いる(TLR3/TRIF 経路ではTRIF でその経路が分岐される)。また、前者のIRF-3 の活性化にはTRAF ファミリーのTRAF3 が、後者のNF-kB の活性化にはTRAF6 が関与していることも報告されている。そこで、TRAF3 あるいはTRAF6 をノックダウンしたときにNS5B による IFN-b および IL-6 の発現誘導がどのように変動するか調べた。その結果、両遺伝子のノックダウンは IFN-b および IL-6 の発現を顕著に亢進させ、さらにはTRAF6 をノックダウンしたPH5CH8/NS5B 細胞では内在性の IRF-3 の二量化が観察されるようになった。

次に、抗二本鎖 RNA 認識抗体を用いる免疫蛍光抗体法により、細胞内での二本鎖 RNA の検出が可能か調べた。最初に、合成二本鎖RNA であるpoly(I-C)あるいは合成二本鎖DNA であるpoly(dA-dT)を細胞内に導入し、この抗体の特異性を確認したところ、poly(I-C)のみ蛍光を検出することができた。次に、全長HCV RNA が持続的に複製されている細胞である0 細胞(HuH-7 細胞系)とその治癒細胞(IFN-a により HCV RNA を排除した細胞)である0c 細胞で比較したところ、0 細胞でのみ、ウイルス由来と思われる二本鎖RNA を検出することができた。また、PH5CH8/NS5B細胞内でも二本鎖 RNA の存在を確認することができた(RNA 依存 RNA ポリメラーゼ活性を欠失させたNS5B 変異体が恒常的に発現するPH5CH8 細胞では確認することはできなかった)。

最後に、PH5CH8 細胞と全長 HCV RNA が持続的に複製されている細胞である 0 細胞や OL 細胞(Li23 細胞系)などのRIG-I、MDA5 やCardif の塩基配列を比較したところ、HuH-7.5 細胞で見られるようなRIG-I の機能を欠失させるような変異はこれらの全長HCV RNA 複製細胞株では見出すことはできなかった。

D. 考察

本研究の結果、HCV NS5B により、ヒト不死化肝PH5CH8 細胞内において産生される二本鎖 RNA は、細胞質に局在する RIG-I や MDA5、あるいは細胞表面やエンドソームに局在する TLR3 のような二本鎖 RNA 認識受容体のいずれによっても認識されることが明らかとなった。RIG-I と MDA5 は、細胞質内に存在する二本鎖 RNA の配列というよりはむしろその長さにおいて認識特異性があることが報告されており、PH5CH8 細胞内においては、様々な長さの二本鎖 RNA が産生されていることが予想される。また、RIG-I や MDA5 のアダプター分子である Cardif は NS5B による IFN-b の産生誘導機構に含まれていたのに対して、TLR3 のアダプター分子である TRIF は含まれていなかった。poly(I-C)のような二本鎖 RNA の細胞外刺激では TLR-3 および TRIF が活性化され、IRF-3 の活性化を経て IFN-b が産生することはすでに報告しており、PH5CH8 細胞においてTRIF が機能していないことは考えにくい。また、RIG-I/MDA5 経路と TLR3 経路の両経路に関与することが報告されているNAP1 もNS5B による IFN-b の産生誘導機構に含まれていたことからも、poly(I-C)の細胞外刺激とは異なる TRIF を介さない経路の存在も示唆される。このような点についても、今後検討していく必要がある。

今回、PH5CH8/NS5B 細胞では活性化された内在性IRF-3 が二量体形成後、核内で IFN-b 遺伝子の転写を活性化した後に分解されている可能性が示唆された。また、TRAF6 をノックダウンしたPH5CH8/NS5B 細胞では内在性の IRF-3 の二量化が観察されるようになった。TRAF6 は E3 ユビキチンリガーゼ活性を持つこと、また、IRF-3 もユビキチン-プロテオソーム系において分解されることがこれまでに報告されており、このような点についても、今後検討していく必要がある。

今回の解析に用いた NS5B(1B-1 株)は肝炎を発症していない HCV キャリアー由来のものであったこと、および HCV にはかなりの遺伝的多様性が認められることから、C 型慢性肝炎患者や肝癌患者由来の NS5B が同じようにヒト肝細胞内にて二本鎖 RNA を産生するという確証は現在のところ得られていない。また、HCV-JFH1 株(2a 型)の NS5B や HCV 近縁の GBV-B 由来の NS5B についても同じように二本鎖 RNA を産生し、IFN-b を産生するかについても、今後検討評価する必要がある。

また、PH5CH8 細胞と全長HCV RNA 複製細胞でRIG-I、MDA5 や Cardif の塩基配列を比較したが、HuH-7.5 細胞で見られるような RIG-I の機能を欠失させるような変異は見出すことはできなかった。今後は、これら以外の自然免疫機構に含まれる分子についてもその塩基配列を調べる必要がある。

自然免疫システムを撹乱するNS5B に対抗する手段を得ることは、HCV の持続感染を断ち切ることができる可能性を示唆しており、今後の重要な研究課題であると考えられる。

E. 結論

今年度の実験結果から、HCV の NS5B は HCV-RNA の複製にかかわらず、肝細胞にて二本鎖RNA を産生する能力があり、自然免疫システムを撹乱している可能性を示した。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1 N. Kato, K. Abe, K. Mori, Y. Ariumi, H. Dansako, T. Wakita, and M. Ikeda. Genetic variability and diversity of intracellular genome-length hepatitis C virus RNA in long-term cell culture.

Arch Virol. 154, 77-85(2009).

2 M. Kuroki, Y. Ariumi, M. Ikeda, H. Dansako, T. Wakita, and N. Kato. Arsenic trioxide inhibits HCV RNA replication through modulation of the glutathione redox system and oxidative stress. J. Virol. in press (2008).

3 Y. Ariumi, M. Kuroki, H. Dansako, K. Abe, M. Ikeda, T. Wakita, and N. Kato: ATM, a DNA damage sensor, is required for hepatitis C virus RNA replication. J. Virol. 82, 9639-9646(2008).

4 H. Dansako, M. Ikeda, K. Abe, K. Mori, K. Takemoto, Y. Ariumi, and N. Kato: A new living cell-based assay system for monitoring genome-length hepatitis C virus RNA replication. Virus Res. 137, 72-79(2008)

5 K. Mori, K. Abe, H. Dansako, Y. Ariumi, M. Ikeda, and N. Kato. New efficient replication system with hepatitis C virus genome derived from a patient with acute hepatitis C. Biochem Biophys Res Commun. 371, 104-109(2008).

6 K. Hirano, T. Ichikawa, K. Nakao, A. Matsumoto, H. Miyaaki, H. Shibata, S. Eguchi, M. Takatsuki, M. Ikeda, H. Yamasaki, N. Kato, T. Kanematsu, N. Ishii, K. Eguchi. Differential effects of calcineurin inhibitors, tacrolimus and cyclosporin A, on interferon-induced antiviral protein in human hepatocyte cells. Liver. Transpl., 14:292-8 (2008).

7 M. Nakamura, H. Saito, M. Ikeda, S. Tada, N. Kumagai, N. Kato, K. Shimotohno, T. Hibi. Possible molecular mechanism of the relationship between NS5B polymorphisms and early clearance of hepatitis C virus during interferon plus ribavirin treatment. J. Med. Virol., 80:632-639 (2008).

8 M. Ando, M. Korenaga, K. Hino, M. Ikeda, N. Kato, S. Nishida, I. Hidaka, I. Sakaida. Mitochondrial electron transport inhibition in full genomic hepatitis C virus replicon cells is restored by reducing viral replication. Liver Int. 28:1158-66 (2008)

2. 学会発表

1 H. Dansako, Y. Ariumi, M. Ikeda, and N Kato. Modulation of dsRNA-induced IFN-beta and inflammatory cytokine productions by HCV NS3-4As derived from patients with different hepatic diseases. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

2 K. Abe, M. Ikeda, H. Tani, Y. Ariumi, H. Dansako, Y. Matsuura, N. Kato. Low permissive cell lines obtained from a high permissive HCV RNA replication cell line by negative selection system: A new strategy for identification of novel host factors. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

3 K. Mori, K. Abe, H. Dansako, Y. Ariumi, M. Ikeda, N. Kato. New efficient replication system with HCV genome derived from a patient with acute hepatitis. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

4 M. Ikeda, K. Abe, M. Kuroki, Y. Ariumi, H. Dansako, N. Kato. Identification of 5-HETE as the anti-HCV molecule among the arachidonic acid metabolites. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

5 N. Kato, K. Mori, K. Abe, H. Dansako, Y. Ariumi, T. Wakita, M. Ikeda. A new human hepatoma cell line enabling persistent reproduction of HCV life cycle and assay for anti-HCV reagents. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

6 Y. Kawai, M. Ikeda, K. Abe, M. Yano, Y. Ariumi, H. Dansako, K. Yamamoto, N. Kato. Genome-length HCV RNA replicating cells possessing IFN-a resistant phenotype for the development of relapse model. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

7 Y. Ariumi, M. Kuroki, M. Ikeda, H. Dansako, T. Wakita, N. Kato. The vacuolar protein sorting pathway is essential for HCV budding. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

8 K. Abe, M. Ikeda, Y. Ariumi, H. Dansako, T. Wakita, N. Kato. Cyclophilins A and B mediate the anti-HCV activity of cyclosporine A in 1b/2a chimeric replicon-harboring cells. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

9 M. Kuroki, Y. Ariumi, M. Ikeda, H. Dansako, T. Wakita, N. Kato. Arsenic trioxide inhibits HCV RNA replication through modulation of oxidative stress. 15th International Symposium on Hepatitis C Virus and Related Viruses, San Antonio, USA. October, 2008.

