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Anti-tuberculosis drug susceptibility testing of *Mycobacterium bovis* BCG Tokyo strain

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SUMMARY

SETTING: The *Mycobacterium bovis* bacille Calmette-Guérin (BCG) vaccine is the only vaccine against tuberculosis (TB), owing to its valuable protective effects and low virulence. However, it can occasionally cause systemic infection in immunocompromised hosts. Isoniazid (INH), rifampicin (RMP), streptomycin (SM) and ethambutol (EMB) are known to be effective anti-tuberculosis drugs and are used for the treatment of BCG infections. Unfortunately, there are few studies of the susceptibility of BCG vaccine strains to these drugs.

OBJECTIVE: To measure the minimum inhibitory concentrations (MICs) of BCG Tokyo vaccine products for anti-tuberculosis drugs and assess vaccine safety in terms of drug susceptibility.

DESIGN: We measured the MIC for one seed and five product lots of BCG Tokyo strain for INH, RMP, SM and EMB using Middlebrook 7H11 agar plates.

RESULTS: The MIC results for INH were 0.06 and 0.125 μg/ml for the product and seed lots, respectively. The MIC results for RMP, SM and EMB were 0.25–0.5, 0.25 and 2–4 μg/ml, respectively.

CONCLUSION: Our results indicate that the BCG Tokyo strain was susceptible to the major anti-tuberculosis drugs and treatable even in cases of severe adverse events, including systemic infection.

KEY WORDS: BCG; minimum inhibitory concentration; drug susceptibility

TUBERCULOSIS (TB) is an infectious disease of international importance that remains a major life-threatening disease worldwide. It is estimated that approximately one third of the world's population is infected with *Mycobacterium tuberculosis*. Every year, approximately 9 million people develop active disease and 1.7 million die of TB.[1]

Bacille Calmette-Guérin (BCG) vaccines are safe, attenuated live bacteria and have been shown to have valuable protective effects against TB. The BCG Tokyo strain is recognised as a low virulence strain among all BCGs,[2] and is widely used in several countries as a vaccine strain. If used properly, it protects against the development of TB and the dissemination of TB bacilli. Few severe complications have been reported.[3] However, systemic BCG infection may occur frequently when it is administered to immunocompromised hosts with congenital or acquired immunodeficiency such as human immunodeficiency virus (HIV) infection.[4,5] BCG is contraindicated in symptomatic HIV diseases. When general BCG infection occurs, patients are treated empirically using anti-tuberculosis drugs because there is limited information about the drug susceptibility of BCG strains. It is therefore very important to evaluate the drug susceptibility of BCG Tokyo strain to ensure the safety of the vaccine.

Isoniazid (INH), rifampicin (RMP), streptomycin (SM) and ethambutol (EMB) are the first-line anti-tuberculosis drugs most commonly used in standard TB treatment regimens. These drugs are currently available even in developing countries. The present study aimed at measuring the minimum inhibitory concentrations (MICs) of these drugs against the BCG Tokyo strain to estimate the effect of clinical treatment in case of infection by the BCG Tokyo strain.

MATERIALS AND METHODS

BCG Tokyo strain

Five lots of vaccine product (number 1003 as 'Lot A', 1960 as 'Lot B', 1036 as 'Lot C', 1061 as 'Lot D', 1998 as 'Lot E') and one seed lot were provided by the Japan BCG Laboratory (Tokyo, Japan) and used in this study. These vaccines were produced by Japan BCG Laboratory for vaccination from the seed lot in 2004. The experiment was carried out in a type II-B

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Minimum inhibitory concentrations

The MICs were measured modifying the proportion method described in M24-A of the Clinical and Laboratory Standards Institute (CLSI, former National Committee for Clinical Laboratory Standards) and in previous reports.[6,7] The following procedure was used: lyophilised BCG Tokyo products were suspended in 1 ml of distilled water and were cultured on Middlebrook 7H10 agar (DIFCO, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) supplemented with oleic acid, albumin, dextrose and catalase (OADC: BBL Prepared Culture Media, Becton Dickinson) at 37°C until sufficient growth was observed. After harvesting colonies from culture media, each lot strain of BCG Tokyo was dispersed by vortex mixing with glass beads (disperser tube: Nichibi, BCG Laboratory, Tokyo, Japan) and two drops of 10% Tween 80 (LC-MS, Santa Fe, CA, USA). After vortex mixing for 30 s, 1 ml of distilled water was added to each sample and they were vortexed again for 10 s. The supernatant of each bacterial suspension was transferred to 10 ml of Middlebrook 7H9 broth supplemented with albumin, dextrose and catalase (BBL Prepared Culture Media, Becton Dickinson), and the suspension density was adjusted to an optical density (OD) of 0.05 at 530 nm. These culture tubes were incubated at 37°C with daily mixing and OD checking. When the OD reached 0.2, they were used as the original bacterial suspension.

To prepare 10^{-2} dilutions, a 100 µl aliquot was transferred into 10 ml of distilled water. In a similar way, 100 µl of the 10^{-2} dilution was added to 10 ml of distilled water for 10^{-4} dilutions. One hundred microlitres of the 10^{-2} dilution were inoculated onto Middlebrook 7H11 agar plates with anti-tuberculosis drugs at the designated concentrations. Final INH concentrations were 0.03, 0.06, 0.125, 0.5, 1.0 and 2.0 µg/ml. RMP (0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 µg/ml), SM (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32 µg/ml) and EMB concentrations (0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32 µg/ml) were adjusted accordingly. The 10^{-2} and 10^{-4} suspensions were inoculated onto Middlebrook 7H11 medium containing no drugs for growth control and 1% proportion measurements. These plates were incubated at 37°C. When the 10^{-2} dilution control showed sufficient growth (>100 visible colonies), the MICs were measured as the lowest concentration of drug that inhibited more than 99% of the bacterial population compared with the number of colonies on drug-containing media and the 10^{-4} growth control. Each test was performed in triplicate.

