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ワクチンを標的としての癌抗原ペプチド

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abstract

ペプチドワクチン療法は，癌免疫療法のなかでも，近年最も脚光を浴びている治療法である．この療法は，担癌患者に投与された腫瘍関連抗原遺伝子由来のHLA拘束性ペプチドを，樹状細胞（dendritic cell：DC）が抗原提示細胞（antigen presenting cell：APC）として取り込み，殺細胞効果があるペプチド特異的細胞傷害性T細胞（cytotoxic T lymphocyte：CTL）を誘導することを利用した治療法である．本邦では，主に4つのグループでワクチンの開発，その投与法の改良，および臨床試験が行われている．しかし，癌細胞のHLAの発現退避，臨床試験の対象症例の免疫機能低下など，克服しなければならない問題は多い．2009年9月に「治療用癌ワクチンについての考察」が，米国食品医薬品局（FDA）から公表され，ペプチドワクチン療法は，欧米でも今後開発競争が進み，免疫療法の中心となっていくと思われる．

I はじめに

ペプチドワクチン療法は，癌免疫療法のなかでも，近年最も脚光を浴びている治療法である．本稿では，この療法の，メカニズム，治療の実際，本邦で現在行われている主な臨床研究の紹介，および本治療の問題点と展望を概説する．

II ペプチドワクチン療法のメカニズム

1991年，van der Bruggenらによって，ヒト腫瘍抗原としてはじめて悪性黒色腫の腫瘍抗原遺伝子（melanoma antigen gene：MAGE）が同定された[1]．以降，多くの腫瘍関連抗原遺伝子が単離され，研究や臨床に利用されてきた．こういった腫瘍に特異的に発現している腫瘍関連抗原はプロテアソームによるプロセシング作用を受けてペプチド断片となり，このペプチドが抗原プロセシング関連トランスポーター（transporter associated with antigen processing：TAP）により小胞体内に運ばれ，HLA分子に結合し，ゴルジ体を介して細胞表面へHLA-ペプチド複合体として表出される．これにより，細胞がこのペプチドを含むタンパク質である腫瘍抗原をつくりだしていることが，細胞外に表示される（図1）[2]．一方，HLA拘束性ペプチドを患者に投与すると，樹状細胞（dendritic cell：DC）が抗原提示細胞（antigen presenting cell：APC）としてこのペプチドを取り込み，HLA上にそのペプチドを提示する．このペプチドを提示したDCが所属リンパ節に移動し抗原提示を行うことにより，ペプチド特異的細胞傷害性T細胞（cytotoxic T lymphocyte：CTL）が誘導される（図2）[3]．

CTLはクローンとして増殖し，リンパ節から腫瘍部位に移動，組織内に浸潤し細胞表面に提示されたHLA-ペプチド複合体を認識して攻撃する．これ

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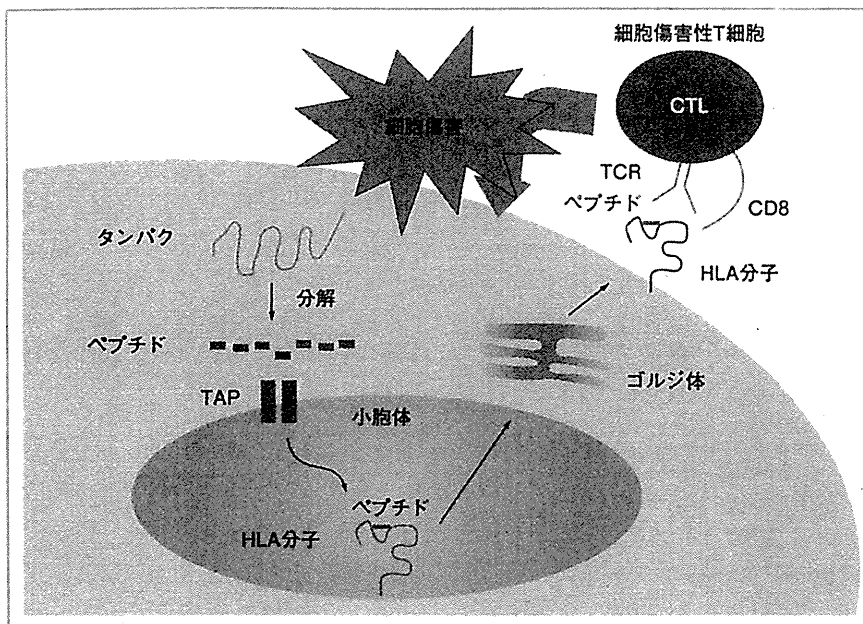

図1
特異的CTLにおける標的認識機構
〔参考文献2）より引用改変〕

によって，CTLが標的腫瘍細胞に対し特異的な殺細胞効果を発揮する（図1・2）．

III ペプチドワクチン療法の実際

現在，ペプチドワクチン療法は，本邦だけでも数多く行われており，そのペプチドの種類，投与方法，対象疾患も多岐にわたる．しかしそれらには，共通することも多い．主な共通項目をペプチドワクチン療法の実際の手技に従い列挙すると次のようになる．

1）腫瘍に高発現し，正常細胞には発現していないペプチドを使用する．多くの癌種に共通して発現しているものもあれば，特定の癌種にのみ高発現しているものもある．その多くは腫瘍細胞と正常精巣細胞にしか発現していない，いわゆる癌精巣抗原であるが，精巣ではHLA分子が発現していないため，CTLは精巣は認識せず腫瘍細胞のみを攻撃し，その結果，化学療法でみられるような正常細胞への副作用は少ないと考えられている．実際，ペプチドワクチン療法の副作用としては，発熱，頭痛，悪寒などのほかには，グレード1または2の局所の注射部位反応（発赤，硬結）は認めるものの，ペプチドに起因する重篤な副作用は認めない．

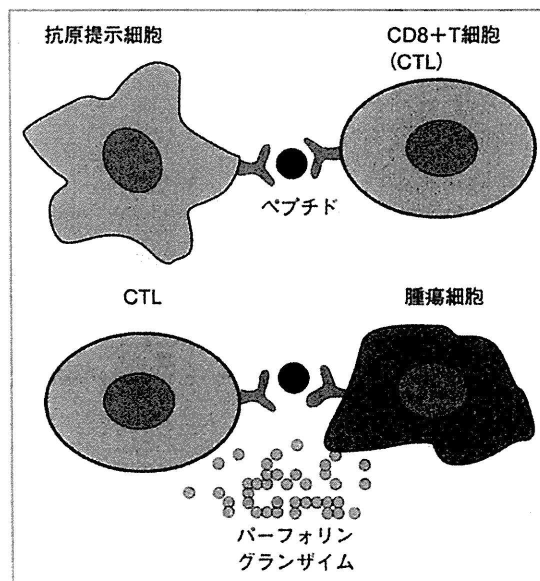

図2 ペプチドワクチンによる腫瘍細胞傷害誘導メカニズム
〔参考文献3）より引用改変〕

2）ペプチドは，化学合成された8～10merのアミノ酸からなり，それぞれの患者のHLAハプロタイプに合わせたペプチドを，アジュバントと混合

ワクチン	開発施設	対象臓器	臨床試験実施施設	主な特徴
Survivin 2B	札幌医大	大腸癌　膵癌 泌尿器科癌 他の固形腫瘍	札幌医大　他	新規アジュバントの開発 サイトカイン併用
中村ワクチン	東大医科研	膵癌，食道癌 大腸癌 他の固形腫瘍	CAPTIVATION Network に所属する全国の施設	網羅的遺伝子解析 抗腫瘍血管新生因子も標的
WT-1	大阪大学	血液悪性疾患 他の固形腫瘍	大阪大学 国立がんセンター　他	樹状細胞療法にも応用
テーラーメイド型	久留米大学	泌尿器科癌 他の固形腫瘍	久留米大学　他	テーラーメイド型ワクチン

表1
本邦で行われている主なペプチドワクチン療法

して皮下に投与する（1～2cc）．アジュバントとは免疫増強剤で，人体が異物と認識して強い免疫応答を起こすもので，不完全フロイントアジュバント（incomplete freund's adjuvant：IFA）などが用いられている．皮下投与する場所は前腕以外に，頸部や腋下，鼠蹊部といったリンパ節近くも（ペプチドを取り込んだDCがリンパ節に早く到達しCTLを誘導できるよう）用いられている．

