24ヶ月または中止時
採取日：2014年12月18日
白血球数：5800/μL
赤血球数：448×10⁴/μL
ヘモグロビン濃度：14.0g/dL
ヘマトクリット：42.8%
血小板：26.3×10⁴/μL
好中球：66.6%
桿状核球：0%
好酸球：2.8%
好塩基球：0.5%
リンパ球：24.4%
単球：5.7%
その他：
AST(GOT)：17IU/L
ALT(GPT)：15IU/L
ALP：209IU/L
総ビリルビン：1.4mg/dL
総タンパク：6.6g/dL
BUN：10mg/dL
クレアチニン：0.61mg/dL
総コレステロール：191mg/dL
LDLコレステロール：96mg/dL
HDLコレステロール：74mg/dL
トリグリセライド：98mg/dL
血糖：83mg/dL
Na：141mEq/L
K：3.8mEq/L
Cl：104mEq/L
pH：7.0
比重：1.011
尿糖：-
尿蛋白：-
亜硝酸塩：-
尿潜血：-
尿中クレアチニン：76.5mg/dL
アルブミン/クレアチニン比：0.91
赤血球：<1/hpf
白血球：50-99/hpf
尿細菌：2+
エストロジェン測定：58.6pg/mL
プロジェストロン測定：1.55ng/mL
テストステロン測定：0.312ng/mL

治療経過：
2012年12月12日よりシロリムスの服薬を開始した。服薬開始後．約1ヵ月で腎盂腎炎を発症し、入院治療した。服薬開始8ヵ月後に憩室炎を発症した。いずれもシロリムスを一時中断し、抗菌薬治療で軽快した。その後、上気道炎1回、尿路感染症で2回休薬したが、2015年2月18日に治験を終了することができた。しかし休薬が多く、服薬率は85～90%であったと推定される。

D.考察
本例では服薬の一時的中断が5回あった。腎盂腎炎をはじめとして、いずれも感染症であった。免疫抑制作用を有するシロリムスによる易感染性の惹起による可能性も否定はできないが、本患者は治験開始以前から尿路感染症の既往があり、しばしば尿沈渣でWBCが多数検出されている。従って尿路感染症とシロリムスの直接的な因果関係はないと考えた。

E.結論
1症例のLAM患者に対し、シロリムスを投与し、有害事象の頻度を主要評価目的とする第Ⅱ相医師主導臨床研究を実施した。

F.健康被害情報
急性腎盂腎炎（複雑性）
2013年1月8日39℃台の発熱・悪寒とともに腎盂腎炎を発症した。LVFXを投与の上、いったん帰宅したが、解熱せず1月9日入院した。シロリムスを中止し、TAZ/PIPCの点滴静注で1月25日回復した。2013年2月5日よりシロリムスを再開した。
本患者はシロリムス治験開始前から尿検査でWBC+++を指摘されており、詳細は不明だが、尿路感染症を繰り返していた可能性がある。
その他に、憩室炎、上気道炎、尿路感染症などで5回一時的な休薬があった。

G.研究発表

18) Histlogical evolution of pleuroparenchymal fibroelastosis. Hirota T, Watanabe K, et al., Histopathology 2015; 66: 545-554.
19) Autopsy analysis in acute exacerbation of idiopathic pulmonary fibrosis. Oda Keishi, Watanabe K, et al. Respiratory Research 2014;15:109.
20) The Thoracic cage becomes flattened in the progression of pleuroparenchymal fibroelastosis. Harada T, Watanabe K, et al. European Respiratory Reviews 2014; 23: 263-266.
21) Chest computed tomography findings in patients with angioimmunoblastic T-cell lymphoma. Ishii H, Watanabe K, et al. Respiratory Investigation 2014; 52: 265-268.

22) Two patients with new granulomatous lung lesions during treatment of Crohn's disease. Takeda S, Watanabe K, et al., Respiratory Medicine Case Reports 2014; 12: 16-18.
23) Two cases of pulmonary lymphangioleiomyomatosis in postmenopausal woman. Ishii H, Watanabe K, et al. Respiratory Investigation 2014; 52: 261-264
24) Treatment guideline for latent tuberculosis infection. The Prevention Committee of the Japanese Society for Tuberculosis. Kato S, Watanabe K, et al. Kekkaku 2014; 89: 21-37.
25) Chapter 7 Physiological manifestation of pulmonary sarcoidosis. In: Sarcoidosis, Intech, Watanabe K. p165-181, 2013,
26) Pleuroparenchymal fibroelastosis as a manifestation of chronic lung rejection? Hirota T, Fujita M, Matsumoto T, Higuchi T, Shiraishi T, Minami M, Okumura M, Nabeshima K, Watanabe K. Eur Respir J. 2013; 41: 243-5.
27) Pleuroparenchymal fibroelastosis: Its clinical characteristics. Watanabe K. Current Pulmonary Medicine Reviews. 2013; 9: 229-237.
28) The Prognostic Significance of Fibroblastic Foci in Usual Interstitial Pneumonia and Nonspecific Interstitial Pneumonia. Harada T, Watanabe K, Nabeshima K, Hamasaki M, Iwasaki H. Respirology 2013; 18: 278-283.
29) A nationwide epidemiological survey of chronic hypersensitivity pneumonitis in Japan. Okamoto T, Miyazaki Y, Watanabe K, et al., Respir Invest 2013; 51: 191-199.
30) Efficacy of aprepitant in patients with advanced or recurrent lung cancer receiving moderately emetogenic chemotherapy. Uchino J, Hirano R, Tashiro N, Yoshida Y, Ushijima S, Matsumoto T, Ohta K, Nakatomi K, Takayama K, Fujita M, Nakanishi Y, Watanabe K. Asian Pac J Cancer Prev. 2012;13(8):4187-90.
31) Elevation of serum C-reactive protein predicts failure of the initial antimicrobial treatment for febrile neutropenia with lung cancer. Matsumoto T, Fujita M, Hirota T, Takeda S, Hirano R, Uchino J, Harada T, Watanabe K. J Infect Chemother. 2013; 19: 202-207.
32) Rapid decrease in forced vital capacity in patients with idiopathic pulmonary upper lobe fibrosis. Watanabe K, Nagata N, Kitasato Y, Wakamatsu K, Nabeshima K, Harada T, Hirota T, Shiraishi M, Fujita M. Respir Invest. 2012; 50(3):88-97.
33) First reported case of hemoglobin lansing in Asia detected by false low oxygen saturation on pulse oximetry. Ishitsuka K, Uchino J, Kato J, Ikuta M, Watanabe K, Matsunaga A, Tamura K. Int J Hematol. 2012; 95: 731-2.
34) The clinical efficacy and safety of micafungin-itraconazole combination therapy in patients with pulmonary aspergilloma. Fujita M, Tao Y, Kajiki A, Ouchi H, Harada E, Ikegame S, Matsumoto T, Uchino J, Watanabe K, Nakanishi Y. J Infect Chemother. 2012; 18(5): 668-74.