RESULTS

The MICs of one seed and five product lots were measured in triplicate. The MICs of the anti-tuberculosis drugs varied slightly with the lots tested, but were identical among the triplicate tests. The MICs for all tested drugs are shown in the Table. The MICs of INH were 0.06 µg/ml and the seed lot MIC was 0.125 µg/ml. The MIC in test 3 of lot A was not determined due to contamination. For RMP, the MICs for lots A, B and C were 0.25 µg/ml; those for lots D and E were 0.5 µg/ml. It was considered that the MICs of RMP were between 0.25 and 0.5 µg/ml. For SM, the MICs were determined to be 0.25 µg/ml in all tests. For EMB, the MICs were 4 µg/ml for lots A, B and C, while the MICs for lots D and E were 2 µg/ml. The MIC of EMB was 2–4 µg/ml.

DISCUSSION

The BCG vaccine was developed by Calmette and Guérin in 1921. All BCG vaccines consist of live attenuated *Mycobacterium bovis* bacteria. BCG vaccination is commonly performed on neonates and infants once or twice in middle to high tuberculosis prevalence countries, and more than 100 million children have received BCG in recent years.[8] Its safety is therefore a priority issue.

BCG vaccination may sometimes cause complications as a pathogen. Local adverse effects of BCG vaccination have at times been observed and usually improve spontaneously, although severe complications in immunocompromised patients have been reported. McKenzie et al. reported systemic haematological dis-

Table MIC values of four first-line drugs for the BCG Tokyo strain

	MIC (µg/ml)											
	INH			RMP			SM			EMB		
Samples	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Lot A	0.06	0.06	cont	0.25	0.25	0.25	0.25	0.25	0.25	4.0	4.0	4.0
Lot B	0.06	0.06	0.06	0.25	0.25	0.25	0.25	0.25	0.25	4.0	4.0	4.0
Lot C	0.06	0.06	0.06	0.25	0.25	0.25	0.25	0.25	0.25	4.0	4.0	4.0
Lot D	0.06	0.06	0.06	0.5	0.5	0.5	0.25	0.25	0.25	2.0	2.0	2.0
Lot E	0.06	0.06	0.06	0.5	0.5	0.5	0.25	0.25	0.25	2.0	2.0	2.0
Seed lot	0.125	0.125	0.125	ND	ND	ND	ND	ND	ND	ND	ND	ND

MIC = minimum inhibitory concentration; BCG = bacille Calmette-Guérin; INH = isoniazid; RMP = rifampicin; SM = streptomycin; EMB = ethambutol; cont = contaminated; ND = not done.

semination of BCG in a child with X-linked severe combined immunodeficiency.[9] Puthanakit et al. reported four cases of BCG infection in HIV-positive children receiving BCG vaccinations at birth; the strain was not indicated.[10]

BCG strains have also been utilised for immunotherapy in addition to TB prevention. BCG is injected into the urinary bladder for intravesical instillation therapy in the early stages of bladder carcinoma.[11,12] The BCG Tokyo strain is popular for such adjuvant therapy in Japan,[13] whereas the Connaught strain is popular in other parts of the world. In a recent study, Mugiya et al. described good, complete response rates of 84% with BCG Tokyo (40 mg administered every 6 weeks) against bladder carcinoma in situ.[14] However, adverse reactions can also occur after instillation therapy. Eichel et al. reported INH-resistant BCG cystitis successfully treated with RMP and EMB.[15]

There is at present no recommended treatment regimen for BCG infection. Anti-tuberculosis drugs are the most potent agents for treating BCG infection. Drug susceptibility testing (DST) of BCG strains has been reported using different methods. Durek et al. evaluated the Connaught BCG strain using a BACTEC 460TB system (Becton Dickinson).[16,17] DST was performed for 31 drugs, including INH, RMP, SM, EMB and rifabutin. The BCG Connaught strain was susceptible to all of the anti-tuberculosis drugs except pyrazinamide (PZA) (BCG has natural/intrinsic resistance to PZA) and some other drugs used for general bacterial infections. The BACTEC 460 TB system employs critical drug concentrations of 0.1, 1.0, 2.0 and 2.5 for INH, RMP, SM and EMB, respectively. Rousseau and Dupuis reported the DST for a seed lot of the BCG Montreal strain by using solid Dubos medium.[18] They showed that this strain was sensitive to INH (0.2 μg/ml), RMP (1.0 μg/ml), SM (2.0 μg/ml) and EMB (5.0 μg/ml). These reports are not, however, comparable because of the differences in testing methods. There is no standard method for the DST of BCG; however, they may be equivalent to each other in the concept of detecting 1% resistance in the strain population. The proportion method with Middlebrook 7H11/OADC media, which is commonly used for the DST of *M. tuberculosis*, was used for this study.

The MICs indicated in the present study were lower than the critical concentrations employed in the previous studies, except for EMB with MIC close to the critical concentration of BACTEC. In the previous studies, the MICs of EMB to *M. tuberculosis* vary between 0.5 μg/ml and 2.0 μg/ml,[19,20] in 7H12 BACTEC broth MIC varies between 0.95 and 3.8 μg/ml and on 7H10 agar between 1.9 and 7.5 μg/ml.[21] Heifets proposed possible guidelines for the interpretation of MIC to *M. tuberculosis* determined in Middlebrook 7H12 broth (radiometric), and MIC 4.0 μg/ml of EMB as moderately susceptible.[22] It is possible that the MIC of BCG Tokyo strain for EMB was higher than wild type *M. tuberculosis*. However, these reports show the tendency of lower MIC in liquid media than solid media. The plasma concentration (C_{max}) of EMB reaches 2.0–5.0 μg/ml[23] and EMB generally works in a time-dependent manner. For this reason it is suggested that EMB could be effective. Although BCG and *M. tuberculosis* are different species, these MICs and pharmacokinetic data would support the potentials of EMB for the treatment of BCG infection. It was therefore considered that, like the BCG Montreal and Connaught strains, the BCG Tokyo strain is susceptible to the four major anti-tuberculosis drugs.

