ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2004, p. 2897–2904 Vol. 48, No. 8
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.8.2897–2904.2004

Impact of Cethromycin (ABT-773) Therapy on Microbiological, Histologic, Immunologic, and Respiratory Indices in a Murine Model of *Mycoplasma pneumoniae* Lower Respiratory Infection

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Received 11 December 2003/Returned for modification 26 February 2004/Accepted 5 April 2004

***Mycoplasma pneumoniae* is a major etiologic agent of acute lower respiratory infections. We evaluated the antimicrobial and immunologic effects of cethromycin (ABT-773), a ketolide antibiotic, for the treatment of *M. pneumoniae* pneumonia in a mouse model. Eight-week-old BALB/c mice were inoculated intranasally once with 10^6 CFU of *M. pneumoniae* on day 0. Treatment was started 24 h after inoculation. Groups of mice were treated subcutaneously with cethromycin at 25 mg/kg of body weight or with placebo daily until sacrifice. Five to ten mice per group were evaluated at days 1, 4, 7, and 10 after inoculation. Outcome variables included bronchoalveolar lavage (BAL) for *M. pneumoniae* quantitative culture and cytokine and chemokine concentration determinations by enzyme-linked immunosorbent assay (tumor necrosis factor alpha [TNF-α], gamma interferon [IFN-γ], interleukin-1β [IL-1β], IL-2, IL-4, IL-12, granulocyte-macrophage colony-stimulating factor, IL-8, monocyte chemoattractant protein 1 [MCP-1], and macrophage inflammatory protein 1α [MIP-1α]), histopathologic score of the lungs (HPS), and pulmonary function tests (PFT) using whole-body, unrestrained plethysmography at the baseline and post-methacholine exposure as indicators of airway obstruction (AO) and airway hyperresponsiveness (AHR), respectively. The cethromycin-treated mice had a greater reduction in *M. pneumoniae* culture titers than placebo-treated mice, reaching statistical significance on days 7 and 10 ($P < 0.05$). HPS was significantly reduced in cethromycin-treated mice compared with placebo-treated mice on days 4, 7, and 10 ($P < 0.05$). Cytokine concentrations in BAL samples were reduced in mice that received cethromycin, and the differences were statistically significant for 7 of the 10 cytokines measured (TNF-α, IFN-γ, IL-1β, IL-8, IL-12, MCP-1, and MIP-1α) on day 4 ($P < 0.05$). PFT values were improved in the cethromycin-treated mice, with AO and AHR significantly reduced on day 4 ($P < 0.05$). In this mouse model, treatment with cethromycin significantly reduced *M. pneumoniae* culture titers in BAL samples, cytokine and chemokine concentrations in BAL samples, histologic inflammation in the lungs, and disease severity as defined by AO and AHR.**

Mycoplasma pneumoniae has been implicated in up to 41% of cases of community-acquired pneumonia in children and adults (6, 13, 22, 28, 38).

Macrolides and tetracyclines are considered the treatment of choice for *M. pneumoniae* respiratory tract infection (6, 12). However, it has been demonstrated that *M. pneumoniae* persists in the airway even after appropriate antibiotic therapy in studies with both humans and animals (8, 11, 20, 43, 44; S. Esposito, F. Blasi, R. Droghetti, S. Bosis, M. T. Panza, L. Allegra, and N. Pricipi, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. G-1542, 2003).

Despite the lack of eradication of the microorganism from the airway, appropriate antibiotic treatment significantly decreases the morbidity of pneumonia and shortens the duration of symptoms (8, 13, 20, 30, 40). Recent data also suggest that therapy with macrolide antibiotics can reduce the rate of recurrent wheezing and abnormal pulmonary function that result from acute *M. pneumoniae* infection (10, 29). The impact of chronic respiratory carriage of *M. pneumoniae* and its association with recurrent wheezing and asthma is being actively investigated (14, 15, 37; S. Biscardi, E. Marc, F. Moulin, E. Nicand, J. L. Iniquez, B. Boutnonnat-Faucher, J. Raymond, F. Brunet, and D. Gendrel, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr G-1550, 2001).