H. 知的財産権の出願・登録状態

1. 特許取得

記載すべきことなし。

2. 実用新案登録

記載すべきことなし。

3. その他

記載すべきことなし。

厚生労働科学研究費補助金（難治性疾患等実用化研究事業）
（総括・分担）研究報告書

ＭＬＳＴＳ医師主導治験の安全性に関する研究

東海大学医学部基盤診療学系臨床薬理学[1]
三上礼子

はじめに

難治性疾患等克服研究事業重点研究分野の研究課題であるＭＬＳＴＳ医師主導治験は、日本人リンパ脈管筋腫症患者に対するシロリムス長期投与の安全性及び有効性を検証する目的で行われた臨床試験である。本試験に先立ち、すでに米国主導の国際共同治験として行われたＭＩＬＥＳ試験[1]では有効性が検証されており、日本人患者も32人が参加していたが、プラセボ対照並行群間比較試験のデザインであったためこのうち実薬（シロリムス）群は13人に留まっていた。また、シロリムスの投与期間も1年間であったことから、製造販売後には長期投与が予測される日本人での安全性データの収集が重要と考えられ、製造販売承認申請に際し、本治験を計画するに至った経緯がある。

現時点ではMLSTSの全安全性データが得られて間もないため固定データの解析は得られていないが、解析可能データから有害事象についてその傾向を分析することとした。

対象と方法

日本人リンパ脈管筋腫症患者のうち、ＭＬＳＴＳ治験参加の同意が得られた71例を対象に2012年8月の投与開始から2014年11月までの有害事象データにつき観察した。また、ＭＩＬＥＳ試験の有害事象データとの比較を試みた。

結果

ＭＬＳＴＳ試験登録開始後、71例で同意を取得、うち63例が服薬開始した。同意取得後中止例は17例であった。服薬中止例は9例あり、理由としては妊娠1例、妊娠希望2例、薬剤性肺障害1例、効果不十分の自己判断により1例、有害事象を理由とした自己判断による中止が3例などであった。2014年11月時点での継続投与例は54例であった。

有害事象の発症件数は2014年9月30日までで1413件、臓器別では胃腸障害466件（口内炎256件・口唇炎21件・口角炎10件・下痢49件）、感染症および寄生虫症233件（鼻咽頭炎120件）、呼吸器、胸膜および縦隔障害144件、皮膚および皮下組織障害142件、神経系障害115件（うち頭痛93件）などであった。重篤有害事象は29件で、その内訳は薬剤性肺障害および気胸各3件、呼吸困難・肺炎各2件、胃腸炎・気管支炎・帯状疱疹・急性腎盂腎炎・急性呼吸不全書く1件であった。

当初より懸念されたシロリムスによる薬剤性肺障害は3例あり、いずれも服薬休止により回復した。このうち2例はシロリムス1mgより服薬再開可能であったが、1例は中止とした。

口内炎・口唇炎・口角炎の罹患者数はのべ287件と多く、患者単位での発症率は約80%以上と高率であった。頭痛は約50%、ざ瘡様皮膚炎は約30%の患者に発生した。これらの発症率はＭＩＬＥＳ試験での日本人患者13人と比較し同様の比率と考えられた。なお、ＭＩＬＥＳ試験では特に言及されていない不規則月経がＭＬＳＴＳ試験では約30%に認められた。

考察

重篤有害事象ではないものの、口内炎やざ瘡様皮膚炎は患者のＱＯＬに影響し服薬継続の障害となることも多いと考えられる。今後固定後データを用いてこれらの有害事象の発現の特徴や傾向を分析する予定である。

結論

ＭＬＳＴＳ試験の重篤有害事象として薬剤性肺障害、気胸、肺炎等を認めた。薬剤性肺障害3例のうち中止に至ったのは1例であった。口内炎、ざ瘡様皮膚炎、頭痛などは服薬行動に影響を与える有害事象として注目される。また、

不規則月経についても注意が必要である。

参考文献

1. McCormack FX, et al. Efficacy and safety of Sirolimus in Lymphangioleiomyomatosis. *N Engl. J Med* 364;17 2011.

平成 26 年度　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	ページ
新井徹 井上義一	リンパ脈管筋腫症	泉　孝英	今日の診療のために ガイドライン外来診療 2014	日経メディカル開発	東京	2014	502-504
井上義一	リンパ脈管筋腫症	弦間昭彦	呼吸器疾患診療　最新ガイドライン	綜合医学社	東京	2014	397-384

平成 26 年度　研究成果の刊行に関する一覧表

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
Saraya T, Kurai D, Nakagaki K, Sasaki Y, Niwa S, Tsukagoshi H, Nunokawa H, Ohkuma K, Tsujimoto N, Hirao S, Wada H, Ishii H, Nakata K, Kimura H, Kozawa K, Takizawa H, Goto H.	Novel aspects on the pathogenesis of Mycoplasma pneumoniae pneumonia and therapeutic implications	*Front Microbiol*	5	410	2014
Serikawa T, Nishiyama K, Tohyama J, Tazawa R, Goto K, Kuriyama Y, Haino K, Kanemura Y, Yamasaki M, Nakata K, Takakuwa K, Enomoto T.	Prenatal molecular diagnosis of X-linked hydrocephalus via a silent C924T mutation in the L1CAM gene	*Congenit Anom (Kyoto)*	54(4)	243-5	2014
Nakagaki K, Nunomura Y, Uchida K, Nakata K, Tazawa R.	Up-regulation of cluster of differentiation (CD) 11b expression on the surface of canine granulocytes with human granulocyte-macrophage colony-stimulating factor (GM-CSF)	*J Vet Med Sci*	76(8)	1173-6	2014