10 加藤 宣之、森 京子、阿部 健一、圍迫 浩方、

有海 康雄、脇田 隆字、池田 正徳. 新しいヒト肝癌細胞株Li23を用いたHCV生活環再現システム. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

11 西村 剛、池田 正徳、有海 康雄、團迫 浩方、中沢 貴秀、加藤宣之. 異なるHCV陽性血清由来の1b型HCVレプリコン複製細胞株の樹立と薬剤感受性の評価. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

12 有海 康雄、黒木 美沙緒、團迫 浩方、阿部 健一、池田 正徳、脇田 隆字、加藤 宣之. DNA損傷センサーATM及びChk2とHCV NS5Bとの相互作用. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

13 團迫 浩方、有海 康雄、池田 正徳、加藤 宣之. 二本鎖RNAによるIFN-b及び炎症性サイトカイン産生誘導に対する肝病態の異なるHCV NS3-4Aの影響. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

14 黒木 美沙緒、有海 康雄、池田 正徳、團迫 浩方、脇田 隆字、加藤 宣之. 亜ヒ酸は酸化ストレスを介してHCV RNAの複製を顕著に抑制する. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

15 池田 正徳、阿部 健一、黒木 美沙緒、有海 康雄、團迫 浩方、加藤 宣之. 抗HCV活性を示すアラキドン酸代謝産物5-HETEの同定. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

16 森 京子、加藤 宣之、阿部 健一、有海 康雄、團迫 浩方、池田 正徳. 新しいヒト肝癌細胞株Li23由来の全長HCV-RNA複製細胞を用いた薬剤評価システム. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

17 阿部 健一、池田 正徳、有海 康雄、團迫 浩方、脇田 隆字、加藤 宣之. Cyclosporine Aに対し抵抗性を示す1b/2aキメラレプリコン. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

18 河合 良成、池田 正徳、阿部 健一、矢野 雅彦、有海 康雄、團迫 浩方、山本 和秀、加藤 宣之. IFN抵抗性全長HCV-RNA複製細胞の特徴および有効な治療法を見出すための治療後再発モデルの構築. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

19 池田 正徳、森 京子、西村 剛、阿部 健一、有海 康雄、團迫 浩方、中沢 貴秀、加藤宣之. 異なる1b型HCV陽性血清由来の全長HCV RNA複製レポーターアッセイ系の開発. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

20 阿部 健一、池田 正徳、谷 英樹、有海 康雄、團迫 浩方、松浦 善治、加藤 宣之. HCV複製に関与する宿主因子探索用細胞株のNegative Selection法による樹立. 第56回日本ウイルス学会学術集会・総会、岡山、2008年10月.

21 森 京子、阿部 健一、團迫 浩方、有海 康雄、池田 正徳、加藤 宣之. 急性C型肝炎患者由来の新しいC型肝炎ウイルスゲノム複製系. 第67回 日本癌学会総会、名古屋、2008年10月.

22 池田 正徳、森 京子、阿部 健一、西村 剛、團迫 浩方、有海 康雄、中沢 貴秀、加藤 宣之. 異なるHCV(1b型)株由来の全長HCV RNA複製レポーターアッセイ系の開発. 第67回 日本癌学会総会、名古屋、2008年10月.

23 有海 康雄、黒木 美沙緒、團迫 浩方、阿部 健一、池田 正徳、脇田 隆字、加藤 宣之. ATM DNA損傷センサーはC型肝炎ウイルスのRNA複製に必要である. 第67回 日本癌学会総会、名古屋、2008年10月.

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（肝炎等克服緊急対策研究事業）
分担研究報告書

病原性を保持したHCVの感染増殖ならびにIRF7を介した免疫抑制機構の解明

研究分担者　土方　誠　京都大学ウイルス研究所　准教授

研究要旨　これまでにC型肝炎ウイルス(HCV)の感染増殖が転写因子であるインターフェロン(IFN)調節因子(IRF)7の機能によって抑制されることを明らかにしてきたが、その制御機構や極めて多様な天然のHCVとの実際の相互作用については全く不明であった。そこで我々が開発した新たな培養細胞実験系を用いて複数の患者血液由来HCVの感染増殖と感染細胞の相互作用について解析をおこなった。この培養細胞実験系は我々が独自に樹立した不死化肝細胞を中空糸に充填することで立体的に培養し、これを用いてHCVの生活環を再現するものである。日本に最も多い遺伝子型1bのHCVが感染している種々の患者血清を用いた結果、大きく分類して2種類の感染増殖パターンが観察された。一つは感染後10日ま、細胞内および培養液中において比較的急激な遺伝子型2aのHCVを用いた場合、感染後細胞にアポトーシスが誘導されたことから、HCVの中には細胞傷害性を有するものが存在することがわかった。

A. 研究目的

血清由来C型肝炎ウイルスが効率良く感染増殖する不死化肝細胞を用いてHCVの感染増殖機構を解析することにより、このウイルスの感染増殖を抑制する自然免疫機構の詳細を明らかにして、これを効果的に亢進させることによる抗HCV戦略構築を目指した。

B. 研究方法

1. 既に樹立している新規ヒト不死化肝細胞を中空糸をもちいて立体培養することにより患者血清由来HCVの感染増殖を効率良くおこなう培養実験系を構築した。この実験系を用いて、異なる患者血清由来HCVの感染増殖ならびに感染した細胞の反応のを解析をおこなった。また長期の継続感染細胞内のHCV-RNA遺伝子の変化ならびに細胞への自然免疫系誘導や細胞死誘導について詳細な解析をおこなった。

（倫理面への配慮）

ヒト初代培養肝細胞は、京都大学附属病院移植外科においておこなわれた先天性代謝異常症患者への生体肝臓移植において切除された患者肝臓組織を用いて作成されたものである。この研究はあらかじめ京都大学医学部医の倫理委員会に申請し、審査の後に承認されたものである。肝臓や血液提供者へのインフォームドコンセントや個人情報の管理は上記委員会の規定通りにおこなわれており、倫理面に関する問題はない。

C. 研究結果

1. 我々が独自に樹立した不死化肝細胞を中空糸に充填することで立体培養した。この培養細胞実験系を用いて複数の患者血液由来HCVの感染増殖と感染細胞の相互作用について解析をおこなったところ、通常の培養皿を用いた場合に比較して細胞内のHCV-RNA量が100倍程度増加することがわかった。また、感染増殖は1ヶ月の長期にわたり維持され観察できることがわかった。
2. 遺伝子型1bのHCVを含む患者血清を用いて感染実験をおこなうと感染10日目まで徐々に細胞内HCV-RNAが上昇するものと7日目までは増加したものの10日目には極度に低下するものがあった。
3. 2.において後者の場合、感染後培養液中にIFN
4. 2.において後者の場合、用いた血清中に大別して2種類の主要なHCVが存在し、その一方が感染後最初に増殖し、IFNを誘導したと推定された。また残りの一方は、長期培養の後増殖が認められ、培養細胞内でHCV株間の動的な変化が観察された。
5. 遺伝子型2aのHCVを含む患者血清を用いて感染実験をおこなった時、感染後10日目に細胞にアポトーシスが誘導されることが示唆されるものがあった。

D. 考察

1. 遺伝子配列や IFN 治療による反応性などから患者血液の天然の HCV は非常に多様であることが分かっていたが、今回中空糸を用いてヒト不死化肝細胞を立体培養する新たな培養細胞系を用いた感染実験によって、これら多様な HCV の感染増殖とそれにともなう種々の細胞側応答を観察することが可能になった。
2. 患者血液の天然のHCVには感染した細胞で増殖する際、細胞のインターフェロン誘導を引きおこすものと起こさないもの、そしてアポトーシスを誘導するものなど多様なものが存在すると考えられた。

E. 結論

1. 中空糸を用いてヒト不死化肝細胞を立体培養する新たな培養細胞系を用いた感染実験によって、患者血液の多様な HCV の感染増殖と自然免疫を含む種々の細胞応答を解析することが可能になった。

F. 研究発表

1. 論文

1 Hussein H Aly, Kunitada Shimotohno, Makoto Hijikata: 3D cultured immortalized human hepatocytes useful to develop drugs for blood-borne HCV. Biochem. Biophys. Res. Commun., 379: 330-334, 2009
2 土方 誠:HCV と肝発癌 医学のあゆみ 224 (9), 693-698 2008
3 宮成 悠介、臼田 信光、土方 誠、下遠野 邦忠: C 型肝炎ウイルスの生活環と発がん 化学と生物 46 (12) 826-831 2008
4 土方 誠、アリ・ハッサン・フセイン、下遠野邦忠: 3D細胞培養系を用いた患者血液由来HCV培養、肝・胆・膵 57 (5), 679-687 2008

2. 学会発表

1 アリ フセイン, 斉月, 山口達哉, 下遠野邦 忠, 土方誠. 不死化肝細胞の中空糸培養によって再現した患者血清由来天然 HCV の感染増殖」 (第 56 回日本ウイルス学会学術集会 2008. 10. 26 岡山コンベンションセンター)
2 Hussein H Aly, Yue Qi, Kunitada Shimotohno, Makoto Hijikata: A prolonged culture system for the study of the entire life cycle and the pathogenesis of natural HCV infection (第 67 回日本癌学会学術総会 2008. 10. 29 名古屋国際会議場)
3 Hussein H Aly, Kunitada Shimotohno, Makot Hijikata: Serum derived HCV infection, replication and particle production in immortalized primary human heopatocytes(XIVth International Congress of Virology 2008. 8. 12 Istanbul)
4 Hussein H Aly, Tatsuya Yamaguchi, Yue Qi, Kunitada Shimotohno, Makoto Hijikata: Development of the novel in vitro system supporting the entire life cycle of natural HCV (15th International Symposium Hepatitis C Virus & Relaed Viruses 2008. 10. 7 San Antonio,)

G. 知的所有権取得状況

1. 特許取得

1 感染性 C 型肝炎ウイルス粒子の製造方法、およびその利用」
発明者／出願者：山口達哉、土方誠、アリ ハッサン フセイン
2008 年 6 月 26 日出願 出願番号 特願 2008-167942

