度で検出されており、小児期を通じて肺炎の病原微生物として重要な役割を果たしているものと考える。

各月群別の検出状況に関しても、インフルエンザ菌は各群の症例より検出されており、インフルエンザウイルス、パラインフルエンザウイルス、RS ウイルスなどの呼吸器ウイルスのような検出率の季節変動は観察されなかった。

細菌性肺炎症例に関して、混合感染例を含めた場合、インフルエンザ菌および肺炎球菌による感染症例の平均年齢、経過中の最高白血球数・好中球数値、最高 CRP 値、発熱期間は類似していた。これは、細菌性肺炎を診療する際の、臨床現場における一般的な認識とも矛盾しないものと考える。一方、単独感染症例については、インフルエンザ菌感染症例の方が、肺炎球菌感染症例より、平均年齢、最高白血球数・好中球数値、CRP 値がやや低い傾向にあったが、統計学的な有意差は得られなかった。さらに、単独感染症例についてのみ検討すると、インフルエンザ菌による症例は *M. pneumoniae* による症例に比べて、経過中の最高白血球数が有意に高値であった。これは、従来より細菌性肺炎と非定型肺炎との鑑別項目として指摘されている項目でもある。

今回検出されたインフルエンザ菌の中で、ABPC 耐性は 10％、中等度耐性は 18％を占め、薬剤耐性状況は今回明らかとなった多種の呼吸器病原性微生物の混合感染とともに、臨床経過に大きな影響を及ぼすものと推察される。今後とも、インフルエンザ菌の薬剤耐性状況ならびに呼吸器病原性微生物の中でのインフルエンザ菌の役割について、経時的に検討していく必要があるものと考える。

E. 結論

インフルエンザ菌は、小児肺炎の原因微生物として最も多く 35.5％で検出されたが、他の微生物との混合感染例も多く、単独で原因微生物と判定されたものは、16 例（10.3％）であった。単独感染症例についてのみ検討すると、インフルエンザ菌による症例は *M. pneumoniae* による症例に比べて、有意に年齢が低く、経過中の最高白血球数は有意に高値であった。

F. 健康危険情報

特記すべきことなし

G. 研究発表

1. 論文発表

1）上原すゞ子、江口博之、尾内一信、岡田賢司、黒崎知道、砂川慶介、春田恒和、満田年宏、山崎　勉：小児呼吸器感染症診療ガイドライン. 日本小児呼吸器疾患学会雑誌, 16: 46, 2005.

2）Yamazaki, T., Kishimoto, T., Shiga, S., Sato, K., Hagiwara, T., Inoue, M., Sasaki, N., Ouchi, K., Hara, Y.: Biosynthesized tea polyphenols inactivate *Chlamydia trachomatis* in vitro. Antimicrob. Agents Chemother., 49: 2501-2503, 2005.

3）Yamano, H., Yamazaki, T., Sato, K., Shiga, S., Hagiwara, T., Ouchi,

K., Kishimoto, T. : In vitro inhibitory effects of hinokitiol on proliferation of *Chlamydia trachomatis*. Antimicrob. Agents Chemother., 49: 2519-2521, 2005.

4) Murayama, K., Yamazaki, T., Ito, A, Uehara, S, Sasaki, N. : Simplified semiquantitative culture using washed sputum from children with lower respiratory tract infections. J. Clin. Pathol., 58: 896, 2005

5) Yamazaki, T., Hagiwara, T., Kishimoto, T., Sasaki, N., Takahashi, S., Ishihara, O., Wangroongsarb, P., Kusum, M., Sirivongrangsan, P. Distribution of *Chlamydia trachomatis* serovars among female prostitutes and non-prostitutes in Thailand, and non-prostitutes in Japan during the mid-90s. Jpn. J. Infect. Dis. 58: 211-213, 2005.

6) Yamaguchi, T., Hashikita, G., Takahashi, S., Itabashi, A., Yamazaki, T., Maesaki, S. In vitro activity of β-lactams, macrolides, telithromycin, and fluoroquinolones against clinical isolates of *Streptococcus pneumoniae*: correlation between drug resistance and genetic characteristics. J. Infect. Chemother. 11: 262-264, 2005.

7) Suzuki, S., Yamazaki, T., Narita, M., Okazaki, N., Suzuki, I., Andoh, T., Matsuoka, M., Kenri, T., Arakawa,

Y., Sasaki, T. Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*. Antimicrob. Agents Chemother., 50: 709-712, 2006.

8) Yamazaki, T., Yamaguchi, T., Inoue, M., Ohtake, A., Sasaki, N., Inoue, M., Sato, K., Ouchi, K., Kishimoto, T. The inhibitory effect of antihyperlipidemic drugs on the growth of *Chlamydia pneumoniae in vitro*. J. Chemother., 18: 107-109, 2006.

9) 橋北義一、山口敏行、舘　良美、岸　悦子、河村　亨、高橋　俊、新井幸枝、小山幸枝、古旗敏文、板橋　明、岡　陽子、山崎　勉、前崎繁文. 肺炎患者における尿中肺炎球菌莢膜抗原迅速検出キットの有用性に関する検討. JARMAM, 16: 153-161, 2005.

１０）山崎　勉：小児に対する抗菌薬投与の注意点. Medical Practice, 22: 2129-2133, 2005.

１１）山崎　勉：感染制御と教育 1. 医療関係者への卒前教育. 小児科臨床, 58: 2559-2569, 2005.

１２）山崎　勉：上気道炎―咽頭炎・扁桃炎、急性喉頭蓋炎―. 小児科診療, 68: 2337-2342, 2005.

１３）山崎　勉：中枢神経系感染症. より良い感染症診療をめざして-中枢神経系感染症・眼科領域感染症-. (菅野治重、相原雅典、監修), p.15-18, ライフ・サイエンス, 東京, 2005.