Hashimoto A, Tanaka T, Itoh Y, Yamagata A, Kitamura N, Tazawa R, Nakagaki K, Nakata K.	Low concentrations of recombinant granulocyte macrophage-colony stimulating factor derived from Chinese hamster ovary cells augments long-term bioactivity with delayed clearance in vitro.	*Cytokine*	68(2)	118-26	2014
Ishii H, Seymour JF, Tazawa R, Inoue Y, Uchida N, Nishida A, Kogure Y, Saraya T, Tomii K, Takada T, Itoh Y, Hojo M, Ichiwata T, Goto H, Nakata K.	Secondary pulmonary alveolar proteinosis complicating myelodysplastic syndrome results in worsening of prognosis: a retrospective cohort study in Japan.	*BMC Pulm Med*	14	37	2014
Kawase T, Uematsu K, Kamiya M, Nagata M, Okuda K, Burns DM, Nakata K, Yoshie H.	Real-time quantitative polymerase chain reaction and flow cytometric analyses of cell adhesion molecules expressed in human cell-multilayered periosteal sheets in vitro.	*Cytotherapy*	16(5)	653-61	2014
Handa T, Nakatsue T, Baba M, Takada T, Nakata K, Ishii H.	Clinical features of three cases with pulmonary alveolar proteinosis secondary to myelodysplastic syndrome developed during the course of Behçet's disease	*Respir Investig*	52(1)	75-79	2014
Uchida K, Nakata K, Carey B, Chalk C, Suzuki T, Sakagami T, Koch DE, Stevens C, Inoue Y, Yamada Y, Trapnell BC.	Standardized serum GM-CSF autoantibody testing for the routine clinical diagnosis of autoimmune pulmonary alveolar proteinosis	*J Immunol Methods*	402(1-2)	57-70	2014
Arai T, Inoue Y, Sugimoto C, Inoue Y, Nakao K, Takeuchi N, Matsumuro A, Hirose M, Nakata K, Hayashi S.	CYFRA 21-1 as a disease severity marker for autoimmune pulmonary alveolar proteinosis.	*Respirology*	19(2)	246-52	2014

Tazawa R, Inoue Y, Arai T, Takada T, Kasahara Y, Hojo M, Ohkouchi S, Tsuchihashi Y, Yokoba M, Eda R, Nakayama H, Ishii H, Nei T, Morimoto K, Nasuhara Y, Ebina M, Akira M, Ichiwata T, Tatsumi K, Yamaguchi E, Nakata K.	Duration of benefit in patients with autoimmune pulmonary alveolar proteinosis after inhaled granulocyte-macrophage colony-stimulating factor therapy.	*Chest*	145(4)	729-37	2014
中田光	稀少肺難病の医師主導治験を行うに至った経緯と戸惑い	*PHARMSTAGE*	13(10)	37-41	2014
Tobino K, Johkoh T, Fujimoto K, Sakai F, Arakawa H, Kurihara M, Kumasaka T, Koike K, Takahashi K, Seyama K.	Computed tomographic features of lymphangioleiomyomatosis: evaluation in 138 patients	*Eur J Radiol*	84(3)	534-41	2014
Suina K, Hayashi T, Mitani K, Suzuki K, Takahashi K, Seyama K.	What's the role of sirolimus on the treatment of lymphangioleiomyomatosis (LAM): Merely tuning up of LAM-associated dysfunctional lymphatic vessels rather than cytoreduction	*Respir Investig*	52(4)	274-6	2014
Haga T, Kataoka H, Ebana H, Otsuji M, Seyama K, Tatsumi K, Kurihara M.	Thoracic endometriosis-related pneumothorax distinguished from primary spontaneous pneumothorax in females	*Lung*	192(4)	583-7	2014
Murakami A, Hayashi T, Terao Y, Mori T, Kumasaka T, Seyama K, Takahashi K.	Cystic, nodular and cavitary metastases to the lungs in a patient with endometrial stromal sarcoma of the uterus	*Intern Med*	53(9)	1001-5	2014

Kuriyama S, Morio Y, Toba M, Nagaoka T, Takahashi F, Iwakami S, Seyama K, Takahashi K	Genistein attenuates hypoxic pulmonary hypertension via enhanced nitric oxide signaling and the erythropoietin system	*Am J Physiol Lung Cell Mol Physiol*	306(11)	L996-L1005	2014
Kumasaka T, Hayashi T, Mitani K, Kataoka H, Kikkawa M, Tobino K, Kobayashi E, Gunji Y, Kunogi M, Kurihara M, Seyama K.	Characterization of pulmonary cysts in Birt-Hogg-Dubé syndrome: histopathological and morphometric analysis of 229 pulmonary cysts from 50 unrelated patients	*Histopathology*	65(1)	100-10	2014
関谷 充晃, 瀬山 邦明, 高橋 和久	【動悸・息切れ-ヤバい病気の見つけ方　そして見つからなかった時の対処法】 動悸・息切れが主訴の患者に出会ったら 慢性呼吸不全を見落とさないために	総合診療	25(1)	26-29	2015
荒野 直子, 関谷 充晃, 守尾 嘉晃, 長岡 鉄太郎, 瀬山 邦明, 高橋 和久, 鈴木 健司, 植草 利公, 菊池 賢	診断に難渋した肺 Mycobacterium xenopi 症の1例	結核	89(12)	841	2014
江花 弘基, 溝渕 輝明, 岩渕 千雅子, 熊坂 利夫, 瀬山 邦明, 栗原 正利	Bedside Teaching 呼吸器科医が遭遇する Birt-Hogg-Dube 症候群	呼吸と循環	62(11)	1087-1094	2014
瀬山 邦明, 安藤 克利, 江花 弘基	【すぐ役に立つ-呼吸器薬の標準的使い方】　その他の呼吸器疾患 リンパ脈管筋腫症	*Medicina*	51(10)	1926-1929	2014
鈴木 一廣, 飛野 和則, 瀬山 邦明, 桑鶴 良平	【症例の比較で学ぶ画像診断　胸部50選】 びまん性肺疾患　嚢胞性疾患　多発肺嚢胞	画像診断	34(11)	148-151	2014
飛野 和則, 芳賀 高浩, 栗原 正利, 熊坂 利夫, 瀬山 邦明	【ブラ・気胸の診断と読影】 女性気胸の胸部CT画像所見の検討	日本気胸・嚢胞性肺疾患学会雑誌	14(1)	30-34	2014

瀬山　邦明	嚢胞はなぜ生じるのか?	日本気胸・嚢胞性肺疾患学会雑誌	14(1)	2-6	2014
長瀬 隆英, 瀬山 邦明, 平井 豊博, 本間 栄	難病対策の見直しをめぐって	呼吸と循環	33(8)	747-753	2014
鈴木 一廣, 瀬山 邦明, 飛野 和則, 桑鶴 良平	呼吸機能障害を来す病態の画像 嚢胞性肺疾患	呼吸と循環	62(9)	868-872	2014
瀬山　邦明	【内科疾患　最新の治療 明日への指針】(第1章)呼吸器 リンパ脈管筋腫症	内科	113(6)	1039-1040	2014
伊藤 佳奈美, 福田 洋, 矢野 友実子, 安藤 知春, 飯田 典宏, 堀 賢, 瀬山 邦明, 佐藤 裕之	大学教職員におけるヘルスリテラシーの関連因子の解析	*CAMPUS HEALTH*	51(1)	189-191	2014
Ishii H, Seymour JF, Tazawa R, Inoue Y, Uchida N, Nishida A, Kogure Y, Saraya T, Tomii K, Takada T, Itoh Y, Hojo M, Ichiwata T, Goto H, Nakata K.	Secondary pulmonary alveolar proteinosis complicating myelodysplastic syndrome results in worsening of prognosis: a retrospective cohort study in Japan	*BMC Pulmonary Medicine*	in press		2014
Akasaka K, (12名略))Tazawa R, (7名略) Nakata K	A mathematical model to predict protein wash out kinetics during whole-lung lavage in autoimmune pulmonary alveolar proteinosis	*Am J Physiol Lung Cell Mol Physiol*	308(2)	105-17	2014
Hashimoto A, Tanaka T, Itoh Y, Yamagata A, Kitamura N, Tazawa R, Nakagaki K, Nakata K.	Low concentrations of recombinant granulocyte macrophage-colony stimulating factor derived from Chinese hamster ovary cells augments long-term bioactivity with delayed clearance in vitro	*Cytokine*	68(2)	118-26	2014