2 C 型肝炎ウイルスの感染増殖性の評価方法、およびその利用」
発明者／出願者：山口達哉、土方誠、アリ ハッサン フセイン
2008 年 6 月 26 日出願 出願番号 特願 2008-16794

2. 実用新案登録 特になし。

3. その他 特になし。

厚生労働科学研究費補助金（肝炎等克服緊急対策研究事業）
分担研究報告書

ウイルスの持続感染機序の解析及びその制御に関する研究

研究分担者　小原道法（財）東京都医学研究機構・東京都臨床医学総合研究所　参事研究員

研究要旨：持続感染機序を解明するためにC型肝炎発症動物モデルを樹立した。スイッチング発現システムにより、急性肝炎、慢性肝炎から肝硬変、肝細胞がんを発症し、HCV感染病態モデルマウスを樹立することに成功した。

A．研究目的

HCV感染ヒト肝臓組織ではHCVが持続的に複製している。持続感染が成立する理由の一つとしてHCVに対して宿主側が免疫寛容状態になっていることが推測される。この持続感染機序の解明にC型肝炎発症動物モデルを樹立し解析を行った。

B．研究方法

HCVの持続感染成立機序、慢性肝炎・肝硬変・肝癌への推移機構解明の為に任意の時期にHCV遺伝子をスイッチング発現することができるCre/loxP systemを用いたHCV構造蛋白質領域発現Tgマウス、HCV全長遺伝子発現Tgマウスを樹立した。このマウスを用いて、HCV遺伝子発現後の経過を経日的に解析した。

（倫理面への配慮）
動物実験は東京都臨床医学総合研究所の実験動物指針に基づいて行った。

C．研究結果

pIpCを1日置きに3回腹腔内投与した。初回投与後0.5日目からHCV core蛋白質の発現が認められ、この発現は12ヶ月を過ぎても同レベルで維持され、慢性肝炎の症状を呈した。

D．考察

スイッチング発現システムを樹立したことにより、HCV感染に似た免疫反応状態をつくることができた。HCV蛋白は完全に排除されることなく、持続的に発現がみられた。さらに、急性肝炎、慢性肝炎から肝硬変、肝細胞がんを発症し、感染者と同様な経過が認められた。

E．結論

スイッチング発現システムを樹立したことにより、受動的に免疫寛容が成立し、HCV感染病態モデルマウスを樹立することに成功した。

F．健康危険情報

特になし

G．研究発表

1．論文発表

1　Yasui F., Kai C., Masahiro Kitabatake, Kohara M.　Prior immunization with SARS-CoV nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. J. Immunology 181(9):6337-48 (2008).

2　Sakamoto N., Tanabe Y., Yokota K., , Kohara M. and Watanabe M.: Inhibition of hepatitis C virus infection and expression in vitro and in vivo by recombinant adenovirus expressing short hairpin RNA.　J. Gastroenterology Hepatology 23(9):1437-47 (2008)

3　Inubushi S.,Nagano-Fujii M., Kitayama K., Kohara M., Sada K. and Hotta H. : Hepatitis C virus NS5A protein interavts with and negatively regulates the non-receptor protein-tyrosine kinase Syk.　J. Gen. Virology　89: 1231 -1242 (2008)

2．学会発表

1　関口　敏、飛田良美、千代智子、小原道法：新規HCV持続感染モデルマウスの作製とその病態解析　第56回日本ウイルス学会学術集会
2008.10.26-28　岡山

H．知的財産権の出願・登録状況

1．特許取得
発明の名称：「C型肝炎ウイルス遺伝子を有する組換えワクシニアウイルス」、特願：2008-05751、発明者：小原道法、村井　深、出願日：2008年3月7日、出願人：東京都医学研究機構、（株）ポストゲノム研究所、（財）化学及血清療法研究所

2．実用新案登録
なし

3. その他
なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	頁
Takeuchi O. & Akira S.	Cytoplasmic Pattern Rectpros (RIG-I and MDA5) and Signaling in Viral Infections	Allen Brasier, Adolfo Garcia-Sastre, Stanley Lemon	Cellular Signaling and Innate Immune Responses to RNA virus Infections	ASM Press	Washington, DC	2008	29-38

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Okamoto K. et al.	Intramembrane processing by signal peptide peptidase regulates the membrane localization of hepatitis C virus core protein and viral propagation.	J. Virol.	82	8349-8361	2008
Okamoto T. et al.	A single amino acid mutation in hepatitis C virus NS5A disrupting FKBP8 interaction impairs viral replication.	J. Virol.	82	3480-3489	2008
Taguwa S. et al.	Human butyrate-induced transcript 1 interacts with hepatitis C virus NS5A and regulates viral replication.	J. Virol.	82	2631-2641	2008
Masaki T. et al.	Interaction of hepatitis C virus nonstructural protein 5A with core protein is critical for the production of infectious virus particles.	J. Virol.	82	7964-7976	2008
Aizaki H. et al.	Critical role of virion-associated cholesterol and sphingolipid in hepatitis C virus infection.	J. Virol.	82	5715-5724	2008
Kanto T. et al.	Virus-associated innate immunity in liver.	Front Bioscience	13	6183-6192	2008
Miyazaki M. et al.	Impaired cytokine response in myeloid dendritic cells in chronic hepatitis C virus infection regardless of enhanced expression of Toll-like receptors and retinoic acid inducible gene-I.	J. Med. Virol.	80	980-988	2008
Kohga K. et al.	Serum levels of soluble major histocompatibility complex (MHC) class I-related chain A in patients with chronic liver diseases and changes during transcatheter arterial embolization for hepatocellular carcinoma	Cancer Sci.	99	1643-1649	2008
Toyama T. et al.	A new prognostic system for hepatocellular carcinoma including recurrent cases: a study of 861 patients in a single institution.	J Clin. Gastroenterol.	42	317-322	2008
Kurashige N. et al.	Initial viral response is the most powerful predictor of the emergence of the YMDD mutant virus in chronic hepatitis B patients treated with lamivudine.	Hepatology Research	38	450-456	2008

Kanada A. et al.	Early emergence of entecavir-resistant hepatitis B virus in a patient with hepatitis B virus/human immunodeficiency virus coinfection	Hepatology Research	38	622-628	2008
Jung A. et al.	Lymphocytoid choriomeningitis virus activates plasmacytoid dendritic cells and induces a cytotoxic T cell response via MyD88.	J. Virol.	82	196-206	2008
Kato H. et al.	Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5.	J. Exp. Med.	205	1601-1610	2008
Saitoh T. et al.	Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production.	Nature	456	264-268	2008
Kawagoe T. et al.	Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2.	Nat. Immunol.	9	684-691	2008
Takeuchi O. & Akira S.	MDA5/RIG-I and virus recognition.	Curr. Opin. Immunol.	20	17-22	2008
Goto A. et al.	Akirins are highly conserved nuclear proteins required for NF-kappaB-dependent gene expression in drosophila and mice.	Nat. Immunol.	9	97-104	2008
Ishii K.J. et al.	Tank-binding kinase-1 delineates innate and adaptive immune responses to DNA vaccines.	Nature	451	725-729	2008
Takahasi K. et al.	Non-self RNA-Sensing Mechanism of RIG-I Helicase and Activation of Antiviral Immune Responses	Molecular Cell	29	428-440	2008
Cui S. et al.	The C-terminal regulatory domain is the RNA 5'-triphosphate sensor of RIG-I	Molecular Cell	29	169-179	2008
Yoneyama M. & Fujita T.	Structural Mechanism of RNA Recognition by the RIG-I-like Receptors	Immunity	29	178-181	2008
Ariumi A. et al.	The DNA damage sensors Ataxia-Telangiectasia mutated kinase and checkpoint kinase 2 are required for hepatitis C virus RNA replication.	J. Virol.	82	9639-9646	2008
Dansako H. et al.	A new living cell-based assay system for monitoring genome-length hepatitis C virus RNA replication.	Virus Res.	137	72-79	2008
Mori K. et al.	New efficient replication system with hepatitis C virus genome derived from a patient with acute hepatitis C.	Biochem. Biophys. Res. Commun.	371	104-109	2008
Hirano K. et al.	Differential effects of calcineurin inhibitors, tacrolimus and cyclosporin A, on interferon-induced antiviral protein in human hepatocyte cells.	Liver. Transpl.	14	292-298	2008
Nakamura M. et al.	Possible molecular mechanism of the relationship between NS5B polymorphisms and early clearance of hepatitis C virus during interferon plus ribavirin treatment.	J. Med. Virol.	80	632-639	2008
Ando M. et al.	Mitochondrial electron transport inhibition in full genomic hepatitis C virus replicon cells is restored by reducing viral replication.	Liver Int.	28	1158-1166	2008

Inubushi S. et al.	Hepatitis C virus NS5A protein interacts with and negatively regulates the non-receptor protein-tyrosine kinase Syk.	J. Gen. Virol.	89	1231-1242	2008
Sakamoto N. et al.	Inhibition of hepatitis C virus infection and expression in vitro and in vivo by recombinant adenovirus expressing short hairpin RNA	J. Gastro. Hepatol.	23	1437-1447	2008
Nishimura T. et al.	Comparative aspects on the role of polypyrimidine tract-binding protein in internal initiation of hepatitis C virus and picornavirus RNAs	Compara. Immu. Microbio. Infect. Dises.	31	435-448	2008

Cellular Signaling and Innate Immune Responses to RNA Virus Infections
Edited by A. R. Brasier et al.

