表1　マイコプラズマによる直接炎症

M. pneumoniae は気管支上皮に達すると，細胞吸着器官（tip 構造）により線毛上皮に付着する。P1蛋白はこの tip の先端部に高濃度に集積し，宿主細胞に直接結合する major attachment protein である。P1蛋白に対する抗体でこの付着は抑制される。また，P1蛋白が欠損した変異株では細胞吸着性が失われ非病原性となる。

⇓

M. pneumoniae が気管支上皮表面で増殖すると（表面感染）

1．線毛運動障害（Ciliostasis）

2．線毛の消失

3．粘膜上皮細胞の破壊

これらは H_2O_2 の産生による

⇓

M. pneumoniae の菌株間に病原性の差異があり，付着能が強いほど病原性が強い

図3　マイコプラズマ肺炎の開胸肺生検病理組織

気管支肺動脈周囲間質には，リンパ球，形質細胞などの炎症細胞の著明な浸潤があり，気管支壁の肥厚として認められる。

与により細胞性免疫が活性化されると，この病変は亢進し，細気管支におけるマクロファージの集積による粒状陰影が増加するが，逆にTヘルパー1細胞を抑制するcyclosporin A（CYA）の投与により細胞性免疫を抑制するとこの病変は抑制され，好中球を主体とした肺胞炎が主な病理組織像であった[7]。Tヘルパー1反応優位マウス(C57B/6)における *Mycoplasma pulmonis* 感染ではIL-2投与と同様な病変パターンを示し，Tヘルパー2反応優位マウス(Balb/c)の感染ではCYA投与と同様の病変となった[8]。

マイコプラズマ肺炎初期には約6割の症例でツベルクリン反応（非特異的な細胞性免疫反応の指標として）が病初期には陰性化する。この陰性化は一時的であり，マウスの *Mycoplasma pulmonis* 感染の検討でも，感染1～3週目では非特異的な細胞性免疫反応の指標の一つである羊赤血球における foot pad 皮内反応が低下し，4週目で感染前のレベルに戻り，感染後6週目では逆に亢進する[9]。

一方，マイコプラズマ肺炎患者の発症1週間以内のツベルクリン皮内反応が陰性の症例では浸潤陰影が優位で，ツベルクリン皮内反

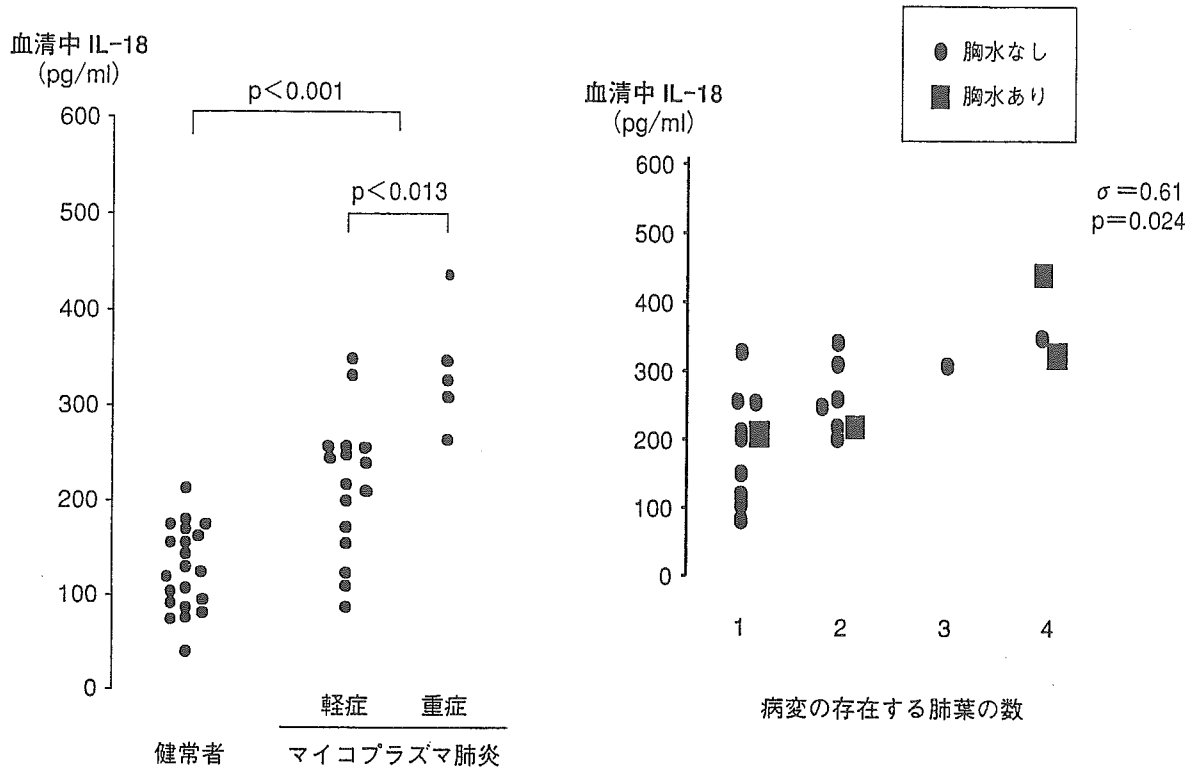

図4　成人マイコプラズマ肺炎重症度と血清中 IL-18値

重症例では血清中 IL-18 値は高値を示す。血清中 IL-18 値と肺炎の存在する肺葉の数とは有意な相関を示す。

（文献12より引用一部改変）

応が陽性を維持していた症例では粒状陰影が優位であった[10]。これらのことは，マイコプラズマ感染により活性化されたリンパ球・単球が気管支・肺に集積するために，末梢組織でリンパ球が不足し一時的にツベルクリン反応が陰性化するが，肺病変の終息と共に末梢リンパ球数の増加によりツベルクリン反応が陽性に戻ると考えられる。個人での宿主反応の一つとしてTヘルパー1反応が有意な場合は肺病変へのリンパ球の浸潤があっても，末梢にツベルクリン反応を陽性にできる程度のTヘルパー1リンパ球が残存するため，ツベルクリン反応が陽性のまま残存すると推測している。

2．気管支喘息との関係

IL-18 は当初，interferon (IFN)-γ 誘導因子として発見されたが，IL-12 の存在下ではTヘルパー1 反応を促進させ，IL-12 の非存在下ではTヘルパー2反応を促進させることが *in vitro* で報告されている。図4に示すようにIL-18がマイコプラズマ肺炎局所で増加し，肺病変の広がりと相関することを我々は報告した[11,12]。IL-12の低下している症例におけるマイコプラズマ肺炎ではIL-18がT2サイトカインを活性化させ喘息様病態になることが推測された。また，喘息患者の発作期には血中IL-18が上昇することも報告している[12]。以前よりマイコプラズマ感染が喘息を悪化させることが知られているが，最近，PCR による検出を用いた検討で，喘息患者のサブグループにおいてマイコプラズマが健常群と比較して有意に検出率が高く，喘息患者の下気道にはマイコプラズマが持続感染しているのではないかと報告されている[13,14]。

最近，山口大学のグループから，小児喘息

の非発作時の咽頭ぬぐい液111名中の14%の症例に，PCR法でマイコプラズマが証明されたというpreliminaryな結果が発表され[15]，今後成人での検討が待たれる。

3．重症化の機序

マイコプラズマ肺炎の多くはself-limitingであるが時に重症化する。欧米での重症マイコプラズマ肺炎の頻度は3～4%と報告されているが[16]，本邦ではかなり低いと思われる。泉川が本邦の重症マイコプラズマ肺炎42例を集計した報告[17]では，発症年齢は20～49歳に多く，70歳以上が6例（14.3%）と，高齢者での急性呼吸不全の原因として認識することが重要と思われる。Chanら[16]は重症，劇症マイコプラズマ肺炎46例の特徴として，基礎疾患のない健常人，男性，喫煙者に多いと報告しているが，泉川らの42例の集計では男女差はなく，基礎疾患を有する症例もあり，重症化のほとんどの症例で発熱，咳嗽などの初発症状出現3～14日後，突然に高熱，呼吸困難を訴えて受診している。臨床所見としては5日以上の発熱(38°C以上)，PaO_2が60Torr以下，胸部XP上両側陰影があり，初期治療はペニシリン，セフェム系または無治療の場合が多い[17]。

マイコプラズマ感染実験において，strainの違いによって病変の程度が異なるが，最近，マイコプラズマ感染のBalb/cマウスとC57BL/6マウスの気管支肺胞洗浄液中のサイトカイン，ケモカインについて検討した報告が発表された[18]。重症化するBalb/cマウスではTヘルパー1サイトカインが上昇しており，TヘルパーサイトカインやGM-CSFのレベルには両者のマウス間に差はなかった（表2）。一方，成人のマイコプラズマ肺炎において，重症では軽症と比較して前述した血中IL-18値が有意に上昇し，血中IL-18値と肺炎の広がりの間には正の相関が認められ[12]，Tヘルパー1サイトカインの産生亢進を起こすIL-18が重症肺炎に関与している可能性があると考えられた。以上のことより宿主のTヘルパー1反応の過剰反応が重症化の機序の一つと考えられる。一方，マイコプラズマのlipoproteinはマクロファージからの一酸化窒素（NO）産生を亢進し，IL-18はIL-2の産生亢進によりマクロファージを活性化するので，NO依存性のマイコプラズマの排除，治癒に関与していると考えられ，本来Tヘルパー1反応は感染に対抗する健常な反応と考えられる。

