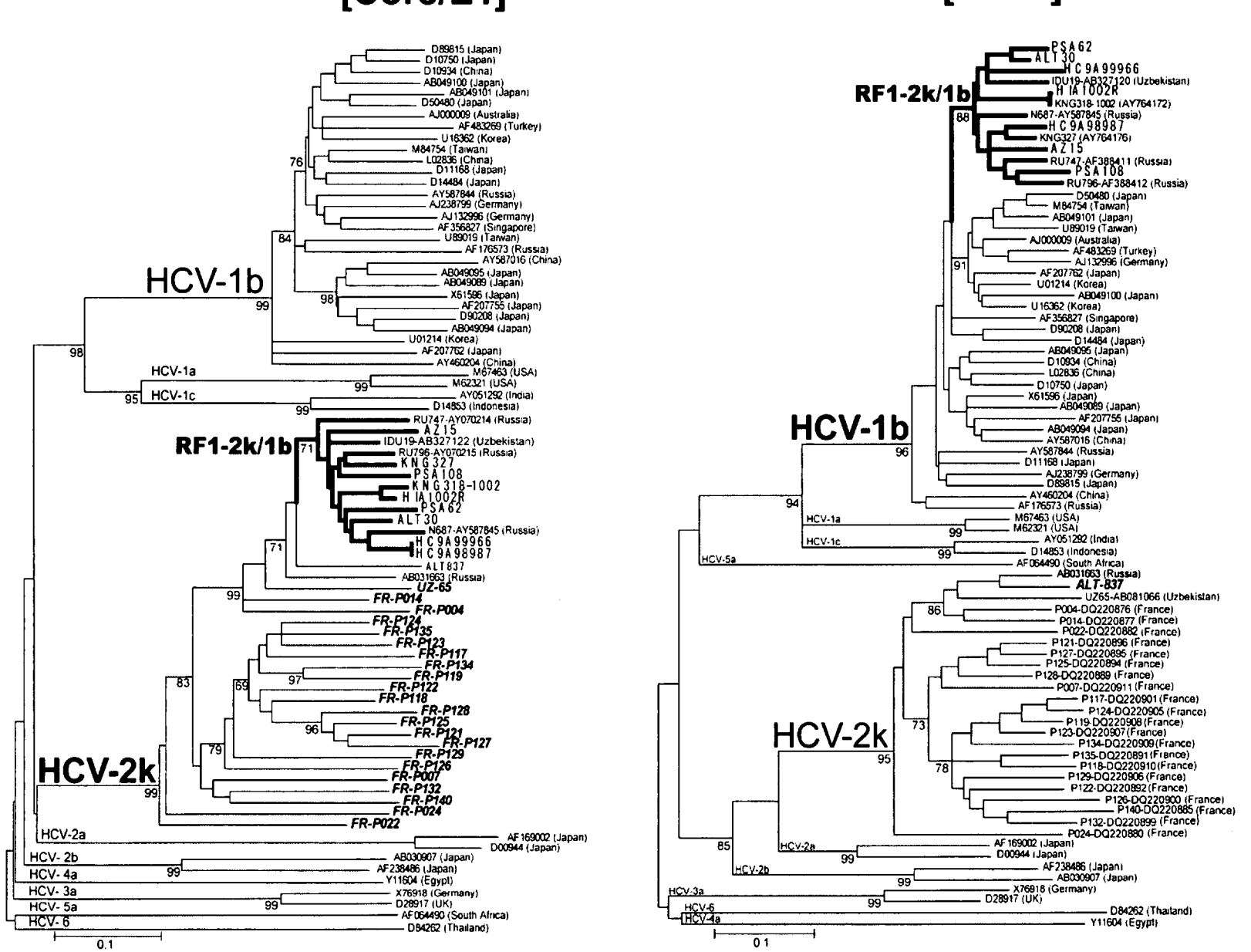
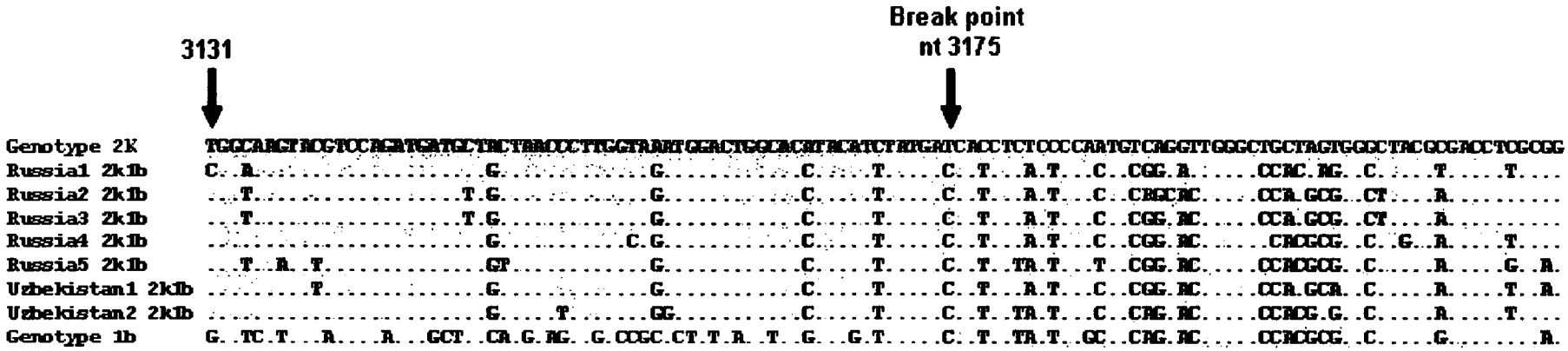
(a)