Cethromycin (ABT-773) belongs to the ketolide family, a new class of antibiotics derived from the macrolides, which represents a class of 14-membered ring macrolide agents characterized by a keto group at position 3 of the macrolactone ring, replacing the L-cladinose moiety of other members of the macrolide group (53). Cethromycin has activity against agents associated with community-acquired pneumonia, including the atypical bacteria (1, 3, 24, 31, 49). In vitro studies have demonstrated that cethromycin has excellent activity against *M. pneumoniae*, with MICs lower than those of macrolides (33, 48). However, there are no in vivo studies evaluating the activity and immunologic impact of therapy with ketolides against *M. pneumoniae* pneumonia.

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The present study was designed to evaluate the in vivo activity of cethromycin against *M. pneumoniae* in a murine pneumonia model (19). The effect of therapy on the pulmonary immune response, as defined by cytokines and chemokines, was also evaluated to gain insight into the immunopathogenesis of *M. pneumoniae* disease and its treatment.

MATERIALS AND METHODS

Organism and growth conditions. *M. pneumoniae* (ATCC 29342) was reconstituted in SP4 broth and subcultured after 24 to 48 h in a flask containing 20 ml of SP4 media at 37°C. When the broth turned an orange hue (approximately 72 h), the supernatant was decanted and 2 ml of fresh SP4 broth was added to the flask. A cell scraper was used to harvest the adherent mycoplasmas from the bottom of the flask. This achieved an *M. pneumoniae* concentration in the range of 10^6 to 10^7 CFU/ml. Aliquots were stored at −80°C. All SP4 media contained nystatin (50 U/ml) and ampicillin (1.0 mg/ml) to inhibit the growth of potential contaminants.

Animals and inoculation. Mice were obtained from commercial vendors (Charles River), who confirmed their mycoplasma- and murine virus-free status. The Animal Resource Center at University of Texas Southwestern Medical Center performed quarterly health surveillance on sentinel mice housed in the mouse storage room. Sentinel mice were analyzed for antibodies against mouse hepatitis virus, Sendai virus, pneumonia virus of mice, reovirus type 3, mouse encephalitis virus (GD-7), mouse rotavirus (EDIM), minute virus of mice, and *Mycoplasma pulmonis*. Sentinel mice were also screened for pinworms and mites. Sentinel mice were free of these pathogens. Mice were housed in filter-top cages and allowed to acclimate to their new environment for 1 week. Methoxyflurane, an inhaled anesthetic, was used for inoculum sedation. Two-month-old female BALB/c mice were intranasally inoculated once (day 0) with 2×10^6 to 7×10^6 CFU of *M. pneumoniae* in 50 μl of SP4 broth. Directly comparable treatment and placebo groups were given *M. pneumoniae* inocula from the same vial. All mice were housed in the same animal room and received identical daily care. Animal guidelines were followed in accordance with the Institutional Care and Research Advisory Committee.

Cethromycin (ABT-773) administration. Laboratory standard cethromycin powder (Abbott Laboratories, Chicago, Ill.) was formulated in 2% ethanol and 5% dextrose, with an adjusted pH of 6.0 to 6.5. Cethromycin (25 mg/kg of body weight; dosage, 0.5 mg in 0.25 ml per mouse) was started 24 h after *M. pneumoniae* inoculation and administered subcutaneously once daily for 10 days.

Since the manufacturer of cethromycin had not established a dosage regimen for humans at the time of our study initiation, we used a dosage that had been used previously in mouse studies (24, 51; I. C. Michelow, R. D. Hardy, K. Olsen, J. Iglehart, B. B. Rogers, H. Jafri, G. H. McCracken, and O. Ramilo, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. B-986, 2001; T. Fujikawa, S. Miyazaki, Y. Ishii, N. Furuya, A. Ohno, T. Matumoto, K. Takeda, and K. Yamaguchi, Abstr 41st Intersci. Conf. Antimcrob. Agents Chemother., abstr B-993, 2001). The pharmacokinetic profile of cethromycin was studied in rats and consisted of a mean peak concentration in plasma of 1.07 μg/ml and an area under the concentration-time curve (AUC) of 12.03 μg · h/ml following an oral dose of 25 mg/kg. It concentrated in the rat lung, with a lung tissue-to-plasma AUC ratio of 29, demonstrating properties similar to those of the macrolides. These pharmacokinetics were comparable between single and multiple doses (31). This dosage has been also evaluated with mice, obtaining a mean peak concentration in plasma of 1.77 μg/ml and a higher AUC of 40.7 μg · h/ml (24).