Hesseling et al. reported that BCG in an HIV co-infected infant who received a BCG Danish 1331 strain vaccination developed INH and RMP resistance following treatment with INH and RMP.[24] The MICs of the original strain were 0.15 and <0.4 μg/ml for INH and RMP, respectively. However, they had risen above 0.3 and 32 μg/ml after treatment. These results suggest that the strain was already clinically resistant to INH (MIC 0.15 μg/ml for INH), and monotherapy with RMP against BCG resulted in RMP resistance. Su et al. reported two general disseminated cases of the BCG Tokyo vaccine strains.[5] One of them was treated using anti-tuberculosis drugs (INH, RMP, SM and EMB) based on the susceptible DST results, and the patient recovered. Another case died following one month's treatment with INH, RMP and EMB. However, no DST data were shown in the mortality case and the infant seemed to have died from severe combined immunodeficiency. The MICs of the BCG Tokyo strain indicated in this study were considered less than or equivalent to those of the previous cases, so it was estimated that BCG Tokyo could be treated successfully even in severe adverse events such as systemic dissemination.

The reason why BCG strains have different phenotypic characteristics with respect to drug susceptibility is not clear. BCG has lost several regions of difference (RD) compared to *M. bovis* as the ancestral strain. In particular, the RD1 deletion made a significant contribution to the attenuation of BCG.[24–26] RD1 encodes a 6 kDa early secreted antigenic target protein (ESAT-6)[27] and a 10 kDa culture filtrate protein (CFP-10)[28] associated with virulence in *M. tuberculosis* complex. The BCG vaccine therefore has attenuated virulence compared to wild *M. bovis* strains. The loss of virulence apparently occurred through repeated passages.

The BCG strains were originally donated by the Pasteur Institute (Paris, France), and have been subcultured by several tuberculosis institutes around the world (Russia, Brazil, Sweden, Denmark, Japan, etc.) since 1924. The donated BCG strains differ from the original BCG strains due to differences in passage cultivation, culture medium and storage conditions. In 1972, Hesselberg found that a Swedish/Norwegian BCG strain became resistant to INH during the period 1953–1964, which was the reason why the serial sub-

culture system was discontinued and a seed lot system was adopted.[29] However, in 2003, low-grade INH-resistant (MIC >0.5 μg/ml) Danish 1331 strains were reported again to the World Health Organization (WHO). The WHO therefore recognises the necessity of a new quality assurance method for BCG vaccines.[30,31]

The BCG Tokyo strain was obtained from Calmette in the Pasteur Institute in 1924. Passage cultivation of BCG Tokyo strain has been performed strictly according to Calmette's original instructions, while some of the other BCG strain passages were tailored to each institute's needs. The BCG Tokyo 172 strain, which has undergone 172 passages since the Second World War II, has been used as the seed lot for lyophilised BCG Tokyo vaccines. In this study, the BCG Tokyo strain proved to be susceptible to the major anti-tuberculosis drugs; however, the results of this study do not apply to all BCG substrains. It will be necessary to ensure the safety of BCG vaccine by checking susceptibility to other antimicrobial agents.

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RÉSUMÉ

CONTEXTE : Le bacille de Calmette et Guérin (BCG) à base de *Mycobacterium bovis* est un vaccin unique contre la tuberculose (TB) en raison de ses effets protecteurs valables et de sa faible virulence. Toutefois, il peut causer occasionnellement une infection systémique chez les sujets en état d'immunodépression. L'isoniazide (INH), la rifampicine (RMP), la streptomycine (SM) et l'éthambutol (EMB) sont des médicaments antituberculeux reconnus comme efficaces et peuvent être utilisés dans le traitement des infections par le BCG. Il n'y a malheureusement que peu d'études concernant la sensibilité des souches de vaccin BCG à l'égard de ces médicaments.
OBJECTIF : Mesurer les concentrations minimales inhibitrices (CMI) du vaccin BCG Tokyo pour les médicaments antituberculeux et évaluer la sécurité du vaccin en ce qui concerne la sensibilité aux médicaments.
SCHÉMA : Nous avons mesuré les CMI sur plaques d'agar Middlebrook 7H11 pour la souche-mère et pour cinq lots de vaccin de la souche BCG Tokyo à la fois pour l'INH, la RMP, la SM et l'EMB.
RÉSULTATS : Les résultats des CMI pour l'INH ont été respectivement de 0,06 et de 0,125 μg/ml pour la souche-mère et pour les lots de vaccin. Les résultats des CMI pour la RMP, la SM et l'EMB ont été respectivement de 0,25–0,5, 0,25 et 2–4 μg/ml.
CONCLUSION : Nos résultats indiquent que la souche BCG Tokyo est sensible à l'égard des médicaments antituberculeux majeurs qui sont efficaces même en cas d'effets indésirables graves, y compris des infections systémiques.