2a/2k primer (2948)
1b primer (3295) 1st PCR
2a/2k primer (2999)
1b primer (3295) 2nd PCR
C
E
NS3
NS4
1
1000
2000
3000
4000
5000
6000
7000
8000
9000
Break point
nt 3175

(b)


2k/1b clone: 10 10² 10³ NC 10 10² 10³ NC 10 10² 10³
ウイルス量 (copy/assay)
1b Clone : – – – – 10⁵ 10⁵ 10⁵ 10⁵ 10⁶ 10⁶ 10⁶


[Core/E1]
HCV-1b
RF1-2k/1b
HCV-2k
HCV-1a
HCV-1c
HCV-2a
HCV- 2b
HCV- 4a
HCV- 3a
HCV- 5a
HCV- 6
0.1
[NS5b]
RF1-2k/1b
HCV-1b
HCV-2k
HCV-1a
HCV-1c
HCV-5a
HCV-2a
HCV-2b
HCV-3a
HCV-5
HCV-4a
0 1


3131
Break point
nt 3175
Genotype 2k
Russia1 2k1b
Russia2 2k1b
Russia3 2k1b
Russia4 2k1b
Russia5 2k1b
Uzbekistan1 2k1b
Uzbekistan2 2k1b
Genotype 1b

厚生科学研究費補助金（日米医学協力研究会肝炎専門部会）
分担研究報告書

C型肝炎における肝脂肪化と酸化ストレス・肝発癌

分担研究者：　小池和彦　東京大学医学部（病）・教授

研究要旨：我々は、マウスモデルを用いてC型肝炎ウイルス(HCV)コア蛋白が肝発癌を引き起こすことを示してきた。この動物モデルでは、明らかな炎症の不在下に肝脂肪化(steatosis)が発生し、その後に肝細胞癌が発生している。このマウスモデルおよびC型肝炎患者の肝に蓄積する脂肪には特徴があり、オレイン酸などのC18一価不飽和脂肪酸が増加しており、HCV 感染により脂質代謝異常が引き起こされることが明らかになっている。また、インスリン抵抗性もHCVにより惹起されることが示され、HCVによる代謝への影響が、HCV による病原性発現において重要な役割を果たしている可能性がある。今回、我々は脂質代謝に関連する核内受容体の一つである peroxisome proliferator-activated receptor (PPAR)αの、HCVコア遺伝子トランスジェニックマウスにおける発現状態を検討した。PPARαタンパクは本マウスモデルの肝において核に蓄積し、cyclin D1 や acyl-CoA oxidase (ACO)等のPPARαターゲット遺伝子産物も同様に増加していた。HCV関連肝発癌におけるPPARα活性化の意義を明らかにするため、PPARαノックアウトマウスとコア遺伝子トランスジェニックマウスを掛け合わせたところ、PPARαの持続的な活性化無しでは肝脂肪化も肝癌も生じないことが明らかになった。肝脂肪化、ミトコンドリア機能障害と肝発癌が密接に関連していることを示すデータであり、HCV の病原性発現を抑制する方策の開発に有用と考えられる。

A. 研究目的

C型慢性肝炎における肝発癌の機序はまだ完全にはわかっていない。チンパンジー以外にC型肝炎の疾患モデル動物がないことも、解明の妨げとなっている。我々は、ＨＣＶのコア蛋白がトランスジェニックマウス(Tg)において肝細胞を誘発することを確認し、このマウスモデルを用いてC型肝炎における病態の解明、肝発癌機序の解明を行ってきた。また、マウスモデルで得られた知見をもとにして、ヒトC型肝炎患者においても検討を行なってきた。

C型肝炎動物モデルであるコア遺伝子Tgにおいては、明らかな炎症の不在下に肝脂肪化(steatosis)が発生し、その後に肝細胞癌（肝癌）が発生している。また、このマウスモデルおよびC型肝炎患者の肝に蓄積する脂肪には特徴があり、オレイン酸、ヴァクセン酸といった一価不飽和脂肪酸が増加していることが明らかになっている。ま