3）ペプチド投与は1～2週間に1回行われる．数種類のペプチドを同時に投与する場合もあるが，その場合は皮下注射の場所を変え，所属リンパ節が違う部位とする．

4）既存の化学療法を併用することが多い．例えば，膵癌に対するペプチドワクチン療法は，ゲムシタビンとの併用療法が多い．

5）ワクチン療法前後で末梢血を採取し，ペプチド特異的CTLが誘導されているかをELISPOT assayやtetramer法によって解析する．

IV 本邦で臨床研究が行われている主なペプチドワクチン療法（表1）

1 Survivin

Survivinはアポトーシス抑制タンパク質（inhibitor of apoptosis：IAP）ファミリーに属し，さまざまな癌細胞に強発現している．札幌医科大学の佐藤らにより合成されたSurvivinペプチド（Survivin 2B 80-88）を用いた臨床試験が，同大学病院を中心に，大腸癌や膵癌，泌尿器科癌などを対象として行われている[4),5)]．またこのグループでは，先述したIFA単独・併用療法だけでなくサイトカイン併用療法も試みられており，新しいアジュバントの開発にも取り組んでいる．

2 （いわゆる）中村ワクチン

東京大学医科学研究所の中村らのグループが中心となって，全国多施設（CAPTIVATION Network）で行っている方法で，腫瘍に特異的な遺伝子発現を網羅的に探索することにより，同定したペプチドワクチンを使用している．

ペプチドは，癌種ごとに腫瘍細胞と正常細胞をlaser microbeam microdissection技術を用いて注意深く切り分け，マイクロアレイを用いた腫瘍特異的な遺伝子発現解析を行うことにより，遺伝子32,000個のなかから，癌で高頻度に発現し，正常臓器ではほとんど発現していない遺伝子を抽出し，その遺伝子由来のタンパク質のアミノ酸配列から，CTLを誘導できるようなHLA拘束性エピトープペプチドを同定することにより作製したものを使用している．また，vascular endothelial growth factor receptor（VEGFR）に対する抗腫瘍血管新生因子のペプチドワクチンも使用している．

現在，膵癌や大腸癌，食道癌などさまざまな固形癌で臨床試験が行われている[2),6)]．

3 WT-1

WT-1は，Wilms Tumor 1遺伝子からつくられるタンパク質で，白血病，肺癌，乳癌，脳腫瘍，胃癌，大腸癌，卵巣癌，胆道癌，膵癌などの広範な腫瘍に発現している．杉山ら大阪大学のグループを中心にWT-1ペプチドワクチンが開発され，その臨床試験が，全国でさまざまな固形癌に対して行われている．また，WT-1ペプチドは*in vitro*で自己のDCにパルス後，患者に投与するいわゆるDCワクチン療法に

・今まで抗癌剤で行われていた臨床試験のデザインとは異なったデザインで臨床試験を行う必要がある

・臨床効果の遅延反応が起こることが多い

・残存病変のない患者や微小な癌をもつ症例にワクチンを投与することにより，免疫活性化のための十分な時間が確保できる

・ほとんどの臨床試験で最大耐性量は認められず，最高用量は毒性ではなく製造上または投与部位の解剖学的な問題により規定される

・ワクチンの遅延効果のため，生存曲線は試験初期では効果を示さない．治療に効果がある場合は，試験後期に生存曲線の乖離が起こる

表2
FDAが公表した治療用癌ワクチンについての考察の要旨

〔参考文献2）より引用，一部改変〕

も使われている[7),8)]．

4　テーラーメイド型

久留米大学の伊東らのグループが行っている方法で，ペプチドワクチン候補31種類のうち，それぞれの患者の血漿中ペプチド特異的IgG抗体の存在が確認されるペプチドに限定し，かつ抗体値上位4種類を投与する方法である．このグループは，HLA-A11，A26，A31，A33陽性患者に適応可能なペプチドワクチンを開発し，汎HLA型に対応したワクチン療法を行っている[9),10)]．

V　ペプチドワクチン療法の問題点

現在行われているペプチドワクチン療法にはいくつかの問題点がある．

第一には，癌細胞上のHLAクラスⅠの30～60%は発現退避を起こしているということである．これらの発現退避を起こしている癌細胞は，ペプチドワクチンを投与してもCTLの標的とはならないので，ペプチドワクチン療法は全く無効となる．この弱点を補う方法としては，化学療法併用や，抗腫瘍血管新生因子のペプチドワクチン併用が考えられる．これらはすでに臨床試験が行われている．

第二には，現在いかなる癌種にも，奏効率に差はあるものの，科学的に有効性が認められている内科的治療法（化学療法や放射線療法）が存在するため，これらよりも有効性が確認されていない（ペプチドワクチン療法のような）治療法の臨床試験（特に第Ⅰ・Ⅱ相試験）は，倫理上既存の治療法の無効例が対象となるということである．しかし，このような症例は，化学療法や放射線の影響により，ペプチドワクチンに反応する免疫系の機能低下を起こしていると考えられ，しかも標的となる癌細胞の数が多いため臨床上の効果が出にくい可能性が高い．また，癌の進行や前治療の副作用により，低栄養や感染症，臓器機能の低下などを合併していることも多く，その結果，治療効果がみられにくいだけでなく，臨床試験中に有害事象が起こる可能性も高くなる．したがって，ペプチドワクチン療法の有効性を証明するためには，切除不能癌に対する第一選択治療や，治癒切除後補助療法といった，免疫機能が維持されていてかつ標的となる癌細胞が少ない症例を対象とした治療に対する臨床試験を行うことが必要となる．しかしこれには，既存の（有効性がすでに証明されている）化学療法や放射線療法との比較試験を行う必要があり，現在の評価法では，有効性を証明することが難しい．また倫理的観点からも問題がある．

第三には化学療法や放射線療法でみられるような腫瘍縮小効果とは違った治療効果（例えばCT画像上で大きさが変わらずSDと判定されていた腫瘍が数カ月後に急速に腫瘍壊死を起こすといった反応）が起こることがあり，現在こういった内科的治療の効果判定に汎用されているResponse Evaluation Criteria In Solid Tumors（RECIST）では，適正に評価できない可能性がある．そのためには，新しい評価法が必要と考えられる．

VI おわりに

このように，ペプチドワクチン療法が癌の標準治療となるためには，まだ克服しなければならない問題は多い．しかしそのようななか，先述した本邦の4つのグループは創意工夫をしながら，臨床研究や臨床試験を進め，その成果を出しているのは特筆に値する．

また，2009年9月に癌ワクチン療法の今後の臨床研究を進めていくうえでの留意点を示した「治療用癌ワクチンについての考察」(**表2**)[2]が，米国食品医薬品局（FDA）から公表された[11]．この考察は，先述したペプチドワクチン療法の問題点に対し言及しており，この治療法に対する米国の期待の高さがうかがえる．ペプチドワクチン療法は，欧米でも今後開発競争が進み，免疫療法の中心となっていくと思われる．

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JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2011, p. 4394–4396 Vol. 49, No. 12
0095-1137/11/$12.00 doi:10.1128/JCM.01240-11
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Case of Secondary Syphilis Presenting with Unusual Complications: Syphilitic Proctitis, Gastritis, and Hepatitis[▽]

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Received 22 June 2011/Returned for modification 22 August 2011/Accepted 28 September 2011

We report the first known case of syphilis with simultaneous manifestations of proctitis, gastritis, and hepatitis. The diagnosis of syphilitic proctitis and gastritis was established by the demonstration of spirochetes with anti-*Treponema pallidum* antibody staining in biopsy specimens. Unusual manifestations of secondary syphilis completely resolved after 4 weeks of antibiotic therapy.

CASE REPORT

A 28-year-old homosexual Japanese man was referred to our hospital for assessment of an abnormal liver function test and circumferential thickening of the rectal wall. The patient presented with a 1-month history of diarrhea, painful defecation, and occasional hematochezia and a 1-week history of mild upper abdominal discomfort. He had been diagnosed with secondary syphilis in a local clinic a week prior to admission based on the serological test for syphilis (STS), the *Treponema pallidum* hemagglutination test (TPHA), and the classical skin lesion. The human immunodeficiency virus type 1 (HIV-1) screening test was negative. Initial laboratory findings were as follows: white blood cell (WBC) count, 7,300 cells/μl (normal range, 3,900 to 9,800 cells/μl); red blood cell (RBC) count, 473×10^4 cells/μl (range, 410×10^4 to 530×10^4 cells/μl); hemoglobin (Hb) level, 13.8 g/dl (range, 13.5 to 17.6 g/dl); platelet count, 29.5×10^4 platelets/dl (range, 12×10^4 to 36×10^4 platelets/dl); total serum protein (TP), 7.6 g/dl (range, 6.5 to 8.0 g/dl); aminotransferase (AST) level, 62 IU/liter (range, 8 to 38 IU/liter); alanine aminotransferase (ALT) level, 74 IU/liter (range, 4 to 44 IU/liter); lactate dehydrogenase (LDH) level, 175 mg/dl (range, 115 to 224 mg/dl); alkaline phosphatase (ALP) level, 486 IU/liter (range, 104 to 338 IU/liter); γ-glutamyl transpeptidase (γ-GTP) level, 62 IU/liter (range, 16 to 73 IU/liter); fasting blood glucose (FBG) level, 84 mg/dl (range, 70 to 107 mg/dl); serological test for syphilis (STS) (latex agglutination assay) results, 155.94 Sysmex units (SU)/ml; *Treponema pallidum* hemagglutination test (TPHA) results, 1,814.50 SU/ml.