表2　マウスにおける *M.pneumoniae* 感染後翌日の肺病変サイトカイン，ケモカイン産生

	Balb/c	C57BL/6
肺炎病理スコア	↑ ↑	↑
気道過敏性	↑ ↑	↑
M. pneumoniae 菌	↑ ↑	↑
BAL中		
TNF-a	↑ ↑	↑
INF-γ	↑ ↑	−
IL-1β	↑ ↑	−
IL-12	↑ ↑	↑
IL-8 (mouse KC)	↑ ↑	−
MIP-1α	↑ ↑	−
IL-6	↑ ↑	↑
IL-4	−	−
IL-5	−	−
GM-CSF	−	−

4．マクロライド耐性マイコプラズマ

1999年以前は，薬剤耐性マイコプラズマはなかったが，2000年以降はマクロライド耐性株が検出され始め（表3），マイコプラズマ野生株の約15%が耐性株である。この耐性株は主に23S rRNA遺伝子の2063または2064番目のAがGやCに点変異しているが，他の部位の点変異も発見されており[4,5]，マクロラ

表3　マクロライド耐性マイコプラズマ

- 1999年以前は薬剤耐性 *M.pneumoniae* はなかった
- 2000年以降はマクロライド耐性菌検出
- *M.pneumoniae* 野生株の約15%が耐性株
- 耐性株による肺炎が必ずしも難治性でない
- これらの耐性菌は主に23S rRNA遺伝子の2063または2064番目のAがGやCに点変異しており，マクロライドの作用部位のドメインVに結合できず耐性となる

イドの作用部位のドメインVに結合できず耐性となるとされている。しかし，この耐性株による肺炎が必ずしも難治性でなく，日常臨床の場では問題となっていないことから，マクロライドによる抗炎症作用により，免疫反応が主体のマイコプラズマによる炎症を抑制しているのではないかと考えられる[5]。

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重症マイコプラズマ肺炎

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特集・呼吸器疾患診療における診断・治療のコツと落とし穴

重症マイコプラズマ肺炎

田中 裕士* 成田 光生** 高橋 弘毅*

Summary

重症マイコプラズマ肺炎は，小児から中年成人に多いが，高齢者でも急性呼吸不全の原因として重要である．重症化の機序は，菌の毒力の差ではなく，宿主の免疫反応の差によるものと考えられる．細胞性免疫(Tヘルパー1反応)が過剰にとなり，急性細気管支炎から低酸素血症を起こす場合と，逆に免疫抑制状態から菌血症となり急性呼吸不全になる場合が推測される．治療は抗生剤とステロイド剤の併用が有効である．

重症マイコプラズマ肺炎の疫学

一般に，*Mycoplasma pneumoniae* (Mp)肺炎の多くは軽症であるが，まれに重症化する場合があり，欧米では全Mp肺炎の3～4%と報告されているが[1]，発生頻度は本邦ではかなり低いと思われる．泉川が本邦重症Mp肺炎42例を集計した結果[2]では，年齢は20～49歳に多く，70歳以上が6例(14.3%)と，高齢者での急性呼吸不全の原因として認識することが重要と思われる．Channら[1]は重症，劇症Mp肺炎46例の特徴として，基礎疾患のない健常人，男性，喫煙者に多いとしているが，泉川らの本邦42例の集計では男女差はほとんどなく，基礎疾患を有する症例もあり，重症化のほとんどの症例で発熱，咳嗽などの初発症状の出現の後に3～14日後，突然に高熱，呼吸困難を訴えて受診している．臨床所見としては5日以上の発熱(38℃以上)，PaO_2が60Torr以下，胸部XP上両側に浸潤陰影，間質性陰影があり，多くの場合初期治療は，ペニシリン系，セフェム系または無治療の場合が多い[2]．

Mp肺炎の感染病態の考え方

Mp菌は細胞壁がないので，リポポリサッカライドやペプチドグリカンなどの細胞壁成分が欠如している．図1に示す様に，経気道的に侵入したMp菌は気管支上皮に達すると，細胞吸着器官(tip構造)により線毛上皮に付着する．P1蛋白(170 kDa)およびP30蛋白(40 kDa)はこのtipの先端部に高濃度に集積し，宿主細胞に直接結合するmajor attachment proteinである．P1蛋白に対する抗体でこの付着は抑制され，P1蛋白が欠損した変異株では細胞吸着性が失われ非病原性となることが知られている．Mp菌による病変形成の機序として直接作用と間接作用がある．直接作用は菌の増殖の過程で産生される過酸化水素や活性酸素による細胞障害があるがそれらの作用は強くなく，間接障害である菌体成分が引き起こす種々の免疫反応がより重要である．Mp菌は，マクロファージなどの貪食細胞上のtoll-like receptor-1,2,6がMp膜由来のlipoproteinを認識し，この受容体を介した自然免疫反応成立を支配していると考えられ，その後interleukin-18 (IL-18), IL-8などを介

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し，Tヘルパー1(Th1)サイトカインを活性化し細胞性免疫反応や炎症反応を亢進させるのではないかと推測している[3]．われわれは，小児および成人Mp肺炎において，胸水中，血中のIL-18やIL-8値が上昇することを報告している[4-6]．一方，Mp膜由来のlipoprotein はラットのマクロファージからのNO産生を亢進し，IL-18はTh1サイトカイン特にIL-2の産生亢進によりマクロファージを活性化するので，NO-dependentのマクロファージによるMp菌の排除に関与していると考えられる(図1)．

われわれは，肺内の免疫反応の場として気管支肺動脈周囲間質病変が重要であると考えている[7]．ヒトのMp肺炎の開胸肺生検組織でも，気管支肺動脈周囲間質にリンパ球を主体とした細胞浸潤を著明に認める．マウスモデルでの実験では，IL-2の投与により非特異的な細胞性免疫を活性化させるとこの病変は亢進し，細気管支と肺胞道のマクロファージの集積が増強した[8]．一方，cyclosporin A (CYA)の投与により細胞性免疫を抑制するとこの病変は抑制され，好中球を主体とした肺胞炎が主体となった(図2)[8]．Th1反応優位マウス(C57B/6)における*Mycoplasma pulmonis*感染ではIL-2投与と同様な病変パターンを示し，Th2反応優位マウス(Balb/c)の感染ではCYA投与と同様の病変となった[9]．Mp肺炎初期には約60%の症例でツベルクリン反応(非特異的な細胞性免疫反応としての指標として)が病期初期には一時的に陰性化する．一方，Mp肺炎患者のツベルクリン皮内反応が陰性の症例では浸潤陰影が優位で，ツベルクリン皮内反応が陽性を維持していた症例では粒状陰影が優位となった[10]．この解釈と