Osamu Takeuchi
Shizuo Akira

3

Cytoplasmic Pattern Receptors (RIG-I and MDA-5) and Signaling in Viral Infections

Virus infections are recognized by the innate immune system, and this recognition elicits initial antiviral responses. Production of type I interferons (IFNs), proinflammatory cytokines, and chemokines by innate immune cells is essential for mounting rapid innate immune responses as well as activating adaptive immunity (1, 4). The innate immune system senses viral invasion via germline-encoded pattern recognition receptors (PRRs). Currently, two classes of PRRs, namely Toll-like receptors (TLRs) and retinoic acid-inducible gene I (RIG-I)-like RNA helicases (RLHs), have been shown to be involved in the recognition of virus-specific components by innate immune cells. Recognition of viral components by these PRRs triggers intracellular signaling cascades that lead to the expression of genes encoding type I IFNs and proinflammatory cytokines. TLRs are transmembrane proteins suitable for detecting microbial components on the outside of cells as well as in the cytoplasmic vacuole after phagocytosis or endocytosis. In contrast, RLHs, which lack a transmembrane domain, are localized in the cytosol and recognize viral RNAs that are translocated into the cytosol or generated during the course of virus replication. Type I IFNs are produced not only by professional innate immune cells, such as dendritic cells (DCs) and macrophages, but also by nonprofessional cells, such as fibroblasts.

Type I IFNs—which comprise multiple IFN-α isoforms, a single IFN-β, and other members, such as IFN-ω, -ε, and -κ—are pleiotropic cytokines that are essential for antiviral immune responses (18). Type I IFNs activate intracellular signaling pathways and regulate the expression of a set of genes by inducing nuclear translocation of transcription factors known as signal transducers and activators of transcription (Stat) proteins. The IFN-inducible genes include protein kinase R (PKR) and 2′,5′-oligoadenylate synthase, among others (62). These IFN-inducible proteins are believed to suppress virus replication by both cleaving viral nucleotides and suppressing the proliferation of virus-infected cells. Importantly, type I IFNs as well as proinflammatory cytokines and chemokines are essential for antibody production in B cells and the induction of cytotoxic T cells and natural killer (NK) cells.

In this review, we will focus on the roles of RLHs in RNA virus recognition and RLH signaling pathways. We also describe the roles of the TLR system with respect to the relationship between these two virus recognition

Osamu Takeuchi and Shizuo Akira, Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, 3–1 Yamadaoka, Suita, Osaka 565-0871, Japan.

mechanisms during the course of RNA virus infections in vivo.

RLHs

RIG-I (also known as DDX58) was identified as a candidate for a cytoplasmic viral RNA detector responsible for the production of type I IFNs (74). RIG-I is composed of two N-terminal caspase recruitment domains (CARDs) followed by a DExD/H box RNA helicase domain. RIG-I forms a family, designated the RLH family, with melanoma differentiation-associated gene 5 (MDA-5; also known as helicard or IFIH1) and LGP2 based on the high similarities among their helicase domains (25, 32, 51, 75). The helicase domains of the RLH family members are highly similar to that of mammalian Dicer. The expression of RLH genes is strongly induced by IFNs. RLHs interact with double-stranded (ds) RNAs through their helicase domains, and this dsRNA stimulation induces their ATP catalytic activity (40). A C-terminal portion of RIG-I, designated the repressor domain (RD), was found to inhibit the triggering of RIG-I signaling in the steady state (54). It has been suggested that RIG-I and LGP2, but not MDA-5, possess a C-terminal RD. The N-terminal CARDs are responsible for activating downstream signaling pathways that mediate dsRNA-induced type I IFN production.

RECOGNITION OF RNA VIRUSES BY RLHs

dsRNA is present in cells infected with dsRNA viruses and also generated during the course of single-stranded (ss) RNA virus replication. Since host cells do not produce dsRNA, the host innate immune system is able to discriminate between host and viral RNAs by the presence of dsRNA. Initially, both RIG-I and MDA-5 were implicated in the recognition of poly(I:C), a synthetic analogue of viral dsRNA. However, analyses of RIG-I$^{-/-}$ and MDA-5$^{-/-}$ mice revealed that MDA-5, but not RIG-I, is responsible for the IFN response to poly(I:C) stimulation (10, 27). Reciprocally, RIG-I, but not MDA-5, recognizes 5′-triphosphate ssRNA (19, 50). RNAs from some viruses are 5′-triphosphorylated and uncapped, whereas the 5′ ends of host mRNAs are capped. Thus, RIG-I discriminates between viral and host RNAs based on differences in the 5′ ends of RNAs. However, it remains unclear whether RIG-I also recognizes dsRNAs in addition to 5′-triphosphate ssRNA. PKR, a serine/threonine kinase containing dsRNA-binding domains, is activated by 5′-triphosphate RNA in a RIG-I-independent manner. PKR phosphorylates eukaryotic initiation factor 2 (eIF2), which inhibits the initiation of translation (46).

RNA viruses are also differentially recognized by RIG-I and MDA-5. RIG-I$^{-/-}$ cells do not produce type I IFNs in response to various RNA viruses, including paramyxoviruses, vesicular stomatitis virus (VSV), and influenza virus (26, 27). In contrast, MDA-5$^{-/-}$ cells do not respond to infections with picornaviruses, such as encephalomyocarditis virus (EMCV) and Theiler's virus. Cells infected with EMCV, but not influenza virus, generate dsRNA (50). Dephosphorylation of the 5′-triphosphate RNA of the influenza genome results in loss of its ability to induce IFNs, suggesting that recognition by RIG-I is mediated through 5′-triphosphate ssRNA. Consistent with the defect in type I IFN production, RIG-I$^{-/-}$ and MDA-5$^{-/-}$ mice are highly susceptible to inoculation with VSV and EMCV, respectively (27). Japanese encephalitis virus and hepatitis C virus (HCV), both of which belong to the *Flaviviridae* family, are recognized by RIG-I (27). Huh7 cells harboring a mutant RIG-I are permissive to HCV infection (64). GB virus, a small primate model of human HCV, is also recognized by RIG-I (6). However, dengue virus and West Nile virus, which also belong to the *Flaviviridae* family, still induce type I IFN production in the absence of RIG-I or MDA-5 (8, 27, 38, 64). Small interfering RNA experiments suggested that dengue virus is recognized by a combination of RIG-I and MDA-5. Vaccine strains of measles virus activate cells in a RIG-I/MDA-5-dependent manner, whereas wild-type measles virus fails to induce type I IFN production (60). It has been suggested that the presence of defective interfering RNAs, which potentially form dsRNA, in the vaccine strains of measles virus is critical for activating innate immune cells. The receptor responsible for detecting poliovirus has not been identified. However, poliovirus is expected to be recognized by MDA-5 because this virus is a member of the *Picornaviridae* family. Interestingly, MDA-5 was reported to be cleaved by a poliovirus-induced protease in infected cells (3). Thus, poliovirus may subvert the MDA-5-mediated recognition system to establish its infection.

RIG-I-mediated signaling is positively and negatively controlled by ubiquitination of RIG-I. First, the CARDs of RIG-I undergo Lys-63-linked ubiquitination by tripartite motif (TRIM) 25, a ubiquitin E3 ligase composed of a RING finger domain, B box/coiled-coil domain, and SPRY domain (9). This ubiquitination is necessary for efficient activation of the RIG-I signaling pathway, and TRIM25$^{-/-}$ cells display impaired production of type I IFNs in response to viral infection. RIG-I also undergoes ubiquitination by the ubiquitin ligase RNF125, which leads to its proteasomal degradation (2). Thus, RIG-1 ubiquitination by RNF125 is considered to inhibit aberrant activation of RIG-I signaling.

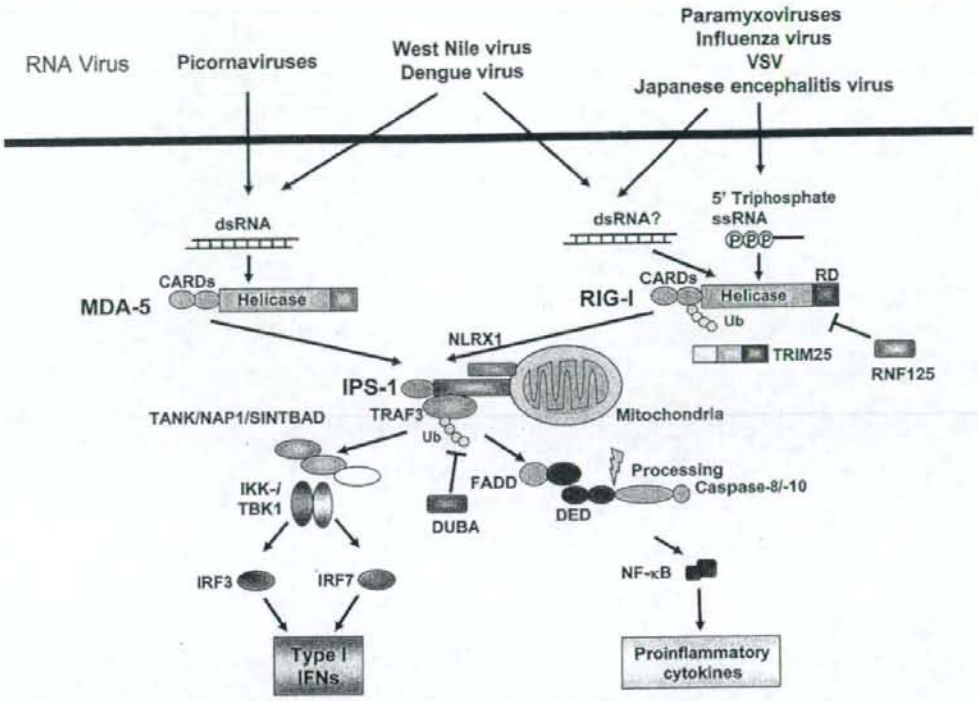

Figure 1 RLH-mediated recognition of RNA viruses. RIG-I and MDA-5 recognize 5′-triphosphate RNA and dsRNA from RNA viruses and interact with IPS-1. TRAF3 is recruited to IPS-1, and Lys-63-type polyubiquitination, which is controlled by the presence of DUBA, is induced. Subsequently, TRAF3 recruits TANK/NAP1/SINTBAD and TBK1/IKK-*i*, which phosphorylate IRF3/IRF7. The phosphorylated IRFs translocate into the nucleus and induce the expression of type I IFN genes. NF-κB is also activated by IPS-1 via the FADD and caspase-8/10-dependent pathway.