１４）Yamazaki, T., Itoh, A., Uehara, S., Sasaki, N., Kishimoto, T., Izutsu, H., Kawagoe, K., Mashida, C.

Detection of *Chlamydia pneumoniae* by polymerase chain reaction method from sputum of children with respiratory infections. *In* Proceedings of the Third joint auspices of Japan Society for Chlamydia Research and Department of Pathobiology, University of Washington (Edited by Kishimoto T., Yamazaki, T., Kuo, CC.), p.22-24, Life Science Co., Ltd., Tokyo, 2005.

１５） Ouchi, K., Kamesaki, S., Yamazaki, T., Ogawa, M., Shiga, S., Kishimoto, T. Short-term effect of azithromycin on severity of asthma attacks in pediatric patients. *In* Proceedings of the Third joint auspices of Japan Society for Chlamydia Research and Department of Pathobiology, University of Washington (Edited by Kishimoto T., Yamazaki, T., Kuo, CC.), p.25-27, Life Science Co., Ltd., Tokyo, 2005.

１６） Yamazaki, T., Yamaguchi, T., Sasaki, N., Inoue, M., Sato, K., Kishimoto, T. The inhibitory effect of disodium cromoglycate on the growth of *Chlamydophila* (*Chlamydia*) *pneumoniae in vitro*. Biol. Pharm. Bull., (in press)

１７） Kobayashi, N., Yamazaki, T., Maesaki, S.: Bacteriological monitoring of water reservoirs in oxygen humidifiers: safety of prolonged and multipatient use of prefilled disposable oxygen humidifier bottles for up to 84 days. Infection Control and Hospital Epidemiology, (in press)

2. 学会発表

１）舘　良美、橋北義一、高橋　俊、板橋　明、岡　陽子、山口敏行、山崎勉、前崎繁文：肺炎球菌尿中抗原迅速診断キットの有用性に関する検討. 第７９回日本感染症学会総会、2005 年 4 月 14-15 日、名古屋

２）鈴木里和、大屋日登美、岡崎則男、山崎　勉、成田光生、荒川宣親、佐々木次男：*Mycoplasma pneumoniae* 感染症におけるマクロライド系抗菌薬の治療効果、マクロライド耐性菌と感受性菌との比較. 第７９回日本感染症学会総会、2005 年 4 月 14-15 日、名古屋

３）岡　陽子、河村　亨、山口敏行、橋北義一、山崎　勉、前崎繁文：埼玉医科大学病院における肺炎球菌の薬剤感受性. 第 53 回日本化学療法学会総会、2005 年 5 月 26-27 日、東京

４）松本哲朗、中浜　力、岡田　淳、朝野和典、村谷哲郎、青木洋介、前崎繁文、山崎　勉、石井良和、岩田　敏、安岡　彰、尾内一信、橋本章司、中森祥隆、水谷　哲、平潟洋一、草野展周、石田　直、田口善夫、斉藤　厚：全国ネットワークによる薬剤感受性サーベイランス実施の試み. 第 54 回日本感染症学会東日本地方会、第 52 回日本化学療法学会東日本支部総会、2005 合同学会、2005 年 10 月 27-28 日、東京

5）伊東敦子、山崎　勉、熊倉理恵、上原すゞ子、佐々木望、前崎繁文：小児の喀痰由来 *H. influenzae* および *S. pneumoniae* の薬剤感受性および耐性遺伝子の検出について．第７８回日本感染症学会総会、平成１６年４月６日、東京

6）伊東敦子、山崎　勉、上原すゞ子、佐々木望、前崎繁文、橋北義一、板橋明：インフルエンザ菌による肺炎患児の臨床的検討．第５３回日本感染症学会東日本地方会総会、平成１６年１０月２１日、新潟

3．対談

1）尾内一信、岩田　敏、山崎　勉、古村　速．外来小児科診療における耐性菌の現状と対策．INFECTION FRONT, 5:31-35, 2005

E．知的財産権の出願・登録状況
（予定を含む）
なし

表1．喀痰より検出された呼吸器病原微生物

病原性微生物	検出数 （%）	単独感染数
細菌	76 (49.7)*	25*
Haemophilus influenzae	54 (35.3)	16
Streptococcus pneumoniae	33 (21.6)	8
Moraxella catarrhalis	3 (2.0)	1
Streptococcus pyogenes	1 (0.7)	0
Mycoplasma pneumoniae	45 (29.4)	23
Chlamydophila pneumoniae	17 (11.1)	2
ウイルス	36 (23.5)*	12*
Influenza virus A	6 (3.9)	4
Influenza virus B	0 (0)	0
Influenza virus C	1 (0.7)	0
Respiratory syncytial virus A	3 (2.0)	0
Respiratory syncytial virus B	3 (2.0)	0
Parainfluenza virus type 1	3 (2.0)	0
Parainfluenza virus type 2	0 (0)	0
Parainfluenza virus type 3	6 (3.9)	2
Parainfluenza virus type 4	2 (1.3)	0
Rhinovirus	12 (7.8)	1
Coronavirus	0 (0)	0
Adenovirus	21 (13.7)	5

*；検出症例数を示す

表2．各年齢群別の微生物検出状況

	年齢				
	< 1	1-2	3-5	6-12	13≦
検体数	20	59	50	22	2
微生物					
H. influenzae	2 (10)	30 (51)	19 (38)	3 (14)	0 (0)
S. pneumoniae	4 (20)	16 (27)	12 (24)	1 (5)	0 (0)
M. catarrhalis	0 (0)	2 (3)	1 (2)	0 (0)	0 (0)
M. pneumoniae	3 (15)	9 (15)	16 (32)	17 (77)	0 (0)
C. pneumoniae	4 (20)	6 (10)	5 (10)	2 (9)	0 (0)
Influenza virus A	2 (10)	4 (7)	0 (0)	0 (0)	0 (0)
RS virus	1 (5)	3 (5)	2 (4)	0 (0)	0 (0)
Parainfluenza virus	2 (10)	2 (3)	3 (6)	4 (18)	0 (0)
Rhinovirus	1 (5)	9 (15)	1 (2)	1 (5)	0 (0)
Adenovirus	4 (20)	9 (15)	6 (12)	1 (5)	1 (50)