RESUMEN

MARCO DE REFERENCIA : *Mycobacterium bovis*, el bacilo de Calmette y Guérin (BCG), es la única vacuna contra la tuberculosis (TB), debido a su valioso efecto de protección y a su baja virulencia. Sin embargo, esta vacuna puede causar en ocasiones infecciones generalizadas en individuos inmunodeprimidos. Isoniazida (INH), rifampicina (RMP), estreptomicina (SM) y etambutol (EMB) son medicamentos antituberculosos eficaces y se emplean en el tratamiento de las infecciones por BCG. Desafortunadamente, existen pocos estudios sobre la sensibilidad de la cepa de la vacuna antituberculosa a estos medicamentos.
OBJETIVO : Medir las concentraciones mínimas inhibitorias (CMI) de los medicamentos antituberculosos contra el BCG de Tokio contenido en las vacunas y evaluar su seguridad toxicológica en la concentración de sensibilidad al medicamento.
MÉTODOS : Se midieron las concentraciones inhibitorias mínimas de INH, RMP, SM y EMB para un lote de siembra y cinco lotes de vacuna de la cepa BCG de Tokio usando cultivos en placas de agar con Middlebrook 7H11.
RESULTADOS : La CMI para INH fue 0,06 con los lotes de siembra y 0,125 μg/ml con los lotes de vacuna. La CMI para los lotes de vacuna con RMP fue de 0,25 a 0,5 ; con SM fue 0,25 ; y con EMB fue de 2 a 4 μg/ml.
CONCLUSIÓN : Estos resultados indican que la cepa BCG de Tokio es sensible a los principales medicamentos antituberculosos y que es posible tratar los casos de reacciones adversas graves, incluida la infección generalizada.

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ウイルス感染とバイオディフェンス

－注目される補中益気湯の可能性－

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ウイルス感染とバイオディフェンス

―注目される補中益気湯の可能性―

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HIV、SARS、鳥インフルエンザ。医学が進歩してもなお、人類はウイルス感染の脅威にさらされ続けている。SARSについては一応の落ち着きをみたが、HIV、そしてインフルエンザのパンデミックに対して、果たして人類は勝利することができるのだろうか。

微生物との緊張感ある闘いのなかで、効果的なバイオディフェンス製剤を求めてさまざまなアプローチが試みられている。既存の薬剤や植物成分の見直しもその一つである。そのなかで、漢方薬も有効性が期待されるものとして研究対象になっている。

ここでは、かねてより免疫賦活作用が報告されている補中益気湯（TJ-41）を中心に、バイオディフェンスの視点で漢方薬の位置付けを考える。

今回、東北大学大学院感染症・呼吸器病態学の服部俊夫氏、千葉大学大学院加齢呼吸器病態制御学の巽浩一郎氏にお話を伺い、岡山大学医学部・歯学部附属病院消化管外科の岩垣博巳氏に資料協力をいただいた。

「Open Sesame」―開けゴマ―ウイルスエントリーの呪文が破られるとき

今年（2006年）、Cell、JAMAに相次いでウイルス学の注目すべき論文が掲載された。Cell掲載の「Virus Entry: Open Sesame」というタイトルを冠した論文[1]は、ウイルス感染経路について最新の知見をレビューしたもので、標的細胞表面にあるウイルス受容体とウイルス膜との結合、ウイルスの刺激による細胞内シグナリング、エンドサイトーシスによるウイルス侵入経路などが明快に解説されている（**図1**）。ちなみに、インフルエンザウイルスはこの図が示すB、風邪の原因ウイ