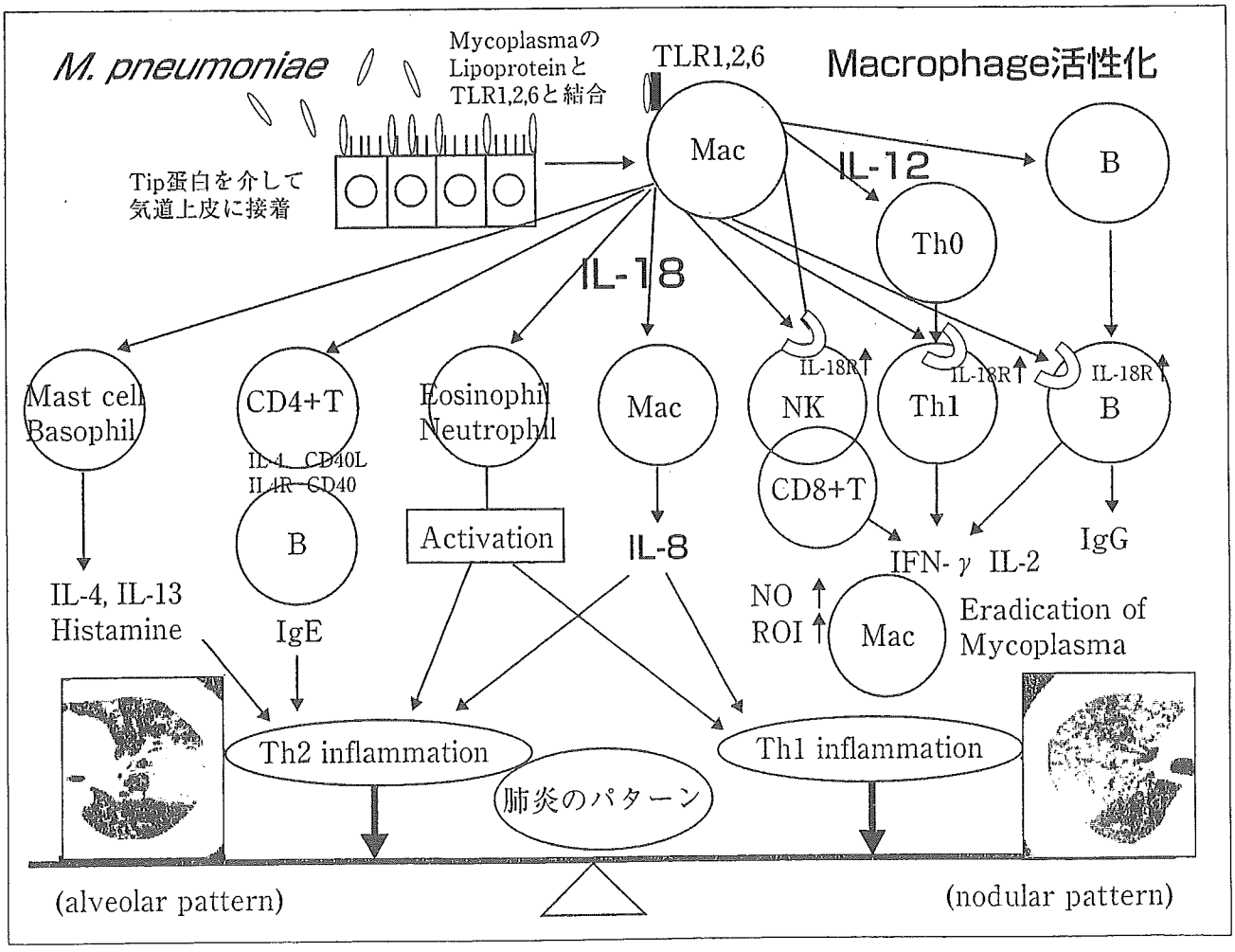

図1　*Mycoplasma pneumoniae*の気道感染に続発する免疫学的反応の仮説　(文献3から引用一部改変)

して，マイコプラズマ感染により惹起されたリンパ球・単球が肺に集積するために，末梢組織でリンパ球が不足し一時的にツベルクリン反応が陰性化するが，肺病変が終息と共に末梢リンパ球数の増加によりツベルクリン反応が陽性に戻ると考えられる．個人特異的にTh1反応が有意な宿主においては肺病変へのリンパ球の浸潤があっても，末梢にツベルクリン反応を陽性にできる程度のTh1リンパ球が残存するため，ツベルクリン反応が陽性のまま残存すると考えている．

重症化の機序

マウスを用いたMp感染実験において，マウスの系統によって軽度の感染しか起こらないstrainがあることが知られていた．*Mycoplasma*

表1　マウスにおける*M. pneumoniae*感染後翌日の肺病変気管支肺胞洗浄液中のサイトカイン，ケモカインレベル

	Balb/c	C57BL/6
肺炎病理スコア	↑↑	↑
気道過敏性	↑↑	↑
M. pneumoniae菌	↑↑	↑
BAL中		
TNF-α	↑↑	↑
INF-ν	↑↑	−
IL-1 β	↑↑	−
IL-12	↑↑	↑
IL-18 (mouse KC)	↑↑	−
MIP-1 α	↑↑	−
IL-6	↑↑	↑
IL-4	−	−
IL-5	−	−
GM-CSF	−	−

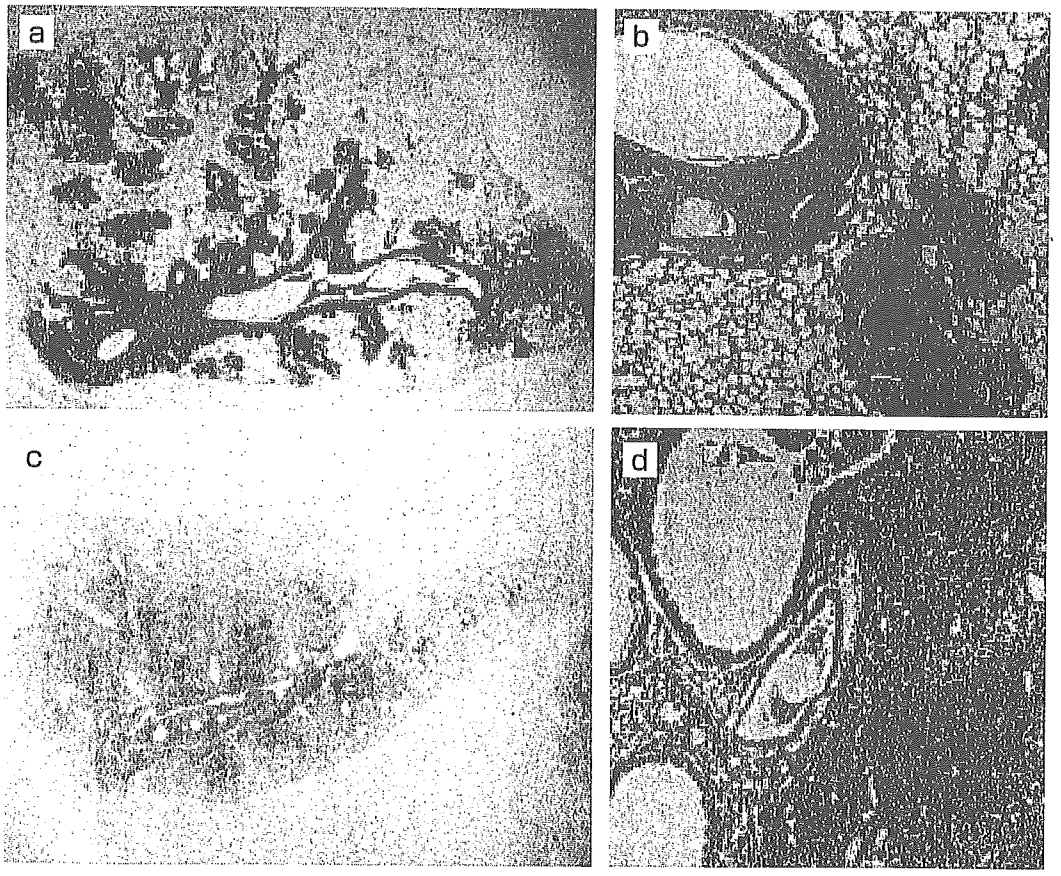

図2　マウス*Mycoplasma pulmonis*肺炎の病理組織像．a, b：Interleukin-2を投与し，Tヘルパー1反応を亢進したICRマウスでは，細気管支肺動脈周囲間質へのリンパ球浸潤が著明で，細気管支から肺胞腔へはマクロファージが集積している．c, d：Cyclosporin Aを投与し，免疫抑制したICRマウスでは，気管支肺動脈周囲間質のリンパ球浸潤は抑制され，肺胞内病変が優位となっている．　(文献8から引用一部改変)

*pulomonis*を用いた感染実験では，Balb/cマウスでは肺病変が重症で肺胞内への浸潤が著明であるが，C57BL/6マウスでは肺病変は軽症で気管支肺動脈周囲間質へのリンパ球浸潤が主体である[9]．この解釈として，Balb/cマウスとC57BL/6マウスの細胞性免疫応答の違いによるものと考えられた．最近，Mp菌感染のBalb/cマウスとC57BL/6マウスの気管支肺胞洗浄液中のサイトカイン，ケモカインについて検討し，重症化するBalb/cマウスではTh1サイトカインが上昇しており，Th2サイトカインやGM-CSFのレベルには両者のマウス間に差はなかった[11]．これらの結果のまとめを表1に示す．一方，成人のMp肺炎において，重症では軽症と比較して血中IL-18値が有意に上昇し，血中IL-18値と肺炎広がりの間には正の相関が認められ[6](図3)，Th1サイトカインの産生亢進を起こすIL-18が重症肺炎に関与している可能性があると考えられた．以上のことより宿主のTh1反応の過剰反応が重症化の機序の一つと考えられる．

公立八女総合病院の吉田らは，基礎疾患のない若年者の重症Mp肺炎で，高エンドトキシン血症を呈した症例を報告し，重症化には血中の高エンドトキシンが関与しているのではないかと推測している[12]．高エンドトキシン血症の機序としては，グラム陰性菌との混合感染というよりは，マイコプラズマ感染によりサイトカインが活性化され腸管壁の透過性亢進のため，エンドトキシンが腸管壁を通り門脈への流れ込み(bacterial translocation)やKupffer細胞の機能低下が起こる事が考えられている．