（　）は、各年齢群の中で各微生物が検出された割合（％）を示す。

表 3. 各月群別の微生物検出状況

| | 月群 | | | |
	1～3 月	4～6 月	7～9 月	10～12 月
検体数	29	43	31	50
微生物				
H. influenzae	9 (31)	18 (42)	11 (35)	7 (14)
S. pneumoniae	4 (14)	9 (21)	5 (16)	9 (18)
M. pneumoniae	6 (21)	9 (21)	13 (42)	17 (34)
C. pneumoniae	1 (3)	5 (12)	0 (0)	3 (6)
Influenza virus A	4 (14)	0 (0)	0 (0)	2 (4)
RS virus	2 (7)	0 (0)	0 (0)	4 (8)
Parainfluenza virus	0 (0)	6 (14)	4 (13)	1 (2)
Rhinovirus	2 (7)	5 (12)	2 (6)	3 (6)
Adenovirus	3 (10)	6 (14)	4 (13)	8 (16)

（　）は、各月群の中で各微生物が検出された割合（%）を示す。

表4. 各微生物による感染症例の平均年齢、入院症例の比率、経過中の最高白血球数、最高CRP値、有熱日数の比較（混合感染症例を含む）

微生物	検出数	平均年齢 (Y)	入院症例の比率(%)	白血球数	好中球数	CRP (mg/dl)	発熱期間 (日)
H. influenzae	54	2.52	63	11,239	6,367	2.81	4.47
S. pneumoniae	33	2.23	55	13,144	7,196	2.36	4.26
M. pneumoniae	47	5.12	81	8,736	5,395	1.68	5.50
C. pneumoniae	17	2.33	71	12,421	6,294	2.08	4.19
Influenza virus A	6	1.18	57	11,181	5,776	2.67	5.00
RS virus	6	2.46	50	11,315	6,266	1.07	3.00
Parainfluenza virus	11	4.20	45	10,503	5,196	1.37	4.10
Rhinovirus	12	2.08	75	12,184	5,488	2.92	4.92
Adenovirus	21	2.81	57	13,743	7,949	2.27	3.85

表5. 各微生物による感染症例の平均年齢，経過中の最高白血球数・好中球数値，最高 CRP 値，有熱日数の比較（単独感染症例のみ）

微生物	検出数	平均年齢 (Y)	白血球数	好中球数	CRP (mg/dl)	発熱期間 (日)
H. influenzae	16	1.85[a]	10,912[d]	5,501	2.07	4.13
S. pneumoniae	8	3.75[b]	13,507	8,261	3.88	3.63
M. pneumoniae	23	6.01[a,c]	7,567[d]	4,684	1.54	5.48
C. pneumoniae	2	0.38	18498	9,911	3.76	7.00
Influenza virus A	4	1.17[b,c]	10.609	4,733	3.39	6.00
RS virus	0	—	—	—	—	—
Parainfluenza virus	2	3.50	11,124	6,295	2.84	6.00
Rhinovirus	1	8.00	12,100	1,0660	2.75	1.00
Adenovirus	5	3.40	16,729	9,869	7.40	4.20

—; 検体なし
a, b, c, d; p<0.05

Ⅲ. 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
詳細は下記参照	詳細は下記参照	佐々木次雄	図　説 呼吸器系細菌感染症：疫学、診断、治療	（株）じほう	東京	2006年5月	約300ページ

監修者
　　　荒川宜親（国立感染症研究所　細菌第二部）
　　　渡辺治雄（国立感染症研究所　細菌第一部）

編集委員会
　　　佐々木次雄（国立感染症研究所　細菌第二部）
　　　高橋元秀（国立感染症研究所　細菌第二部）
　　　堀内善信（国立感染症研究所　細菌第二部）
　　　山下和予（国立感染症研究所　感染症情報センター）
　　　和田昭仁（国立感染症研究所　細菌第一部）

執筆者
　　　百日咳菌
　　　　　　大塚正之（江東微生物研究所）
　　　　　　岡田賢司（国立病院機構福岡病院　小児科）
　　　　　　蒲地一成（国立感染症研究所　細菌第二部）
　　　　　　中野貴司（国立病院機構三重病院　小児科）
　　　　　　堀内善信（国立感染症研究所　細菌第二部）
　　　ジフテリア菌
　　　　　　岩城正昭（国立感染症研究所　細菌第二部）
　　　　　　小宮貴子（国立感染症研究所、細菌第二部）
　　　　　　高橋元秀（国立感染症研究所、細菌第二部）
　　　肺炎マイコプラズマ
　　　　　　岡崎則男（神奈川県衛生研究所　微生物部）
　　　　　　見理　剛（国立感染症研究所　細菌第二部）
　　　　　　佐々木次雄（国立感染症研究所　細菌第二部）

成田光生(札幌鉄道病院　小児科)

インフルエンザ菌

荒川宜親(国立感染症研究所　細菌第二部)

肺炎クラミジア

井上美由紀(埼玉医科大学　小児科)

岸本寿男(国立感染症研究所　ウイルス第一部)

佐藤　梢(国立感染症研究所　ウイルス第一部)

山口徹也(埼玉医科大学　小児科)

山崎　勉(埼玉医科大学　小児科)

レジオネラ菌

倉　文明(国立感染症研究所　細菌第一部)

常　彬(国立感染症研究所　細菌第一部)

前川純子(国立感染症研究所　細菌第一部)