On admission, the patient's body temperature was 37.8°C. The general physical examination was basically normal except for bilateral inguinal lymphadenopathy without pain and small, nonconfluent, erythematous, macular lesions on the trunk, back, arms, and face. The patient admitted to recent, unprotected, receptive anal intercourse. There were no detectable anal lesions, but rectal examination showed circumferential thickening of the rectal wall. The colonoscopy showed an indurated nodular mucosa around the rectal lumen, which initially suggested a submucosal tumor. A barium enema showed similar findings (Fig. 1A and B). Histologic findings of the rectal mucosa revealed severe inflammatory cell infiltration, predominantly by plasma cells. No malignant cells were identified. Immunostaining of rectal biopsy specimens with anti-*Treponema pallidum* polyclonal antibodies identified numerous spirochetes, and the diagnosis of syphilitic proctitis was confirmed.

The patient had been complaining of upper abdomen discomfort. His antecedent medical records, including stomach disease, were not remarkable. The gastroduodenoscopy showed multiple erosive lesions in the whole gastric mucosa, and numerous spirochetes were identified by immunostaining the biopsy specimens (Fig. 2A and B). Histologic examination of the mucosa showed mild infiltration of neutrophils with superficial necrosis and fibropurulent exudates. There was no evidence of carcinoma, lymphoma, or *Helicobacter pylori* infection.

A liver function test at admission showed elevated AST (480 IU/liter), ALT (607 IU/liter), ALP (2,493 IU/liter), LDH (420 IU/liter), γ-GTP (774 IU/liter), and total bilirubin (TB) (1.1 mg/dl) levels. Acute viral hepatitis was initially suspected, but the following serologic markers of acute viral infection were all negative: IgM anti-hepatitis A virus (IgM-HAV), the hepatitis B virus (HBV) surface antigen (HBsAg), the IgM anti-HBV core antigen (IgM-HBc), hepatitis C virus antibodies (HCV-Ab), IgM anti-hepatitis E virus (IgM-HEV), IgM anticytomegalovirus (IgM-CMV), and the IgM anti-viral capsid antigen of the Epstein-Barr virus (IgM-VCA EBV). On the other hand, serological markers for IgG-CMV, the IgG-VCA EBV, and the EBV nuclear antigen (EBNA) were all positive, sug-

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▽ Published ahead of print on 12 October 2011.

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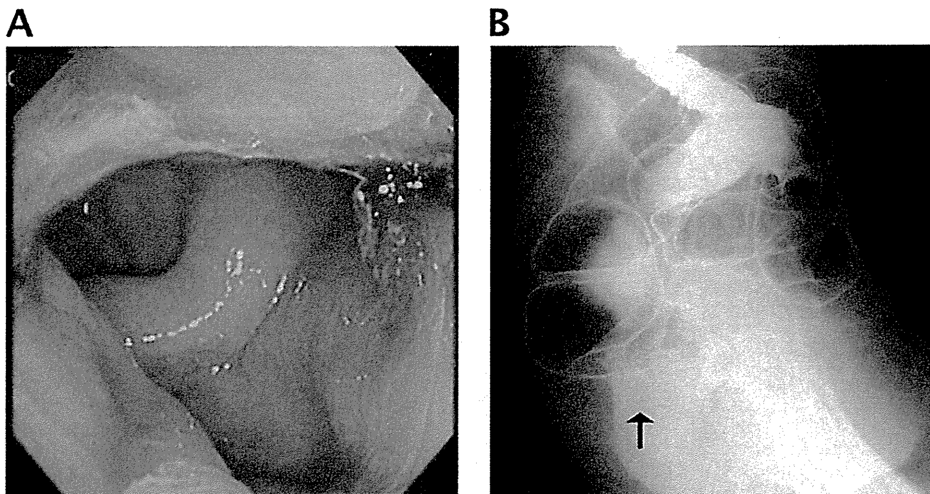

FIG. 1. (A) Sigmoidoscopy findings indicating multiple chancres and indurated nodular mucosa located on the wall of the lower rectum. (B) Barium enema results showing circumferential thickening of the lower rectal wall and multiple nodular mucosa.

gesting that the patient was previously infected with CMV and EBV. HBV DNA, HCV RNA, and HIV RNA were not detected. The results for both the antimitochondrial M2 antibody and anti-smooth muscle antibodies were negative. The immunoglobulin levels, including the IgE level, were all normal. The antinuclear antibody (ANA) was positive at a titer of 1:40 (speckled pattern). However, autoimmune hepatitis was ruled out by other laboratory data and did not fulfill the criteria proposed by the international autoimmune hepatitis group (9). Abdominal ultrasonography did not reveal any evidence of chronic liver diseases. There was no history of alcohol abuse, intravenous drug abuse, oral illicit drug use, or smoking. The levels of ALP, γ-GTP, and TP were progressively elevated to 5,358 IU/liter, 1,103 IU/liter, and 1.9 mg/dl, respectively, 4 days after admission. A liver biopsy was not done because the patient's consent was not obtained. Although the etiology of the liver enzyme abnormalities remained unclear, alternative causes of hepatic damage were excluded and syphilitic hepatitis was strongly suspected. There were no cerebrospinal fluid (CSF) abnormalities, including the results of the STS and TPHA. Oral administration with 2.25 g/day amoxicillin hydrate (AMOX) was initiated according to the guidelines proposed by The Japanese Society for Sexually Transmitted Diseases. A Jarisch-Herxheimer reaction, consisting of fever and skin rash deterioration, developed 6 h after the first oral administration of AMOX but resolved spontaneously in 24 h. The liver function tests improved gradually and became normal after 4 weeks of AMOX administration. The patient's fever resolved promptly, and his skin lesions disappeared in a few days. Circumferential thickening of the rectal wall and upper abdominal discomfort were completely resolved; in addition, the STS results decreased to 5.28 SU/ml 2 months after the initiation of AMOX.

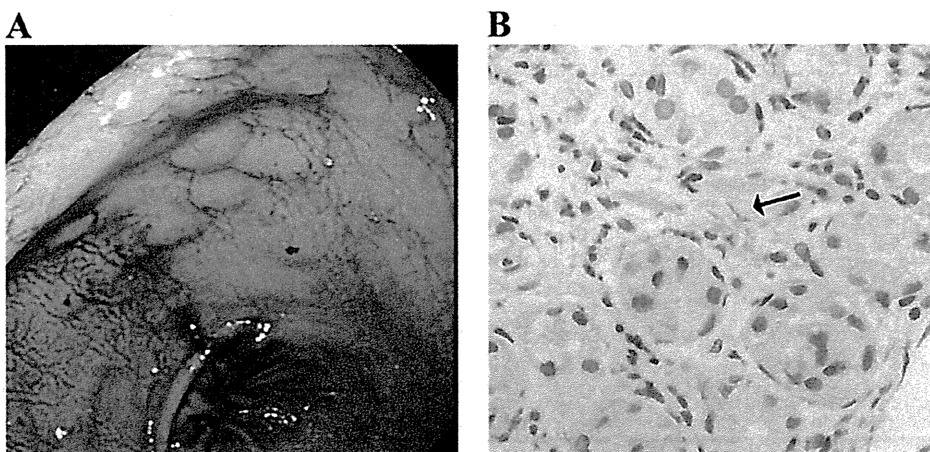

FIG. 2. (A) Gastroscopic findings demonstrating multiple erosive lesions in the whole gastric mucosa (indigo carmine dye contrast). (B) A gastric biopsy specimen with antibody staining showing numerous brown stained spirochetes (arrow) in the interstitium (*Treponema pallidum* polyclonal antibody stain). Magnification, ×400.

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T. pallidum, the etiologic agent of syphilis, is known to affect a wide variety of organs. Involvements of the skin, genital organs, retinas, and central nervous system are well described, but occasionally, unusual manifestations, such as gastrointestinal lesions and liver and renal dysfunction, can occur. We highlight *T. pallidum* here as an important but often unrecognized agent that could involve the rectum, stomach, and liver simultaneously. In a search of the MEDLINE database since 1960, this is the first case report which manifested three unusual complications, proctitis, gastritis, and hepatitis, in secondary syphilis. Syphilitic proctitis is rarely reported but is being recognized more frequently due to the recently increased incidence of syphilis among men who have sex with men (MSM). A retrospective review of clinical proctitis in MSM showed that syphilis was found in 2% of clinical patients presenting with rectal symptoms (6). Anorectal primary syphilis is easily overlooked because of the absence of anal lesions in some cases. In addition, it is difficult to diagnose because clinical manifestations of syphilitic proctitis have been shown to mimic amoebiasis, Crohn's disease, malignant lymphoma, or carcinoma (4).