Mp肺病変のXP像・CT像

Mp肺炎の胸部XP所見は種々の陰影を呈し，浸潤陰影からびまん性粒状陰影[13]まで示し，この違いは宿主細胞性免疫反応の違いを反映していると考えている．われわれは，Mp肺炎における肺病変の場を以下の3つに分けて検討している[7,14]．1)気管支肺動脈周囲間質病変は，気管支周囲および併走する肺動脈周囲間質におけるリンパ球，形質細胞などの炎症細胞浸潤であり，レントゲン所見では気管支壁の肥厚像として認められる．2)細気管支とその周囲病変は，細気管支壁への炎症細胞浸潤と細気管支内腔へのマクロファージの集積および肉芽性病変であり，胸部レントゲンおよびCT像では，細気管支陰影に連続する細葉，小葉中心性の粒状陰影として見られる．3)肺胞腔内の滲出液，炎症細胞の浸潤病変は他の細菌性肺炎と共通な浸潤陰影として認められ，細気管支および細気管支内腔病変(いわゆるbronchiolitis obliterance with organizing pneumonia様病変)のため容積減少を伴いやすいのが特徴である[7]．成人

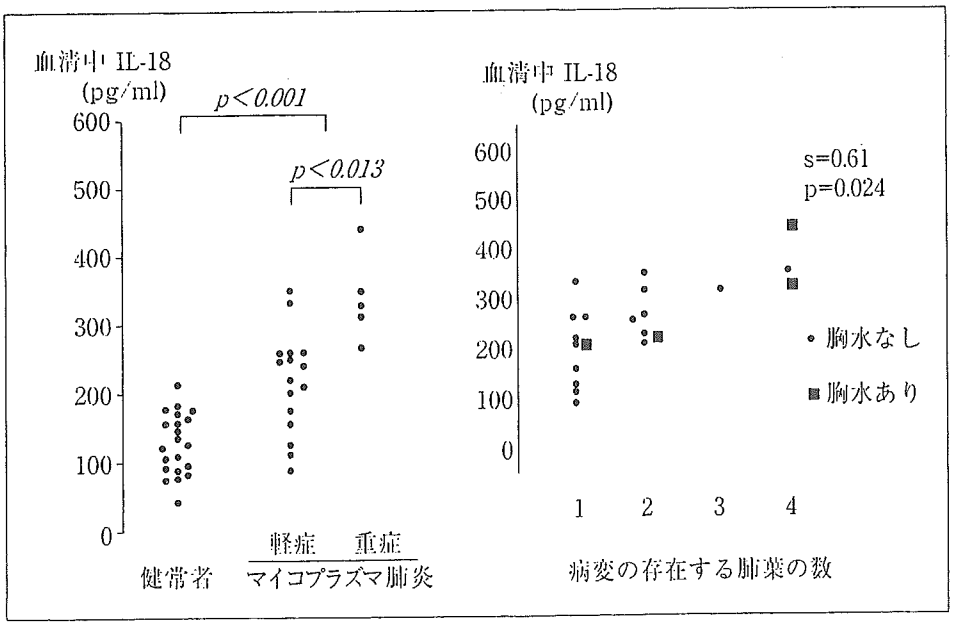

図3　成人マイコプラズマ肺炎重症度と血清中IL-18値．重症例では血清中IL-18値は高値を示す．血清中IL-18値と肺炎の存在する肺葉の数とは有意な相関を示す．
(文献6から引用一部改変)

表2　重症化の機序の可能性

免疫学的な機序で起こる肺病変は，炎症性サイトカインとTh1サイトカインで起こり，Th2サイトカインの関与は少ない．Th1反応の過剰が肺炎の悪化，重症化を起こす．
細胞性免疫反応が抑制されている症例やステロイド剤投与中の症例における*M. pneumoniae*の全身撒布による重症化．
サイトカインの活性が原因の腸管壁の透過性亢進(bacterial translocation)による血中endotoxin高値が重症化を起こす．

Mp肺炎95例のCT像を用いた解析では[4]，気管支肺動脈周囲間質病変が75％の症例に認められ，肺野浸潤陰影のない部位でも認められることが特徴である．また，細葉，小葉中心性の粒状陰影は65%に見られ，特に浸潤陰影が消失した後も4週間以上CT像で観察すると残存していることがある．呼吸機能では末梢気道閉塞所見を呈し，気道過敏性亢進が長く続く．重症化の場合Th1過剰反応による細気管支病変がびまん性に存在するものと推測しており，これが閉塞性換気障害や低酸素血症を起こすのではないかと思われる．このような症例には特に短期間のステロイド剤の投与が有用と考える．

重症Mp肺炎の治療

治療はマクロライド系，ミノサイクリン，ニューキノロン系などの抗菌剤とステロイド剤の投与である．われわれは，マウスのMp肺炎において，ミノサイクリンの単独治療群，ミノサイクリンとプレドニンの併用治療群，プレドニン単独治療群で比較したところ，ミノサイクリンとプレドニンの併用治療群がミノサイクリン単独治療群よりも肺炎の治癒率が良かった．また，プレドニン単独群では菌が全身に散布してしまったことから，プレドニンの単独治療は危険であり，有効な抗生物質の併用が必須であると考えられた[5]．また，免疫抑制剤を投与する場合には感染初期の方がより有利であることが示された．早期に確定診断が難しいこともあり，呼吸不全を伴う重症例に対しては，過剰な免疫反応を抑制する目的で，抗生剤の併用下での短期間大量ステロイド薬の併用が必要と思われる．

結　語

重症Mp肺炎の機序，重症化要因についてはまだ十分に解明されていない．これまでの検討では病原体の毒力の差というよりは，宿主細胞性免疫反応の過剰反応によるものと推測される．表2に重症化の機序の可能性を列挙したが，今後更なる検討が必要と思われる．

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* * *

PostScript

CORRESPONDENCE

Simplified semiquantitative culture using washed sputum from children with lower respiratory tract infections

Microbiologic diagnosis of bacterial lower respiratory tract infection in children is difficult because invasive diagnostic approaches, such as bronchoscopy or lung biopsy,[1] are usually not available for children. Previously, we showed the usefulness of a semiquantitative culture using sputum obtained from children.[2] However, this method requires sputum to be washed three times to reduce bacteria from the upper respiratory tract, and is tedious to perform as routine laboratory work. The purpose of our present study was to evaluate a simplified culture method for identifying causative pathogens in childhood lower respiratory tract infection.

We studied 268 children who were admitted to Saitama Medical School, Japan from February 1999 to August 2001 with the diagnosis of lower respiratory tract infection. Sputum was obtained by inducing the children to cough, as described previously.[2] The specimens were classified according to the Geckler classification[3] and washed in sterile saline by vigorous stirring using a bacterial loop. The core part of the sputum was collected and inoculated on to agar plates and incubated. For the simplified method, colonies were identified and bacteria with almost pure growth or with colony numbers of more than 50% on the plate were defined as pathogens. For 60 specimens we used sputum remaining from the simplified method, which was washed in fresh saline twice more and cultured. This was the same methodology used in the original method.[2] Results were compared between the two methods. Informed consent was obtained from the parents of all children.

Most specimens were classified into Geckler 5: fewer than 10 squamous epithelial cells and more than 25 neutrophils for each low power (×100) field. The pathogens identified by the original/simplified methods were as follows: *Haemophilus influenzae*, 17/19; *Streptococcus pneumoniae*, eight/eight; *Pseudomonas aeruginosa*, six/three; methicillin resistant *Staphylococcus aureus*, six/three; *Streptococcus agalactiae*, two/two; and *Stenotrophomonas maltophilia*, one/one. No significant pathogens were identified in 21 and 24 specimens by the simplified and original methods, respectively. Mixed pathogens were identified in three and one specimen, respectively. In 56 samples, results were the same with both methods, with an agreement rate of 93% (56 of 60).

Pathogens identified by the simplified method in 268 children were as follows: *H influenzae* 33%, *S pneumoniae* 16%, and *S aureus* 3%. No significant pathogens were identified in 123 specimens (46%).

The diagnostic value of sputum in children is not clear because expectorated sputum is difficult to obtain. We successfully obtained sputum by inducing the children to cough. In addition, the simplified semiquantitative culture using washed sputum showed a high agreement rate with the original method, which involved three washes. We recommend this simplified method as a less invasive method to clarify bacterial pathogens in lower respiratory tract infections among children.

Our result showed that *H influenzae* was the most frequent pathogen. Although the pathological role of non-typable *H influenzae* in respiratory tract infections has not been highly evaluated in children,[1] Shann[1] and Korppi and colleagues[5] reported a pathogenic role for non-typable *H influenzae*. We think these discrepancies could be a result of the diagnostic methods used.

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BOOK REVIEWS

A Handbook of Anatomical Pathology

Edited by R A Burnett. Royal Institute of Public Health and Hygiene, 2004, £15.00 (paperback). ISBN 1 90166 011 7145

Students of the Certificate or Diploma in Anatomical Pathology Technology will find this an excellent source of operational knowledge; mortuary managers will want to buy the editor a drink for the bibliography alone; pathologists will be reminded just how difficult it is to operate a mortuary in these days of Department of Health reports, Health and Safety Executive guidelines, and CPA visits. Such big praise for such a relatively small book, but then discussion about the size and colour of the publication surprisingly takes up about half of the introduction by the editor; the theme shall be maintained!