Azoulay-Dupuis et al. demonstrated with a mouse pneumonia model that cethromycin (ABT-773) had much higher maximum concentration in serum, AUC, and half-life in the lung than in the serum of *Streptococcus pneumoniae*-infected and noninfected Swiss mice after a single dose of 12.5 or 25 mg/kg by either gavage or the subcutaneous route, reaching lung-to-serum ratios of around 10. They also showed that the maximum concentration in serum/MIC and AUC/MIC ratios were higher in the lungs than in serum for the different strains of *S. pneumoniae* used (E. Azoulay-Dupuis, J. P. Bedos, P. Moine, J. Mohler, and C. Carbon, Abstr. Int. Congress Chemother. 2001, abstr. P19.023, 2001).

The placebo groups received the same treatment regimens with an identical solution not containing cethromycin.

The mycoplasma inoculum was sent to the Diagnostic Mycoplasma Laboratory (Birmingham, Ala.) to check for susceptibilities. The MIC of cethromycin was 0.000004 μg/ml, and those of azithromycin, clarithromycin, and erythromycin were 0.000125, 0.000125, and 0.02 μg/ml, respectively.

Experimental design and sample collection. Groups of 5 to 10 mice per cethromycin treatment group at each time point and 5 to 15 mice per placebo group at each time point were sampled for *Mycoplasma* cultures, histopathology scores (HPS), cytokines, and chemokines. The pulmonary function tests had 4 to 8 mice per cethromycin treatment group at each time point and 4 to 12 mice per placebo group at each time point. Not all outcome variables were available for some mice due to culture contamination or insufficient quantity of bronchoalveolar lavage (BAL) fluid for cultures, cytokines, and chemokines.

Mice were anesthetized with an intraperitoneal injection of 75 mg of ketamine/kg and 5 mg of acepromazine/kg before cardiac puncture. BAL specimens were obtained by infusing 0.5 ml of SP4 broth through a 25-gauge needle into the lungs, via the trachea, followed by aspiration of this fluid into a syringe. Whole-lung specimens (including the trachea and both lungs) were collected and fixed with a 10% buffered formalin solution for histologic evaluation.

Culture. Twenty-five microliters of undiluted sample and serial 10-fold dilutions in SP4 broth of BAL fluid (50 μl of undiluted sample was used for the initial dilution) were immediately cultured on SP4 agar plates at 37°C while the remainder of the undiluted BAL specimens were stored at −80°C. Quantification was performed by counting colonies on plated specimens and expressed as $\log_{10}$ CFU/milliliter.

Histopathology. The HPS was determined by a single pathologist who was unaware of the treatment status of the animals from which specimens were taken. The HPS was based on the grading of peribronchiolar or bronchial infiltrate, bronchiolar or bronchial luminal exudate, perivascular infiltrate, and parenchymal pneumonia (neutrophilic alveolar infiltrate). This HPS system assigned values from 0 to 26 (the greater the score, the greater the inflammatory changes in the lung) and has been validated previously with the animal model (7, 19, 20, 21). The variation in HPS when the same slide was scored by the same pathologist on multiple occasions has been found to be 0 to 1.

BAL cytokines and chemokines. BAL specimens were assessed for concentrations of cytokines and chemokines by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, Minn.) or with the mouse cytokine multiplex antibody bead kit (Luminex; Biosource International). When ELISA was used, the limits of detection were as follows: tumor necrosis factor alpha (TNF-α), 5.1 pg/ml; mouse KC (functional interleukin-8 [IL-8]), 2.0 pg/ml; JE/monocyte chemoattractant protein 1 (MCP-1), 2.0 pg/ml; macrophage inflammatory protein 1α (MIP-1α), 1.5 pg/ml. For the mouse cytokine multiplex, the lower limits of detection were the following: IL-1β, 10 pg/ml; IL-2 (p40/p70), 15 pg/ml; IL-4, 5 pg/ml; IL-12, 15 pg/ml; granulocyte-macrophage colony-stimulating factor (GM-CSF), 10 pg/ml; gamma interferon (IFN-γ), 1 pg/ml. For statistical analysis, samples with optical density readings below the limit of the standard curve of the assay were assigned a value one-half that of the lowest detectable value. Correlations between results obtained with the mouse cytokine multiplex antibody bead kit (Luminex; Biosource International) and results obtained with the Biosource ELISA ranged from 0.86 to 0.95 for the different cytokines measured.