た、インスリン抵抗性もHCVにより惹起されることが示され、HCVによる代謝への影響がHCVによる病原性発現において重要な役割を果たす可能性がある。

また、我々はこれまでに脂質代謝に関連の深いRXR (retinoid X receptor) αがHCVコア蛋白とDNA結合領域で結合して、RXRα反応性エレメントをもつ遺伝子の発現を活性化することを示してきた。更に、RXRαとheterodimerを形成するPPAR (peroxisome proliferator-activated receptor) αへの反応性エレメントをもつ遺伝子の発現も、コア蛋白によって活性化されることも示してきた。本年度、我々は脂肪酸の輸送と異化に関連が深いPPARα発現の状態を本マウスモデルにおいて検討した。

B. 方法

本研究の対象動物として用いたTgは、HCVタンパク質そのものが肝発癌活性を有することを証明する上で重要な動物モデルである。このマウスモデルにおいては、2か月齢でインスリン抵抗性を、3か月齢で肝脂肪化を、16か月齢で肝癌を発生する。また、このマウスでは若年時から肝細胞のミトコンドリアに機能障害を起こし、特に電子伝達系コンプレックスIの傷害によって酸化ストレスの過剰産生を起こす事が明らかになっている。

PPARαを始めとするタンパクの発現は、ウエスタンブロッティングあるいは免疫組織染色を行ない発現レベルを確認した。また、ノーザンブロッティング、Taqman PCRも適宜施行した。

さらに、米国NIHのFrank Gonzalez博士との共同研究により、PPARαノックアウト(KO)マウスとコア遺伝子本Tgとの掛け合わせを行なった。

C. 結果

(1)HCVコア遺伝子Tg肝においては、PPARαタンパクが増加していた。主に核内に蓄積しており、またmRNAレベルには変化が認められなかった。Pulse-chase実験によって、コア蛋白の存在によってPPARαの安定性が増加することが核内PPARαタンパク増加の機序と考えられた。

(2) HCVコア遺伝子Tg肝において、PPARαターゲット遺伝子であるcyclin D1、CDK4、acyl-CoA oxidase、peroxisome thiolase、liver-fatty acid binding protein (L-FABP)等のタンパク、mRNAはともに増加していた。

(3) HCVコア蛋白による病原性発現におけるPPARα活性化の意義を明らかにするために、PPARα KOマウスとコア遺伝子Tgを掛け合わせてハイブリッドマウスを作製した。このCoreTg/PPARα KOマウスにおいては、肝脂肪化が認められなかった。

(4) 更に、CoreTg/PPARα KOマウスにおいてはPPARαターゲット遺伝子の発現は消失・低下し、ミトコンドリア障害も軽減していた。

(5) CoreTg/PPARα KOマウスにおいては肝癌も発生しなかった。これに対してCoreTg/PPARα intactマウスでは、これまでの報告通りに雄の約35%で肝癌を発生した。

(6)PPARαがヘテロのコア遺伝子Tgにお

いても肝脂肪化、肝癌は発生しなかった。これらの事実は、コア蛋白による病原性発現のためにはPPARαの存在ではなく、持続的な活性化が必要であることを示している。

（7）PPARαヘテロのマウスにclofibrate (peroxisome proliferator agonist)を24ヶ月にわたり投与したところ、コア遺伝子（＋）PPARαヘテロマウスでのみ肝脂肪化を生じ、肝発癌もコア遺伝子（-）PPARαヘテロマウスに比し有意に高率であった。

D. 考察

今回の研究において、PPARαの持続的な活性化がHCVコア蛋白による病原性発揮に必要であることが示唆された。しかしながら、PPARαの活性化のみでは肝脂肪化は起こらず、また肝発癌も低頻度である。HCコア蛋白とPPARα活性化の共存がこの様な表現型をもたらすと推測された。

コア蛋白によって肝脂肪化、脂肪酸の増加がもたらされると、これがPPARα活性化をもたらす。また、RXRαを介して、コア蛋白はPPARαを活性化する。PPARα活性化自体は通常は脂肪の異化をもたらすが、PPARα活性化は同時にACO活性化を介して酸化ストレスを増加させ、ミトコンドリア機能を障害する。つまり、コア蛋白によって既に誘発されているミトコンドリア機能障害、電子伝達系の障害がPPARα活性化によって更に増悪し、脂肪酸を介した「負のループ」の形成、いわば「脂肪酸スパイラル」とでも呼ぶべきものが起こり、肝脂肪化、脂肪酸増加、酸化ストレス産生、細胞増殖遺伝子発現増加という一連の現象を次々と惹起し、細胞増殖遺伝子発現増加と相まって、最終的に肝癌の発生へと至ると考えられる。

HCVによる病原性発現において、コア蛋白によるミトコンドリア機能の修飾は大きな意義を保持していること、PPARαの活性化はそれを更に増悪する因子として機能することが示唆された。C型肝炎における病態解明と病変進行の予防法の開発において重要な発見と考える。