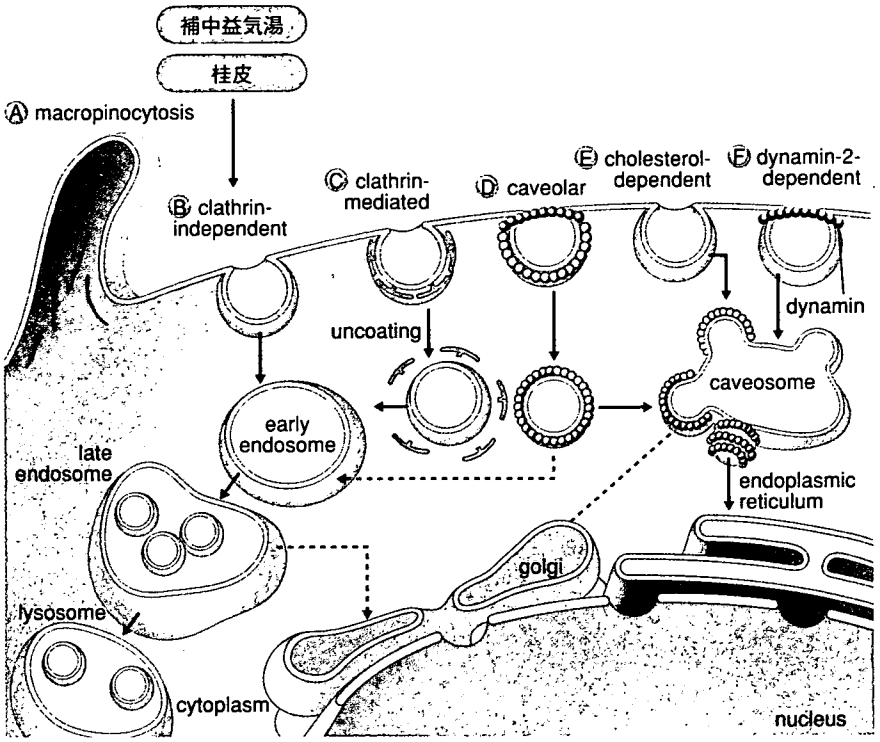


図1　ウイルス進入経路 文献1より引用、改変

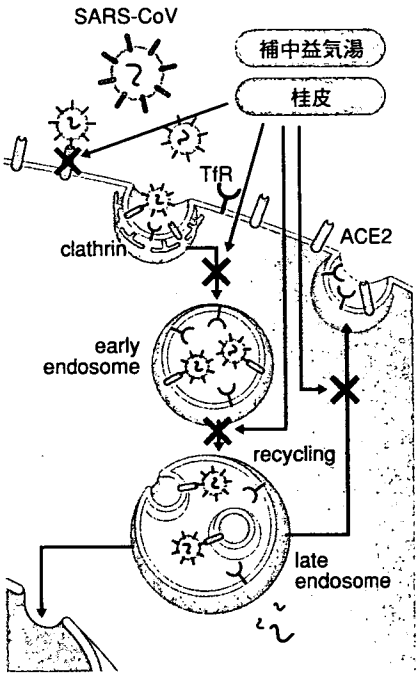


図2　生薬によるエンドサイトーシスの調節の可能性

ルスで最も多いとされるコロナウイルスは主にDの経路で細胞内に侵入すると考えられている。

こうしたウイルスエントリー、すなわちウイルスが細胞内に侵入する経路の解明は、エントリーインヒビターという新しいアプローチの抗ウイルス薬開発には欠かせない。例えば、HIV感染はウイルス表面のスパイク蛋白gp120と標的細胞表面の受容体CD4が結合し、gp120-CD4複合体がさらに細胞表面のケモカイン受容体と結合することでウイルス膜と標的細胞膜との融合が起こる。この融合を、ケモカイン受容体をブロックすることで阻害しようという設計で、いくつかの薬剤が開発段階にあり、そのうち一つはすでに上梓に至っている。ウイルスエントリー経路の詳細な分析は、ウイルスインヒビターのターゲットをより細かく絞り込み、特異的な阻害作用を得ることになる。

服部氏は、これまで一貫してウイルスエントリー阻害による抗ウイルス作用に焦点を当てて、さまざまな成分について精力的な研究を進めてきた。検討した成分には、感染防御作用が指摘される漢方薬の構成生薬や、HIV感染患者に対する臨床効果が報告されているアフリカ原産の植物エキスなども含まれている。また、技術面でもウイルスエントリーを高感度に測定する実験系も確立している。これはルシフェラーゼ遺伝子を組み込んだレポーター遺伝子にウイルスの外膜糖蛋白遺伝子を導入して外膜のスパイク蛋白を発現する疑似ウイルスを使うもので、この実験系ではルシフェラーゼ活性を測定することでウイルスエントリーを詳細に評価することができる。

こうした研究アプローチによって服部氏はこれまでに、漢方薬の補中益気湯や生薬の桂皮などがウイルス感染の抑制効果を示すこと、なかでも桂皮および丁子エキスがHIV/SARS疑似ウイ

ルス感染に対し濃度依存性に抑制効果を示すことを明らかにしている[2]。

特に桂皮エキスの抑制効果は大きく、多種類のウイルス感染に対し阻害作用を示すことから、桂皮エキスは「ウイルス感染の共通部分に作用している可能性」が考えられる。そこで服部氏は、桂皮成分がエンドサイトーシスの諸相に抑制的に作用しているとの仮説を立て、より詳細な研究を進めている（**図2**）。