It is bigger than the 1991 "Red Book" which, according to its introduction—"in many mortuaries it takes pride of place on the bookshelf along with the much bigger format Yellow Book..."—well, not in mine it doesn't, because it was/is such an annoying little book to use, with a scanty contents page and no index. The 2004 Red and White Book (should have kept to one colour to maintain the chromatic flow) has undoubtedly expanded in its scope and amount of text and, as such, is a better source of information. However, because of the barely improved contents page and persistently non-existent index, it is still difficult to use; the contents page in this handbook takes you to the country, then to a region and strands you there. I don't need the sophistication of satellite navigation to find my way around a book, but even the cheapest road atlas has an index to take you straight to the town. It troubles me that the introduction prides itself in the fact that each "part" is designed to be complete in itself, with minimal cross referencing and, therefore, some duplication and repetition is inevitable; who really wants to read the whole book over and over again to spot the duplications—that is, facts pertaining to the same subject area? I don't, and neither I suspect does any busy APT or pathologist. This is a shame because there is a veritable mine of information here. The price of £15 (including post and packaging) is easily affordable for the most cash strapped of organisations (even UK NHS hospital trusts). Having said that, on publishing the second edition, please will the Royal Institute of Public Health as publishers invest in a better word processing package with an index facility (even Microsoft Word will do it on a desk top) and pass the few pence on to the reader; it will be money well spent.

S C Biddolph

Biology and Management of Multiple Myeloma

Edited by J R Berenson. Humana Press, 2004, $125.00 (hardback), pp 376. ISBN 0 89603 706 1

This multiauthor book provides a useful comprehensive account of our current state of knowledge of multiple myeloma, encompassing epidemiology and aetiology, diagnosis, cytogenetic and molecular genetic abnormalities, prognosis, and treatment. There are chapters on renal lesions, bone lesions, and anaemia and monoclonal gammopathy of undetermined significance has also been discussed. The authors have been drawn from Europe, North America, and Australia and the approach taken is therefore generally applicable. The text has been well edited (or perhaps the book was well planned) so that there is not a great deal of duplication; some duplication could have been avoided between the chapter on cytogenetic and molecular genetic analysis and that on prognosis. The book will probably be of use to clinicians because it not only gives an account of relevant scientific advances but also provides an up to date review of current and future treatment options. It should also be useful to pathologists, providing an update of the clinical context in which they are reporting. The standard of production is high—acid free paper and a good clear font that makes for easy reading.

B J Bain

CALENDAR OF EVENTS

Full details of events to be included should be sent to Maggie Butler, Technical Editor JCP, The Cedars, 36 Queen Street, Castle Hedingham, Essex CO9 3HA, UK; email: maggie.butler2@btopenworld.com

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PEDIATRICS®

Epidemiology of Community-Acquired Pneumonia in Children

Tsutomu Yamazaki, Kei Murayama, Atsuko Ito, Suzuko Uehara and Nozomu Sasaki

Pediatrics 2005;115;517-
DOI: 10.1542/peds.2004-2055

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doi:10.1542/peds.2004-2625

Epidemiology of Community-Acquired Pneumonia in Children

To the Editor.—

We read the recent article by Michelow et al,[1] "Epidemiology and Clinical Characteristics of Community-Acquired Pneumonia in Hospitalized Children," with great interest. One hundred fifty-four children were enrolled in this study, and blood or pleural fluid cultures, pneumolysin-based polymerase chain reaction assays, and serologic tests were used to clarify the epidemiology. The study indicated that *Streptococcus pneumoniae, Mycoplasma pneumoniae, Chlamydia pneumoniae,* and respiratory viruses were the major pathogens involved in community-acquired pneumonia (CAP). Although these results are informative for pediatricians, it does not seem appropriate that *Haemophilus influenzae* was not found to be a causative pathogen of CAP in this study. In the guidelines[2] published by the American Thoracic Society, *H influenzae* is recognized as an etiologic pathogen that causes CAP. There may be some discrepancy between adults and children over whether *H influenzae* is a respiratory pathogen of CAP. As it is stated by the authors in their discussion, however, such a discrepancy in the epidemiology might be the result of a lack of specific tests for *H influenzae* (and *Moraxella catarrhalis*). Because most respiratory infections of *H influenzae* are caused by nontypeable strains, analysis by serologic methods should be improved to include antigens made by local strains of *H influenzae*. We have been using a semiquantitative culture method of detection on washed sputum samples for a couple of decades and have found that *H influenzae* is one of the most frequent causative pathogens found in lower respiratory tract infections in children. This method is not difficult for pediatricians and is less invasive for children. Although expectorated sputum is difficult to obtain from younger children, it can be obtained successfully from children repeatedly by inducing cough. In this method, the sputum obtained is washed in sterile saline three times to reduce the effect of microorganisms from the upper respiratory tract that are usually present on the outside of the specimens. Using this method, Uehara[3] emphasized the significant etiologic role of *H influenzae* in children with CAP, and our recent data also indicate that nearly 30% of lower respiratory infections in children are related to *H influenzae* (unpublished data). We would suggest that this semiquantitative culture analysis of washed sputum be compared with an improved serologic method for *H influenzae* in the future.

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In Reply.—

The incidence of *Haemophilus influenzae* infection was not specifically studied in our recent publication.[1] Although non-type b and nontypeable *H influenzae* have been confirmed as causes of pneumonia in children, the frequency of infection in the United States is poorly defined. Since the introduction of the conjugated polysaccharide *H influenzae* type b vaccine in 1990, the incidence of invasive disease by that pathogen has declined dramatically. A limited number of publications have demonstrated lower frequencies of non-type b and nontypeable *H influenzae* pneumonia in children compared with adults and in developed countries compared with developing countries.[2] These strains have also been isolated from patients with underlying respiratory disorders including acute exacerbations of chronic bronchitis, bronchiectasis, and cystic fibrosis.[3]

H influenzae commonly colonizes the upper respiratory tract of children. These commensal organisms can gain access to the lungs by contiguous spread or aspiration but are less likely to invade the blood stream than *H influenzae* type b. Therefore, to confirm infection with non-type b or nontypeable *H influenzae*, culture of percutaneous lung aspirates are necessary.[4,5] Because these invasive procedures are rarely undertaken in children, the incidence of pneumonia caused by these strains presumably is underestimated.

Other diagnostic approaches have not been adequately validated in children. Specifically, acute and convalescent serology may indicate acute infection with *H influenzae*, but serologic assays may also reflect a nonspecific anamnestic response to a previous infection or a response to colonizing organisms during a viral infection.[6,7] Sputum collection in young children is unreliable, and semiquantitative cultures of sputum samples to diagnose causes of pneumonia in children have not been adequately validated by other investigators. Because *H influenzae* frequently colonizes the nasopharynx of children, there is a substantial risk of overestimating *H influenzae* pneumonia based on nasopharyngeal cultures.[5]

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doi:10.1542/peds.2004-2480

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Clinical Evaluation of Macrolide-Resistant *Mycoplasma pneumoniae*

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Macrolide-resistant *Mycoplasma pneumoniae* (MR *M. pneumoniae*) has been isolated from clinical specimens in Japan since 2000. A comparative study was carried out to determine whether or not macrolides are effective in treating patients infected with MR *M. pneumoniae*. The clinical courses of 11 patients with MR *M. pneumoniae* infection (MR patients) treated with macrolides were compared with those of 26 patients with macrolide-susceptible *M. pneumoniae* infection (MS patients). The total febrile days and the number of febrile days during macrolide administration were longer in the MR patients than in the MS patients (median of 8 days versus median of 5 days [$P = 0.019$] and 3 days versus 1 day [$P = 0.002$], respectively). In addition, the MR patients were more likely than the MS patients to have had a change of the initially prescribed macrolide to another antimicrobial agent (63.6% versus 3.8%; odds ratio, 43.8; $P < 0.001$), which might reflect the pediatrician's judgment that the initially prescribed macrolide was not sufficiently effective in these patients. Despite the fact that the febrile period was prolonged in MR patients given macrolides, the fever resolved even when the initial prescription was not changed. These results show that macrolides are certainly less effective in MR patients.

Mycoplasma pneumoniae is a common pathogen causing community-acquired respiratory tract infection mainly in children and young adults. Macrolides are generally considered to be the first-choice agents for treatment of *M. pneumoniae* infection. Although tetracyclines and fluoroquinolones are effective against *M. pneumoniae*, these agents are not recommended for children because of their toxicity. Tetracyclines can cause depression of bone growth, permanent gray-brown discoloration of the teeth, and enamel hypoplasia when given during tooth development. Although the clinical importance of fluoroquinolones has not been demonstrated, they produce cartilage erosion in young animals. Thus, these agents should be given only when there is no alternative (15).

As reported by Lucier et al. (9) and Okazaki et al. (14), an A-to-G transition or A-to-C transversion at position 2063 or 2064 of domain V of the *M. pneumoniae* 23S rRNA gene results in resistance to macrolide antibiotics. We have previously reported the isolation of macrolide-resistant (MR) *M. pneumoniae* from ca. 20% of clinical specimens collected from pediatric patients in Japan (11). Most of those isolates were highly resistant to 14-membered ring macrolides (MIC, >256 μg/ml) and moderately resistant to 15- and 16-membered ring macrolides.