E. 結論

PPARαタンパクはHCVコア遺伝子Tg肝において核に蓄積し、cyclin D1やACO等のPPARαターゲット遺伝子産物も同様に増加していた。PPARαの持続的な活性化無しでは肝脂肪化も肝癌も生じないことが明らかになった。コア蛋白がもたらすミトコンドリア機能障害、電子伝達系の障害がPPARα活性化によって更に増悪し、脂肪酸を介した「負のループ」が形成され、肝脂肪化、脂肪酸増加、酸化ストレス産生、細胞増殖遺伝子発現増加という一連の現象を次々と惹起して、最終的に肝癌の発生へと至ると考えられる。HCVの病原性発現を抑制する方策の開発に有用と考えられる。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況

該当なし

厚生労働科学研究費補助金（国際医学協力研究事業）
（総括・分担）研究報告書

C型肝炎ウイルスと樹状細胞

（主任又は分担）研究者　考藤　達哉　大阪大学大学院医学系研究科樹状細胞制御治療学准教授

研究の要旨

樹状細胞（DC）にはウイルス感知系であるToll様受容体（TLR）、RIG-I、MDA-5が発現しており、I型IFNや炎症性サイトカインなどの発現を介して先天免疫、獲得免疫の活性化に関与する。C型慢性肝炎におけるDCのTLR/RIG-I/MDA-5の発現と機能及び病態における意義を解析した。C型慢性肝炎患者のミエロイドDC(MDC)では、非感染者と比較してTLR2、TLR4、RIG-Iの発現は増加しているが、TLR3、MDA-5の発現には差を認めなかった。C型慢性肝炎患者MDCでは、TLR3、TLR4アゴニストによるIFN-β、TNF-α、IL-12などの産生が非感染者に比べ低下していた。その機序としてTLR3、TLR4のアダプター分子であるTRIF、TRAF6の発現低下が示唆された。C型肝炎患者MDCにおけるTLR/RIG-Iの機能低下は、DCがHCV感染を十分に感知できず、効果的に免疫系を活性化できない可能性を示唆している。

A.研究目的

樹状細胞（DC）は強力な抗原提示細胞であり、ウイルス、癌に対する免疫応答の中心的役割を果たしている。我々の検討によりC型慢性肝炎患者ではミエロイドDC（MDC）とプラスマサイトイドDC（PDC）が減少しており、MDCのTh1誘導能やPDCのIFN産生能が低下していることが明らかになった。DCにはToll様受容体（TLR）やRIG-I、MDA-5が発現しており、ウイルス感染を感知して免疫応答を効果的に発動させる。HCV感染においてもこれらのウイルス感知系が免疫病態に関与していると想定されるが、その発現と機能については明らかではない。本研究ではC型慢性肝炎のDCにおけるTLR/RIG-I/MDA-5の発現と機能を解析し、DCの機能制御によるHCV排除の治療法を確立することを目的とする。

B.研究方法

C型慢性肝炎患者および非感染者の末梢血よりMDCを分離し、TLR/RIG-I/MDA-5の発現をReal-time PCR法を用いて検討した。また各TLRに特異的なアゴニストを用いてMDCを刺激し、DCのサイトカイン産生能を検討した。HCV感染によるTLR/RIG-I系のシグナル伝達異常の有無を評価するために、TLR/RIG-Iのシグナル伝達関連分子をPCR-Arrayを用いて網羅的に解析した。また多数例でTLR/RIG-Iのアダプター分子の発現を比較した。

（倫理面への配慮）

本研究は大阪大学医学部倫理委員会の承認を受けており、事前に被験者の同意を得ており倫理的問題はないと考える。

C.研究結果

MDCにおけるTLR2、TLR4、RIG-Iの発現はC型慢性肝炎患者で亢進していたが、TLR3、MDA-5の発現は非感染者と同程度であった。TLR3、TLR4のアゴニスト刺激によりMDCのIFN-β、TNF-α、IL-12の発現は亢進したが、その程度はC型慢性肝炎患者で低かった。C型患者MDCではMAPK系やNF-κB系関連分子の発現が低下していた。またIPS-1の発現はC型慢性肝炎患者MDCで高値であったが、TRIF、TRAF6は患者MDCで低値であった。

D.考察

C型慢性肝炎患者MDCではTLR3、TLR4下流のシグナル伝達が強く抑制されていることが示された。その機序としてTRIF、TRAF6の発現低下が関与している可能性が示唆された。C型慢性肝炎MDCにおけるTLRの機能低下は、DCがHCV感染を感知して効果的に免疫系を活性化できない可能性を示唆している。

E.結論

C型慢性肝炎患者MDCではTLR2、TLR4、RIG-Iの発現が亢進しているにも関らず、TLR3、TLR4の刺激によるサイトカイン誘導は低下しており、下流でのシグナル伝達阻害機構の存在が示された。TLR3-TRIF-TRAF6はDC機能活性化のための治療標的になる可能性が示唆された。