Tazawa R, (10名略) Ishii H, (8名略) Nakata K.	Duration of benefit in patients with autoimmune pulmonary alveolar proteinosis after inhaled granulocyte-macrophage colony-stimulating factor therapy.	*Chest*	145(4)	729-37	2014
Suzuki M, Makita H, Östling J, Thomsen LH, Konno S, Nagai K, Shimizu K, Pedersen JH, Ashraf H, Bruijnzeel PL, Maciewicz RA, Nishimura M; Hokkaido COPD Cohort Study; Danish Lung Cancer Screening Trial Investigators.	Lower leptin/adiponectin ratio and risk of rapid lung function decline in chronic obstructive pulmonary disease	*Ann Am Thorac Soc*	11(10)	1511-9	2014
Taniguchi N, Konno S, Isada A, Hattori T, Kimura H, Shimizu K, Maeda Y, Makita H, Hizawa N, Nishimura M.	Association of the CAT-262C>T polymorphism with asthma in smokers and the nonemphysematous phenotype of chronic obstructive pulmonary disease.	*Ann Allergy Asthma Immunol*	113(1)	31-36	2014
Konno S, Hizawa N, Makita H, Shimizu K, Sakamoto T, Kokubu F, Saito T, Endo T, Ninomiya H, Iijima H, Kaneko N, Ito YM, Nishimura M; J-Blossom Study Group.	The effects of a Gly16Arg ADRB2 polymorphism on responses to salmeterol or montelukast in Japanese patients with mild persistent asthma	Pharmacogenet Genomics	24(5)	246-55	2014
Kambara K, Shimizu K, Makita H, Hasegawa M, Nagai K, Konno S, Nishimura M.	Effect of lung volume on airway luminal area assessed by computed tomography in chronic obstructive pulmonary disease.	PLoS One	9(2)	e90040	2014
Yoshida T, Nagai K, Inomata T, Ito Y, Betsuyaku T, Nishimura M.	Relationship between neutrophil influx and oxidative stress in alveolar space in lipopolysaccharide-induced lung injury.	*Respir Physiol Neurobiol*	191	75-83	2014

Suzuki M, Makita H, Ito YM, Nagai K, Konno S, Nishimura M; Hokkaido COPD Cohort Study Investigators.	Clinical features and determinants of COPD exacerbation in the Hokkaido COPD cohort study.	*Eur Respir J*	43(5)	1289-97	2014
鈴木　雅	肺の構造・機能解析におけるCTの役割	呼吸と循環	62(8)	7868-92	2014
Sato T, Watanabe A, Kondo H, Kanzaki M, Okubo K, Yokoi K, Matsumoto K, Marutsuka T, Shinohara H, Teramukai S, Kishi K, Ebina M, Sugiyama Y, Meinoshin O, Date H	Japanese Association for Chest Surgery Long-term results and predictors of survival after surgical resection of patients with lung cancer and interstitial lung diseases	*J Thorac Cardiovasc Surg*	149(1)		2014
Ono M, Ohkouchi S, Kanehira M, Tode N, Kobayashi M, Ebina M, Nukiwa T, Irokawa T, Ogawa H, Akaike T, Okada Y, Kurosawa H, Kikuchi T, Ichinose M.	Mesenchymal Stem Cells Correct Inappropriate Epithelial-mesenchyme Relation in Pulmonary Fibrosis Using Stanniocalcin	*Mol Ther*	23(3)	549-60	2014
Sato T, Kondo H, Watanabe A, Nakajima J, Niwa H, Horio H, Okami J, Okumura N, Sugio K, Teramukai S, Kishi K, Ebina M, Sugiyama Y, Kondo T, Date H.	A simple risk scoring system for predicting acute exacerbation of interstitial pneumonia after pulmonary resection in lung cancer patients	*Gen Thorac Cardiovasc Surg*	63(3)	164-72	2014
Sato T, Teramukai S, Kondo H, Watanabe A, _ Ebina M, Kishi K, Fujii Y, Mitsudomi T, Yoshimura M, Maniwa T, Suzuki K, Kataoka K, Sugiyama Y, Kondo T, Date H	Japanese Association for Chest Surgery. Impact and predictors of acute exacerbation of interstitial lung diseases after pulmonary resection for lung cancer.	*J Thorac Cardiovasc Surg*	147(5)	1604-1611	2014

Horimasu Y, Hattori N, Ishikawa N, Tanaka S, Bonella F, Ohshimo S, Guzman J, Costabel U, Kohno N.	Differences in serum SP-D levels between German and Japanese subjects are associated with SFTPD gene polymorphisms	*BMC Med Genet*	15	4	2014
Ohshimo S, Ishikawa N, Horimasu Y, Hattori N, Hirohashi N, Tanigawa K, Kohno N, Bonella F, Guzman J, Costabel U.	Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis	*Respir Med*	108	1031-1039	2014
渡辺　憲太朗	上葉肺線維症	呼吸器内科	25(2)	263-266	2014
Seiya KATO Members: Nobuo NISHIMURA, ShingoTAKANASHI, Hidetoshi IGARI, Tomokazu INAGAKI, Saburo IZUMI, Akira IKARI, Osamu TOKUNAGA, Jiro OKIMOTO, and Kentaro WATANABE	TREATMENT GUIDELINES FOR LATENT TUBERCULOSIS INFECTION	結核	89(1)	21-37	2014
Satoshi Takeda a,c,*, Takanori Akagi a,c, Hiroyuki Miyazaki a, Masaru Kodama a,c, Satoshi Yamamoto b, Takahiro Beppu d, Takashi Nagahama d, Toshiyuki Matsui d, Kentaro Watanabe c, Nobuhiko Nagata	Two patients with new granulomatous lung lesions during treatment of Crohn's disease	*Respiratory Medicine Case Reports*	12	16-18	2014
青山　崇、田中　誠、廣田貴子、石井　寛、藤田昌樹、渡辺憲太朗	両肺の多発性陰影と膿痂疹を呈したLemierre症候群の１例	日本呼吸器雑誌	3(2)	260-264	2014
澤田芳雄、北里裕彦、渡辺憲太朗、岡元昌樹、岩間映二、吉見通洋、岡林　寛、高田昇平、川山智隆、星野友昭	病理組織学的にＵＩＰとＲＢがオーバーラップした喫煙関連間質性肺炎疾患の１例	日本胸部臨床	73(5)	580-586	2014