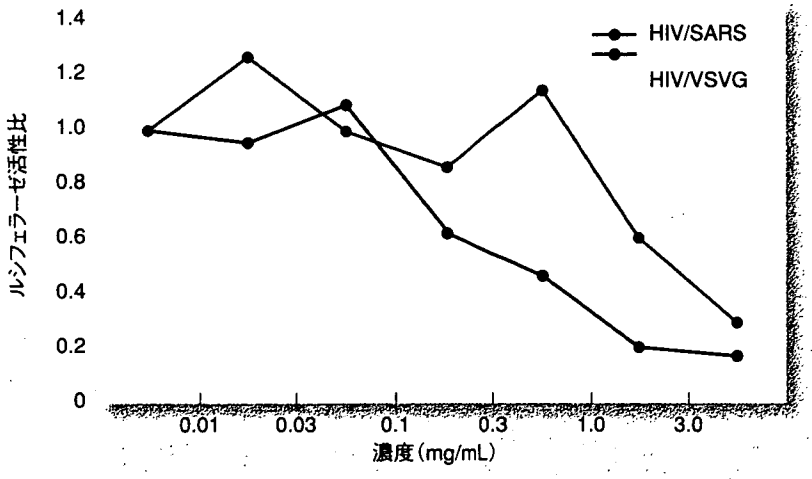


図3　HIV/SARS疑似ウイルスに対する補中益気湯の抑制効果

補中益気湯はHIV/SARSおよびHIV/VSVGを用量依存的に抑制した。（庄 敏・服部俊夫）文献2より引用

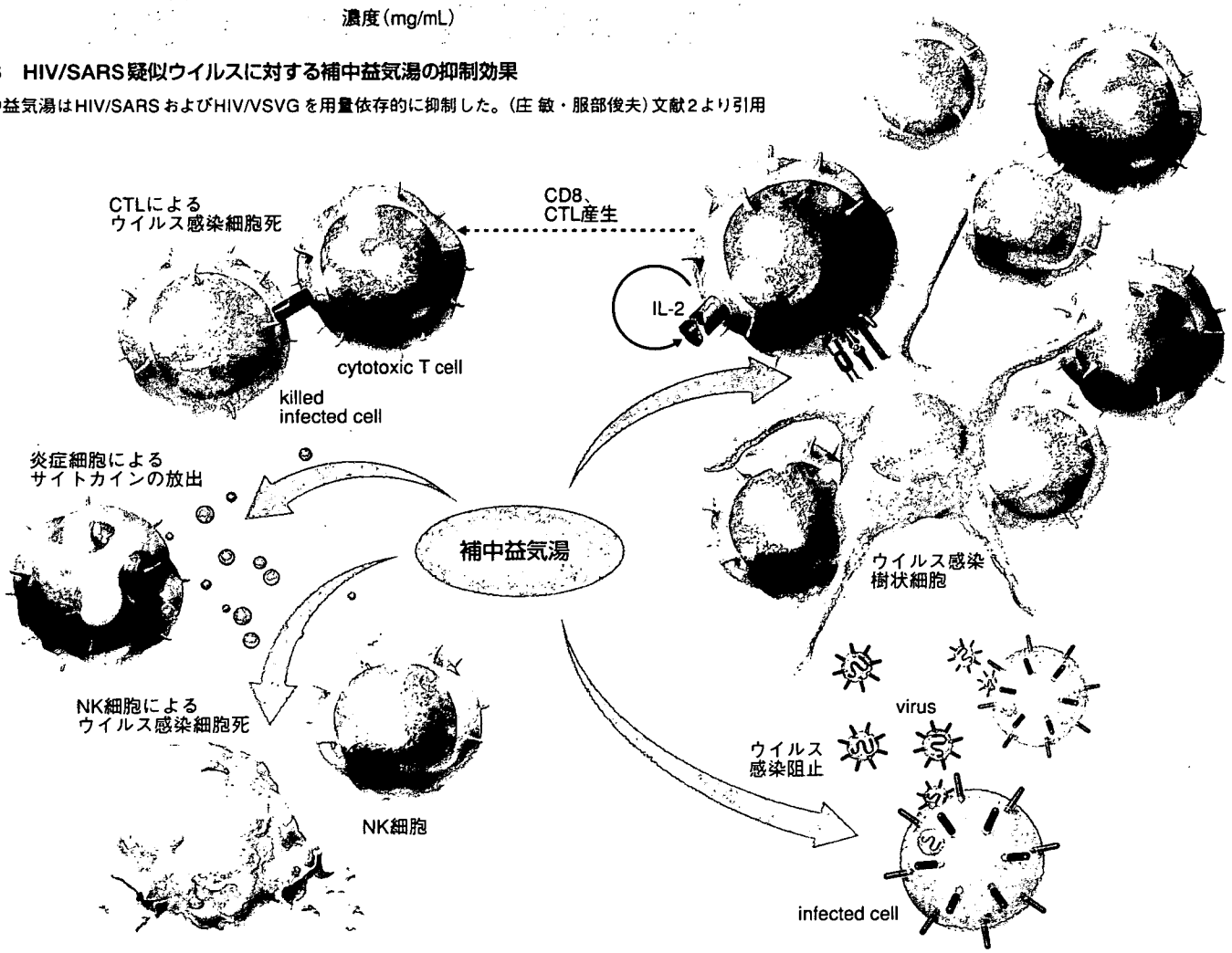


イラスト　補中益気湯の作用機序（監修：服部俊夫）

さらに同様の実験系で補中益気湯についても検討を行ない、桂皮と同様のウイルス感染抑制効果を補中益気湯に認めている（**図3**）。これについて服部氏は補中益気湯の構成成分である柴胡・黄蓍が特に抗ウイルス作用に関与している可能性を指摘している。

「ウイルス量はHIV感染予後因子にあらず」の衝撃

もう一つの論文は、今年のJAMA 9月27日号に載った「Predictive Value of Plasma HIV RNA Level on Rate of CD4 T-Cell Decline in Untreated HIV Infection」である[3]。これは、1984-2004年間に行なわれた約3000人対象のコホートスタディで明らかになった、HIV感染者の未治療段階での血清ウイルス量はその後のAIDS発症の予後予測因子にはならない、という解析結果である。この論文に対し、かつてHIV感染者のウイルス量がAIDS発症と生命予後を規定するとの論文を掲載したScienceが9月29日号でコメントを発表したのをみても、その論文の衝撃の大きさがうかがえる。JAMA掲載の論文の考察では、CD4T細胞の減少を規定するのはウイルス量ではなく他の因子-まだ確定はできないがおそらくは長期間の炎症持続状態の関与を示唆している。

服部氏はこの論文について「ウイルス量と予後は必ずしも一致しないということは、多くの臨床家が感じていたことで、その間を埋める因子として、全身性炎症の持続が指摘されたことになる」と評価。慢性ウイルス感染におけるバイオディフェンスを考えるうえで、炎症反応という新たなターゲットが提示されたのである。

全身性炎症抑制作用を補中益気湯で確認

全身性炎症の持続については、一見ウイルス感染とは関係のない領域でも興味深い報告がある。

その一つが、慢性閉塞性肺疾患（COPD）を全身性炎症としてとらえ、その対策として補中益気湯の有用性を検討した千葉大学の巽氏の研究である。

かつてのCOPDの疾患概念は、気道炎症と肺胞破壊という局所病変を背景にした、進行性で非可逆性の一秒量低下を病態の本質としてとらえるものだった。しかし、2004年にCelliにより、%FEV1・BMI・運動能力・運動時の息切れの4項目を指標としたBODE indexがCOPD患者の予後予測因子となることが報告[4]された頃から、COPDは全身性疾患として認識されるようになり、特に栄養状態と予後の関係が注目されるようになってきた。