F.健康危険情報　なし

G.研究発表

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H.知的財産権の出願・登録状況

特に予定なし

III.研究成果の刊行に關する一覧表

平成19年度厚生労働科学研究費補助金(社会保障国際協力推進研究事業)
主にアジアに蔓延するウイルス性肝疾患の制御に資する為の日米合作的肝炎ウイルス基礎研究

研究成果の刊行に関する一覧表

Wenny Astuti Achwan, Zainul Muttaqin, Edy Zakaria, Sulaiman Amangongu Depamede, Mulyanto, Suwignyo Sumoharjo, Fumio Tsuda, Kazuaki Takahashi, Natsumi Abe, Shunji Mishiro. Epidemiology of Hepatitis B, C, and E Viruses and Human Immunodeficiency Virus Infections in Tahuna, Sangihe-Talaud Archipelago, Indonesia. Intervirology 2007;50:408-411

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（和文は省略）

IV. 研究成果の刊行物・別刷

Original Paper

Intervirology

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Epidemiology of Hepatitis B, C, and E Viruses and Human Immunodeficiency Virus Infections in Tahuna, Sangihe-Talaud Archipelago, Indonesia

Wenny Astuti Achwan[a–c] Zainul Muttaqin[b] Edy Zakaria[b]
Sulaiman Amangongu Depamede[d] Mulyanto[d, e] Suwignyo Sumoharjo[a, b]
Fumio Tsuda[f] Kazuaki Takahashi[f] Natsumi Abe[f] Shunji Mishiro[f]

[a]Department of Internal Medicine, [b]Biomedical Research Unit, [c]HIV-AIDS Voluntary Counseling and Testing Clinic, Mataram General Hospital, [d]Immunobiology Laboratory, School of Medicine, Mataram University, and [e]West Nusa Tenggara Hepatitis Laboratory, Mataram, West Nusa Tenggara, Indonesia; [f]Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan

Key Words
Hepatitis B virus · Hepatitis C virus · Hepatitis E virus · Human immunodeficiency virus · Epidemiology · HBV genotypes · Vaccination · Indonesia

Abstract
Background/Aims: The epidemiology of hepatitis B, C, and E viruses (HBV, HCV, HEV) and human immunodeficiency virus (HIV) has been obscure in Indonesia, particularly in its remote areas. ***Methods:*** We undertook serological surveys for HBV/HCV/HEV/HIV infections in the general population of Tahuna, the capital city of Sangihe-Talaud Archipelago, an outlier in the northeastern part of Indonesia. ***Results:*** Of 581 sera collected in April 2005, 1.4% was reactive for HBsAg, 0.2% for anti-HCV, and 5.9% for anti-HEV, but none for anti-HIV. All the HBsAg-positive sera were also positive for HBV DNA, the nucleotide sequence of which is segregated within subgenotype C5. Most of the preschool children were positive for anti-HBs as a result of an HB immunization initiated in 1997. The titer of anti-HCV in the only individual detected was very low, with a negative result of HCV RNA detection, suggesting a nonspecific reaction. Anti-HEV was significantly more frequent in those over 30 years of age than in the younger age group (24 vs. 1.9%, $p < 0.0001$). ***Conclusion:*** Thus, it seems that HCV and HIV have fortunately not made it as far as the Sangihe-Talaud Archipelago. Although HBV infection remains a major problem in adults (with the HBsAg-positive rate at 4.9%), HB immunization has begun to protect the younger generation.

Introduction

Indonesia consists of thousands of islands inhabited by hundreds of ethnic groups. Accordingly, the prevalence of hepatitis B and C virus (HBV and HCV) infections varies widely from one part to another, ranging from 5 to 10% for HBsAg and 1 to 4% for anti-HCV [1–5]. Tahuna is the capital city of the Sangihe-Talaud Archipelago, in a relatively isolated island (Sangihe) with a

The nucleotide sequences reported in this paper have been deposited in DDBL/EMBL/GenBank databases under accession numbers AB301078-85.

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Dr. Shunji Mishiro
Department of Medical Sciences, Toshiba General Hospital
6-3-22 Higashi Oh-i, Shinagawa-ku
Tokyo 140-8522 (Japan)
Tel. +81 3 3764 0511, Fax +81 3 3764 8992, E-Mail shunji.mishiro@po.toshiba.co.jp

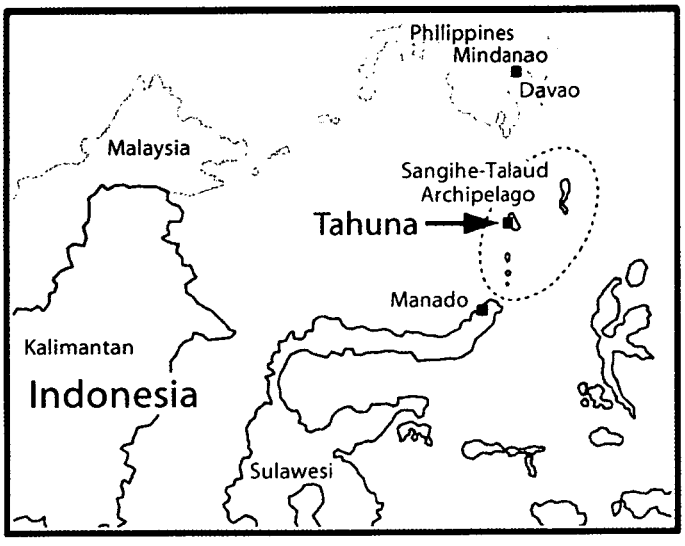

Fig. 1. Location of Tahuna.