Syphilitic gastritis is found in less than 1% of patients with syphilis and is seldom reported (4). The gastroduodenoscopy features described in previous case reports are multiple erosive or ulcerative lesions in the whole gastric mucosa. Endoscopic and microscopic findings can mimic gastric cancer or lymphoma (7). To make a definitive diagnosis of syphilitic gastritis and proctitis, it is necessary to identify *T. pallidum* histologically. Immunohistochemistry staining with anti-*T. pallidum* polyclonal antibodies can be used to detect *T. pallidum* in tissue, as it was in the present case. Recently, better sensitivity and a more rapid diagnosis of gastric syphilis were achieved by using real-time PCR (3).

Hepatitis, as well as proctitis and gastritis, is a rare complication of syphilis. Liver dysfunction occurring in early syphilis presents a diagnostic challenge. Clinical manifestations of syphilitic hepatitis described in previous reports showed that levels of alkaline phosphatase were disproportionately elevated relative to those of bilirubin and transaminases (2). These features are consistent with those of our case. Mullick et al. proposed the following criteria for the diagnosis of acute syphilitic hepatitis (10): abnormal liver enzyme levels indicating hepatic involvement, serological evidence for syphilis with a positive TPHA titer in conjunction with an acute clinical presentation consistent with secondary syphilis, exclusion of alternative causes of hepatic injury, and improvements in liver enzyme levels with an appropriate antimicrobial therapy. Our case met all of these criteria, and we therefore attributed this patient's liver dysfunction to the involvement of *T. pallidum* even though a liver biopsy was not performed. A liver biopsy specimen is likely to have a lower yield for detection of *T. pallidum* than a biopsy specimen of gastrointestinal tissue. The majority of reported cases since 1975 failed to reveal treponemes in liver biopsy specimens (1, 2, 8, 11). Although the precise mechanism involving three different organs in this case remains unclear, we believe that the relationship between proctitis and hepatitis is related to the venous drainage pathway from the rectal area into the portal system. This postulation is supported by the fact that syphilitic hepatitis occurs often in conjunction with syphilitic proctitis (5) and is seen frequently in persons who engage in anal intercourse (2).

Syphilitic involvement of the stomach, rectum, and liver is easily overlooked and has not been described in the modern literature to our knowledge. These are all reversible conditions, and appropriate antibiotic treatment results in rapid resolution. Therefore, the diagnosis of these complications is important to prevent progression to a severe condition and to avoid unnecessary investigations. Clinicians should bear in mind the possibility of syphilitic involvement in patients at risk for sexually transmitted diseases who present with either gastrointestinal discomfort or liver dysfunction.

We thank Kei Ouchi for his comments on drafts of the manuscript.

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International Scholarly Research Network
ISRN Gastroenterology
Volume 2011, Article ID 509251, 4 pages
doi:10.5402/2011/509251

Clinical Study

Is Surveillance Endoscopy Necessary after Colectomy in Ulcerative Colitis?

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Received 25 March 2011; Accepted 15 May 2011

Academic Editor: G. H. Kang

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The role of surveillance endoscopic followup in colectomized patients with long standing total colitis is controversial. Here, we aimed to clarify its usefulness for the early detection of dysplasia and cancer in this group of patients. Ninety-seven colectomised UC patients followedup by surveillance endoscopy were retrospectively investigated by reviewing the pathological reports. Patients had received either subtotal colectomy and ileo-rectal anastomosis (IRA) or total proctocolectomy and ileal anal anastomosis (IPAA). Definite dysplasia was diagnosed in 4 patients, who had received IRA; among them, 2 were carcinoma with submucosal invasion, and one was a high-grade dysplasia. Postoperative surveillance endoscopy is useful for the detection of early cancer in the remaining colonic mucosa of UC patients, and those receiving IRA, in which rectal mucosa is left intact, would be good candidates. However, its effectiveness for patients receiving IPAA, in which the rectal mucosa is resected, needs further investigation.

1. Introduction

Long-standing extensive ulcerative colitis (UC) is reported to be a risk factor for the development of colorectal cancer (CRC) [1–3]. Surveillance colonoscopy instead of prophylactic proctocolectomy is generally recommended for those with total colitis for more than 8 years after the onset or left-sided colitis for more than 15 years [4, 5].

Subtotal colectomy with ileo-rectal anastomosis (IRA) had been the surgical treatment of choice for UC until pouch operation was established, but patients undergoing subtotal colectomy have also been reported to carry a certain risk of developing carcinoma in the rectal remnant [6, 7]. Furthermore, Johnson et al. reported that most of them were found in an advanced stage [7]. Although the importance of surveillance colonoscopy for the rectal remnant has been emphasized, few reports describe the effectiveness of surveillance colonoscopy in the colectomized population.

Since the 1980s, total proctocolectomy and ileal pouch-anal anastomosis (IPAA) has become the surgical treatment of choice for UC [8, 9]. Although total proctocolectomy eliminates the risk of colorectal cancer, several cases with cancer in the rectal remnant or ileal pouch have been reported. The major methods of IPAA are stapled IPAA without mucosectomy and handsewn IPAA with mucosectomy. Stapled IPAA is a safer and less complicated method than handsewn IPAA, but the rectal remnant of a few centimeters may retain a malignant potential. Mucosectomy could theoretically remove all the rectal mucosa that might have malignant potential. However, the resected specimens of the patients undergoing pouch excision following mucosectomy revealed that isolated rectal mucosa might remain [10, 11]. Indeed, several cases of "rectal" carcinoma after mucosectomy have been reported [12–14]. Moreover, several cases of dysplasia or cancer in the ileal pouch have been reported after IPAA [15–17].

Although those cases of UC who have undergone colectomy may be at risk of carcinoma in the rectal remnant or the ileal pouch, the effectiveness of surveillance endoscopy after colectomy is still controversial. The aim of this study is to clarify the effectiveness of surveillance endoscopy after colectomy in UC.

2. Methods

2.1. Patients. Ninety-seven UC patients who received colectomy and postoperative surveillance endoscopy in our surgical department, in the period between January 1979 and December 2008, were retrospectively analyzed. Among them, 29 had received IRA, and 68 IPAA (stapled without mucosectomy in 47 and hand-sewn with mucosectomy in 21).

2.2. Endoscopy. Surveillance endoscopy, using flexible endoscopes, was conducted regularly in most of the cases, and in addition to the conventional observation by the experienced colonoscopist, the dye spray method was performed for the better visualization of mucosal lesions. Only those patients who had at least one postoperative biopsy were included. Biopsy specimens were taken from the lesions suggestive of dysplasia as well as from the apparently normal flat rectal mucosa of patients who had received IRA or stapled IPAA (either with or without mucosectomy). Pathological reports from all patients were retrospectively reviewed for the patients' clinicopathological features, the surgical procedures, and the colonoscopic and histological diagnosis.

2.3. Pathology. Dysplasia was graded, according to the Riddell' classification, into high-grade (HGD), low-grade (LGD), indefinite (IND), or negative for dysplasia [18].

2.4. Evaluation. Histopathological reports were retrospectively reviewed, and the patient's clinicopathological features, such as age, duration after onset of UC, time after colectomy, and histopathological diagnosis of the resected surgical specimen, were analyzed according to the presence or absence of dysplasia.

3. Results

A total of 531 surveillance endoscopies were performed. The median followup time after operation was 5.3 and 15.6 years for those who had received IPAA and IRA, respectively. Results of postoperative surveillance endoscopy are summarized in Figure 1.

By the surveillance endoscopy, 4 patients who had received IRA were diagnosed as definite dysplasia (Table 1, Figure 2). Among them, 2 had received hand-sewn IPAA with mucosectomy, and one rectal excision. One patient was not operated on due to the presence of various extracolonic complications, but the subsequent surveillance endoscopy revealed no abnormalities. Histopathological examination of the resected specimen revealed adenocarcinoma invading the submucosa in 2 of them, and in 1, HGD was diagnosed.

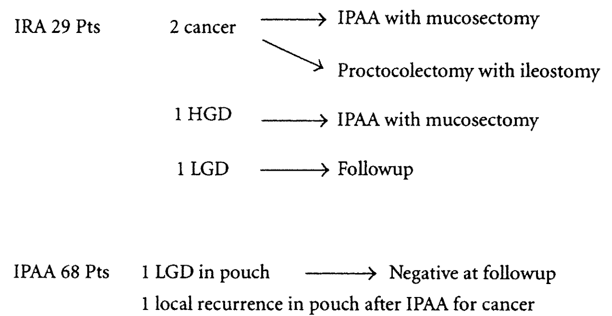

Figure 1: Results of postoperative surveillance endoscopy after colectomy in patients with ulcerative colitis.