RNase L, an endonuclease originally thought to cleave viral ssRNA, was reported to be involved in the production of IFN-β in response to RNA virus infection or dsRNA stimulation (39). Furthermore, 2′,5′-linked oligoadenylate generated by virus infection was found to activate RNase L for cleavage of self-RNA, resulting in the generation of small RNA products that are responsible for RIG-I/MDA-5-mediated recognition and subsequent production of type I IFNs. However, the precise structures of these small RNAs generated by RNase L require further investigation.

Since LGP2 lacks a CARD, it is suggested to function as a negative regulator of RIG-I/MDA-5 signaling. Overexpression of LGP2 inhibits Sendai virus and Newcastle disease virus (NDV) signaling (51, 54, 75). LGP2 also contains an RD, which was found to interact with the RD of RIG-I and suppress RIG-1 self-association. Recently, *Lgp2*$^{-/-}$ mice were generated and analyzed by Barber and colleagues (71). *Lgp2*$^{-/-}$ mice show highly elevated induction of type I IFNs in response to poly(I:C) stimulation, as well as modestly increased IFN production in response to VSV infection. On the other hand, these mice exhibit partially impaired type I IFN production in response to EMCV infection. The authors proposed that LGP2 is a negative regulator of RIG-I, but not MDA-5. However, given that both poly(I:C) and EMCV are recognized by MDA-5, the difference cannot simply be explained by differential usage of LGP2 for RIG-I and MDA-5 signaling.

RLH SIGNALING PATHWAYS

The CARDs of RIG-I and MDA-5 are responsible for initiating signaling cascades (Fig. 1). RIG-I and MDA-5 associate with an adaptor protein, IFN-β promoter stimulator-1 (IPS-1; also known as MAVS, VISA, or

Cardif), which also contains a CARD (29, 44, 58, 72). Overexpression of IPS-1 induces the activation of IFN promoters as well as nuclear factor-κB (NF-κB). IPS-$1^{-/-}$ mice are defective in the production of type I IFNs and proinflammatory cytokines in response to all RNA viruses recognized by either RIG-I or MDA-5 (35, 65). These findings indicate that IPS-1 plays an essential role in RIG-I/MDA-5 signaling. Interestingly, this protein is present in the outer mitochondrial membrane, suggesting that mitochondria may be important for IFN responses, in addition to their roles in metabolism and cell death (58). Recently, IPS-1 and RIG-I were shown to associate with a conjugate of Atg5 and Atg12, which are essential components for the autophagic process (23). $Atg5^{-/-}$ mouse embryonic fibroblasts (MEFs) show increased type I IFN production in response to RNA virus infection, suggesting that the autophagic machinery directly affects the RIG-I signaling pathway in addition to autophagosome formation. IPS-1 is known to be cleaved by an HCV protease, NS3/4A, in HCV-infected cells.

IPS-1 associates with tumor necrosis factor receptor-associated factor (TRAF) 3, an E3 ubiquitin ligase assembling the Lys-63-linked polyubiquitin chain, through its C-terminal TRAF domain (12, 48, 53). Both the N-terminal RING finger domain and C-terminal TRAF domain are required for the function of TRAF3 to activate the type I IFN promoter (53). $TRAF3^{-/-}$ cells exhibit severely impaired production of type I IFNs in response to virus infection. In addition to the regulation of IFN responses, TRAF3 negatively regulates the noncanonical NF-κB pathway (14). $TRAF3^{-/-}$ mice show early postnatal lethality, and this phenotype is rescued by codisruption of the noncanonical NF-κB p100 gene (13). Recently, a deubiquitinase designated DUBA was found to deubiquitinate TRAF3 and suppress RLH signaling (30).

TRAF3 recruits and activates two IκB kinase (IKK)-related kinases, namely TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1) and inducible IκB kinase (IKK-*i*; also known as IKK-ε), which phosphorylate IFN regulatory factor (IRF) 3 and IRF7 (7, 15, 59). Although TBK1, but not IKK-*i*, is important for controlling IFN responses in MEFs, these two kinases function redundantly for inducing type I IFN production in macrophages and conventional DCs (cDCs) (41, 49). However, IKK-*i*/IKK-ε was reported to be additionally activated by IFN-β to directly phosphorylate Stat1, thereby controlling a set of IFN-inducible genes, such as dsRNA-activated adenosine deaminase gene (*Adar1*) (69).

Recently, an IPS-1/MAVS-interacting protein, designated NLRX1 (also known as NOD-9), was identified (45). NLRX1 is composed of a nucleotide-binding domain and leucine-rich repeats, and localizes on the mitochondrial outer membrane. Overexpression of NLRX1 inhibits virus-induced IFN-β promoter activation by disrupting the interaction of RIG-I or MDA-5 with IPS-1. Reciprocally, knockdown of NLRX1 leads to augmentation of virus-induced type I IFN production. Although the precise role of NLRX1 in RLH signaling remains to be clarified, NLRX1 is suggested to function as a modifier of IPS-1/MAVS, rather than an innate immune receptor, in contrast to other NOD-like receptors reported to directly recognize bacterial components in the cytoplasm.

TBK1 and IKK-*i* interact with TANK, NAK-associated protein 1 (NAP1), and similar to NAP1 TBK1 adaptor (SINTBAD) (11, 52, 55). These molecules contain a TBK1-binding motif and show similarities among their coiled-coil domains. Although knockdown of either TANK, NAP1, or SINTBAD impairs RLH signaling, the relationship between these molecules in RLH signaling is not yet fully understood.

Phosphorylation of IRF3 or IRF7 by these kinases induces the formation of homodimers and/or heterodimers (17), which translocate into the nucleus and bind to IFN-stimulated response elements (ISREs), resulting in the expression of type I IFNs and a set of IFN-inducible genes.

Fas-associated death domain-containing protein (FADD) interacts with caspase-8, caspase-10, and IPS-1, and the FADD-dependent pathway is responsible for the activation of NF-κB downstream of IPS-1 (67). Although FADD has also been implicated in RLH-mediated IFN responses, $FADD^{-/-}$ MEFs are still capable of producing IFN-β in response to NDV infection (75).

RECOGNITION OF VIRAL COMPONENTS BY THE TLR SYSTEM

In addition to RLHs, TLRs are also important in recognizing virus infections. TLRs comprise leucine-rich repeats, a transmembrane domain, and a cytoplasmic domain designated the Toll/interleukin-1 receptor (IL-1R) homology (TIR) domain (1, 4, 42). To date, 12 and 13 TLRs have been reported in humans and mice, respectively, and the microbial components recognized by each TLR have mostly been identified. Among the TLRs, TLR3, TLR7, and TLR9 are localized on cytoplasmic vesicles, such as endosomes and the endoplasmic reticulum, and recognize microbial nucleotides. Specifically, TLR3 detects dsRNA, while TLR7 and TLR9 recognize ssRNA and DNA with a CpG motif, respectively. In addition, TLR2 and TLR4, located on the cell surface, recognize RNA virus envelope proteins. Since the leucine-rich repeats, which are responsible for

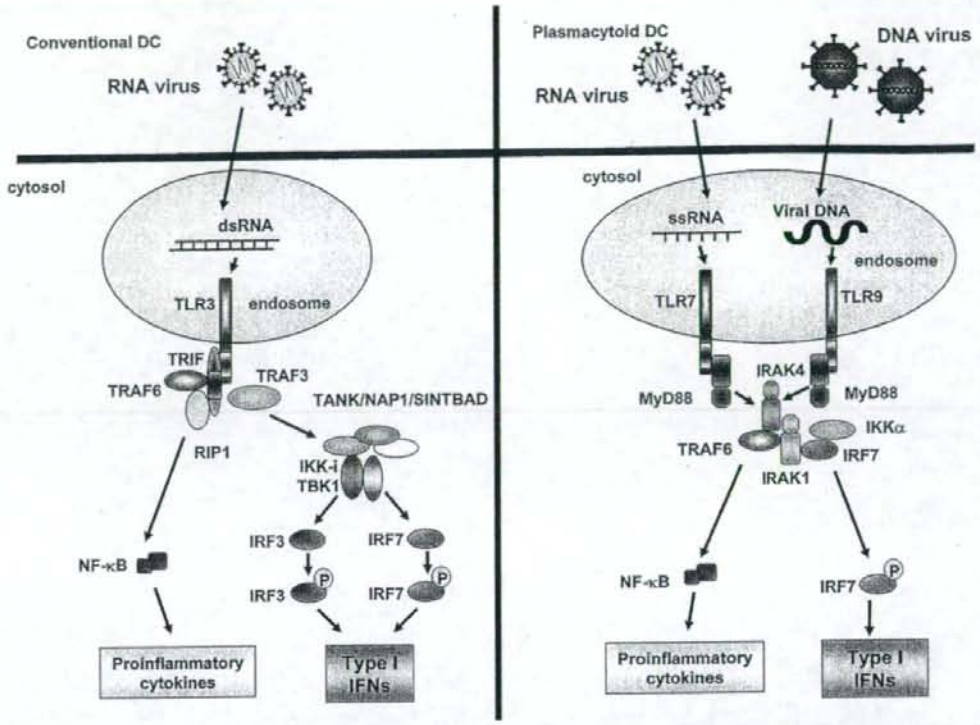

Figure 2 TLR signaling pathways leading to the production of type I IFNs. TLR3 and TLR7/9 activate distinct intracellular signaling pathways. TLR3 recruits TRIF as an adaptor molecule. TRIF associates with TRAF3, TRAF6, and RIP-1, which are responsible for the activation of IRF3, IRF7, and NF-κB. Downstream of TRAF3, TLR3 and RLHs share a common signaling cascade. On the other hand, TLR7 and TLR9 activate specialized signaling cascades in pDCs. In response to RNA and DNA viruses, TLR7 and TLR9 recruit MyD88 as an adaptor and activate IRAK-4, IRAK-1, and TRAF6, resulting in activation of the IKK complex and nuclear translocation of NF-κB, which in turn initiates the expression of proinflammatory cytokine genes. IRAK-1 and IKK-α activate IRF7 together with TRAF3 and IKK-α, and induce nuclear translocation of IRF7. Finally, transcription of type I IFN genes occurs.

microbial component recognition, face the extracellular space or endosomal lumen, TLRs are not suitable for recognizing viruses that have infected cells and are localized in the cytoplasm or nucleus.