population of 35,000, located 150 km north of Manado, Sulawesi, Indonesia, and 200 km south of Davao, Mindanao, Philippines (fig. 1). There has been no previous report on the prevalence of HBV and HCV infections in the population, let alone for hepatitis E virus (HEV) and human immunodeficiency virus (HIV) infections. Thus, this is the first report on the epidemiology of these viruses in the Sangihe-Talaud Archipelago. Another interest of our current study lay in the possibility that we might find a new genotype/subgenotype of HBV, since it had been suggested by our previous study [6] that a diverse variety of subtypes/subgenotypes/genotypes of HBV exist in Indonesia.

Subjects and Methods

The majority of subjects in this study were primary and secondary school children (aged 7–15 years, n = 402), along with less numbers of subjects belonging to different age groups: preschool children (aged 1–6, n = 15), young adults (aged 16–30, n = 60), middle-aged (aged 31–50, n = 62), and elderly (aged 51–86, n = 42). Serum samples were collected in April 2005. HBsAg, anti-HBs, anti-HCV, and anti-HIV were tested by commercially available kits based on hemagglutination or particle-agglutination methods (Mycell2-HBsAg and Mycell2-anti-HBs, Institute of Immunology; Ortho HCV Ab PA test, Ortho Clinical Diagnostics; and Genedia HIV-1/2 mix PA, Fujirebio), while anti-HEV was detected by an in-house system as reported previously [5]. Only repeatedly reactive samples were regarded as positive in these assays. HBV DNA and HCV RNA were tested in samples positive for HBsAg and anti-HCV, respectively, by methods described previously [7, 8] with some modifications. HBsAg-positive samples were subjected to polymerase chain reaction (PCR) to amplify a 433-nt fragment (corresponding to nt positions 403–835 of the prototype HBV subgenotype C5 sequence from the Philippines [9]) covering a 3′ part of the small S gene of HBV DNA, which were then sequenced for phylogenetic analyses.

Table 1. Prevalence of the IgG class antibodies to HEV compared between different age groups

Age	Male	Female	Male + female
1–10 years	0/84	2/66 (3.0)	2/150 (1.3)
11–20 years	0/134	4/169 (2.4)	4/303 (1.3)
21–30 years	1/5 (20)	2/19 (11)	3/24 (13)
31–40 years	5/9 (56)	2/20 (10)	7/29 (24)
41–50 years	4/14 (29)	3/19 (16)	7/33 (21)
51–60 years	2/9 (22)	3/16 (19)	5/25 (20)
61– years	1/4 (25)	5/13 (38)	6/17 (35)
Total	13/259 (5.0)	21/322 (6.5)	34/581 (5.9)

Figures in parentheses are percentage.

Results

HCV and HIV Infections

Anti-HCV was detected positive in one sample. But the titer of anti-HCV in this serum was very low, and HCV RNA could not be amplified by PCR. Anti-HIV was negative in all samples.

HEV Infection

IgG class anti-HEV was positive in a substantial proportion of the studied population (table 1). Notably, there was a significant difference in the positive rate by age: in particular, the difference between 1–30 years (1.9%) and over 30 years (24%) was statistically significant ($p < 0.0001$).

HBV Infection

HBsAg and anti-HBs were tested for HBV infection, and compared between different age groups. As shown in figure 2, HBsAg was positive in 8 of the adult age groups, while none in the younger generations. By contrast, younger subjects (particularly the preschool children) were highly positive for anti-HBs. HBV DNA sequences obtained from the 8 subjects who were positive for HBsAg clustered within the subgenotype C5, together with known isolates from the Philippines [9] and Vietnam [10] (fig. 3).