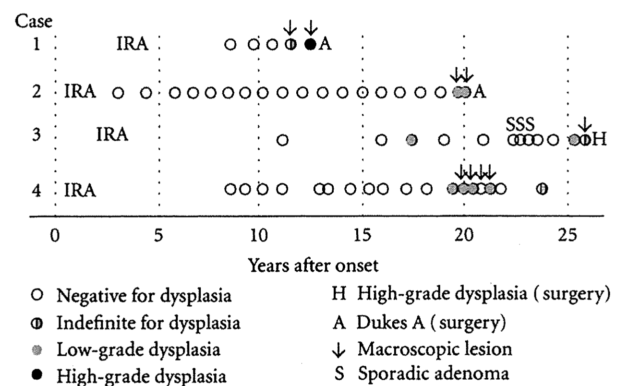

Figure 2: Results of surveillance colonoscopy in patients with definite dysplasia after ileo-rectal anastomosis.

One patient, who had received hand-sewn IPAA, had an ulcerative lesion in the staple line of the pouch, and was diagnosed as LGD. However, the subsequent surveillance revealed no abnormalities. No other patients receiving IPAA had definite dysplasia or cancer detected during the postoperative surveillance.

One patient, who had received IPAA with mucosectomy due to colitic cancer, was diagnosed as HGD during the pouch surveillance conducted 2 years after the operation. The ileal pouch was surgically resected, and the permanent ileostomy was left. The histopathology revealed transmurally invasive signet-ring cells, which was compatible with recurrence of the colitic cancer. No other dysplastic or neoplastic lesions were found during the pouch surveillance.

4. Discussion

It is well recognized that patients receiving subtotal colectomy remain at a risk of developing carcinoma in the remnant rectum; most of the carcinomas of the remnant rectum are diagnosed in an advanced stage. The development of rectal cancer is reported to be associated with the duration of ulcerative colitis and with poor surveillance, and most patients who developed rectal cancer presented with an advanced tumour stage (III and IV) [19]. In our series, by the performance of a meticulous surveillance colonoscopy,

Table 1: Cases of definite dysplasia or cancer after ileo-rectal anastomosis.

Case	Age at onset	Age at IRA	IRA duration	Grade of dysplasia at endoscopy	Postoperative diagnosis	Survival
1	29	33	9	HGD	sm	Alive
2	57	58	19	LGD	sm	Alive
3	22	24	23	LGD	m(HGD)	Alive
4	56	58	20	LGD	—	Alive

HGD: high-grade dysplasia: IRA, ileo-rectal anastomosis: LGD: low-grade dysplasia;M: mucosa; sm: submucosa.

definite dysplasia and cancer were found in four (14%) out of 29 patients who had received IRA. Analysis of the resected specimens revelead carcinoma with submucosal invasion in two of them, and HGD in one. Therefore, surveillance colonoscopy was considered effective for the detection of dysplasia or cancer at an earlier stage.

Stapled IPAA is a safer method, with lower incidence of complications than hand-sewn IPAA, but the rectal remnant of few centimeters will theoretically retain the risk of malignant transformation [15]. In a study conducted by the British group, involving 135 patients who had received IPAA with double-stapling technique (DST), and were followed by surveillance colonoscopy for a median period of 56 months [20], no cases of dysplasia or carcinoma were found; thus, it was concluded that cuff surveillance in the first decade after IPAA with DST is not necessary. On the other hand, a study from the Cleveland Clinic diagnosed 2 LGD and 4 HGD among the 178 patients who had received stapled IPAA [21]. They concluded that patients with dysplasia of the colon or the upper third of the rectum can be efficiently managed with stapled IPAA, provided that a postoperative surveillance program is adequately indicated. In our series, no cases of dysplasia of the remnant rectum were found among the patients who had received stapled IPAA. Therefore, taking together our results and those previously reported, we can speculate that the risk of carcinoma development in the remnant rectum is relatively low. On the other hand, although not common, carcinoma or dysplastic lesion may occur in those patients who had received stapled IPAA, since remnant rectum is left similar to those who receive IRA. Although the risk of carcinoma of the remnant rectum is relatively low, we believe that surveillance colonoscopy should be performed in those patients who had received stapled IPAA. The early detection of carcinoma or dysplastic lesion by the surveillance program will allow the indication of anal sphincter saving procedures, such as the pouch advancement method.

Mucosectomy is a technique to remove all the rectal mucosa with malignant potential. In our series, no cases of dysplasia of the rectal remnant were found among those patients who had received hand-sewn IPAA. However, the histological analysis of the resected specimens of patients who had received pouch excision following mucosectomy revealed the presence of remaining isolated rectal mucosa [10, 11]. Moreover, several cases of "rectal" carcinoma after IPAA with mucosectomy have been reported [12–14]. Therefore, mucosectomy is not enough to completely remove the rectal mucosa, in order to eliminate the risk of cancer.

Recently, the risk of carcinoma of the ileal pouch is a major concern among colorectal surgeons. In our series, one patient who had received stapled IPAA was diagnosed as LGD of the staple line ulcer of the pouch, but the subsequent surveillance endoscopic followup of this case revealed no remaining dysplastic lesions. Gullberg et al. found 5 cases of dysplasia in the ileal pouch by the surveillance endoscopy, and all these cases had persistent severely atrophic mucosa [13]. Several reports have shown the detection of dysplasia or cancer in the ileal pouch or in the rectal remnant after restorative proctocolectomy [21, 22]. However, several authors consider that the risk of carcinoma development in the ileal pouch is not high. Hulten et al. conducted a 30-year followup study of the Kock pouch, and concluded that it was very unlikely for invasive carcinoma to develop in the ileal pouch [23]. Thompson-Fawcett et al. examined a retrospective cohort consisting of 1221 patients with ileal pouches, and found only one patient with LGD, who had never had pouchitis [24]. Herline et al. found only one LGD out of 222 biopsies from the ileal pouch [25]. Although the risk of carcinoma development in the ileal pouch is controversial, it is of note that most of the patients with carcinoma of the ileal pouch were diagnosed at an advanced stage. We consider that the appropriate surveillance endoscopy should be indicated for those patients receiving IPAA, until definitive conclusions can be drawn.

In summary, postoperative surveillance endoscopy for UC is useful to detect cancer at an early stage, which will allow the inidication of curative restorative proctocolectomy. Patients receiving IPAA are those who mostly should receive surveillance endoscopy. Although the total risk of cancer development in patients who had undergone IPAA seems to be relatively low, surveillance should be indicated until further investigations deny its effectiveness.

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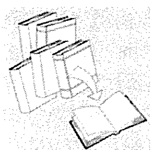
Rev. Med. Virol. 2011; 21: 67–77.
Published online 10 February 2011 in Wiley Online Library
(wileyonlinelibrary.com)
Reviews in Medical Virology
DOI: 10.1002/rmv.680

REVIEW



Antiviral responses induced by the TLR3 pathway

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SUMMARY

Antiviral responses are successively induced in virus-infected animals, and include primary innate immune responses such as type I interferon (IFN) and cytokine production, secondary natural killer (NK) cell responses, and final cytotoxic T lymphocyte (CTL) responses and antibody production. The endosomal Toll-like receptors (TLRs) and cytoplasmic RIG-I-like receptors (RLRs), which recognize viral nucleic acids, are responsible for virus-induced type I IFN production. RLRs are expressed in most tissues and cells and are primarily implicated in innate immune responses against various viruses through type I IFN production, whereas nucleic acid-sensing TLRs, TLRs 3, 7, 8 and 9, are expressed on the endosomal membrane of dendritic cells (DCs) and play distinct roles in antiviral immunity. TLR3 recognizes viral double-stranded RNA taken up into the endosome and serves to protect the host against viral infection by the induction of a range of responses including type I IFN production and DC-mediated activation of NK cells and CTLs, although the deteriorative role of TLR3 has also been reported in some virus infections. Here, we review the current knowledge on the role of TLR3 during viral infection, and the current understanding of the TLR3-signalling cascade that operates via the adaptor protein TICAM-1 (also called TRIF). Copyright # 2011 John Wiley & Sons, Ltd.