Even in the cases of patients infected with MR *M. pneumoniae*, some pediatricians had the impression that there was a good response to macrolide therapy (11). There is a similar debate about the management of infection due to pneumococci. As noted in The Infectious Diseases Society of America (IDSA) guidelines for community-acquired pneumonia management (10), despite the increase of resistant isolates, a corresponding increase has not been seen in the number of clinical treatment failures.

One possible explanation for this is the nonantimicrobial effects of macrolides. It is known that macrolides have beneficial immunomodulating effects (1, 4, 6, 20), and they are clinically effective in hypersecretory conditions such as diffuse panbronchiolitis (7, 8) and cystic fibrosis (16). Thus, macrolides could be clinically effective even in MR *M. pneumoniae* infections.

It is important to know the clinical significance of MR *M. pneumoniae* infection, because in vitro susceptibility testing for *M. pneumoniae* is not available for daily management of patients. If macrolides are clinically effective against MR *M. pneumoniae* infection, pediatricians do not need to consider the use of tetracyclines or fluoroquinolones, even if the prevalence of MR *M. pneumoniae* rises in the future. Therefore, we performed a comparative study to determine whether or not MR *M. pneumoniae* influences the clinical outcome in patients treated with macrolides.

MATERIALS AND METHODS

Study population and sample collection. Three pediatric clinics in three different areas in Japan participated in this study. Sera and throat swabs or sputa taken from inpatients or outpatients suspected of *M. pneumoniae* infection were subjected to the laboratory tests.

Isolation. Isolation and identification of *M. pneumoniae* was carried out as described in a previous report (11).

PCR detection of *M. pneumoniae*. Sputa were obtained from patients, suspended in a small amount of saline, mixed well, and centrifuged at 2,000 rpm for 15 min, and then DNA was extracted from the supernatant with a QIAamp DNA Mini kit (QIAGEN K. K., Tokyo, Japan) according to the manufacturer's instructions. *M. pneumoniae* DNA was detected by the nested PCR method with primer sets for amplification of the P1 gene as previously described (17). The first

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TABLE 1. Prevalence of macrolide-resistant *M. pneumoniae* in Japan

Year	Isolation method		DNA detection method	
	No. of isolates	No. of resistant isolates (%)	No. of specimens with positive *M. pneumoniae* DNA (no. of examined specimens)	No. of specimens with macrolide resistance mutation in *M. pneumoniae* DNA (%)
1999	296	0	12 (630)	0
2000	10	1 (10.0)	9 (92)	4 (44.4)
2001	6	2 (33.3)	28 (384)	3 (10.7)
2002	12	3 (25.0)	44 (352)	5 (11.4)
2003	54	7 (13.0)	10 (236)	1 (10.0)
2004	6	1 (16.7)	8 (183)	2 (25.0)
5-year total (2000–2004)	88	14 (15.9)	99 (1247)	15 (15.2)

primer set was ADH2F (5'-GGC AGT GGC AGT CAA CAA ACC ACG TAT-3') and ADH2R (5'-GAA CTT AGC GCC AGC AAC TGC CAT-3'). The second primer set was ADH3F (5'-GAA CCG AAG CGG CTT TGA CCG CAT-3') and ADH3R (5'-GTT GAC CAT GCC TGA GAA CAG TAA-3').

Serological diagnosis. Particle agglutination (PA) antibody titers for *M. pneumoniae* were assayed by using Serodia-MYCO II (Fuji Rebio Ltd., Tokyo, Japan), which is manufactured using artificial gelatin particles, sensitized with cell membrane components of *M. pneumoniae*, according to the manufacturer's instructions.

Detection of resistance point mutations in domain V of 23S rRNA. MR *M. pneumoniae* isolates were screened on the basis of MIC of erythromycin (ERY), and identification of point mutations in domain V of 23S rRNA for ERY-resistant *M. pneumoniae* was performed according to our previously reported methods (11). For PCR-positive samples of *M. pneumoniae* DNA, the detection of a point mutation is indicative of a resistant phenotype because there is only a single rRNA operon in the genome (2). Neither plasmids with *erm* genes to mediate ribosomal modification nor any enzymes that inactivate macrolides have been found in *M. pneumoniae*. Thus, the prevalence of MR *M. pneumoniae* detected by the PCR methodology should reflect the true incidence of resistant strains.

Patient extraction for comparison of clinical courses. Clinical information was collected for the patients from whom *M. pneumoniae* had been isolated or its DNA detected. Patients who fulfilled the following criteria were extracted: (i) *M. pneumoniae* infection was laboratory confirmed, (ii) macrolides were prescribed during the illness, and (iii) complete information about prescribed antimicrobial agents and febrile days was available from the medical record. Laboratory-confirmed *M. pneumoniae* infection was defined as (1-a) isolation of *M. pneumoniae* from throat swabs or (1-b) detection of *M. pneumoniae* DNA from the sputum by PCR methods and serologically positive results, i.e., fourfold or greater rise of antibody titer in paired serum samples or titer higher than 1:640 in a single-serum sample by PA assay.

Patients infected with *M. pneumoniae* showing a point mutation in domain V of the 23S rRNA gene were defined as MR *M. pneumoniae*-infected patients (MR patients), and those infected with *M. pneumoniae* without the mutation were defined as macrolide-susceptible *M. pneumoniae*-infected patients (MS patients). MS patients were selected from the same study population as MR patients, and there were approximately twice as many of them as MR patients.

Measurement of clinical efficacy. To compare the clinical courses of MR and MS patients, we adopted the number of febrile days as a main outcome measurement. A febrile day was defined as a day during which the body temperature exceeded 38.0°C at least once. Total febrile days and the number of febrile days during macrolide administration were assessed. As these parameters would be affected by the time of commencement of macrolide administration, the number of febrile days before macrolide administration was also assessed. Other clinical symptoms and signs, such as cough and chest roentgenogram findings, were not taken into account in this study on account of the difficulty of objective and unified assessment through a retrospective review of medical records.

The numbers of patients whose prescribed antibiotic was changed were also compared. We speculated that a change in prescribed antimicrobial agent might reflect the pediatrician's clinical decision that the initial therapy had insufficient efficacy based on the general clinical condition of the patients. The pediatricians had no information about the susceptibility of *M. pneumoniae* at the time of clinical decision-making.

TABLE 2. Characteristics of enrolled patients

Characteristic	MR patients (n = 11)	MS patients (n = 26)	P
Age (yr)			
Median (range)	9.0 (0–13)	5.5 (1–14)	0.30
Mean	7.6	6.5	
Sex, male/female	4/7	14/12	0.33
No. of patients prescribed 14-membered ring macrolides (%)	8 (72.7)	7 (26.9)	0.025

Statistical analysis was performed using SPSS software, version 9.05 for Windows (SPSS, Inc., Chicago). Differences in categorical variables were assessed with the two-tailed Fisher's test, and for the comparison of medians the exact Wilcoxon rank-sum test was used. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Prevalence of MR *M. pneumoniae*. The prevalence of MR *M. pneumoniae* among clinical isolates and specimens with positive *M. pneumoniae* DNA is shown in Table 1. Before 1999, no MR *M. pneumoniae* was found among 296 clinical isolates. In 2000, however, MR *M. pneumoniae* appeared in 10% of isolates, and its prevalence rose to 33.3% in 2001. The overall prevalence of MR *M. pneumoniae* during 2000 to 2004 was 15.9%. All MR *M. pneumoniae* isolates had a resistance point mutation in domain V of 23S rRNA. A similar trend was seen in specimens with PCR-positive *M. pneumoniae*. Although the number of positive specimens before 1999 was limited (n = 12), no MR *M. pneumoniae* was detected. The prevalence of MR *M. pneumoniae* during 2000 to 2004, based on PCR-positive specimens, was 15.2%.

Comparison of the clinical courses between MR and MS patients. Eleven MR patients were selected for the analysis according to the criteria given above, and 26 MS patients were used as controls.

The patients' characteristics are summarized in Table 2. All patients were outpatients at the time of onset and had no severe underlying disease that might have influenced the clinical course. MR patients tended to be older and had a lower male/female ratio than MS patients, but the differences lacked statistical significance. Most patients were first prescribed β-lactam antimicrobial agents by primary physicians, followed by prescription of macrolides after attendance at a hospital. The prescribed macrolides differed among MR and MS patients. Significantly more MR patients than MS patients were prescribed 14-membered ring macrolides (72.7% versus 26.9%; P = 0.025). The majority of MS patients (19 out of 26 [73.1%]) were prescribed only 15-membered ring macrolides (azithromycin [AZM]).