Harada T, Yoshida Y, Kitasato Y, Tsuruta N, Wakamatsu K, Hirota T, Tanaka M, Tashiro N, Ishii H, Shiraishi M, Fujita M, Nagata N and Watanabe K.	The thoracic cage becomes flattened in the progression of pleuroparenchymal fibroelastosis	*European Respiratory review*	23	263-266	2014
Ishii H, Kushima H, Watanabe K, Kadota J.	Two cases of pulmonary lymphangioleiomyomatosis in postmenopausal women	*Respiratory Investigation.*	52	261-264	2014
Ishi H, Kushima H, Komiya K, Okada F, Watanabe K, Kadota J.	Chest computed tomography findings in patients with angioimmunoblastic T-cell lymphoma	*Respiratory Investigation.*	52	265-268	2014
渡辺憲太朗、柳澤　純、白石武史、藤田昌樹、白石素公、石井　寛、北里裕彦、松本武格、廣田貴子、岩﨑昭憲、竹下盛重、鍋島一樹	肺移植に続発した肺胞蛋白症	*THE LUNG perspectives*	22(3)	230-234	2014
石井　寛、渡辺憲太朗	全身性疾患の肺病変　第5章　他臓器疾患の肺病変「炎症性腸疾患の肺病変」	最新医学・別冊新しい診断と治療のＡＢＣ85	第5章	177-182	2014
渡辺憲太朗	ATS/ERS 2013　IIP分類と今後の課題、ＩＰＰＦＥの臨床と病理・病態	日本胸部臨床	73(11)	1307-1319	2014
Takako Hirota1, Yuji Yoshida1, Yasuhiko Kitasato, Michihiro Yoshimi, Takaomi Koga, Nobuko Tsuruta, Masato Minami, Taishi Harada1, Hiroshi Ishii1, Masaki Fujita1, Kazuki Nabeshima, Nobuhiko Nagata and Kentaro Watanabe	Historogical evolution of pleuroparenchymal fibroelastosis	*Histopathology*	66(4)	545-554	2014
井形文保、石井　寛、平野涼介、廣田貴子、白石素公、渡辺憲太朗	非小細胞癌に対するペメトレキセドによる維持療養中に生じた薬剤性肺炎の2例	*呼吸*	34(2)	199-203	2014

厚生労働科学研究費補助金

難治性疾患等実用化研究事業(難治性疾患実用化研究事業)

リンパ脈管筋腫症に対するシロリムスの安全性確立のための医師主導治験

平成26年度 総括・分担研究報告書

研究成果の刊行物・別刷

(2/2冊)

研究代表者　中田　光

平成27(2015)年3月

厚生労働科学研究費補助金

難治性疾患等実用化研究事業（難治性疾患実用化研究事業）

リンパ脈管筋腫症に対するシロリムスの安全性確立のための医師主導治験

平成２６年度　総括・分担研究報告書
研究成果の刊行物・別刷

(2/2冊)

研究代表者　中田　光

平成２７（２０１５）年3月

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 11 August 2014
doi: 10.3389/fmicb.2014.00410

Novel aspects on the pathogenesis of Mycoplasma pneumoniae pneumonia and therapeutic implications

Takeshi Saraya[1], Daisuke Kurai[1], Kazuhide Nakagaki[2], Yoshiko Sasaki[3], Shoichi Niwa[3], Hiroyuki Tsukagoshi[3], Hiroki Nunokawa[1], Kosuke Ohkuma[1], Naoki Tsujimoto[1], Susumu Hirao[1], Hiroo Wada[1], Haruyuki Ishii[1], Koh Nakata[4], Hirokazu Kimura[5], Kunihisa Kozawa[3], Hajime Takizawa[1] and Hajime Goto[1]*

[1] Department of Respiratory Medicine, Kyorin University School of Medicine, Mitaka, Japan
[2] Department of Virology and Immunology, College of Veterinary Medicine, Nippon Veterinary and Animal Science University, Mitaka, Japan
[3] Gunma Prefectural Institute of Public Health and Environmental Sciences, Maebashi, Japan
[4] Bioscience Medical Research Center, Niigata University Medical and Dental Hospital, Niigata, Japan
[5] Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

Edited by:
Evangelos Giamarellos-Bourboulis, University of Athens, Greece

Reviewed by:
George Dimopoulos, Attikon University Hospital, Greece
Garyfallia Poulakou, Athens University School of Medicine, Greece

*Correspondence:
Hajime Goto, Department of Respiratory Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
e-mail: h510@ks.kyorin-u.ac.jp

Mycoplasma pneumoniae (Mp) is a leading cause of community acquired pneumonia. Knowledge regarding Mp pneumonia obtained from animal models or human subjects has been discussed in many different reports. Accumulated expertise concerning this critical issue has been hard to apply clinically, and potential problems may remain undiscovered. Therefore, our multidisciplinary team extensively reviewed the literature regarding Mp pneumonia, and compared findings from animal models with those from human subjects. In human beings, the characteristic pathological features of Mp pneumonia have been reported as alveolar infiltration with neutrophils and lymphocytes and lymphocyte/plasma cell infiltrates in the peri-bronchovascular area. Herein, we demonstrated the novel aspects of Mp pneumonia that the severity of the Mp pneumonia seemed to depend on the host innate immunity to the Mp, which might be accelerated by antecedent Mp exposure (re-exposure or latent respiratory infection) through up-regulation of Toll-like receptor 2 expression on bronchial epithelial cells and alveolar macrophages. The macrolides therapy might be beneficial for the patients with macrolide-resistant Mp pneumonia via not bacteriological but immunomodulative effects. This exhaustive review focuses on pathogenesis and extends to some therapeutic implications such as clarithromycin, and discusses the various diverse aspects of Mp pneumonia. It is our hope that this might lead to new insights into this common respiratory disease.

Keywords: Mycoplasma pneumoniae pneumonia, animal models, epidemiology, pathology, pathogenesis

INTRODUCTION

Mycoplasma pneumoniae (Mp) was first isolated in tissue culture from the sputum of a patient with primary atypical pneumonia by Eaton et al. (1944). This "Eaton's agent" was shown to be a Mycoplasma species in 1961. Chanock et al. succeeded in culturing Eaton's agent in mammalian cell-free medium and proposed the taxonomic designation Mp in 1963 (Chanock et al., 1962; Chanock, 1963). Mp is a unique organism that lacks a cell wall in any circumstances, and does not need a host cell for replication. This organism causes a variety of clinical presentations, from self-limiting to life-threatening. The disease severity seems to depend on the degree of host's defenses. In this review, we focused on the pathogenesis of Mp pneumonia from the perspective of host defenses, based on findings from our mouse models.