肺炎球菌

和田昭仁(国立感染症研究所　細菌第一部)

A 群溶血レンサ球菌

池辺忠義(国立感染症研究所　細菌第一部)

結核菌

慶長直人(国立国際医療センター　研究所　呼吸器疾患研究部)

小林信之(国立国際医療センター病院　呼吸器科)

山崎利雄(国立感染症研究所　ハンセン病研究センター　病原微生物部)

山本三郎(国立感染症研究所　細菌第二部)

モラキセラ・カタラーリス

黒崎知道(千葉市立海浜病院　小児科)

髄膜炎菌

高橋英之(国立感染症研究所　細菌第一部)

付属資料

病原体検出情報システム

山下和予(国立感染症研究所　感染症情報センター)

小児呼吸器感染症治療ガイドライン

上原すゞ子(千葉大学・埼玉医科大学小児科)

病原体の入手方法

佐々木次雄(国立感染症研究所　細菌第二部)

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
佐々木次雄	1) マクロライド耐性マイコプラズマ感染症に関する研究.	Jpn. J. Antibiotics	58	133-137	2005
	2) Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*.	Antimicrob Agents Chemother	50	709-712	2006
	3) Epidemiological studies on *Bartonella quintana* infections among homeless peoples in Tokyo, Japan.	Jpn. J. Infect. Dis.	59	31-35	2006
	4) Genetic and Phenotypic characterization of *Haemophilus influenzae* type b isolated from children with meningitis and their family members in Vietnam.	Jpn. J. Infect. Dis.	59	(in press)	2006
堀内善信	1) Development and evaluation of loop-mediated isothermal amplification method for rapid diagnosis of *Bordetella pertussis* infection.	J.Clin. Microbiol.		(in press)	2006
見理　剛	1) Involvement of P1 adhesin in gliding motility of *Mycoplasma pneumoniae* as revealed by the inhibitory effects of antibody under optimized gliding conditions.	J. Bacteriol	187	1875-1877	2005
	2) ワンポイント臨床細菌学 「マイコプラズマ属」	感染と抗菌薬	8	334-336	2005
	3) Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*.	Antimicrob Agents Chemother	50	709-712	2006
荒川宜親	1) マクロライド耐性マイコプラズマ感染症に関する研究.	Jpn. J. Antibiotics	58	133-137	2005
	2) Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*.	Antimicrob Agents Chemother	50	709-712	2006
	3) Epidemiological studies on *Bartonella quintana* infections among homeless peoples in Tokyo, Japan.	Jpn. J. Infect. Dis.	59	31-35	2006
	4) Genetic and Phenotypic characterization of *Haemophilus influenzae* type b isolated from	Jpn. J. Infect. Dis.	59	(in press)	2006

		children with meningitis and their family members in Vietnam.				
菊池　賢	1)	Prospective surveillance of community-onset and healthcare-associated methicillin-resistant Staphylococcus aureus isolated from a university-affiliated hospital in Japan.	Microbiol. Immunol.	49	959-970	2005
	2)	Detection of new methicillin-resistant Staphylococcus aureus clones containing the toxic shock syndrome toxin 1 gene responsible for hospital- and community-acquired infections in France.	J. Clin. Microbiol.	44	847-853	2006
成田光生	1)	Cytokines involved in CNS manifestations caused by Mycoplasma pneumoniae.	Pediatr Neurol	33	105-109	2005
	2)	マクロライド耐性マイコプラズマ感染症に関する研究.	Jpn. J. Antibiotics	58	A133-137	2005
	3)	小児期マイコプラズマ感染症診断におけるマイコプラズマ特異的 IgG, IgA, IgM 抗体検出 enzyme- linked immunosorbent assay キットの有用性に関する検討.	感染症学雑誌	79	457-463	2005
	4)	マイコプラズマ肺炎－診断と耐性菌に関する話題を中心に－	日本胸部臨床	64	778-786	2005
	5)	「せき」のある子供の急性感染症.	日本医事新報	4255		2005
	6)	気道系，呼吸器系のマイコプラズマの現況	感染と抗菌薬	8	386-392	2005
			クリニカ	32	333-338	2005
	7)	重症マイコプラズマ肺炎	Antimicrob	50	709-712	2006
	8)	Clinical evaluation of macrolide-resistant Mycoplasma pneumoniae	Agents Chemother	医学書	163	2006
	9)	マイコプラズマ感染症	今日の治療指針 2006年版	院		
山崎　勉	1)	In vitro activity of β-lactams, macrolides, telithromycin, and fluoroquinolones against clinical isolates of Streptococcus pneumoniae: correlation between drug resistance and genetic characteristics.	J. Infect. Chemother.	11	262-264	2005
	2)	Epidemiology of community-acquired	Pediatrics	2006/3/2	517	2006

		pneumonia in children.				
	3)	Clinical evaluation of macrolide-resistant *Mycoplasma pneumoniae*	Antimicrob Agents Chemother	50	709-712	2006
	4)	Genetic and Phenotypic characterization of *Haemophilus influenzae* type b isolated from children with meningitis and their family members in Vietnam.	Jpn. J. Infect. Dis.	59	(in press)	2006

IV. 研究成果の刊行物・別冊

Involvement of P1 Adhesin in Gliding Motility of *Mycoplasma pneumoniae* as Revealed by the Inhibitory Effects of Antibody under Optimized Gliding Conditions

Shintaro Seto,[1]† Tsuyoshi Kenri,[2] Tetsuo Tomiyama,[3] and Makoto Miyata[1,4]*

Department of Biology, Graduate School of Science, Osaka City University,[1] and PRESTO, JST,[4] Sumiyoshi-ku, Osaka, and Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Musashimurayama,[2] and Tomiyama Laboratories, Nerima-ku,[3] Tokyo, Japan

To examine the participation of P1 adhesin in gliding of *Mycoplasma pneumoniae*, we examined the effects of an anti-P1 monoclonal antibody on individual gliding mycoplasmas. The antibody reduced the gliding speed and removed the gliding cells from the glass over time in a concentration-dependent manner but had only a slight effect on nongliding cells, suggesting that the conformational changes of P1 adhesin and its displacement are involved in the gliding mechanism.