巽氏は、COPD患者では気道炎症反応で生じた炎症性サイトカインが他組織におよび、全身性の炎症反応を惹

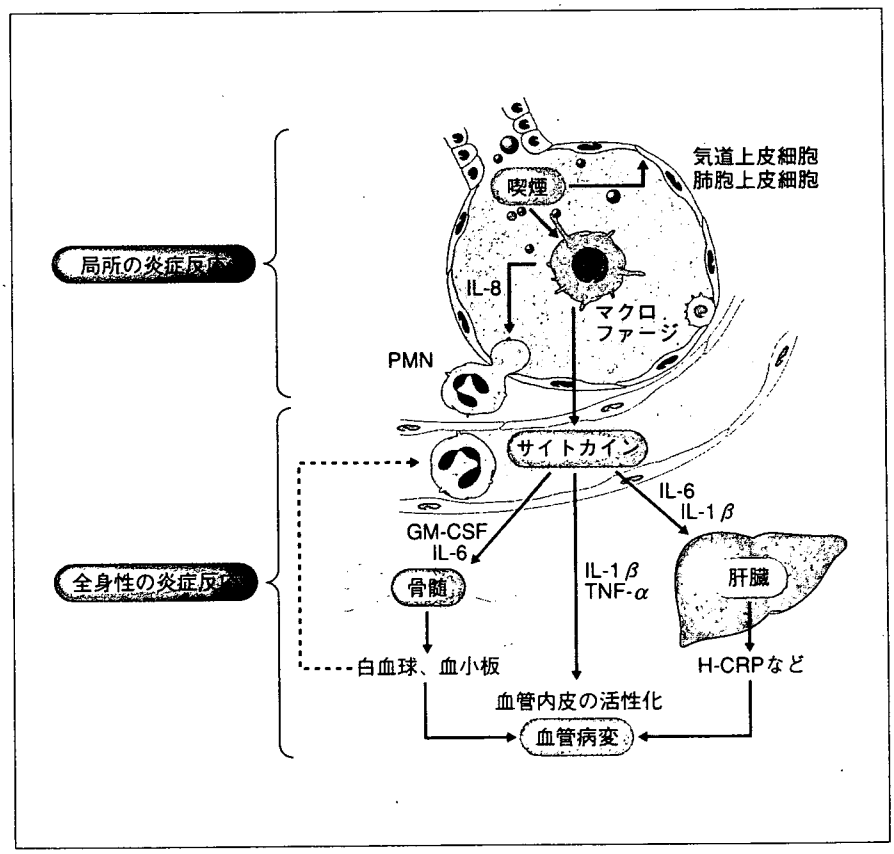


図4　COPDと全身性炎症

起しているとの考えに立ち(図4)、呼吸器疾患症状(SGRQのSymptom score)、漢方医学的な体調評価(気虚スコア)、感冒回数、急性増悪回数、炎症指標[5](高感度CRP、TNF-α、IL-6)、栄養指標(プレアルブミン、レプチン)、動脈硬化指標(高分子量アディポネクチン)を評価項目として、COPD患者に対する補中益気湯の効果を調べた。対象はCOPD症例35例で、うち17例に補中益気湯を投与し、18例を対照群とした。その結果、補中益気湯投与群ではSGRQのSymptom scoreおよび気虚スコアが改善し、感冒罹患頻度、急性増悪頻度が抑制され(図5)、高感度CRPおよびTNF-α値の低下が認められた。また、%一秒量と各種炎症指標とは負の相関がみられ、COPDが重症化するほど全身性炎症反応が強まっていることが示唆された。こうした結果は、COPDの病態は栄養状態および全身性炎症反応と深く関与し、補中益気湯はCOPDの栄養状態を改善し、全身性炎症反応を抑制している可能性を示唆するものといえる。

また、動脈硬化指標のアディポネクチン値も補中益気湯群で改善が認められた。これについて巽氏は、「近年、動脈硬化を血管壁の炎症反応としてとらえるようになり、また欧米ではCOPD患者に心血管イベントの発症頻度が高いという指摘がある。そういう意味でもCOPDを全身性炎症反応とする考え方は示唆に富んでいる」と、炎症反応に関与している可能性を指摘した。

過剰な炎症を抑え、免疫抑制を是正

このように、全身性炎症反応の抑制効果が指摘された補中益気湯だが、その一方で従来からNK細胞の活性化やCTL免疫誘導といった免疫賦活作用も知られている(図6)。

ここで一つの疑問が生じる。一般に免疫応答は炎症反応をともなうものである。果たして補中益気湯の抗炎症作用と免疫賦活作用は両立するものなのだろうか。

これについては、岡山大学の岩垣氏の周術期患者に補中益気湯を投与したデータが一つの答えを示している。進行胃・大腸癌患者を対象とし、補中益気湯の術前投与が術後の全身性炎症反応症候群(SIRS)とそれに引き続き起こる代償性抗炎症反応症候群(CARS)への影響をみたもので、これによると、補中益気湯群では手術後の体温上昇が非投与群に対し抑えられ、術後感染症併発率(術後予防投与に用いた抗菌薬とは異なる抗菌薬を術後2週間以内に投与した症例を術後感染症併発と定義)も補中益気湯群で有意