Plethysmography. Whole-body, unrestrained plethysmography (Buxco, Troy, N.Y.) was utilized to monitor the respiratory dynamics of mice in a quantitative manner both before and after methacholine exposure (baseline plethysmography determined airway obstruction and methacholine plethysmography determined airway hyperreactivity). Prior to methacholine exposure, mice were allowed to acclimate to the chamber and then plethysmography readings were recorded to establish enhanced pause (P_{enh}) baseline values. Next, the mice were exposed to aerosolized methacholine (50 mg/ml), and P_{enh} values were recorded again. P_{enh} is a dimensionless value that represents a function of the ratio of peak expiratory flow to peak inspiratory flow and a function of the timing of expiration. P_{enh} correlates with pulmonary airflow resistance or obstruction. P_{enh}, as measured by plethysmography, has been previously validated in animal models of airway hyperresponsiveness (15, 17, 19–21, 41, 18–20, 47).

Statistics. For all statistical analysis, Sigma Stat 2000 software (SPSS Science) was used. The *t* test was used to compare values for cethromycin-treated animals versus placebo-treated animals at the same time point, if the data were normally distributed. In the instances where the data were not normally distributed, the Mann-Whitney rank sum test was used for comparisons. A comparison was considered statistically significant if the *P* value was ≤0.05.

The following variables had a normal distribution: *Mycoplasma* cultures, airway hyperreactivity, IL-4, IL-8, IL-12, and GM-CSF. Hence, the *t* test was used for these variables. HPS, airway obstruction, and the rest of the cytokines evaluated (TNF-α, IFN-γ, MCP-1, MIP-1α, IL-1, and IL-2) were not normally distributed; the Mann-Whitney rank sum test was used for these variables. The variables that had a normal distribution are presented as means with standard deviations, and the variables not normally distributed are presented as medians with 25th to 75th percentiles. Since some of the variables evaluated had normal

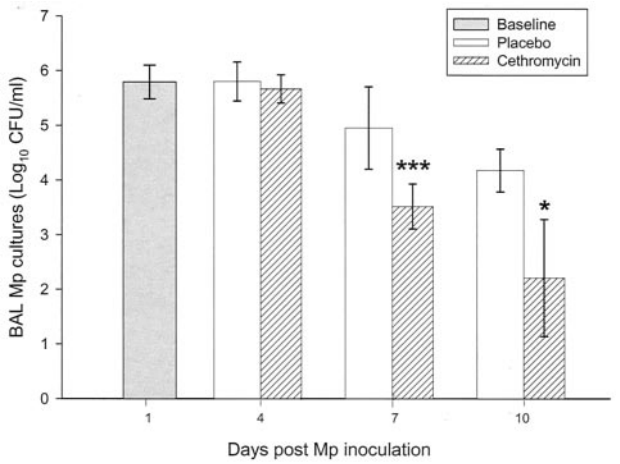

FIG. 1. Mean CFU of *M. pneumoniae* (Mp) in BAL cultures of infected mice treated with either cethromycin (25 mg/kg/day subcutaneously) or placebo for 10 days (therapy was started 1 day after infection). Error bars represent standard deviations. *, $P < 0.05$; ***, $P < 0.001$. $n = 5$ to 10 mice per cethromycin treatment group and 5 to 15 mice per placebo group.

distribution and others were not normally distributed, the correlations were measured by using Spearman rank order.

RESULTS

Visual. No visual differences could be detected between the infected mice treated with cethromycin and the mice that received the placebo.

BAL culture. Quantitative *M. pneumoniae* BAL cultures of specimens from infected mice were lower at all times in the cethromycin treatment groups than in the placebo groups, reaching statistical significance on days 7 and 10 after infection. Despite these significant reductions, *M. pneumoniae* was not eradicated from the animals' airways in either group (Fig. 1).

Histopathology. Mice treated with cethromycin had a significantly reduced HPS on days 4, 7, and 10 compared with mice in the placebo group (Fig. 2). In contrast to BAL cultures, lung inflammation was almost completely resolved by 10 days after inoculation in cethromycin-treated mice.