The clinical courses in the MR and MS patients are summarized in Table 3. The total febrile days and the number of febrile days during macrolide administration were significantly greater in MR patients than in MS patients (median of 8 days versus 5 days [P = 0.019] and 3 days versus 1 day [P = 0.002], respectively). Febrile periods before macrolide administration, which consist of antimicrobial-free and mostly β-lactam-ad-

TABLE 3. Comparison of clinical courses in MR patients and MS patients

Characteristic	MR patients (n = 11)	MS patients (n = 26)	P
Febrile days			
Median (range)	8 (4–19)	5 (2–9)	0.019
Mean	9.3	5.5	
Febrile days during macrolide administration			
Median (range)	3 (1–11)	1 (1–5)	0.002
Mean	4.3	1.4	
Febrile days before macrolide administration			
Median (range)	3 (1–10)	4 (1–8)	0.402
Mean	3.8	4.1	
No. of patients with a febrile period exceeding 48 h after macrolide administration (%)	8 (72.7)	5 (19.2)	0.006
No. of patients with a change of prescription after macrolide administration (%)	7 (63.6)	1 (3.8)	<0.001

TABLE 4. Comparison of patients prescribed 14-membered ring macrolides

Characteristic	MR patients (n = 8)	MS patients (n = 7)	P
Total febrile days			
Median (range)	10.0 (4–19)	6.0 (4–9)	0.152
Mean	10.4	6.6	
Febrile days during macrolide administration			
Median (range)	3.5 (1–11)	1.0 (1–2)	0.004
Mean	4.9	1.1	
Febrile days before macrolide administration			
Median (range)	3.0 (1–10)	5.0 (3–8)	0.152
Mean	4.0	5.4	
No. of patients with a febrile period exceeding 48 h after macrolide administration (%)	7 (87.5)	1 (14.3)	0.01
No. of patients with a change of prescription after macrolide administration (%)	6 (75.0)	0	0.007

ministered days, showed no statistically significant difference (median of 3 days versus 4 days, P = 0.402).

The MR patients were more likely to have had the initially prescribed macrolide changed to another antimicrobial agent by their pediatricians (63.6% versus 3.8%; odds ratio, 43.8; P < 0.001). Among seven MR patients whose prescriptions were changed, all but one were changed to minocycline.

The results were similar for patients to whom 14-membered ring macrolides were administered (Table 4). Among these 15 patients (8 MR patients and 7 MS patients), 9 patients were prescribed clarithromycin, while the remaining 6 were prescribed ERY. Presumably due to the fact that the number of febrile days during macrolide administration was greater in MR patients than in MS patients (median of 3.5 days versus 1.0 day, P = 0.004), the initially prescribed macrolide was more frequently changed among MR patients than MS patients (75% versus 0%, P = 0.007). Although there was no statistical significance, there was a prolongation of total febrile days for MR patients (median of 10 days versus 6 days, P = 0.152).

When we focused on patients given 15-membered ring macrolides, 2 MR patients and 19 MS patients, the differences were not clear. Although there were only two MR patients in this group, their total febrile days and number of febrile days during macrolide administration were not different from those of MS patients (medians of 4.5 days versus 5.0 days and 1.0 day versus 1.0 day, respectively).

DISCUSSION

There are few reports on the isolation of MR *M. pneumoniae* from clinical specimens, and most of the isolates were obtained following ERY treatment (13, 19). In our survey, MR *M. pneumoniae* was not found in any of 296 clinical isolates or 12 *M. pneumoniae* PCR-positive specimens collected between 1983 and 1999, but it has been found in 15% to 20% of clinical isolates or PCR-positive specimens since 2000. MR *M. pneumoniae* first appeared in 2000 and rapidly spread throughout Japan (11, 12). Thus, it is important to evaluate the clinical significance of MR *M. pneumoniae*.

In our study, when patients infected with MR *M. pneumoniae* were treated with macrolides, the total febrile period was 3 days longer than that of patients with MS *M. pneumoniae*. Although we did not assess other clinical outcome variables, such as chest roentgenogram findings, a higher frequency of changes in prescription was observed in MR patients than in MS patients. This might reflect the pediatrician's judgment, based on the patient's clinical condition, that the initially prescribed macrolide was not sufficiently effective, even though the pediatricians had no information about the susceptibility of isolates at the time of clinical management. This tendency was also seen in patients who were treated only with 14-membered ring macrolides.

It was difficult to assess the immunomodulatory effects of macrolides in patients with *M. pneumoniae* infection in this study, because all the patients enrolled were prescribed macrolides according to the inclusion criteria. To evaluate the immunomodulatory effects of macrolides, it will be necessary to compare the clinical outcomes among MR patients treated with and without macrolides. An alternative is to compare the number of febrile days of MR patients with that of patients without antimicrobial agent therapy in the literature. According to review articles, fever might persist for about a week in the natural course of *M. pneumoniae* infection (3, 18). Kingston et al. (5) evaluated the effect of demethylchlortetracycline in a double-blind study, and the mean duration of fever in the treated group was 2.13 days, while it was 8.14 days in the placebo group. They started to count the number of febrile days not at the point of onset but only after entry into the study. In our study, the mean number of febrile days of MR patients was 9.2, which is similar to that of the placebo group

in Kingston's study. This implies that the antimicrobial effect is dominant over immunomodulatory effects in macrolide therapy, at least as far as duration of fever in *M. pneumoniae* infection is concerned. On the other hand, we did not assess the duration of other symptoms, such as malaise, sore throat, and cough, and it is possible that the immunomodulatory effects of macrolides can shorten these symptoms even in MR *M. pneumoniae* infection.

A difference of three febrile days in MR patients might not have a great impact in the management of *M. pneumoniae* infection, because it is often a mild and self-limiting disease, and the fever resolved even when the initially prescribed macrolide was not changed. However, it is reasonable to consider the use of alternative antimicrobial agents, such as minocycline, when macrolides are less effective than expected in patients more than 8 years old with possible *M. pneumoniae* infection.

The criteria for *M. pneumoniae* infection used in this study were stringent enough to confirm acute *M. pneumoniae* infection. This was a retrospective study based on a review of medical records, and patients with incomplete records were excluded. In general, clinical records of patients showing mild illness with *M. pneumoniae* infection were incomplete, and their clinical evaluation was excluded from this study.

In conclusion, we compared clinical outcomes in 11 MR patients and 26 MS patients given macrolide therapy. The MR patients showed more febrile days (by a median of 2 days) during the initial macrolide therapy than MS patients. On the other hand, no apparent treatment failure or serious illness was reported for MR patients. The influence of the emergence of MR *M. pneumoniae* on the treatment for *M. pneumoniae* infection deserves further study.

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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

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Methicillin-resistant *Staphylococcus aureus* (MRSA) clones harboring the toxic shock syndrome toxin 1 (*tst*) gene have been detected in France and in Switzerland since 2002. During a passive survey conducted between 2002 and 2003, we collected 103 *tst*-positive *S. aureus* isolates from 42 towns in France, of which 27 were resistant to methicillin. The *tst*-positive MRSA belonged to two clones: a major clone comprising 25 isolates of sequence type (ST) 5 and *agr* group 2 and a minor clone comprising two isolates of ST30 and *agr3*. The *tst*-positive MRSA clones were associated with both hospital-acquired (12 cases) and community-acquired (8 cases) infections. The MRSA clones were mainly isolated from children (overall median age, 3 years). They caused a variety of clinical syndromes, including toxic shock syndrome and suppurative infections. Both clones were found to harbor a type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) and to have similar antibiotic resistance profiles (usually resistant to oxacillin, kanamycin, and tobramycin and with intermediate resistance to fusidic acid). The origin of these clones is unclear. The *tst*-positive *agr2* MRSA clone has the same sequence type (ST5) of two pandemic nosocomial MRSA clones, namely, the Pediatric clone and the New York/Japan clone. These findings suggest that all these clones are phylogenetically related. The pulsotype of the *tst*-positive MRSA clones differed from that of methicillin-sensitive *S. aureus* (MSSA) clones by a single band involving the SCC*mec* element. These findings suggest that the *tst*-positive MRSA clones may have emerged from their respective MSSA counterparts.

Staphylococcus aureus is an important human pathogen in both hospitals and the community. The first methicillin-resistant *S. aureus* (MRSA) isolates were detected in the hospital setting in the early 1960s. A number of pandemic nosocomial clones have been characterized by molecular methods (3, 24, 25, 34). These epidemic MRSA strains of hospital origin have also been detected in the community, infecting patients with risk factors associated with hospital-acquired MRSA infection (H-MRSA), such as recent hospitalization. The epidemiology of MRSA has changed radically since 1999; in particular, true community-acquired MRSA (C-MRSA) infections have been reported in patients with no clear risk factors (2). These C-MRSA clones predominantly infect young and previously healthy patients and have now spread throughout the world (29). They produce Panton-Valentine leucocidin (PVL) and harbor a type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) element (1, 4, 5, 13, 22, 37).