EPIDEMIOLOGY

Mp is one of the most common pathogens of community-acquired pneumonia (CAP) in adults (Table 1). In general, both regional differences and varying periods of surveillance may influence the results of etiological studies of infectious diseases. Table 1 summarizes the proportions of adult Mp pneumonia among CAP populations enrolled in several large-scale studies conducted in various countries (Marston et al., 1997; Ngeow et al., 2005; Arnold et al., 2007; Von Baum et al., 2009; Gilloniz et al., 2011). Mp pneumonia accounted for 10.6–17.0 and 3.0–20.8% of CAP in out- or in-patients settings, respectively, and the frequency of ICU admission was relatively low (2–3.6%). Arnold et al. showed that Mp is the most common atypical pneumonia pathogen, accounting for 11–13% of CAP throughout the world (Arnold et al., 2007). Serological studies in Denmark over a 30-year period showed that Mp infections exhibit epidemic periodicity every 3–5 years, but this trend now seems to be getting obscured (Lind et al., 1997). Mp pneumonia occurs at any age, but the incidence is less common in elderly, as compared with young, adults (Lim et al., 2009), and is highest among school-aged children (Foy et al., 1979).

Macrolides were recommended for treatment of microbiologically defined Mp pneumonia. However, macrolide-resistant

Table 1 | Prevalence of Mycoplasma pneumoniae pneumonia in CAP.

Author	Country	Year	N	Out-Pts%	Ward%	ICU%	Total%	Mortality%
Cilloniz	Spain	1996-2008	1463	17	3	2	4	3.1
Ngeow	Asia	2001-2002	926	ND	ND	3.6	11.4	ND
Baum	German	2002-2006	4532	10.6	4.7		6.8	0.7
Marston	USA	1991	1938	ND	20.8** (5.4*)		20.8* (5.4*)	ND
Arnold	Whole world	2001-2006	4337	ND	12		12	ND

(Marston et al., 1997; Ngeow et al., 2005; Arnold et al., 2007; Von Baum et al., 2009; Cilloniz et al., 2011).

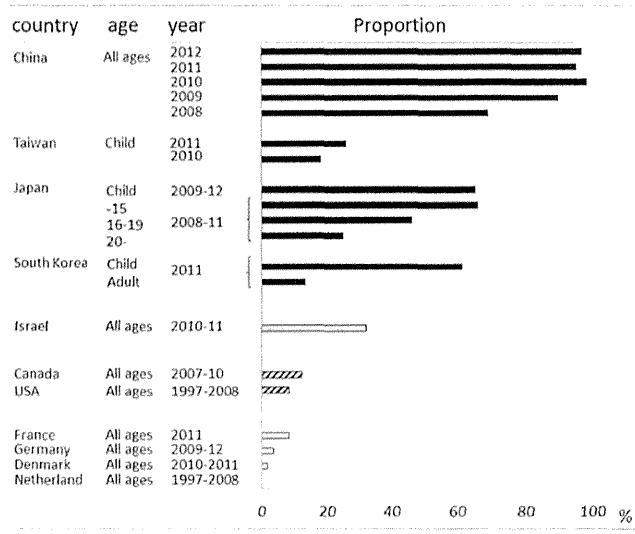
*definite case.

**definitive and possible cases.

N, number; ND, not determined; Out-Pts, out patients.

Mp was isolated from Japanese children, and the incidence was increasing in the early 2000s (Matsuoka et al., 2004). There was a major concern that macrolide-resistant Mp had increased locally and was spreading throughout the world. In East Asia, macrolide-resistant Mp rapidly increased and became the cause of the majority of clinically-proven Mp in both children and adults. The prevalence of macrolide-resistant Mp varies among countries and age groups (Averbuch et al., 2011; Akaike et al., 2012; Miyashita et al., 2012; Spuesens et al., 2012; Uldum et al., 2012; Yamada et al., 2012; Yoo et al., 2012; Dumke et al., 2013; Eshaghi et al., 2013; Pereyre et al., 2013; Wu et al., 2013; Zhao et al., 2013) (Table 2). For example, over 90% of isolated Mp in China was macrolide resistant, while no macrolide-resistant Mp was found in the Netherlands. Generally, it became highly prevalent in East Asian countries including China, Japan and South Korea, while being a medium or low prevalent in North America and Europe, respectively. Macrolide-resistant Mp is reportedly more prevalent in children, and the predominant point mutation found was A2063G in domain V of 23S rRNA. Aside from geographical and racial differences between individual studies, the application of different diagnostic techniques or criteria might affect the epidemiology of Mp pneumonia in each study.

Table 2 | Proportions of macrolide-resistant Mycoplasma pneumonia.



HUMAN PATHOLOGY AND BRONCHOALVEOLAR LAVAGE FLUID

PATHOLOGY

Studies focused on the pathological description of human Mp pneumonia have rarely been reported. However, pathological examinations have been conducted on several different types of specimens that were sampled using different techniques; e.g., autopsy specimens (Parker et al., 1947; Maisel et al., 1967; Benisch et al., 1972; Meyers and Hirschman, 1972; Halal et al., 1977; Kaufman et al., 1980; Koletsky and Weinstein, 1980), open lung biopsy specimens (Coultas et al., 1986; Rollins et al., 1986; Llibre et al., 1997; Ebnother et al., 2001; Wachowski et al., 2003), video-assisted thoracic surgery (VATS) specimens (Chan et al., 1999) and transbronchial lung biopsy specimens (Ganick et al., 1980; Nakajima et al., 1996; Ohmichi et al., 1998). According to these reports, the most characteristic pathological feature of human Mp pneumonia is a marked plasma cell-rich lymphocytic infiltration in the peri-bronchovascular areas (PBVAs), with accumulations of macrophages, neutrophils, and lymphocytes in the alveolar spaces (Parker et al., 1947; Coultas et al., 1986; Rollins et al., 1986). The presence of plasma cells in PBVAs might reflect up-regulation of humoral immunity via Mp infection.

BRONCHOALVEOLAR LAVAGE FLUID (BALF) FINDINGS

There have been several case series focused on BALF obtained from human Mp pneumonia patients (Hayashi et al., 1986, 1993, 1998; Yano et al., 2001); those studies demonstrated varying levels of monocytes, polymorphonuclear leukocytes (PMNs), lymphocytes, eosinophils, and total cell counts. Among them, PMNs and lymphocytes counts were relatively more increased than the other cell types. The CD4 to CD8 ratios in the BALF were also elevated, and ranged from 2.1 (Hayashi et al., 1986) to 3.5 ±2.1 (Hayashi et al., 1993), irrespective of the sampling timing.