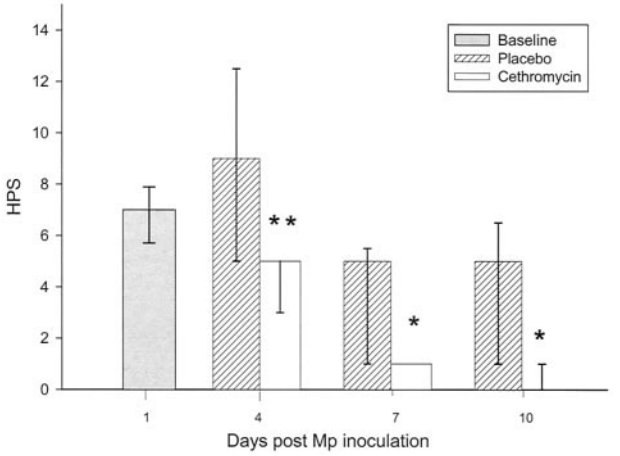

FIG. 2. Median HPS of mice infected with *M. pneumoniae* (Mp) and treated with either cethromycin or placebo. Error bars represent 75th to 25th percentiles. *, $P < 0.05$; **, $P < 0.01$. $n = 5$ to 10 mice per cethromycin treatment group and 5 to 15 mice per placebo group.

BAL specimen cytokines and chemokines. Cytokines and chemokines were measured in BAL samples on days 1 and 4 after inoculation. The *M. pneumoniae*-infected mice treated with cethromycin had significantly reduced concentrations of TNF-α, IL-1β, IL-12, and IFN-γ in BAL fluid at day 4 compared with mice in the placebo group (Fig. 3a, b, d, and e). IL-2, IL-4, and GM-CSF were not significantly modified by therapy with cethromycin (Fig. 3c, f, and g). Among the chemokines measured, KC (functional mouse IL-8), JE/MCP-1, and MIP-1α were significantly lower in treated mice than in placebo group mice at day 4 (Fig. 3h, i, and j).

Plethysmography. Airway obstruction, defined by baseline P_{enh} values, in mice inoculated with *M. pneumoniae* was significantly reduced in cethromycin-treated animals compared with placebo group mice on days 4 and 10 (Fig. 4).

Airway hyperreactivity, defined by P_{enh} values after exposure to methacholine, was reduced in mice treated with cethromycin compared with placebo group mice on days 4 and 7 and reached statistical significance on day 4 (Fig. 5).

Correlations (day 4). The correlations between *M. pneumoniae* BAL cultures, HPS, pulmonary function, and cethromycin therapy are shown in Table 1. Correlations between the cytokines and chemokines with BAL *M. pneumoniae* cultures, HPS, pulmonary function, and cethromycin therapy are shown in Table 2. Correlations of interest among the cytokines and chemokines are shown in Table 3.

Cethromycin therapy inversely correlated with HPS and airway obstruction (Table 1). Regarding the BAL cytokines and chemokines measured, cethromycin correlated inversely with IL-1β, TNF-α, IL-12, IFN-γ, IL-8, MCP-1, and MIP-1α (Table 2).

DISCUSSION

Therapy with cethromycin resulted in statistically significant improvement of microbiological, histologic, respiratory, and immunologic markers of disease severity in the murine model of *M. pneumoniae* pneumonia.

Even though the BAL *M. pneumoniae* quantitative cultures were significantly reduced in the mice treated with cethromycin, *M. pneumoniae* was not eradicated from the airway, as has been previously documented, in response to other antibiotic regimens (3, 9, 20, 45). The significance of *M. pneumoniae* persistence in the airway is still not well defined, but it is hypothesized to play a role in chronic respiratory conditions, such as asthma (14, 15, 17, 18, 26, 50).

Cethromycin had a more pronounced effect on lung inflammation than on *M. pneumoniae* cultures at all of the time points evaluated in these experiments. On day 4, while *M. pneumoniae* cultures were not significantly reduced, HPS was significantly decreased. On day 10, lung histology had normalized while *M. pneumoniae* cultures were still positive. Similarly on day 4, cethromycin therapy had a significant effect on a broad range of pulmonary cytokines with significant reductions in concentrations of the proinflammatory cytokines, IL-1β, TNF-α, TH1, IFN-γ, and IL-12, and chemotactic cytokines, MIP-1α, MCP-1, and IL-8, without any significant effect on the concentrations of IL-2 and the TH2 cytokines evaluated, IL-4 and GM-CSF.