Received: 16 October 2010; Revised: 21 December 2010; Accepted: 21 December 2010

INTRODUCTION

Mammalian cells possess several defense strategies against viral infection, of which, the type I interferon (IFN) system is most important for innate and adaptive antiviral responses [1,2]. Type I IFN induces an antiviral state in uninfected host cells by upregulating IFN-stimulated genes (ISGs) through IFN-a/b receptor signalling, and also activates innate and adaptive immune cells, such as dendritic cells (DCs), natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) [3]. Intrinsic double-stranded RNA (dsRNA) sensors, dsRNA-binding protein kinase R and 2(–5(oligoadenylate synthetase, are both ISGs, which trigger the shutdown of protein translation and induce RNA degradation within virus-infected cells, respectively [4,5]. Recent progressive studies have demonstrated that the endosomal Toll-like receptors (TLRs) and cytoplasmic retinoic acid inducible gene-I (RIG-I)-like receptors (RLRs) are responsible for virus-induced type I IFN production [6–8]. These receptors recognize viral nucleic acids and induce type I IFN, inflammatory cytokine and chemokine production and DC maturation. TLR3 recognizes virus-derived dsRNA and its synthetic analogue, polyriboinosinic:polyribocytidylic acid (poly (I:C)) [9–11]. dsRNA is found in some virus particles as a viral genome and can be generated

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Abbreviations:
CT, C-terminal; CTL, cytotoxic T lymphocytes; CVB3, coxsackievirus group B serotype 3; dsRNA, double-stranded RNA; DC, dendritic cell; DUBA, deubiquitinating enzyme A; ECD, ectodomain; EMCV, encephalomyocarditis virus; HCV, hepatitis C virus; HSV-1, herpes simplex virus-1; IAV, influenza A virus; IFN, interferon; INAM, IRF-3-dependent NK-activating molecule; ISG, IFN-stimulated gene; LRR, leucine-rich repeat; MCMV, murine cytomegarovirus; MDA5, melanoma differentiation associated gene 5; NAK, NF-kB activating kinase; NAP1, NAK-associated protein 1; NK, natural killer; NT, N-terminal; NTD, N-terminal domain of TICAM-1; pDC, plasmacytoid DC; poly(I:C), polyriboinosinic:polyribocytidylic acid; PVR, poliovirus receptor; RIG-I, retinoic acid inducible gene-I; RIP1, receptor-interacting protein 1; ss, single-stranded; TBK1, TANK-binding kinase 1; TICAM-1, TIR-containing adaptor molecule-1; TIR, Toll-IL-1 receptor; TLR, Toll-like receptor; RLR, RIG-I-like receptor; WNV, West Nile virus.

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during the process of positive-stranded RNA virus and DNA virus replication [12]. TLR7 and TLR8 recognize virus-derived single-stranded (ss) RNA, while TLR9 recognizes non-methylated CpG-containing DNA that is found in some microbes [13–15]. Since these TLRs localize to the endosomal membranes of myeloid or plasmacytoid DCs (pDCs), they appear to detect extracellular viral nucleic acids released from infected cells or virus particles. However, the mechanism by which TLRs encounter virus-derived nucleic acids in endosomes remains to be determined. Interestingly, a recent report showed that TLR7-mediated IFN-a secretion by pDCs in response to ssRNA virus infection requires the transport of cytosolic viral RNA into the lysosome via the process of autophagy [16]. Whether this autophagy-dependent viral recognition is applicable to TLRs 3, 8 and 9 remains unclear.

By contrast, RLRs are expressed in most tissues and cells and detect viral nucleic acids in the cytoplasm. RIG-I recognizes viral RNA genomes bearing 5(-triphosphates and panhandle structures and also short-length dsRNAs [17–21], while melanoma differentiation-associated gene 5 (MDA5) detects long-length dsRNAs and poly(I:C) [22]. Studies using gene-disrupted mice and cells revealed that RIG-I is essential for the detection of various negative-stranded RNA viruses including influenza A virus (IAV), Sendai virus and vesicular stomatitis virus and a positive-stranded RNA virus, hepatitis C virus (HCV), whereas MDA5 plays a key role in sensing encephalomyocarditis virus, a member of Picornavilridae family [23–26]. Thus, multiple innate immune pathways are implicated in dsRNA responses and each pathway plays a distinct role in antiviral responses. In this review, we focus on TLR3, whose antiviral function has been controversial, but recent studies have demonstrated the critical role of the TLR3–TICAM-1 pathway in antiviral responses and the induction of adaptive immunity.

Expression and subcellular localization of TLR3

Human TLR3 mRNA has been detected in various tissues including the placenta, pancreas, lung, liver, heart and brain [27]. Interestingly, in the human central nervous system, TLR3 is expressed constitutively in neurons, astrocytes and microglia, suggesting a role in the response to viruses causing encephalopathy [28–30]. In immune cells, only myeloid DCs and macrophages express TLR3. The pDCs, which express TLR7 and TLR9 and secrete large amounts of IFN-a in response to viral infection, do not express TLR3 [31–35]. TLR3 is also expressed in fibroblasts and a variety of epithelial cells, including airway, corneal, cervical, biliary and intestinal cells [10,36–38], which are target sites of virus infection. TLR3 localizes both on the cell surface and endosomes in fibroblasts, macrophages and some of epithelial cell lines. Cell surface-expressed TLR3 participates in dsRNA recognition, as shown by the finding that an anti-human TLR3 monoclonal antibody (mAb) (TLR3.7) inhibits poly(I:C)-induced IFN-b production by fibroblasts [10]. By contrast, myeloid DCs only express TLR3 intracellularly [35]. Subcellular localization analysis showed that endogenous human TLR3 localizes to the early endosome but not to late endosomes/lysosomes in HeLa cells [39], while transfected human TLR3 predominantly localizes to multivesicular bodies in the mouse B-cell line Ba/F3, in which TLR3 was stably expressed at high levels. In any case, TLR3 signalling arises in the endosomal compartment, requiring endosomal maturation [35]. The 'linker' region consisting of 26 a.a. between the transmembrane domain and the Toll-IL-1 receptor (TIR) domain of TLR3, determines intracellular localization of TLR3 [40,41]. An unidentified molecule associating with the linker region may regulate the endosomal retention of TLR3 in myeloid DCs.

Notably, TLR3 expression is upregulated by viral infection and the exogenous addition of poly(I:C) or type I IFN [42]. The IFN-responsive element is located at approximately 30 bp in the human TLR3 promoter region [43,44].

Recognition of dsRNA by TLR3

TLR3 recognizes dsRNA through its ectodomain (ECD), which induces receptor dimerization required for adaptor-mediated signal transduction [45]. TLR3 consists of an ECD formed by 23 leucine-rich repeats (LRRs) and N- and C-terminal flanking regions, known as the LRR N-terminal (LRR-NT) and C-terminal (LRR-CT) regions, the transmembrane domain and the cytoplasmic TIR domain [46] (Figure 1A). TLR3–ECD possesses 15 putative carbohydrate-binding motifs. Structural analyses

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DOI: 10.1002/rmv

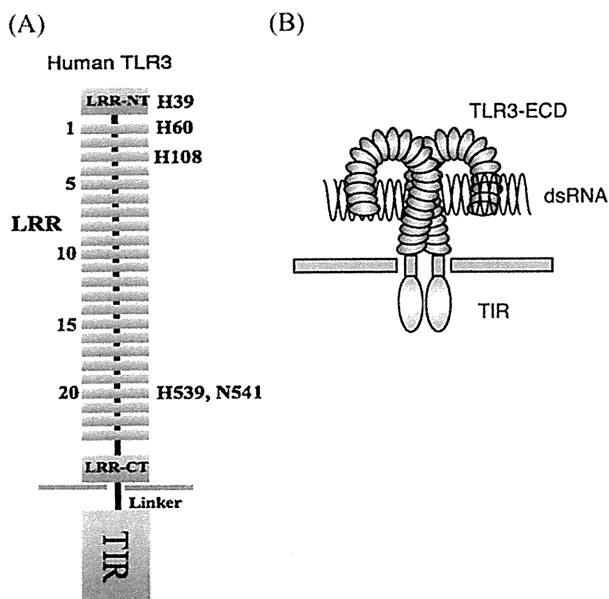

Figure 1. (A) Schematic structure of human TLR3. TLR3 is a type I transmembrane protein of 904 a.a. TLR3 consists of an ECD formed by 23 LRRs and N- and C-terminal flanking regions (LRR-NT and LRR-CT), the transmembrane domain, cytoplasmic linker region and the TIR domain. H539 and N541 in TLR3-LRR20, H39 in the LRR-NT, H60 in LRR1 and H108 in LRR3 are essential for dsRNA-binding. (B) Model of the dsRNA–TLR3-signalling complex. dsRNA interacts with both an N- and a C-terminal binding site on the glycan-free surface of each TLR3–ECD, which are located on opposite sides of the dsRNA (53).

of human TLR3–ECD revealed that the LRRs form a large horseshoe-shaped solenoid of which one face is largely masked by carbohydrate, while the other face is unglycosylated [47,48]. By point mutation analysis, Bell et al. [49] demonstrated that the His539 and Asn541 residues in TLR3–LRR20, located on the glycan-free lateral face, are critical amino acids for dsRNA binding and signalling. Wild-type TLR3–ECD protein directly binds poly(I:C) at pH7.6, while mutant proteins H539E and N541A fail to bind poly(I:C). Based on the observation that an acidic pH (pH 6.0 and below) is required for TLR3 recognition of dsRNA, the N-terminal conserved histidine residues, His39 in the LRR-NT, His60 in LRR1 and His108 in LRR3, were identified as a second binding site for dsRNA [50,51]. Protonation of these imidazole groups under acidic conditions, such as those found in endosomes, appears to generate an ionic interaction between the histidine residues and the negatively charged phosphate backbone of dsRNA.