PATHOGENESIS

ANIMAL MODELS

The incidence of Mp pneumonia is relatively low among the elderly over 70 years old or children less than 5 years old. This led to the hypothesis that elderly persons must be repeatedly exposed to and respond immunologically to the organism with clinical or subclinical progression. Indeed, as for cellular immunity, Brunner et al. have suggested that the occurrence of clinical

disease in adults is favored by prior sensitization induced by infection at an early age, causing large or small mononuclear cell reactions (Brunner et al., 1973). This cellular response, lasting several years, could be proved by Mp antigen-induced lymphocyte transformation of cell suspensions from previously infected patients (Biberfeld et al., 1974; Biberfeld, 1974). It is important for us to understand immune responses attributed to Mp pneumonia.

We designed five different mouse models for Mp pneumonia (Figure 1) to examine the resulting pathology in animals having various immune status (Saraya et al., 2007b, 2011; Saraya, 2013). Animals were peritoneally immunized with various regimens (one per model) once a week (on days −14 and −7), then 1 week after the last immunization the animals were intratracheally (IT) challenged with sonicated Mp antigen, as previously reported (Saraya et al., 2011). Among those models, only groups immunized with Mp antigen and alum adjuvant (Figure 1E) or CpG (Figure 1C) developed severe lymphocytic infiltration into PBVAs at 96 h after IT (Figures 2C,E) while, no inflammatory cells were seen on models A and B (Figures 2A,B). However, the pathognomonic feature for human Mp pneumonia was reconstructed only in models D and E, in which lymphoplasmacytic infiltration into PBVAs occurred 96 h post-IT (Figures 2D,E). Those results suggest that enhanced host immune responses, as occurred in models C and E, against Mp antigen are required for persistent inflammation in the lung, as well as Th2 characteristics (produced by use of Th2 adjuvant, as in models D and E) causing plasma cell infiltration into the PBVAs, but not Th1 characteristics (produced by use of the Th1 adjuvant, CPG, as in the model depicted in Figure 1C). Aluminum hydroxide adjuvant, named alum, is well-known for initiating strong antigen-specific Th2 responses in the absence of interleukin(IL)-4- or IL-13-mediated signaling (Brewer et al., 1999); Th2 predominant characteristics might be required to generate typical Mp pneumonia, even in humans. Previous studies showed that the histopathological score of Mp pneumonia is significantly higher in infected BALB/c mice (Th2 predominant) than in C57BL/6 mice (Th1 predominant) through the late phase, suggesting differences in host reactions against intranasally-inoculated live Mp (Fonseca-Aten et al., 2005). Tanaka et al. (1996) describe the different pathological findings in an M. pulmonis-infected mouse model for treatment with IL-2 (Th1 up-regulated) vs. cyclosporine A (Th1 down-regulated).

Thus, the severity of Mp pneumonia seems to depend on the host immune response to the infection through a complexity of various mechanisms, including an allergic reaction to Mp, Mp virulence, host defenses, and polarization toward Th1 or Th2 predominance, to name a few. In the context of allergic reaction, IgE antibodies specific to Mp were detected in serum samples from patients with Mp pneumonia, suggestive of IgE-mediated hypersensitivity (Tipirneni et al., 1980; Yano et al., 1994; Seggev et al., 1996) as well as an involvement in asthma attacks (Henderson et al., 1979; Biscardi et al., 2004). In this review, we will further discuss the pathomechanisms of Mp pneumonia from the perspective of the virulence of Mp and presumed host defenses based on findings obtained from our experimental mouse models.

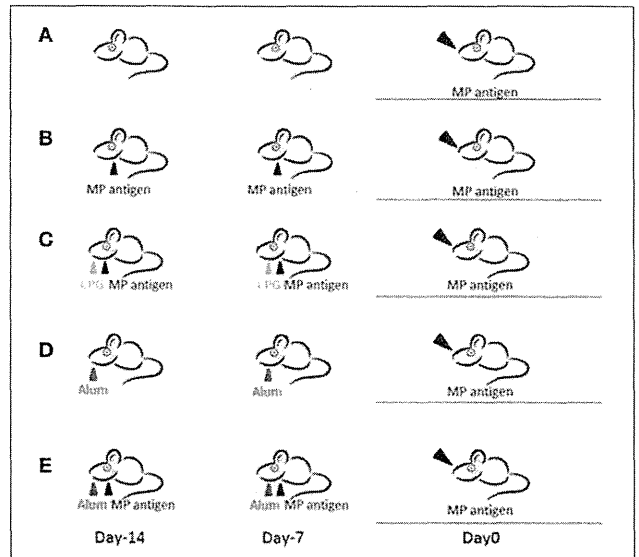


FIGURE 1 | Female BALB/c (7-week-old) mice were inoculated intratracheally with Mp antigen with or without pre-immunization. Pre-immunization was carried out by intraperitoneal injection at 7 and 14 days prior to the intratracheal (IT) challenge. Model A: IT without pre-immunization. Model B: IT after twice pre-immunizing with Mp antigen alone. Model C: IT after twice pre-immunizing with Mp antigen plus CpG. Model D: IT after twice pre-immunizing with alum alone. Model E: IT after twice pre-immunizing with Mp antigen plus alum. One week following the last immunization, mice underwent IT with 50 μg of Mp antigen. Bronchoalveolar lavage fluid (BALF) and lung specimens were obtained before this process and 8, 24, 48, 96, and 168 h after IT. Mp: mycoplasma.

VIRULENCE OF MP

Lipoproteins

Lipoproteins from various Mycoplasma species have potent inflammatory properties. Three lipoproteins/lipopeptides of M. fermentans origin, macrophage-activating lipopeptide-2 (MALP-2), P48, and M161Ag (identical to MALP-404), reportedly modulate the host immune system via Toll-like receptor (TLR)-2/TLR-6 signaling (Takeuchi et al., 2000; Luhrmann et al., 2002; Seya and Matsumoto, 2002). Genes for more than 30 different Mp lipoproteins have been reported (Himmelreich et al., 1997). Shimizu et al. reported that the mycoplasma-derived lipoproteins N-ALP1/N-ALP2 (Shimizu et al., 2008) and F_0F_1-ATPase (Shimizu et al., 2005) activated NF-κβ via TLR-1, 2 or TLR-1, 2, 6 signaling, respectively. Stimulation of these TLRs has been known to be related to production of chemokines (Brant and Fabisiak, 2008; Andrews et al., 2013) that promote lymphocyte and neutrophil trafficking and inflammation in the lung.