Mycoplasma gliding. Mycoplasmas are parasitic, occasionally pathogenic, small-genome bacteria lacking a peptidoglycan layer (20). Several mycoplasma species, including *Mycoplasma pneumoniae*, *M. genitalium*, *M. pulmonis*, *M. gallisepticum*, and *M. mobile*, have distinct cell polarity and exhibit gliding motility in the direction of the tapered end (2, 10, 13). The mechanisms underlying gliding motility are intrinsically different from those of other motility systems and are not well understood (8, 12–15, 26).

M. pneumoniae **and P1 adhesin.** *M. pneumoniae*, a human pathogen, forms a membrane protrusion, an attachment organelle, at a cell pole (11, 13). The cell surface of the attachment organelle exhibits clustering of a 170-kDa transmembrane protein, P1 adhesin, which is responsible for binding to animal cells and glass surfaces (4, 6, 19, 24). It shares structural similarities with the adhesion proteins of other mycoplasma species, such as MgPa of *M. genitalium* (7) and GapA of *M. gallisepticum* (5), but not with Gli349 of *M. mobile*, the fastest species (26). It is known that an antibody raised against P1 can block the binding of *M. pneumoniae* to animal cells and glass (4, 6, 19, 24), but the effects on glass binding or gliding have not been observed for individual cells (4, 6). Here we analyzed the effects of such an antibody on individual cells under conditions optimized for gliding.

Optimizing conditions for gliding. In previous work, we found that a greater proportion of *M. pneumoniae* cells glided at higher speeds in a phosphate-buffered saline (PBS) solution containing serum than in the growth medium (9). Accordingly, in the present study we examined the effects of medium on gliding. *M. pneumoniae* M129 cells grown in Aluotto medium (1, 17) were suspended in fresh medium, dispersed as previously described (21, 22), put on a clean coverslip, and incu-

bated at 37°C for 60 min to let the cells bind to the glass. The coverslip with mycoplasma cells was then assembled into a tunnel slide, as previously described (12, 26). After incubation of the cells on a microscope stage chamber at 37°C for 10 min, the growth medium was replaced by PBS containing 10% horse serum or by a fresh medium. The microscopic images were recorded and analyzed (15–17, 26). Since all cells are not always gliding (9), we examined both the proportion of gliding cells in relation to the total cells and the gliding speeds to evaluate the effects of the various conditions. The gliding activity presented by the two parameters did not change when the medium was replaced by fresh medium, but it increased in response to the replacement with PBS containing 10% serum. The proportion of gliding cells was 0 out of 406 cells at time zero but increased with time and reached 0.37 at 60 min, when the growth medium was replaced by PBS containing 10% serum. This proportion stayed at 0, however, when the growth medium was replaced with fresh medium. The gliding speed in PBS containing 10% serum also increased with time and plateaued at 0.93 μm/s at 15 min, although it did not change in the fresh medium. The average gliding speed of *M. pneumoniae* was originally reported to be as fast as 0.4 μm/s in a medium, comparable to the speed observed here in the PBS containing serum (3, 18). The content of the Aluotto medium used here was slightly different from that of the Hayflick medium used in the previous studies. We did try the Hayflick medium, but no difference in the gliding results was observed. These observations may suggest that the active gliding of *M. pneumoniae* is induced by starvation, which was unexpectedly achieved in the previous studies (3, 18).

We next examined the effects of serum concentrations, temperature, and gelatin. Once cells were bound to glass with 10% horse serum, gliding continued even in its absence but was better in concentrations ranging from 5 to 20%. The number of cells that glided was approximately the same over a temperature range of 27 to 42.5°C, but their speed increased linearly with temperature over this range from approximately 0.5 to 0.8 μm/s, as previously observed in the gliding of the fastest mycoplasma species, *M. mobile* (15). The addition of 1 to 5%

* Corresponding author. Mailing address: Department of Biology, Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan. Phone: 81(6)6605 3157. Fax: 81(6)6605 3158. E-mail: miyata@sci.osaka-cu.ac.jp.
† Present address: Department of Oral Microbiology, Meikai University School of Dentistry, Keyakidai, Sakado, Saitama 350-0283, Japan.

gelatin did not prevent cells from leaving the glass during gliding (9, 18). Therefore, the effects of antibody were examined in PBS plus 10% horse serum without gelatin at 37°C.

Inhibition of gliding by anti-P1 adhesin antibody. We made a monoclonal antibody by immunizing mice with a recombinant protein comprising 1,160 to 1,518 amino acids of a whole P1 molecule of 1,627 amino acids, which is known to have a site responsible for cell and glass binding (19). The specificity of antibody was confirmed by immunoblotting, immunofluorescence microscopy of fixed cells with and without permeabilization, and immunofluorescence microscopy of living cells (12, 22, 23, 26).

The effects of the antibody on gliding of individual cells were examined (Fig. 1 and 2). Cultured mycoplasma cells were resuspended in PBS containing 10% serum and bound to a clean coverslip at 37°C for 70 min. Then, PBS containing 10% serum was replaced by PBS containing 10% serum and various concentrations of the antibody, ranging from 0 to 300 μg/ml at time zero, and cells bound to glass with and without gliding motility were counted separately, as presented in Fig. 1A and B, respectively. The addition of antibody removed the gliding cells from the glass over time in a concentration-dependent manner (Fig. 1A). However, the antibody affected the glass binding of nongliding cells only slightly (Fig. 1B). These observations indicate that the displacement of a cell along a glass surface during gliding is essential to cell removal by the antibody. The effects of antibody on the gliding speed were examined (Fig. 2). The average speed of gliding cells was found to be reduced by the addition of antibody in a concentration-dependent manner, an effect similar to that for the inhibition of glass binding, indicating that the binding of antibody reduces the gliding speed.