In addition, Leonard et al. [52], showed that TLR3–ECD binds as a dimer to 40–50 bp length of dsRNA, and multiple TLR3–ECD dimers bind to long dsRNA strands. Binding affinities increase with both buffer acidity and dsRNA length. At the pH within early endosomes (6.0–6.5), >90-bp length of dsRNA is required to form a stable complex with TLR3. However, at the pH within late endosomes (5.5 and below), 40–50-bp length of dsRNA forms stable complex with dimeric TLR3, suggesting that dsRNA-induced TLR3-mediated signalling depends on the length of the dsRNA and the TLR3 localization site [52]. Finally, structural analysis of the complex of two mouse TLR3–ECDs and one 46-bp dsRNA oligonucleotide revealed that dsRNA interacts with both an N- and a C-terminal binding site on the glycan-free surface of each mTLR3–ECD, which are located on opposite sides of the dsRNA [53] (Figure 1B). The dsRNA in the complex retains a typical A-form DNA-like structure. dsRNA has been predicted to adopt a right-handed A-form helix with 11 bp per helical turn and a 28 Å helical pitch [54]. Therefore, two helical turns would fit between the N- and C-terminal binding sites of TLR3 [53]. In addition, the two LRR-CT domains are brought into proximity and form a series of protein–protein interactions, which facilitate the dimerization of the cytoplasmic TIR domain. Funami et al. [40], reported that the Phe732, Leu742 and Gly743 residues in the TLR3 cytoplasmic linker region are essential for TLR3 signalling, suggesting that the linker region controls the dimerization of the TLR3–TIR domain.

TLR3–TICAM-1-signalling pathway

TLR3 mediates signalling via an adaptor protein, TIR-containing adaptor molecule-1 (TICAM-1; also called TRIF) [55,56] (Figure 2A). TICAM-1 activates the transcription factors IRF-3, NF-kB and AP-1, leading to the induction of type I IFN, cytokine/chemokine production and DC maturation, which then enables the activation of NK cells and CTLs. TLR3 also associates with c-Src tyrosine kinase on endosomes in response to dsRNA [57]. The Src kinase inhibitor markedly inhibits dsRNA-elicited phosphorylation of Akt, a downstream target of phosphatidylinositol 3-kinase (PI3-K). In addition, PI3-K is required for full phosphorylation and activation of IRF-3 by dsRNA [58]. The precise role of c-Src in IRF-3 activation via the PI3-K-Akt pathway requires further elucidation.

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DOI: 10.1002/rmv

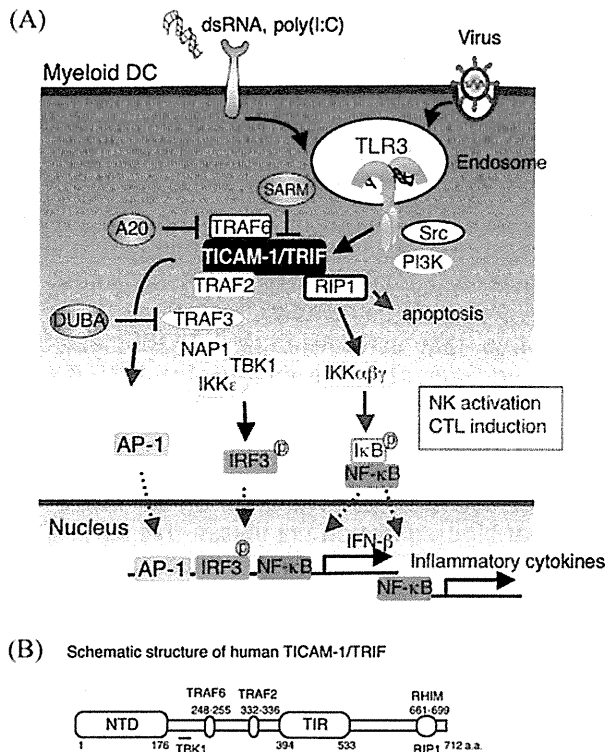

Figure 2. (A) TLR3–TICAM-1-signalling pathway. In myeloid DCs, TLR3 is expressed in the endosomal compartments and recognizes extracellular viral dsRNA and its synthetic analogue poly(I:C). Once TLR3 is dimerized by dsRNA, it recruits the adaptor protein TICAM-1/TRIF that activates the transcription factors, IRF3, NF-kB and AP-1. RIP1 associates with TICAM-1 via the PHIM domain in the C-terminal region and acts as an NF-kB activator and apoptosis mediator in TICAM-1-mediated signalling. TRAF3 and NAP1 participate in the recruitment and activation of the IRF-3 kinases TBK1 and IKKε. Phosphorylated IRF-3 translocates into the nucleus and together with NF-kB and AP-1 induces IFN-b gene transcription. The TICAM-1-mediated AP-1 activation pathway is unclear. (B) Schematic structure of human TICAM-1/TRIF. N-terminal domain (NTD) (1–176 a.a.), TIR domain (394–533 a.a.), RHIM domain (661–699 a.a.), TRAF6-binding site (248–256 a.a.), TRAF2-binding site (332–336 a.a.) and TBK1-binding site (under line) are shown.

TICAM-1 consists of an N-terminal region, a TIR domain and a C-terminal region (Figure 2B). The TIR domain of TICAM-1 is essential for binding to the TIR domain of TLR3 and also to the TLR4 adaptor TICAM-2 (also called TRIF-related adaptor molecule) [59,60]. TICAM-1 is expressed at a low level in most tissues and cells and is diffusely localized in the cytoplasm of resting cells [39]. When endosomal TLR3 is activated by dsRNA, TICAM-1 transiently co-localizes with TLR3, then dissociates from the receptor and forms speckled structures that co-localize with downstream-signalling molecules [39]. Homo-oligomerization through the Pro434 residue in the TIR domain and the C-terminal region is essential for TICAM-1-mediated activation of NF-kB and IRF-3 [61]. Once TICAM-1 is oligomerized, the serine-threonine kinases, TANK-binding kinase 1 (TBK1; also called NAK or T2K) and IkB kinase-related kinase-ε (IKK-ε; also called IKK-i), are activated and phosphorylate IRF-3 [62,63]. The ubiquitin ligase of the TRAF family members, TRAF2, TRAF3 and TRAF6, are downstream-signalling molecules of TICAM-1. TRAF2 and TRAF6 directly bind to the N-terminal region of TICAM-1 [64,65] (Figure 2B). The Lys63-linked autoubiquitination of TRAF3 is required for IRF-3 activation [66,67]. Furthermore, NF-kB-activating kinase (NAK)-associated protein 1 (NAP1) participates in the recruitment of IRF-3 kinases to the N-terminal region of TICAM-1 [68]. Although both TRAF3 and NAP1 associate with oligomerized TICAM-1 and serve as a critical link between TICAM-1 and downstream IRF-3 kinases, there is no evidence that they bind directly to TICAM-1. Interestingly, recent reports showed that direct binding of TBK1 to TICAM-1 is necessary for IRF-3 activation [69]. The Leu194 residue in the N-terminal region is critical for TBK1 binding to TICAM-1. In addition, the Ser189, Arg195 and Ser196 residues are involved in TBK1–TICAM-1 binding.

The N-terminal 176 a.a. of TICAM-1 form a protease-resistant structural domain, designated NTD (Figure 2B). Because the crucial amino acids for TRAF2-, TRAF6- and TBK1-binding reside between the NTD and the TIR domain, naive TICAM-1 may have a closed conformation that covers these binding sites. Indeed, protein–protein interaction analysis revealed that the NTD interacts with the N-terminus of TICAM-1–TIR [69]. Thus, the NTD folds into the TIR domain structure to maintain the naive conformation of TICAM-1. Upon stimulation of TLR3 or TLR4, TICAM-1 oligomerizes through the TIR domain and the C-terminal region, possibly breaking the intramolecular association and inducing a conformational change that allows TBK1 access to TICAM-1.