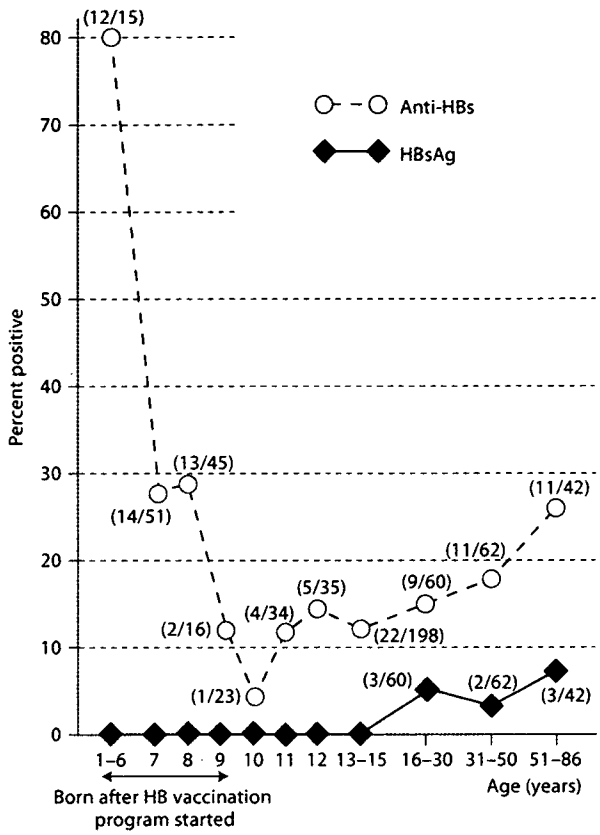

Fig. 2. Prevalence of HBsAg and anti-HBs in different age groups. Vaccine-induced anti-HBs is notable in those younger than 10 years of age, while naturally acquired anti-HBs is found more frequently in older age groups.

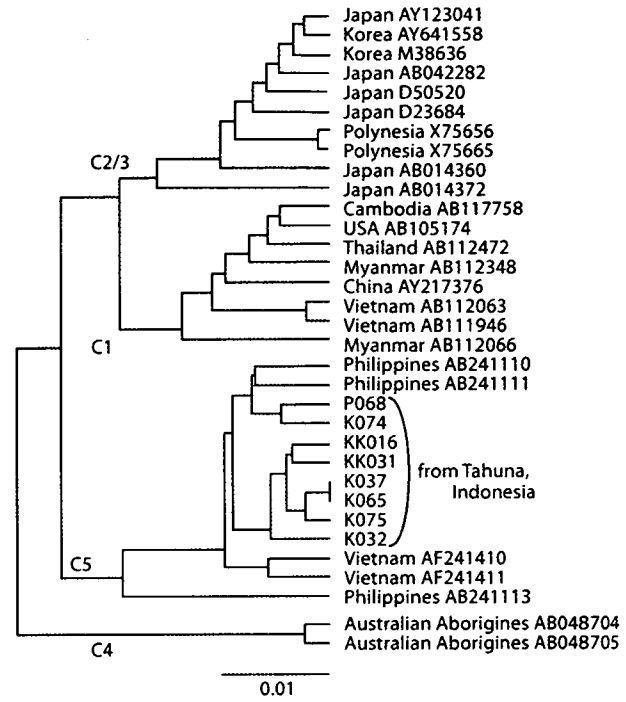

Fig. 3. Phylogenetic tree of HBV isolates. Tahuna-derived HBV sequences co-clustered with two each from the Philippines and Vietnam previously reported as strains of subgenotype C5.

Discussion

Our study indicated an absence or scarcity of HCV and HIV infections in Tahuna. This is noteworthy in view of a slow but steady increase in the prevalence of these viruses in other areas of Indonesia [11]. For example, we have identified more than 50 individuals who are infected with HIV in Lombok Island, which is also an outlier in Indonesia as is Sangihe Island [our unpubl. results]. However, Lombok is more easily accessible from the outside world than Sangihe. Having being difficult for outsiders to reach, Sangihe seems to have been protected from the importation of HCV and HIV, which are usually carried by illicit drug users.

Pertaining to HEV infection, it is of interest that the positive rate of anti-HEV in the adult population of Tahuna (about 20%) was comparable to that reported for Hindu people living in Bali, Indonesia [5]. Anti-HEV was positive in about 20% of Hindu pregnant women whereas only in about 2% of Muslim pregnant women in Bali, and this great difference might be associated with the fact that Balinese Hindu people like to eat pork whereas Muslim people are prohibited to eat and/or even have close contact with pigs. Indeed, HEV RNA was recovered from pigs in Bali [12]. About 70% of people in Tahuna are Christians (the others are Muslim), and their fondness, especially in adults, for eating pork may explain the difference between adults and children with respect to anti-HEV positive rates.

As expected, HBV was the most prevalent virus in Tahuna among the 4 viruses examined in this study. In particular, those older than 50 years of age had a higher prevalence of HBsAg (7.1%), compared with those of younger generations. This implies that HBV infection has been endemic in this area until recently. The extraordinarily high rate of anti-HBs in preschool children (80%) and the relatively higher rate in the 7- to 8-year-old primary

school children (about 30%) compared to the children born before 1997 (about 10%) are most likely caused by the hepatitis B immunization initiated in 1997, even if the coverage of vaccination still remains insufficient.