Involvement of P1 adhesin in gliding. The dependence of the antibody's ability to inhibit glass binding during gliding on its concentration indicates that P1 is responsible not only for static binding but also for that during gliding (Fig. 1A).

The obvious difference in resistance to the antibody between gliding cells (Fig. 1A) and nongliding cells (Fig. 1B) suggests that P1 induces conformational changes in gliding. In other words, the P1 molecules should be in a state where the accessibility of the antibody is significantly reduced when the cell is not gliding on glass, compared to the states occurring in gliding. This observation can be explained by an assumption that the P1 molecule itself is involved in a "power stroke" that propels a cell like a leg.

The binding of antibody to a cell was found to decrease the gliding speed (Fig. 2), consistent with the observation of *M. mobile* with anti-Gli349 antibody (26). The results of the present study can be explained by one of the following three hypotheses, based on the assumption of a power stroke of the P1 molecule. The first hypothesis is that the binding of antibody reduces the rate of release of P1 molecules from the glass, resulting in generation of a drag force, and also blocks rebinding after the release, as discussed for *M. mobile* (refer to Fig. 7 of reference 26). The second hypothesis is that only a fraction of P1 molecules are in the propelling cycle, while others are in a state of static binding, keeping the cells on the glass and also causing a drag force in normal gliding. In this case, the binding of antibody causes a decrease in the number of P1 molecules in the cycle, resulting in a shortage of propel-
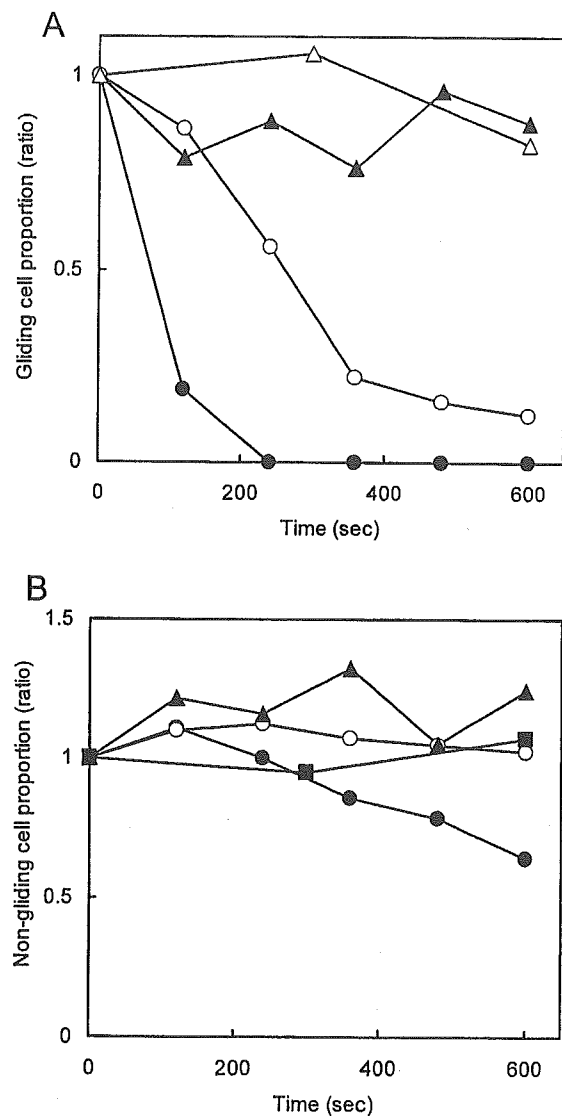

FIG. 1. Decrease in the number of bound cells after the addition of antibody. The number of bound cells relative to the initial number in a field of 9,600 μm² is shown. (A) The ratio of gliding cells remaining on the glass is shown for each time point after the addition of antibody to 300 (closed circles), 30 (open circles), 3 (closed triangles), and 0 (open triangles) μg/ml relative to the number of cells gliding at time zero. (B) The number of nongliding cells remaining on the glass is shown relative to the number of nongliding cells at time zero. The same symbols as those in panel (A) are used. More than 100 cells were analyzed to determine the total number of gliding and nongliding cells at the zero time point.

ling force for a cell with the normal speed. In the third hypothesis, a fraction of P1 molecules are in the propelling cycle, as proposed in the second hypothesis, but the drag force is not large enough to balance the propelling force exerted through a P1 molecule in the cycle. However, the duration of a stroke is short, and the speed of a cell depends on the sum of stroke durations. In this case, the decrease in the number of P1 molecules in the cycle directly reduces the cell's gliding speed.

Conclusions. We showed that P1 is involved in the gliding motility of *M. pneumoniae*. This finding may suggest that the
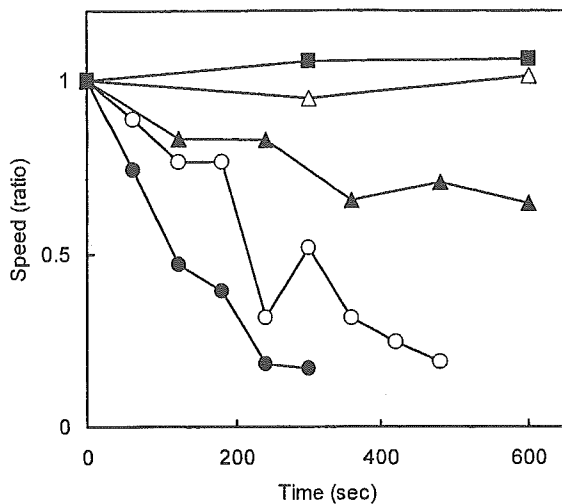
FIG. 2. Gliding speed after the addition of antibody. The gliding speeds normalized according to the initial speed are presented for each time point after the addition of antibody to 100 (closed circles), 30 (open circles), 10 (closed triangles), 3 (open triangles), and 0 (closed squares) μg/ml.

gliding of other mycoplasma species sharing an adhesion protein structure with P1 (5, 7, 25) also depends on their adhesion molecules.