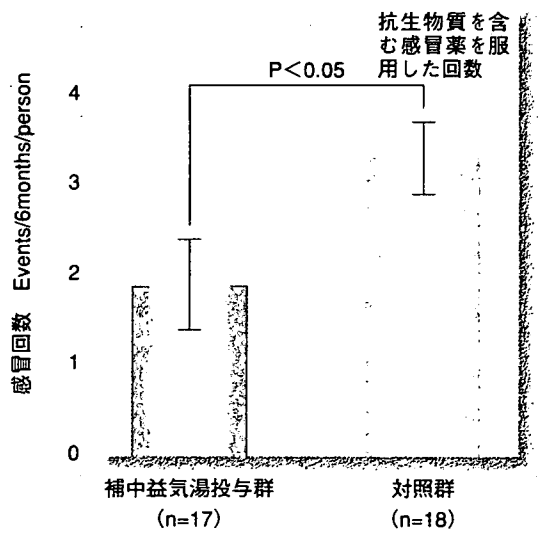


図5 COPD患者に対する補中益気湯投与群と対照群での感冒罹患頻度

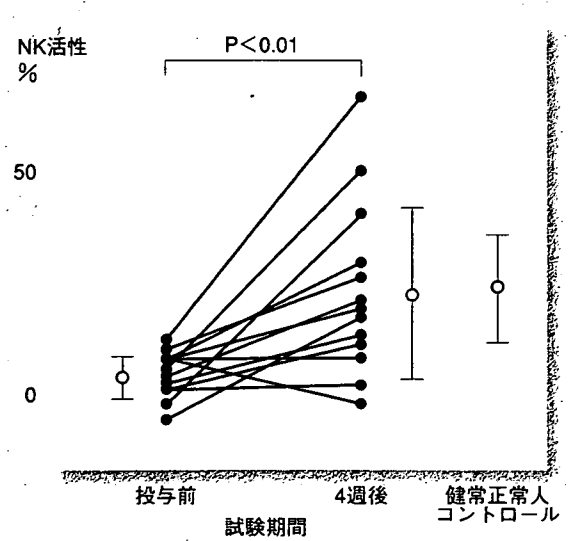


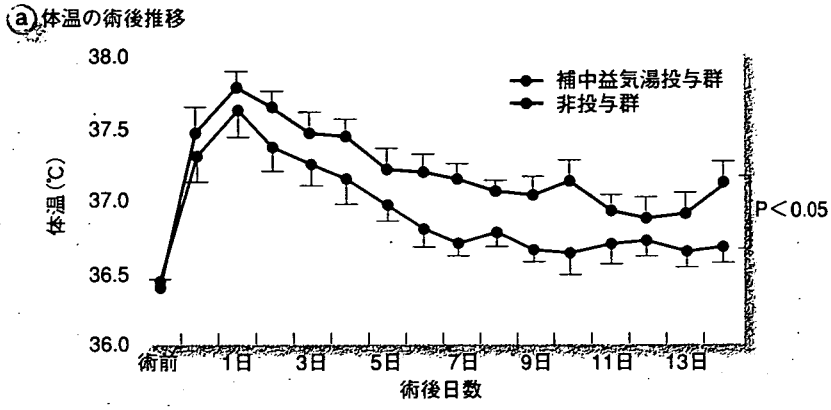
図6 NK活性低値例における補中益気湯の効果

に減少していた(図7)。このデータは、補中益気湯が術後の過度の炎症反応および免疫低下をどちらも改善していることを示すものといえる(図8)。

こうした補中益気湯がもつ作用の多面性について服部氏はこう述べる。「漢方は体内の環境バランスを整える方向で作用します。補中益気湯が免疫賦活作用・抗ウイルス作用・抗炎症作用を併せもつことを示すこれらのデータは、同剤が生体防御に有利な状態に体内環境を整える方剤であることを示しているといえます」。

ここで紹介したデータの他にも、補中益気湯についてはインフルエンザ感染マウスの生存率向上、感冒(ライノウイルス)感染抑制、ヘルペスウイルス感染抑制、タバコ刺激に対する抗炎症効果、などの報告もある。こうした作用は同剤の多面的作用によるバイオディフェンス増強作用によるものといえるだろう。

ひたひたと着実に広がりを見せる鳥インフルエンザの人への感染例、そしてHIV感染は世界的に増加の一途をたどっている。バイオディフェンスを増強する手段は、選択肢は多いほど人類にとっては好ましい。服部氏らが取り組む、こうした漢方薬の薬理効果の新たな解明に、多くの期待が集まっている。



ⓑ術後感染症併発率

	補中益気湯投与群(n=22)	非投与群(n=26)	p値
術後感染症併発率	3/22(13.6%)	11/26(42.3%)	p<0.05

図7　体温の術後推移と術後感染症併発率に対する補中益気湯の効果

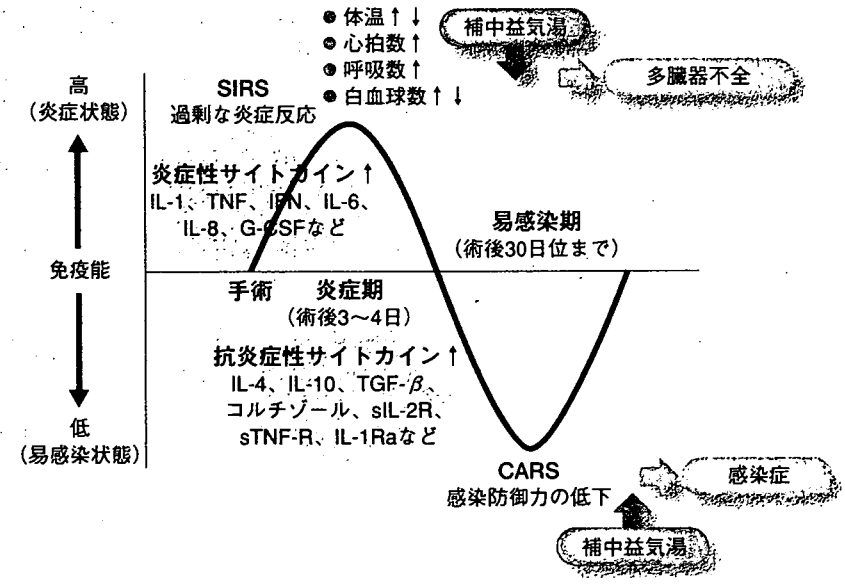


図8　術後侵襲による炎症反応・感染防御力低下に対する補中益気湯の可能性

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※図7は別刷制作時に追記したものです。