The TIR domains of TLRs are responsible for triggering intracellular signaling pathways that lead to the expression of genes encoding proinflammatory cytokines and type I IFNs (Fig. 2). Upon stimulation of TLRs, TIR domain-containing adaptor molecules are recruited to TLRs. Currently, five adaptors (MyD88, TRIF, TIRAP/MAL, TRAM, and SARM) have been identified, and all of these except SARM are involved in TLR signaling in mice (1, 31).

In response to stimulation with poly(I:C), TLR3 recruits TIR-domain-containing adaptor inducing IFN-β (TRIF; also known as TICAM-1) (73). Subsequently, TRIF associates with TRAF3, TRAF6, and receptor-interacting protein 1 (RIP-1) (43, 48, 56). The RLH and TLR3 signaling pathways share TRAF3 for inducing the expression of type I IFNs. Simultaneously, TRAF6 and RIP-1 are responsible for activating NF-κB, thereby leading to the expression of proinflammatory cytokines. However, the role of TLR3 in type I IFN production in vivo in response to dsRNA stimulation is questionable, since mice lacking TLR3 or TRIF show normal IFN-α production in response to systemic poly(I:C) administration (27). On the other hand, the production of IL-12p40 in serum is dependent on TLR3 signaling. Reciprocally, mice lacking MDA-5 show abrogated IFN production together with normal IL-12p40 production,

suggesting that TLR3 and MDA-5 function to control proinflammatory cytokines and type I IFNs, respectively.

Plasmacytoid DCs (pDCs) produce extremely large amounts of type I IFNs in response to virus infections (61). TLR7 and TLR9 are highly expressed on pDCs, and stimulation with viral RNA or DNA efficiently induces the production of type I IFNs (22). TLR7 and TLR9 recruit MyD88, an adaptor protein comprising a death domain and TIR domain. MyD88 then interacts with IL-1R-associated kinase-1 (IRAK-1), IRAK-4, and IRF7. IRAK-1 and IκB kinase a (IKK-α) have been identified as potential IRF7 kinases (16, 20, 28, 70). Phosphorylated IRF7 dissociates from the MyD88-containing complex and translocates into the nucleus to initiate the expression of IFN-inducible genes. TRAF3 is also required for this signaling cascade. TLR9 stimulation also activates cDCs for IFN-β production, although the amounts of IFN-β are not comparable to those produced by pDCs. In cDCs, IRF1, but not IRF7, is responsible for the IFN responses (47, 57).

An endoplasmic reticulum membrane protein, UNC-93B, was identified as an essential molecule for signaling by TLR3, TLR7, and TLR9 by forward genetic screening of mice (66). Recently, an autosomal recessive mutation in UNC-93B in humans was found to result in impaired immune responses against herpes simplex virus (HSV)-1 encephalitis (5).

Autophagy is also responsible for the induction of type I IFNs in response to VSV or HSV infections in pDCs (37). Some ssRNA viruses, such as VSV, appeared to require live virus infection to induce the production of type I IFNs. Autophagosomes are constitutively formed in pDCs, and pDCs lacking *Atg5*, a gene critical for autophagosome formation, show severely impaired production of type I IFNs in response to VSV and HSV infections, indicating that autophagosome formation is critical for the recognition of some viruses in pDCs (37). Since TLR7 is responsible for the production of type I IFNs in response to VSV infection in pDCs, autophagosomes containing VSV components may fuse to organelles containing TLR7. Interestingly, CpG-DNA-induced IFN-α production, but not IL-12p40 production, is also impaired in $Atg5^{-/-}$ pDCs, suggesting that Atg5 modifies the TLR9 signaling pathway leading to the production of type I IFNs.

TYPE I IFN-PRODUCING CELLS IN RESPONSE TO VIRAL INFECTION

Although RLHs play essential roles in the production of type I IFNs and cytokines in various cell types, such as fibroblasts and cDCs, pDCs produce these cytokines in the absence of RLH signaling (26). pDCs produce huge amounts of type I IFNs in response to virus infections, and TLR signaling is essential for this IFN production. Although the importance of pDCs as a source of type I IFNs in vivo has been emphasized, direct identification of IFN-producing cells in vivo has not been carried out. Analysis of reporter mice expressing green fluorescent protein (GFP) under the control of the IFN-α6 gene ($Ifna6^{GFP/+}$) revealed that distinct cell types produce IFN-α in response to systemic versus local RNA virus infection (34). Although pDCs were highly potent in expressing GFP upon systemic NDV infection, lung infection of $Ifna6^{GFP/+}$ mice with NDV resulted in increased numbers of GFP^+ alveolar macrophages and cDCs, but not pDCs (34). Thus, cells other than pDCs can be a source of type I IFNs depending on the route of infection. pDCs started to produce IFN-α when alveolar macrophages were depleted, suggesting that pDCs function when the first line of defense is broken.

ROLES OF RLHs AND TLRs IN THE ACTIVATION OF ADAPTIVE IMMUNE RESPONSES TO VIRUSES

Innate immediate immune responses are important for mounting acquired immune responses to viral infections. However, it is not clear how the innate PRRs are involved in the activation of acquired immunity. Recently, two different virus infection models have been analyzed to examine the roles of RLHs and TLRs in the activation of acquired immune responses. The first model virus is lymphocytic choriomeningitis virus (LCMV), an ambisense ssRNA virus belonging to the *Arenaviridae* family, which is known to induce a cytotoxic-T-lymphocyte (CTL) response in a type I IFN-dependent manner (24). Analyses of $MyD88^{-/-}$ and $IPS\text{-}1^{-/-}$ mice revealed that the serum levels of type I IFNs and proinflammatory cytokines are mainly dependent on the presence of MyD88, but not IPS-1. Furthermore, the generation of virus-specific CTLs is critically dependent on MyD88, but not IPS-1. Analysis of $Ifna6^{+/GFP}$ reporter mice revealed that pDCs are the major source of IFN-α in LCMV infection. These results suggest that recognition of LCMV by pDCs via TLRs is responsible for the production of type I IFNs in vivo. Furthermore, TLRs, but not RLHs, appear to be important for mounting CTL responses to LCMV infection.

Influenza virus has also been used to study the activation of adaptive immune responses (33). Induction of type I IFNs in response to intranasal influenza A virus infection was found to be abrogated in the absence of both MyD88 and IPS-1, although mice lacking either of

these molecules were capable of producing IFNs. Induction of B cells or CD4 T cells specific to viral proteins was dependent on the presence of MyD88, but not IPS-1, whereas induction of nuclear protein antigen-specific CD8 T cells was not impaired in the absence of either MyD88 or IPS-1. These results suggest that the adaptive immune responses to influenza A virus are governed by TLRs.

As described above, poly(I:C) is recognized by MDA-5 and TLR3. The contributions of these two systems to the activation of T-cell responses have been examined using mice deficient in IPS-1 or TRIF, adaptor molecules responsible for the signaling of MDA-5 and TRIF, respectively (36). Enhancement of antigen-specific antibody responses as well as CD8 T-cell expansion in response to poly(I:C) stimulation is impaired in IPS-1-deficient mice. Although the responses of TRIF-deficient mice are modestly impaired, IPS-1/TRIF doubly deficient mice are almost unresponsive to poly(I:C) treatment, suggesting that both MDA-5 and IPS-1 contribute to mounting acquired immune responses to poly(I:C) stimulation.

The virus infection models tested to date support roles for TLRs, rather than RLHs, in instructing the adaptive immune system. However, further studies are required since these two PRR systems contribute differently depending on the viruses involved, and their contributions may also depend on the route of infection.

CONCLUSIONS

Recent progress in studies of RLHs and their signaling pathways has revealed that the RLH system is essential for inducing innate immune responses in response to RNA viruses infecting cells. On the other hand, TLRs play critical roles in producing type I IFNs in pDCs—professional type I IFN-producing cells—in response to virus infection. Although recent studies have clarified the functions and signaling pathways of RLHs, the molecular structures of the RNAs recognized by MDA-5 are not fully understood. Although recognition of 5′-triphosphate RNA by RIG-I is established, it is unclear whether RIG-I also detects dsRNA without a 5′-triphosphate end. Thus, further investigations are required to fully clarify the structures of the RIG-I and MDA-5 ligands.

Furthermore, the mechanisms of DNA virus recognition are not well understood. Although TLR9 is essential for detecting HSV and mouse cytomegalovirus, the presence of a TLR-independent DNA virus recognition mechanism has been predicted. The receptors involved are supposed to recognize viral genomic DNA (21, 63), and a protein named DAI has been proposed as a candidate for such a sensor (68). However, MEFs and DCs from DAI/ZBP1-deficient mice show normal IFN-β production as well as IFN-inducible gene expression in response to dsDNA stimulation. Thus, another unknown receptor system may be responsible for the detection of viral DNA. Although we focused on the mechanisms of innate immune responses and T-cell activation in response to RNA viruses in this review, many other cell types, such as NK cells and NK T cells, are involved in antiviral responses in vivo. Furthermore, various immune cells cooperate in order to establish optimized antiviral immune responses. Thus, studies monitoring immune responses in vivo could be vital to fully clarify the mechanisms of antiviral immune responses.