REFERENCES

1. **Aluotto, B. B., R. G. Wittler, C. O. Williams, and J. E. Faber.** 1970. Standardized bacteriologic techniques for the characterization of mycoplasma species. Int. J. Syst. Bacteriol. **20:**35–58.
2. **Bredt, W.** 1979. Motility, p. 141–145. *In* M. F. Barile, S. Razin, J. G. Tully, and R. F. Whitcomb (ed.), The mycoplasmas, vol. 1. Academic Press, New York, N.Y.
3. **Bredt, W.** 1968. Motility and multiplication of *Mycoplasma pneumoniae.* A phase contrast study. Pathol. Microbiol. (Basel) **32:**321–326.
4. **Feldner, J., U. Gobel, and W. Bredt.** 1982. *Mycoplasma pneumoniae* adhesin localized to tip structure by monoclonal antibody. Nature **298:**765–767.
5. **Goh, M. S., T. S. Gorton, M. H. Forsyth, K. E. Troy, and S. J. Geary.** 1998. Molecular and biochemical analysis of a 105 kDa *Mycoplasma gallisepticum* cytadhesin (GapA). Microbiology **144:**2971–2978.
6. **Hu, P. C., R. M. Cole, Y. S. Huang, J. A. Graham, D. E. Gardner, A. M. Collier, and W. A. Clyde, Jr.** 1982. *Mycoplasma pneumoniae* infection: role of a surface protein in the attachment organelle. Science **216:**313–315.
7. **Inamine, J. M., S. Loechel, A. M. Collier, M. F. Barile, and P. C. Hu.** 1989. Nucleotide sequence of the MgPa (mgp) operon of *Mycoplasma genitalium* and comparison to the P1 (mpp) operon of *Mycoplasma pneumoniae.* Gene **82:**259–267.
8. **Jaffe, J. D., M. Miyata, and H. C. Berg.** 2004. Energetics of gliding motility in *Mycoplasma mobile.* J. Bacteriol. **186:**4254–4261.
9. **Kenri, T., S. Seto, A. Horino, Y. Sasaki, T. Sasaki, and M. Miyata.** 2004. Use of fluorescent-protein tagging to determine the subcellular localization of *Mycoplasma pneumoniae* proteins encoded by the cytadherence regulatory locus. J. Bacteriol. **186:**6944–6955.
10. **Kirchhoff, H.** 1992. Motility, p. 289–306. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas—molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
11. **Krause, D. C., and M. F. Balish.** 2004. Cellular engineering in a minimal microbe: structure and assembly of the terminal organelle of *Mycoplasma pneumoniae.* Mol. Microbiol. **51:**917–924.
12. **Kusumoto, A., S. Seto, J. D. Jaffe, and M. Miyata.** 2004. Cell surface differentiation of *Mycoplasma mobile* visualized by surface protein localization. Microbiology **150:**4001–4008.
13. **Miyata, M.** 2005. Gliding motility of mycoplasmas—the mechanism cannot be explained by current biology. *In* A. Blanchard and G. Browning (ed.), Mycoplasmas: pathogenesis, molecular biology, and emerging strategies for control, in press. Horizon Scientific Press, Norwich, United Kingdom.
14. **Miyata, M., and J. Petersen.** 2004. Spike structure at interface between gliding *Mycoplasma mobile* cell and glass surface visualized by rapid-freeze and fracture electron microscopy. J. Bacteriol. **186:**4382–4386.
15. **Miyata, M., W. S. Ryu, and H. C. Berg.** 2002. Force and velocity of *Mycoplasma mobile* gliding. J. Bacteriol. **184:**1827–1831.
16. **Miyata, M., and A. Uenoyama.** 2002. Movement on the cell surface of gliding bacterium, Mycoplasma mobile, is limited to its head-like structure. FEMS Microbiol. Lett. **215:**285–289.
17. **Miyata, M., H. Yamamoto, T. Shimizu, A. Uenoyama, C. Citti, and R. Rosengarten.** 2000. Gliding mutants of *Mycoplasma mobile*: relationships between motility and cell morphology, cell adhesion and microcolony formation. Microbiology **146:**1311–1320.
18. **Radestock, U., and W. Bredt.** 1977. Motility of *Mycoplasma pneumoniae.* J. Bacteriol. **129:**1495–1501.
19. **Razin, S., and E. Jacobs.** 1992. Mycoplasma adhesion. J. Gen. Microbiol. **138:**407–422.
20. **Razin, S., D. Yogev, and Y. Naot.** 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. **62:**1094–1156.
21. **Romero-Arroyo, C. E., J. Jordan, S. J. Peacock, M. J. Willby, M. A. Farmer, and D. C. Krause.** 1999. *Mycoplasma pneumoniae* protein P30 is required for cytadherence and associated with proper cell development. J. Bacteriol. **181:**1079–1087.
22. **Seto, S., G. Layh-Schmitt, T. Kenri, and M. Miyata.** 2001. Visualization of the attachment organelle and cytadherence proteins of *Mycoplasma pneumoniae* by immunofluorescence microscopy. J. Bacteriol. **183:**1621–1630.
23. **Seto, S., and M. Miyata.** 2003. Attachment organelle formation represented by localization of cytadherence protein and formation of the electron-dense core in wild-type and mutant strains of *Mycoplasma pneumoniae.* J. Bacteriol. **185:**1082–1091.
24. **Svenstrup, H. F., P. K. Nielsen, M. Drasbek, S. Birkelund, and G. Christiansen.** 2002. Adhesion and inhibition assay of *Mycoplasma genitalium* and *M. pneumoniae* by immunofluorescence microscopy. J. Med. Microbiol. **51:**361–373.
25. **Tham, T. N., S. Ferris, E. Bahraoui, S. Canarelli, L. Montagnier, and A. Blanchard.** 1994. Molecular characterization of the P1-like adhesin gene from *Mycoplasma pirum.* J. Bacteriol. **176:**781–788.
26. **Uenoyama, A., A. Kusumoto, and M. Miyata.** 2004. Identification of a 349-kilodalton protein (Gli349) responsible for cytadherence and glass binding during gliding of *Mycoplasma mobile.* J. Bacteriol. **186:**1537–1545.

© Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases 2005

NOTE

Toshiyuki Yamaguchi · Giichi Hashikita · Shun Takahashi
Akira Itabashi · Tsutomu Yamazaki · Shigefumi Maesaki

In vitro activity of β-lactams, macrolides, telithromycin, and fluoroquinolones against clinical isolates of *Streptococcus pneumoniae*: correlation between drug resistance and genetic characteristics

Abstract The in vitro activity of antimicrobial agents against *Streptococcus pneumoniae* was determined using 16 strains of penicillin-susceptible *S. pneumoniae* (PSSP) and 26 strains of penicillin intermediately resistant *S. pneumoniae* (PISP) + penicillin-resistant *S. pneumoniae* (PRSP) in Japan. The minimum inhibitory concentrations (MICs) of potent antibiotics, including eight β-lactams (benzylpenicillin, ampicillin, cefotiam, cefepime, cefditoren, faropenem, panipenem, and biapenem), three macrolides (erythromycin, clarithromycin, and azithromycin), telithromycin, and three fluoroquinolones (ciprofloxacin, levofloxacin, and gatifloxacin), were determined. Twenty-three strains exhibited genetic variations at *pbp1a* + *pbp2x* + *pbp2b*, which are genetic-PRSP (g-PRSP). g-PISP strains accounted for 62.5% (10/16) of the PSSP strains. The existence of an abnormal *pbp* gene conferred not only penicillin resistance but resistance to cephems; however, panipenem and biapenem had potent in vitro efficacy against alterations. Regarding the macrolide resistance mechanisms (*mefA* or *ermB*): 16 isolates had only *mefA*, 18 isolates had *ermB*, and 2 isolates had both *mefA* and *ermB*. There was no correlation between the existence of an abnormal *pbp* gene and the existence of the *mefA* gene or the *ermB* gene.

Key words PRSP · PBP · *mefA* · *ermB*

Streptococcus pneumoniae is an important pathogen in many cases of community-acquired pneumonia, acute otitis media, and purulent meningitis. In Japan, rates of penicil-

T. Yamaguchi (✉) · T. Yamazaki · S. Maesaki
Department of Infectious Disease and Infection Control, Saitama Medical School, 38 Morohongo, Moroyama-machi, Saitama 350-0495, Japan
Tel./Fax: +81-49-276-2032
e-mail: toshi-ngs@umin.ac.jp

G. Hashikita · S. Takahashi · A. Itabashi
Department of Laboratory Medicine, Saitama Medical School, Saitama, Japan

lin-resistant *S. pneumoniae* (PRSP) are reported to be 30% to 46%.[1] Currently, the antibiotic resistance patterns of *S. pneumoniae* isolates vary widely. Using polymerase chain reaction (PCR) methods, mechanisms of penicillin resistance and macrolide resistance can be obtained. Determination of the *pbp* genotype or the existence of the *mefA* gene or the *ermB* gene is useful in assessing β-lactams and macrolide resistance.[2]

In the present study, we examined the in vitro activity of β-lactams, macrolides, telithromycin, and fluoroquinolones against 16 strains of defined penicillin-susceptible *S. pneumoniae* (PSSP) and 26 strains of penicillin intermediately resistant *S. pneumoniae* (PISP) + PRSP. The study was conducted at Saitama Medical School Hospital, a 1483-bed hospital in Saitama, Japan. We used clinical pneumococcal strains isolated from patients with infectious diseases between April and September 2002. The minimum inhibitory concentrations (MICs) of potent antibiotics against *S. pneumoniae*, including benzylpenicillin, ampicillin, cefditoren, and biapenem (Meiji Seik, Tokyo, Japan), cefotiam (Takeda Chemical Industries, Osaka, Japan), cefepime (Bristol Myers KK, Tokyo, Japan), faropenem (Yamanouchi Pharmaceutical, Tokyo, Japan), panipenem (Sankyo, Tokyo, Japan), erythromycin (Abbott Laboratories, Chicago, IL, USA), clarithromycin (Taisho Pharmaceutical, Tokyo, Japan), azithromycin (Pfizer Japan, Tokyo, Japan), telithromycin (Aventis Pharma, Tokyo, Japan), ciprofloxacin (Bayer Yakuhin, Osaka, Japan), levofloxacin (Daiichi Pharmaceutical, Tokyo, Japan), and gatifloxacin (Kyorin Pharmaceutical, Tokyo, Japan) were measured. The MICs were determined by the broth microdilution method described by the National Committee for Clinical Laboratory Standards (NCCLS).[3] Microtiter plates containing 5.0×10^4 CFU/well were incubated with antibiotic at 35° for 18h, and the lowest concentration of drug that prevented visible growth was considered the MIC. The medium used was Mueller-Hinton broth (BBL Microbiology System, Cockeysville, MD, USA), supplemented with 5% lysed horse blood, according to the recommendations of the NCCLS. Daily quality control testing was conducted with *S. pneumoniae* ATCC 49619 (American Type Culture