Whereas the N-terminal region is crucial for TICAM-1-mediated IRF-3 activation, the C-terminal region of TICAM-1 is involved in NF-kB activation and apoptosis. Receptor-interacting protein 1 (RIP1), a kinase containing a death domain, associates with

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DOI: 10.1002/rmv

TICAM-1 via the RIP homotypic interaction motif domain in the C-terminal region and acts as an NF-kB inducer and apoptosis mediator in TICAM-1-mediated signalling [70–72]. TRAF6 has also been implicated in NF-kB activation by TICAM-1 in a cell-type-dependent manner [64,73].

TLR3–TICAM-1-mediated signalling is negatively regulated by a fifth TIR adaptor protein SARM [74]. SARM and TICAM-1 have been shown to interact and SARM strongly suppresses NF-kB activation, as well as IRF-3 activation by TICAM-1. Moreover, deubiquitinating enzyme A (DUBA) negatively regulates TLR3-mediated type I IFN production. DUBA selectively cleaves the Lys63-linked polyubiquitin chains on TRAF3, resulting in its dissociation from the downstream-signalling molecules [75]. In addition, the ubiquitin-modifying enzyme A20 inhibits TICAM-1-mediated NF-kB activation by deubiquitinating TRAF6 [76]. However, the precise mechanisms by which TRAF3 and TRAF6 are ubiquitinated and their interaction with downstream-signalling molecules are unknown.

Antiviral function of TLR3

The role of TLR3 in viral infection is complex (Table 1). Studies in TLR3-deficient (TLR3 /) mice showed that the immune response to different viruses, including lymphocytic choriomeningitis virus (an ambisense RNA virus), vesicular stomatitis virus (a negative-stranded RNA virus), murine cytomegarovirus (MCMV, a dsDNA virus) and reovirus (a dsRNA virus), was unaffected in these mutant mice compared with wild-type mice [77].

By contrast, Hardarson et al. [78] reported that TLR3 is important in host defense against encephalomyocarditis virus (EMCV, a positive sense ssRNA virus belonging to the Picornaviridae family). When mice were inoculated intraperitoneally with 50 plaque-forming units EMCV, TLR3 / mice were more susceptible to EMCV infection and had a significantly high viral load in the heart compared with wild-type mice. Opposing to these data, Kato et al. [24] showed that MDA5 but not TLR3 plays an important role in host defense against EMCV infection, when mice were infected with 100 plaque-forming units EMCV intraperitoneally. It is unclear why these different results were obtained from similar EMCV infection studies.

Table 1. The role of TLR3 in antiviral responses

	References
Protection	
Flaviviridae [þ, ss]	
West Nile virus (WNV)	[84]
Picornaviridae [þ, ss]	
Encephalomyocarditis virus (EMCV)	[78]
Poliovirus	[79,80]
Coxsackievirus group B serotype 3 (CVB3)	[82]
Herpesviridae [dsDNA]	
Murine cytomegarovirus (MCMV)	[90]
Herpes simplex virus 1 (HSV-1)	[101]
Deterioration	
Flaviviridae [þ, ss]	
West Nile virus (WNV)	[83]
Orthomyxoviridae [, ss]	
Influenza A virus (IAV)	[88]
Bunyaviridae [, ss]	
Phlebovirus	[89]

More recently, the essential role of the TLR3–TICAM-1 pathway in protection from poliovirus infection, a virus belonging to the Picornaviridae family, has been demonstrated [79,80]. Poliovirus receptor (PVR)-transgenic/TICAM-1-deficient mice are more susceptible than PVR-transgenic mice to intraperitoneal or intravenous inoculation with a low titre of poliovirus [79,80]. Forty-eight hours after infection, virus titres in serum dramatically increased and mortality greatly decreased compared with PVR-transgenic or PVR-transgenic/IPS-1 (RLR adaptor)-deficient mice. It is well known that in cultured mammalian cells, poliovirus infection results in inhibition of cellular protein synthesis so-called 'shut-off' event [81]. Therefore, mRNA upregulation of RIG-I and MDA5 by type I IFN does not link to protein synthesis at an early stage of virus infection. Thus,

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DOI: 10.1002/rmv

it appears that the inhibitory effects of viral multiplication on host cells depend on the TLR3–TICAM-1 pathway, but not the RLR–IPS-1 pathway.

In addition, Negishi et al. [82] showed that TLR3$^{/}$ mice are more vulnerable to coxsackievirus group B serotype 3 (CVB3, a virus belonging to the Picornaviridae family) than wild-type mice, in terms of higher mortality and acute myocarditis. The expression of IL-12p40, IL-1b and IFN-g mRNAs, but not IFN-b mRNA, was impaired in the hearts of CVB3-infected TLR3-deficient mice compared with those of wild-type mice infected with CVB3. By contrast, expression of TLR3 by transgene protects mice from lethal CVB3 infection and hepatitis even in the absence of type I IFN signalling. Antibody blocking studies revealed that TLR3–TICAM-1-dependent type II IFN (IFN-g) production is critical for host defense against CVB3 infection [82].

Remarkably, Wang et al. [83] demonstrated that TLR3 is involved in the viral pathogenesis of West Nile virus (WNV, a positive-stranded RNA virus). TLR3$^{/}$ mice showed impaired cytokine production and enhanced viral loads in the periphery, whereas in the brain, the viral load, inflammatory responses and neuropathology were reduced compared with wild-type mice [83]. TLR3-mediated peripheral inflammatory cytokine production is critical for disruption of the blood–brain barrier, which facilitates viral entry into the brain causing lethal encephalitis. Therefore, TLR3$^{/}$ mice are more resistant to lethal WNV infection. In contrast, Daffis et al. [84] reported the protective role of TLR3 in sublethal WNV infection. The absence of TLR3 enhances WNV mortality in mice and increases viral burden in the brain after inoculation with the pathogenic New York strain of WNV, although there are little differences in WNV-specific antibody responses, CD8^{b} T-cell activation, blood–brain barrier permeability and IFN-a/b induction in draining lymph nodes and serum, between wild-type and TLR3$^{/}$ mice [84]. The reason why TLR3 shows the opposite function against WNV infection remains to be determined.

In other RNA viral infections such as respiratory syncytial virus, IAV and phlebovirus (all negative-stranded RNA viruses), TLR3-dependent inflammatory cytokine and chemokine production also appears to affect virus-induced pathology and host survival [85–89]. TLR3$^{/}$ mice infected with IAV exhibited reduced inflammatory mediators, leading to increased survival [88]. It is notable that experimental conditions using high viral doses may lead to the over-production of inflammatory cytokines and chemokines. However, what type of TLR3-expressing cells that respond to virus-derived dsRNA in vivo has not been shown in these studies.

Cellular immunity induced by the TLR3–TICAM-1 pathway

In addition to type I IFNs, CTLs and NK cells are also principal effector cells in antiviral immunity. The contribution of TLR3 to antiviral responses has been shown in MCMV infection [90], during which virus clearance is partly dependent on NK cell activation. TLR3$^{/}$ mice are hypersusceptible to MCMV infection. Cytokine (type I IFN, IL-12p40 and IFN-g) production, and NK cell and NKT cell activation are impaired in TLR3$^{/}$ mice compared with wild-type mice.

Selective TLR3 expression in myeloid DCs but not in pDCs raises the possibility that TLR3 also plays a key role in the antiviral response by induction of adaptive immune responses rather than primary IFN-a/b production (Table 2). Myeloid DCs are the most effective professional antigen-presenting cells, possessing several antigen processing and transporting pathways [91,92]. One of the most notable features of myeloid DCs is the cross-presentation of exogenous antigens to CD8^{b} T cells. This pathway is important for effective host CTL induction against viruses that do not directly infect DCs. Among the myeloid DC subsets, the splenic CD8a^{b} DC subset in mice and the CD141(BDCA3)^{b}DNGR-1(CLEC9A)^{b} DC subset in humans highly express TLR3 and display a superior capacity for cross-presenting apoptotic and necrotic cell antigens after TLR3 stimulation [93–97]. Using TLR3-deficient mice, Schultz et al. [98] clearly showed that TLR3 plays an important role in cross-priming. Mouse CD8a^{b} DCs are activated by phagocytosis of apoptotic bodies from virally infected cells or cells containing poly(I:C) in a TLR3-dependent manner. Furthermore, immunization with virally infected cells or cells containing poly(I:C), both carrying ovalbumin antigen, induces ovalbumin-specific CD8^{b} T-cell responses, which are largely dependent on TLR3-expressing DCs [98]. In many cases, virally infected cells produce IFN-a/b which activates DCs to

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DOI: 10.1002/rmv