We thank M. Hashimoto for excellent secretarial assistance. This work was supported in part by grants from the Special Coordination Funds of the Japanese Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labour and Welfare in Japan; the 21st Century Center of Excellence Program of Japan; and the National Institute of Health (P01 AI070167).

References

1. **Akira, S., S. Uematsu, and O. Takeuchi.** 2006. Pathogen recognition and innate immunity. *Cell* 124:783–801.
2. **Arimoto, K., H. Takahashi, T. Hishiki, H. Konishi, T. Fujita, and K. Shimotohno.** 2007. Negative regulation of the RIG-I signaling by the ubiquitin ligase RNF125. *Proc. Natl. Acad. Sci. USA* 104:7500–7505.
3. **Barral, P. M., J. M. Morrison, J. Drahos, P. Gupta, D. Sarkar, P. B. Fisher, and V. R. Racaniello.** 2007. MDA-5 is cleaved in poliovirus-infected cells. *J. Virol.* 81:3677–3684.
4. **Beutler, B., C. Eidenschenk, K. Crozat, J. L. Imler, O. Takeuchi, J. A. Hoffmann, and S. Akira.** 2007. Genetic analysis of resistance to viral infection. *Nat. Rev. Immunol.* 7:753–766.
5. **Casrouge, A., S. Y. Zhang, C. Eidenschenk, E. Jouanguy, A. Puel, K. Yang, A. Alcais, C. Picard, N. Mahfoufi, N. Nicolas, L. Lorenzo, S. Plancoulaine, B. Senechal, F. Geissmann, K. Tabeta, K. Hoebe, X. Du, R. L. Miller, B. Heron, C. Mignot, T. B. de Villemeur, P. Lebon, O. Dulac, F. Rozenberg, B. Beutler, M. Tardieu, L. Abel, and J. L. Casanova.** 2006. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 314:308–312.
6. **Chen, Z., Y. Benureau, R. Rijnbrand, J. Yi, T. Wang, L. Warter, R. E. Lanford, S. A. Weinman, S. M. Lemon, A. Martin, and K Li.** 2007. GB virus B disrupts RIG-I signaling by NS3/4A-mediated cleavage of the adaptor protein MAVS. *J. Virol.* 81:964–976.
7. **Fitzgerald, K. A., S. M. McWhirter, K. L. Faia, D. C. Rowe, E. Latz, D. T. Golenbock, A. J. Coyle, S. M. Liao, and T. Maniatis.** 2003. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. *Nat. Immunol.* 4:491–496.
8. **Fredericksen, B. L., B. C. Keller, J. Fornek, M. G. Katze, and M. Gale, Jr.** 2008. Establishment and maintenance of

the innate antiviral response to West Nile virus involves both RIG-I and MDA-5 signaling through IPS-1. *J. Virol.* 82:609–616.

9. Gack, M. U., Y. C. Shin, C. H. Joo, T. Urano, C. Liang, L. Sun, O. Takeuchi, S. Akira, Z. Chen, S. Inoue, and J. U. Jung. 2007. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature* 446:916–920.
10. Gitlin, L., W. Barchet, S. Gilfillan, M. Cella, B. Beutler, R. A. Flavell, M. S. Diamond, and M. Colonna. 2006. Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. *Proc. Natl. Acad. Sci. USA* 103: 8459–8464.
11. Guo, B., and G. Cheng. 2007. Modulation of the interferon antiviral response by the TBK1/IKKi adaptor protein TANK. *J. Biol. Chem.* 282:11817–11826.
12. Hacker, H., V. Redecke, B. Blagoev, I. Kratchmarova, L. C. Hsu, G. G. Wang, M. P. Kamps, E. Raz, H. Wagner, G. Hacker, M. Mann, and M. Karin. 2006. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* 439:204–207.
13. He, J. Q., B. Zarnegar, G. Oganesyan, S. K. Saha, S. Yamazaki, S. E. Doyle, P. W. Dempsey, and G. Cheng. 2006. Rescue of TRAF3-null mice by p100 NF-kappa B deficiency. *J. Exp. Med.* 203:2413–2418.
14. He, J. Q., S. K. Saha, J. R. Kang, B. Zarnegar, and G. Cheng. 2007. Specificity of TRAF3 in its negative regulation of the noncanonical NF-kappa B pathway. *J. Biol. Chem.* 282:3688–3694.
15. Hemmi, H., O. Takeuchi, S. Sato, M. Yamamoto, T. Kaisho, H. Sanjo, T. Kawai, K. Hoshino, K. Takeda, and S. Akira. 2004. The roles of two IkappaB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. *J. Exp. Med.* 199:1641–1650.
16. Honda, K., H. Yanai, T. Mizutani, H. Negishi, N. Shimada, N. Suzuki, Y. Ohba, A. Takaoka, W. C. Yeh, and T. Taniguchi. 2004. Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. *Proc. Natl. Acad. Sci. USA* 101:15416–15421.
17. Honda, K., H. Yanai, H. Negishi, M. Asagiri, M. Sato, T. Mizutani, N. Shimada, Y. Ohba, A. Takaoka, N. Yoshida, and T. Taniguchi. 2005. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature* 434:772–777.
18. Honda, K., A. Takaoka, and T. Taniguchi. 2006. Type I interferon gene induction by the interferon regulatory factor family of transcription factors. *Immunity* 25:349–360.
19. Hornung, V., J. Ellegast, S. Kim, K. Brzozka, A. Jung, H. Kato, H. Poeck, S. Akira, K. K. Conzelmann, M. Schlee, S. Endres, and G. Hartmann. 2006. 5′-Triphosphate RNA is the ligand for RIG-I. *Science* 314:994–997.
20. Hoshino, K., T. Sugiyama, M. Matsumoto, T. Tanaka, M. Saito, H. Hemmi, O. Ohara, S. Akira, and T. Kaisho. 2006. IkappaB kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. *Nature* 440:949–953.
21. Ishii, K. J., C. Coban, H. Kato, K. Takahashi, Y. Torii, F. Takeshita, H. Ludwig, G. Sutter, K. Suzuki, H. Hemmi, S. Sato, M. Yamamoto, S. Uematsu, T. Kawai, O. Takeuchi, and S. Akira. 2006. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. *Nat. Immunol.* 7:40–48.
22. Iwasaki, A., and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol* 5:987–995.
23. Jounai, N., F. Takeshita, K. Kobiyama, A. Sawano, A. Miyawaki, K. Q. Xin, K. J. Ishii, T. Kawai, S. Akira, K. Suzuki, and K. Okuda. 2007. The Atg5-Atg12 conjugate associates with innate antiviral immune responses. *Proc. Natl. Acad. Sci. USA* 104:14050–14055.
24. Jung, A., H. Kato, Y. Kumagai, H. Kumar, T. Kawai, O. Takeuchi, and S. Akira. 2008. Lymphocytoid choriomeningitis virus activates plasmacytoid dendritic cells and induces a cytotoxic T-cell response via MyD88. *J. Virol.* 82:196–206.
25. Kang, D. C., R. V. Gopalkrishnan, Q. Wu, E. Jankowsky, A. M. Pyle, and P. B. Fisher. 2002. *mda-5*: an interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. *Proc. Natl. Acad. Sci. USA* 99: 637–642.
26. Kato, H., S. Sato, M. Yoneyama, M. Yamamoto, S. Uematsu, K. Matsui, T. Tsujimura, K. Takeda, T. Fujita, O. Takeuchi, and S. Akira. 2005. Cell type-specific involvement of RIG-I in antiviral response. *Immunity* 23: 19–28.
27. Kato, H., O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K. J. Ishii, O. Yamaguchi, K. Otsu, T. Tsujimura, C. S. Koh, C. Reis e Sousa, Y. Matsuura, T. Fujita, and S. Akira. 2006. Differential roles of MDA-5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 441:101–105.
28. Kawai, T., S. Sato, K. J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. Inoue, S. Uematsu, O. Takeuchi, and S. Akira. 2004. Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat. Immunol.* 5:1061–1068.
29. Kawai, T., K. Takahashi, S. Sato, C. Coban, H. Kumar, H. Kato, K. J. Ishii, O. Takeuchi, and S. Akira. 2005. IPS-1, an adaptor triggering RIG-I- and MDA-5-mediated type I interferon induction. *Nat. Immunol.* 6:981–988.
30. Kayagaki, N., Q. Phung, S. Chan, R. Chaudhari, C. Quan, K. M. O'Rourke, M. Eby, E. Pietras, G. Cheng, J. F. Bazan, Z. Zhang, D. Arnott, and V. M. Dixit. 2007. DUBA: a deubiquitinase that regulates type I interferon production. *Science* 318:1628–1632.
31. Kim, Y., P. Zhou, L. Qian, J. Z. Chuang, J. Lee, C. Li, C. Iadecola, C. Nathan, and A. Ding. 2007. MyD88–5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. *J. Exp. Med.* 204:2063–2074.
32. Kovacsovics, M., F. Martinon, O. Micheau, J. L. Bodmer, K. Hofmann, and J. Tschopp. 2002. Overexpression of Helicard, a CARD-containing helicase cleaved during apoptosis, accelerates DNA degradation. *Curr. Biol.* 12: 838–843.
33. Koyama, S., K. J. Ishii, H. Kumar, T. Tanimoto, C. Coban, S. Uematsu, T. Kawai, and S. Akira. 2007. Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. *J. Immunol.* 179:4711–4720.