Toxic shock syndrome toxin 1 (TSST-1) is a superantigenic toxin secreted by some *S. aureus* isolates. TSST-1, encoded by the *tst* gene, is a major virulence factor in toxic shock syndrome (TSS), staphylococcal scarlet fever, and neonatal toxic shock-like exanthematous diseases (NTED) recently described in Japan and France (11, 16, 38). TSS was first described in 1978 by Todd et al. as a multisystem disease characterized by rapid onset of fever, hypotension, erythematous rash, and mucosal hyperemia, followed by desquamation and multiorgan involvement. TSS was initially linked to tampon use by young women, but non-menstruation-associated TSS now predominates, occurring both in the community and in hospitals secondary to local *S. aureus* infection (10). Musser et al. showed that *tst*-positive *S. aureus* strains were clonal by comparing their isoenzymatic profiles (21), and studies based on multilocus sequence typing (MLST) have recently shown that these strains belong to sequence type (ST) 30 (27). Jarraud et al. reported that most *tst*-positive *S. aureus* strains are genetically related and have a type 3 accessory gene regulator (*agr*) allele (15). The isolates in these studies were associated with community- and hospital-acquired diseases and were all methicillin-sensitive *S. aureus* (MSSA). There have been few reports of MRSA isolates producing TSST-1 in Japan or Germany (12, 30). In 2003, we observed the first French case of NTED due to TSST-1-producing methicillin-resistant *S. aureus* (16, 38).

In order to characterize TSST-1-producing *S. aureus* isolates in France, we retrospectively typed all *tst*-positive isolates sent to the French National Reference Center for Staphylococci in

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2002 and 2003 and collected relevant epidemiological and clinical data. We observed the presence of new *tst*-positive MRSA clones responsible for both hospital- and community-acquired infections.

MATERIALS AND METHODS

Bacterial isolates. Among the 1,550 unconstrained strains sent to the French National Reference Center for Staphylococci during 2002 and 2003, 103 isolates from 42 towns were *tst* positive. As controls we used nine *tst*-positive MRSA isolates from Australia (one isolate, provided by Graeme Nimmo), Switzerland (three isolates), and Japan (five isolates causing neonatal toxic shock-like exanthematous diseases, TWCC3812, TWCC390861, TWCC4082, TWCC4382, and TWCC4410) (16). We also used an isolate representative of the Pediatric clone and an isolate representative of the New York/Japan clone.

Data collection. For each *S. aureus* strain we collected relevant clinical information (age, sex, type, and site of infection) by using a standard form provided by the French National Reference Center for Staphylococci. TSS, staphylococcal scarlet fever, and neonatal toxic shock syndrome-like NTED were diagnosed by using published criteria (11, 21, 30, 32). For this study, MRSA infection was considered to be community acquired if the specimen was obtained outside the hospital setting or less than 2 days after hospital admission of a patient with no direct or indirect exposure to the healthcare system in the previous year (2).

DNA extraction. Strains were grown on brain heart infusion agar or in brain heart infusion broth at 37°C overnight. Genomic DNA was extracted with a standard procedure, and its concentration was estimated spectrophotometrically (18). Amplification of *gyrA* was used to confirm the quality of each DNA extract and the absence of PCR inhibitors. All PCR products were analyzed by electrophoresis on ethidium bromide-stained 1% agarose gels (Sigma, France).

Identification of *agr* alleles. The *agr* group (*agr1* to *-4*) was determined by PCR as previously described (15).

Detection of the *mecA* gene and SCC*mec* typing. The *mecA* gene coding for methicillin resistance was detected by PCR as described by Murakami et al. (20). The staphylococcal chromosomal cassette *mec* (SCC*mec* I to IV) was detected by using the method of Oliveira et al. (23). The following reference strains, kindly provided by Herminia de Lencastre and Alexander Tomasz, were used as controls: COL (SCC*mec* I), BK2464 (SCC*mec* II), HU106 (SCC*mec* III), and BK2529 (SCC*mec* IV).

Detection of toxin and adhesin genes. Sequences specific for staphylococcal enterotoxin genes (*sea-e* and *seg-o*), the toxic shock syndrome toxin gene (*tst*), exfoliative toxin genes (*eta* and *etb*), PVL genes (*lukS*-PV-*lukF*-PV), the LukE-lukD leukocidin genes (*lukE-lukD*), the class F *lukM* leukocidin gene (*lukM*), and hemolysin genes (gamma [*hlg*], gamma variant [*hlgv*], and beta [*hlb*]) and for nine MSCRAMM genes (microbial surface components recognizing adhesive matrix molecules), bone sialoprotein binding protein (*bsp*), clumping factors A and B (*clfA* and -B), collagen binding protein (*cna*), elastin binding protein (*ebpS*), laminin binding protein (*eno*), fibronectin binding proteins A and B (*fnbA* and *-B*), and extracellular fibrinogen binding protein (*efb*), were detected by PCR as described elsewhere (15, 23, 26, 27, 35, 37).

Antimicrobial susceptibility testing. Susceptibility tests were performed with the ATB System (bioMérieux, France).

Capsular typing. Capsular serotyping was performed for all MRSA strains and for randomly selected MSSA strains. The strains were grown for 24 h at 37°C on Columbia agar plates containing 2% $MgCl_2$ and 0.5% $CaCl_2$. Several colonies of each strain were suspended in 0.9% saline and tested by slide agglutination with rabbit polyclonal antibodies specific for capsular polysaccharide types 5 and 8 (8, 9).

Fingerprinting by PFGE. SmaI macrorestriction patterns were obtained by using a contour-clamped homogeneous electric field DR-II apparatus (Bio-Rad), as described elsewhere (19). Strain NCTC 8325 was used as a pulsed-field gel electrophoresis (PFGE) control. Resolved macrorestriction patterns were compared as recommended by Tenover et al. (33). Isolates were assigned to a single clonal group if they differed by less than six bands. PFGE patterns with more than six band differences (<75% similarity) were considered to correspond to different types.

The *mecA* gene was tested for in one of the PFGE bands, as follows: the fragment was cut out from the agarose gel, DNA was extracted by using the MinElute gel extraction kit protocol (QIAGEN), and PCR with the *mecA* primers and multiplex PCR for SCC*mec* typing were performed on the extract as described above.

***spa* typing.** *spa* typing was performed on MRSA isolates and on *agr2* MSSA isolates, as previously described (14). The x region of the *spa* gene was amplified by PCR. *spa* types were determined with Ridom Staph Type software (Ridom GmbH, Germany), which automatically detects *spa* repeats and assigns a *spa* type.

MLST. MLST was performed on strains representative of each clonal group, as described elsewhere (6, 36). The allelic profile of each strain was obtained by sequencing internal fragments of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) and entering them on the MLST home page (http://saureus.mlst.net), where seven numbers depicting the allelic profile were assigned which defined an ST (6). To determine genetic relationships, MLST data were examined with BURST software (based upon related sequence types; details are available from http://www.mlst.net/BURST/burst.htm). The algorithm places STs that share five out of seven MLST alleles in a common clonal complex (7).

TABLE 1. Distribution of the *mecA* gene and *agr* alleles among 103 French *S. aureus* isolates containing the *tst* gene collected between 2002 and 2003

agr allele type	No. (%) of isolates		
	mecA$^+$ (n = 27)	*mecA* deficient (n = 76)	Total
1	0 (0)	1 (1)	1
2	25 (93)	5 (7)	30
3	2 (7)	70 (92)	72
4	0 (0)	0 (0)	0

RESULTS

Distribution of isolates according to methicillin resistance and *agr* group. Among the 103 *tst*-positive *S. aureus* isolates, 27 were methicillin resistant (*mecA*$^+$), and 76 were methicillin susceptible (*mecA* deficient) (Table 1). Twenty-five *tst*-positive MRSA isolates had *agr* allele type 2, and two had *agr* allele type 3. Seventy *tst*-positive MSSA isolates had *agr* allele type 3, five isolates had *agr* allele type 2, and one isolate had *agr* allele type 1.

Clinical characteristics of *tst*-positive MRSA infections. The median age of the 27 patients with *tst*-positive MRSA infections was 3 years (range, <1 month to 84 years), and the sex ratio was 1. Five patients had toxic shock syndrome, two had NTED (31), and one had staphylococcal scarlet fever; nine patients had toxic shock syndrome but did not fulfill all the criteria of a TSST-1-mediated syndrome (i.e., fever and rash without shock) (Table 2). Five skin infections occurred in patients with varicella. Eight patients had deep-seated infections (pneumonia or osteoarthritis), and no clinical information was available for two other patients. Two deaths occurred. The isolates were recovered from skin and soft tissues (14 isolates), blood (7 isolates), the umbilicus (2 isolates from cases of NTED), bronchopulmonary secretions (2 isolates), a prosthesis (1 isolate), and a ligament (1 isolate).

Information on the hospital or community acquisition of the infection was available for 20 MRSA infections and 51 MSSA infections. The origin of MRSA infection was unknown in seven cases. Eight of the 27 patients with *tst*-positive MRSA isolates had no known link to healthcare facilities and no known risk factors for MRSA acquisition; these cases were considered to be community acquired. Twelve cases were hospital acquired.

Microbiological characteristics of *tst*-positive MRSA isolates. The 25 *tst*-positive *agr2* MRSA strains all harbored the *sec*, *sed*, *sel*, *sem*, *seo*, *lukDE*, and *hlgv* toxin genes and the *clfA-B*, *ebpS*, *eno*, and *efb* adhesin genes (Table 3). These