Interestingly, Tahuna-derived HBV DNA sequences segregated, co-clustering with those from Philippines [9] and Vietnam [10], to a subgenotype within genotype C, namely C5 (fig. 3). Anthropological evidence [13] indicates that there is a very close relationship between Filipino and Vietnamese. Thus, although it is yet to be known, whether the people now living in the Sangihe Island, geographically near to the Philippines, are genetically close to Filipino as well is intriguing. In any case, the subgenotype C5 strain of HBV found in Tahuna is a kind of 'endangered species', since hepatitis B vaccination has begun to show its effects in eradicating the virus.

Conclusion

In Tahuna of the Sangihe Island, an outlying tiny part of Indonesia, HBV and HEV infections are of epidemiological importance, particularly in adults, yet with a scarcity of HCV and HIV infections. HBV immunization towards full coverage of all infants should be encouraged. The HBV subgenotype C5 strain thereof may be worth considering for full-genome sequencing.

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Journal of Medical Virology 80:268–276 (2008)

Epidemiological and Clinical Evaluation of Hepatitis B, Hepatitis C, and Delta Hepatitis Viruses in Tajikistan

Anis Khan,[1] Fuat Kurbanov,[1] Yasuhito Tanaka,[1] Abeer Elkady,[1] Masaya Sugiyama,[1] Abdusamad Dustov,[2] and Masashi Mizokami[1]*

[1]*Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya, Japan*
[2]*Institute of Gastroenterology of the Academy of Sciences of the Republic of Tajikistan, Dushanbe, Republic of Tajikistan*

The implication of genotypes is recognized increasingly in the clinical course of hepatitis B virus (HBV) and in response to anti-viral drugs of hepatitis C virus (HCV). Genotypic prevalence of both etiological agents varies geographically and no data are available for Tajikistan. To investigate the epidemiology and clinical significance of HBV and HCV genotypes in chronic hepatitis (group 1) and liver cirrhosis/hepatocellular carcinoma (HCC) (group 2) patients in Tajikistan, 124 patients with chronic liver disease (group 1 = 84 and group 2 = 40) were enrolled. Genotypes of HBV, HCV, and delta hepatitis virus (HDV) were determined by sequencing. The overall prevalence of anti-HCV, HCV core antigen (HCVcAg) and HBsAg was 46% (57/124) and 41.1% (51/124), respectively. Coinfection of HCV/HBV, HBV/HDV, and HCV/HBV/HDV was found in 4.8% (6/124), 11.2% (12/124), and 0.8% (1/124) of cases, respectively. HDV genotype 1 was found in 19.6% (10/51) of HBsAg-positive patients. The HBV/HDV coinfection was relatively high in group 2 compared to group 1 (15% vs. 7.1%). HCV/1b detected in 84.6% (44/52) of HCV RNA-positive patients, followed by 3a (7.6%), 2a (5.7%), and 2c (1.9%). HBV/D was detected in 94.1% (48/51) of HBsAg-positive patients, followed by HBV/A [5.8% (3/51)]. T1762/A1764 double mutation was associated with liver cirrhosis/HCC in HBV-infected patients ($P = 0.0004$). This is the first study on the molecular epidemiology of hepatitis viruses among chronic liver diseases patients in Tajikistan. Among HBV-infected patients, the T1762/A1764 mutation was associated with liver cirrhosis/HCC. ***J. Med. Virol. 80:268–276, 2008.***

KEY WORDS: Tajikistan; HBV; HCV; HDV; genotype; molecular epidemiology; mutations BCP

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are common etiological agents of viral hepatitis. Approximately 350 million people are infected with HBV worldwide and the World Health Organization (WHO) estimates that approximately 170 million people are infected with HCV. Chronic HBV and/or HCV infection can progress to liver cirrhosis and hepatocellular carcinoma (HCC).

HCV displays a high degree of genetic variability and is classified into six major genotypes that show sequence similarities of only 66–69% [Pawlotsky, 2003a]. Each of the genotypes contains multiple subtypes with >75% nucleotide sequence similarity [Simmonds et al., 2005]. The HCV genotypes have different geographical distributions, that is, some strains are distributed worldwide, whereas others are found only in specific geographical regions [Pawlotsky, 2003a; Verbeeck et al., 2005]. Investigation of circulating HCV genotypes is useful as an epidemiological tool, and it is helpful for improvement of diagnostic tests and treatment efficiency [Zein, 2000; Hui et al., 2003; Pawlotsky, 2003b]. Similarly, HBV has been classified into eight major genotypes (A–H) by sequence divergence in the entire genome in excess of 8% and are distributed geographically [Miyakawa and Mizokami, 2003; Norder et al., 2004]. A number of studies have demonstrated that HBV genotypes influence the course of disease, therapeutic responsiveness and clinical outcomes [Chu et al., 2002; Miyakawa and Mizokami, 2003; Norder et al., 2004; Schaefer, 2005]. Previous reports have indicated that

*Correspondence to: Masashi Mizokami, MD, PhD, Prof., Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Kawasumi 1, Mizuho, Nagoya 467-8601, Japan.
E-mail: mizokami@med.nagoya-cu.ac.jp

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