34. **Kumagai, Y., O. Takeuchi, H. Kato, H. Kumar, K. Matsui, E. Morii, K. Aozasa, T. Kawai, and S. Akira.** 2007. Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. *Immunity* 27:240–252.
35. **Kumar, H., T. Kawai, H. Kato, S. Sato, K. Takahashi, C. Coban, M. Yamamoto, S. Uematsu, K. J. Ishii, O. Takeuchi, and S. Akira.** 2006. Essential role of IPS-1 in innate immune responses against RNA viruses. *J. Exp. Med.* 203:1795–1803.
36. **Kumar, H., S. Koyama, K. J. Ishii, T. Kawai, and S. Akira.** 2008. Cutting edge: cooperation of IPS-1- and TRIF-dependent pathways in poly IC-enhanced antibody production and cytotoxic T cell responses. *J. Immunol.* 180:683–687.
37. **Lee, H. K., J. M. Lund, B. Ramanathan, N. Mizushima, and A. Iwasaki.** 2007. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 315:1398–1401.
38. **Loo, Y. M., J. Fornek, N. Crochet, G. Bajwa, O. Perwitasari, L. Martinez-Sobrido, S. Akira, M. A. Gill, A. Garcia-Sastre, M. G. Katze, and M. Gale, Jr.** 2008. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J. Virol.* 82:335–345.
39. **Malathi, K., B. Dong, M. Gale, Jr., and R. H. Silverman.** 2007. Small self-RNA generated by RNase L amplifies antiviral innate immunity. *Nature* 448:816–819.
40. **Marques, J. T., T. Devosse, D. Wang, M. Zamanian-Daryoush, P. Serbinowski, R. Hartmann, T. Fujita, M. A. Behlke, and B. R. Williams.** 2006. A structural basis for discriminating between self and nonself double-stranded RNAs in mammalian cells. *Nat. Biotechnol.* 24:559–565.
41. **Matsui, K., Y. Kumagai, H. Kato, S. Sato, T. Kawagoe, S. Uematsu, O. Takeuchi, and S. Akira.** 2006. Cutting edge: role of TANK-binding kinase 1 and inducible IκB kinase in IFN responses against viruses in innate immune cells. *J. Immunol.* 177:5785–5789.
42. **Medzhitov, R.** 2007. Recognition of microorganisms and activation of the immune response. *Nature* 449:819–826.
43. **Meylan, E., K. Burns, K. Hofmann, V. Blancheteau, F. Martinon, M. Kelliher, and J. Tschopp.** 2004. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. *Nat. Immunol.* 5:503–507.
44. **Meylan, E., J. Curran, K. Hofmann, D. Moradpour, M. Binder, R. Bartenschlager, and J. Tschopp.** 2005. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 437:1167–1172.
45. **Moore, C. B., D. T. Bergstralh, J. A. Duncan, Y. Lei, T. E. Morrison, A. G. Zimmermann, M. A. Accavitti-Loper, V. J. Madden, L. Sun, Z. Ye, J. D. Lich, M. T. Heise, Z. Chen, and J. P. Ting.** 2008. NLRX1 is a regulator of mitochondrial antiviral immunity. *Nature* 451:573–577.
46. **Nallagatla, S. R., J. Hwang, R. Toroney, X. Zheng, C. E. Cameron, and P. C. Bevilacqua.** 2007. 5′-triphosphate-dependent activation of PKR by RNAs with short stem-loops. *Science* 318:1455–1458.
47. **Negishi, H., Y. Fujita, H. Yanai, S. Sakaguchi, X. Ouyang, M. Shinohara, H. Takayanagi, Y. Ohba, T. Taniguchi, and K. Honda.** 2006. Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. *Proc. Natl. Acad. Sci. USA* 103:15136–15141.
48. **Oganesyan, G., S. K. Saha, B. Guo, J. Q. He, A. Shahangian, B. Zarnegar, A. Perry, and G. Cheng.** 2006. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* 439:208–211.
49. **Perry, A. K., E. K. Chow, J. B. Goodnough, W. C. Yeh, and G. Cheng.** 2004. Differential requirement for TANK-binding kinase-1 in type I interferon responses to Toll-like receptor activation and viral infection. *J. Exp. Med.* 199:1651–1658.
50. **Pichlmair, A., O. Schulz, C. P. Tan, T. I. Naslund, P. Liljestrom, F. Weber, and C. Reis e Sousa.** 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. *Science* 314:997–1001.
51. **Rothenfusser, S., N. Goutagny, G. DiPerna, M. Gong, B. G. Monks, A. Schoenemeyer, M. Yamamoto, S. Akira, and K. A. Fitzgerald.** 2005. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *J. Immunol.* 175:5260–5268.
52. **Ryzhakov, G., and F. Randow.** 2007. SINTBAD, a novel component of innate antiviral immunity, shares a TBK1-binding domain with NAP1 and TANK. *EMBO J.* 26: 3180–3190.
53. **Saha, S. K., E. M. Pietras, J. Q. He, J. R. Kang, S. Y. Liu, G. Oganesyan, A. Shahangian, B. Zarnegar, T. L. Shiba, Y. Wang, and G. Cheng.** 2006. Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. *EMBO J.* 25:3257–3263.
54. **Saito, T., R. Hirai, Y. M. Loo, D. Owen, C. L. Johnson, S. C. Sinha, S. Akira, T. Fujita, and M. Gale. Jr.** 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc. Natl. Acad. Sci. USA* 104:582–587.
55. **Sasai, M., M. Shingai, K. Funami, M. Yoneyama, T. Fujita, M. Matsumoto, and T. Seya.** 2006. NAK-associated protein 1 participates in both the TLR3 and the cytoplasmic pathways in type I IFN induction. *J. Immunol.* 177:8676–8683.
56. **Sato, S., M. Sugiyama, M. Yamamoto, Y. Watanabe, T. Kawai, K. Takeda, and S. Akira.** 2003. Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling. *J. Immunol.* 171:4304–4310.
57. **Schmitz, F., A. Heit, S. Guggemoos, A. Krug, J. Mages, M. Schiemann, H. Adler, I. Drexler, T. Haas, R. Lang, and H. Wagner.** 2007. Interferon-regulatory-factor 1 controls Toll-like receptor 9-mediated IFN-beta production in myeloid dendritic cells. *Eur. J. Immunol.* 37:315–327.
58. **Seth, R. B., L. Sun, C. K. Ea, and Z. J. Chen.** 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 122:669–682.
59. **Sharma, S., B. R. tenOever, N. Grandvaux, G. P. Zhou, R. Lin, and J. Hiscott.** 2003. Triggering the interferon antiviral response through an IKK-related pathway. *Science* 300:1148–1151.
60. **Shingai, M., T. Ebihara, N. A. Begum, A. Kato, T. Honma, K. Matsumoto, H. Saito, H. Ogura, M. Matsumoto, and T. Seya.** 2007. Differential type I IFN-inducing abilities of wild-type versus vaccine strains of measles virus. *J. Immunol.* 179:6123–6133.

61. **Shortman, K., and Y. J. Liu.** 2002. Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol.* 2:151–161.
62. **Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber.** 1998. How cells respond to interferons. *Annu. Rev. Biochem.* 67:227–264.
63. **Stetson, D. B., and R. Medzhitov.** 2006. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity* 24:93–103.
64. **Sumpter, R., Jr., Y. M. Loo, E. Foy, K. Li, M. Yoneyama, T. Fujita, S. M. Lemon, and M. Gale, Jr.** 2005. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. *J. Virol.* 79:2689–2699.
65. **Sun, Q., L. Sun, H. H. Liu, X. Chen, R. B. Seth, J. Forman, and Z. J. Chen.** 2006. The specific and essential role of MAVS in antiviral innate immune responses. *Immunity* 24:633–642.
66. **Tabeta, K., K. Hoebe, E. M. Janssen, X. Du, P. Georgel, K. Crozat, S. Mudd, N. Mann, S. Sovath, J. Goode, L. Shamel, A. A. Herskovits, D. A. Portnoy, M. Cooke, L. M. Tarantino, T. Wiltshire, B. E. Steinberg, S. Grinstein, and B. Beutler.** 2006. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. *Nat. Immunol.* 7:156–164.
67. **Takahashi, K., T. H. Kawai, S. Sato, S. Yonehara, and S. Akira.** 2006. Roles of caspase-8 and caspase-10 in innate immune responses to double-stranded RNA. *J. Immunol.* 176:4520–4524.
68. **Takaoka, A., Z. Wang, M. K. Choi, H. Yanai, H. Negishi, T. Ban, Y. Lu, M. Miyagishi, T. Kodama, K. Honda, Y. Ohba, and T. Taniguchi.** 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* 448:501–505.
69. **tenOever, B. R., S. L. Ng, M. A. Chua, S. M. McWhirter, A. Garcia-Sastre, and T. Maniatis.** 2007. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. *Science* 315:1274–1278.
70. **Uematsu, S., S. Sato, M. Yamamoto, T. Hirotani, H. Kato, F. Takeshita, M. Matsuda, C. Coban, K. J. Ishii, T. Kawai, O. Takeuchi, and S. Akira.** 2005. Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-α induction. *J. Exp. Med.* 201:915–923.
71. **Venkataraman, T., M. Valdes, R. Elsby, S. Kakuta, G. Caceres, S. Saijo, Y. Iwakura, and G. N. Barber.** 2007. Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. *J. Immunol.* 178:6444–6455.
72. **Xu, L. G., Y. Y. Wang, K. J. Han, L. Y. Li, Z. Zhai, and H. B. Shu.** 2005. VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol. Cell* 19:727–740.
73. **Yamamoto, M., S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Sanjo, O. Takeuchi, M. Sugiyama, M. Okabe, K. Takeda, and S. Akira.** 2003. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science* 301:640–643.
74. **Yoneyama, M., M. Kikuchi, T. Natsukawa, N. Shinobu, T. Imaizumi, M. Miyagishi, K. Taira, S. Akira, and T. Fujita.** 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol.* 5:730–737.
75. **Yoneyama, M., M. Kikuchi, K. Matsumoto, T. Imaizumi, M. Miyagishi, K. Taira, E. Foy, Y. M. Loo, M. Gale, Jr., S. Akira, S. Yonehara, A. Kato, and T. Fujita.** 2005. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA-5, and LGP2 in antiviral innate immunity. *J. Immunol.* 175:2851–2858.