CARDS (Community Acquired Respiratory Distress Syndrome) toxin

Kannan et al. first demonstrated the possibility that Mp produces the CARDS toxin that is involved in the mediation of disease (Kannan et al., 2005). The CARDS toxin is an ADP-ribosylating and vacuolating toxin, with homology to the S1 subunit of pertussis toxin, that has a high affinity for surfactant protein-A, suggesting a physiological role for the toxin in the pulmonary compartment. In mice, intranasal inoculation

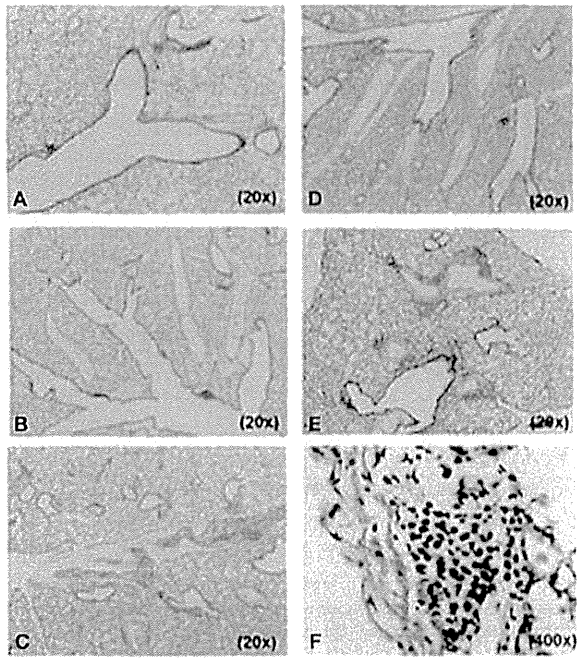


FIGURE 2 | Histopathological examination of lung specimens. At 96 h post-intratracheal (IT) challenge, no inflammatory cells were seen in model (A) A or (B) B specimens. Mild to moderate lymphocyte infiltration was observed in the peribronchovascular area (PBVA) of (D) model D tissue. Models (C) C and (E) E had more severe lymphocyte infiltration in the PBVA. Plasmacyte infiltration within the PBVA was only recognized in models D and E, and the number of infiltrating plasmacytes was significantly higher in model E than model D. (F) High power magnification of E. Hematoxylin and eosin stain. A-F, 200x; E, 400x.

of recombinant CARDS toxin caused an increased level of pro-inflammatory cytokines IL-1α 1β 6, 12, 17, Tumor necrosis factor(TNF)-α and Interferon-gamma (IFN) -γ together with elevation of Keratinocyte chemoattractant(KC), IL-8, regulated on activation, normal T cell expressed and secreted (RANTES), and G-CSF (Hardy et al., 2009). However, to our knowledge, there have been no reports of CARDS toxin identified in human respiratory specimens.

Other factors

Mp produces a soluble hemolysin (Somerson et al., 1963, 1965), hydrogen peroxide and superoxide radicals, which produce oxidative stress in the respiratory epithelium, resulting in both structural and functional deterioration of cilia (Waites and Talkington, 2004). Stimulation of human respiratory epithelial cells (A549 cells) in vitro with Mp lysate (MPL) induced IL-8 production (Sohn et al., 2005). MPL induced IL-8 release in a time- and dose-dependent manner together with activation of extracellular signal-regulated kinase (ERK), which was inhibited by PD98059, a specific inhibitor of ERK. Chmura et al. (2003) reported that the Mp membrane fraction induced IL-8 on BEAS-2B human bronchial epithelial cells. Our report (Hirao et al., 2011) also demonstrated activation of mitogen-activated protein kinase (MAPKs) on the alveolar macrophage-like cell line, RAW264.6, by stimulation with Mp antigen, as confirmed by significant suppression of IL-6 and TNF-α production after preceding treatment with an MAPKs inhibitor such as parthenolide (PAR: NF-kB inhibitor), SB20580 (SB, p38-linked signal of inhibitor), or LY294002 (LY, PI-3K inhibitor). Thus, Mp antigen or live Mp can induce inflammatory cytokines in bronchial epithelial cells and in alveolar macrophages (AMs).

HOST DEFENSES

Cellular immunity

Biberfeld et al. reported that the peripheral lymphocyte response to a sonicate of Mp organisms or a membrane fraction was significantly higher in recently infected patients than in healthy patients (Biberfeld et al., 1974). The positive responsiveness to sonicated Mp antigen was demonstrable up to 10 years after infection. Others also reported on the in vitro response of human peripheral lymphocytes to Mp antigen (Fernald, 1972; Biberfeld, 1974), while tuberculin anergy in patients with Mp pneumonia was noted soon or fairly soon after onset. This has been speculatively explained by the possibility that (1) lymphocytes and macrophages needed for the skin reaction to tuberculin are engaged in the immune response to the infecting agent, or (2) a transient change of the T lymphocyte population occurs (Biberfeld and Sterner, 1976). Tanaka et al. reported that the rate of positive tuberculin tests during the acute stage of Mp pneumonia was higher in patients with the nodular type of pulmonary lesions on thoracic computed tomography than those having the consolidation pattern. This finding suggests that the level of current cell-mediated immunity might influence the pattern of pulmonary lesions. Another study showed that delayed hypersensitivity was noted on skin testing with Mp antigen of patients with Mp pneumonia (Mizutani et al., 1971).

However, to our knowledge, no direct evidence from patients with Mp pneumonia has been reported regarding the reactivity of BALF lymphocytes to Mp antigen. In other words, it is still under debate whether the lung inflammation of Mp pneumonia is a specific reaction to the Mp antigen.

In consideration of this question, Saraya et al. (2011) demonstrated a lack of specific response of lymphocytes in the BALF to Mp antigen 96 h post-IT using the 3H-thymidine uptake test in an Mp pneumonia mouse model (Figures 1D,E). The BALF cells in the lymphocyte gate were 35.8% CD3 positive and 57.6% CD3 negative. Among the CD3 positive cells, CD4−/CD8− cells were predominant. The CD4 to CD8 ratio was 0.02, which was a lower value than that of human Mp pneumonia patients (Hayashi et al., 1986, 1993), and the CD8 positive cells consisted of naïve cells (CD62L+ ^{hi}/ CD44+ ^{lo}), effector memory cells (CD62L+ ^{lo}/CD44+ ^{hi}), and central memory cells (CD62L+ ^{hi}/CD44+ ^{hi}), in that order (Saraya et al., 2007a) (Figure 3). Cellular immunity seemed to play an important role in development of Mp pneumonia (Foy et al., 1973; Broughton, 1986); the results given above might indicate that non-specific reactions to Mp antigen govern the severity of lung inflammation.