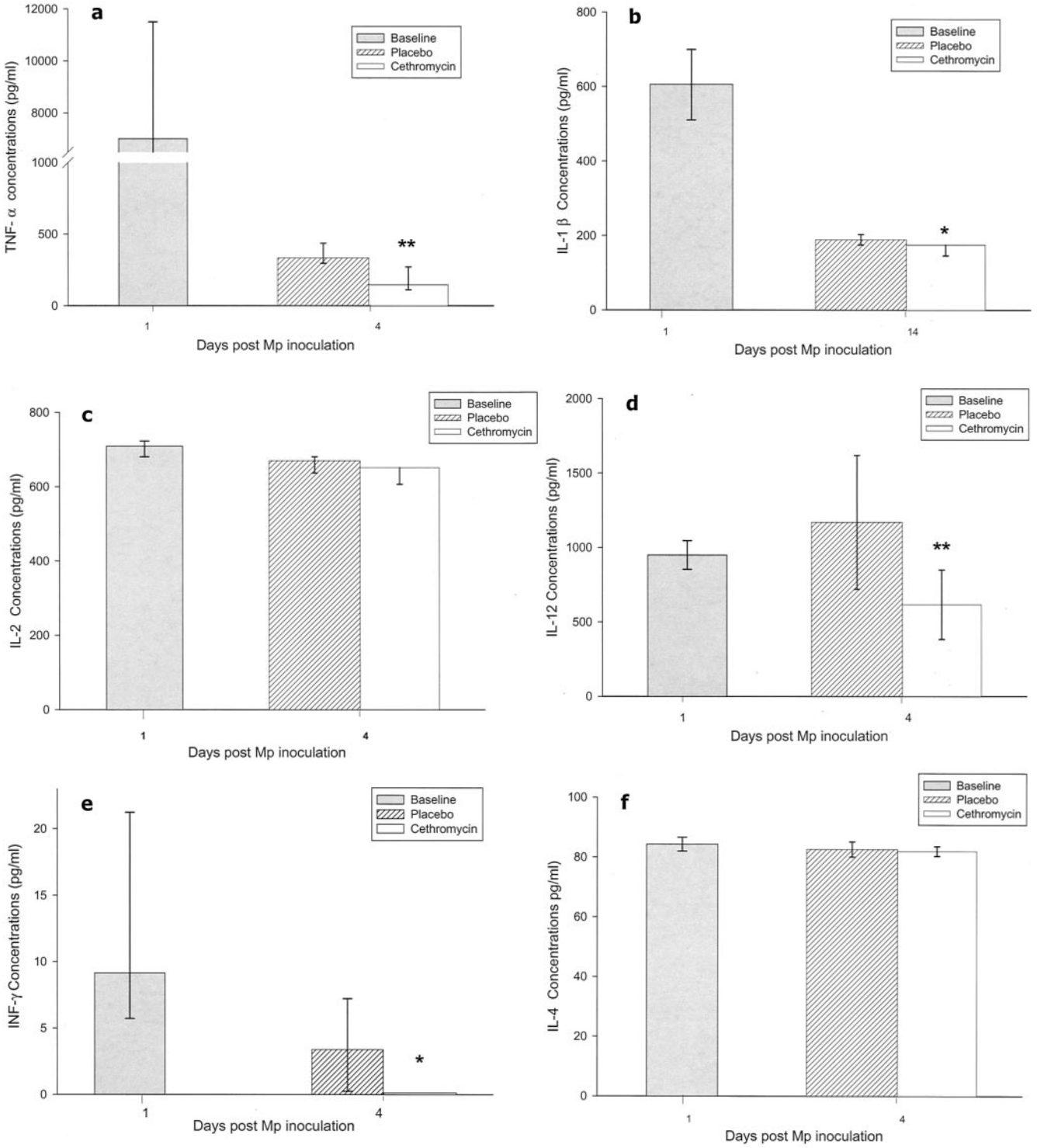

FIG. 3. (a to j) Mean and median cytokine and chemokine concentrations in BAL fluid of mice infected with *M. pneumoniae* (Mp) and treated with either cethromycin or placebo. Error bars represent standard deviations when variables have a normal distribution and 75th to 25th percentiles when variables do not have a normal distribution. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. $n = 5$ to 10 mice per cethromycin treatment group and 5 to 15 mice per placebo group.

The cytokines that were reduced most significantly by therapy with cethromycin were among those with the strongest correlation with lung inflammation, suggesting that these cytokines play a significant role in orchestrating the acute pulmonary inflammatory response.

Among the proinflammatory cytokines, TNF-α was previ-

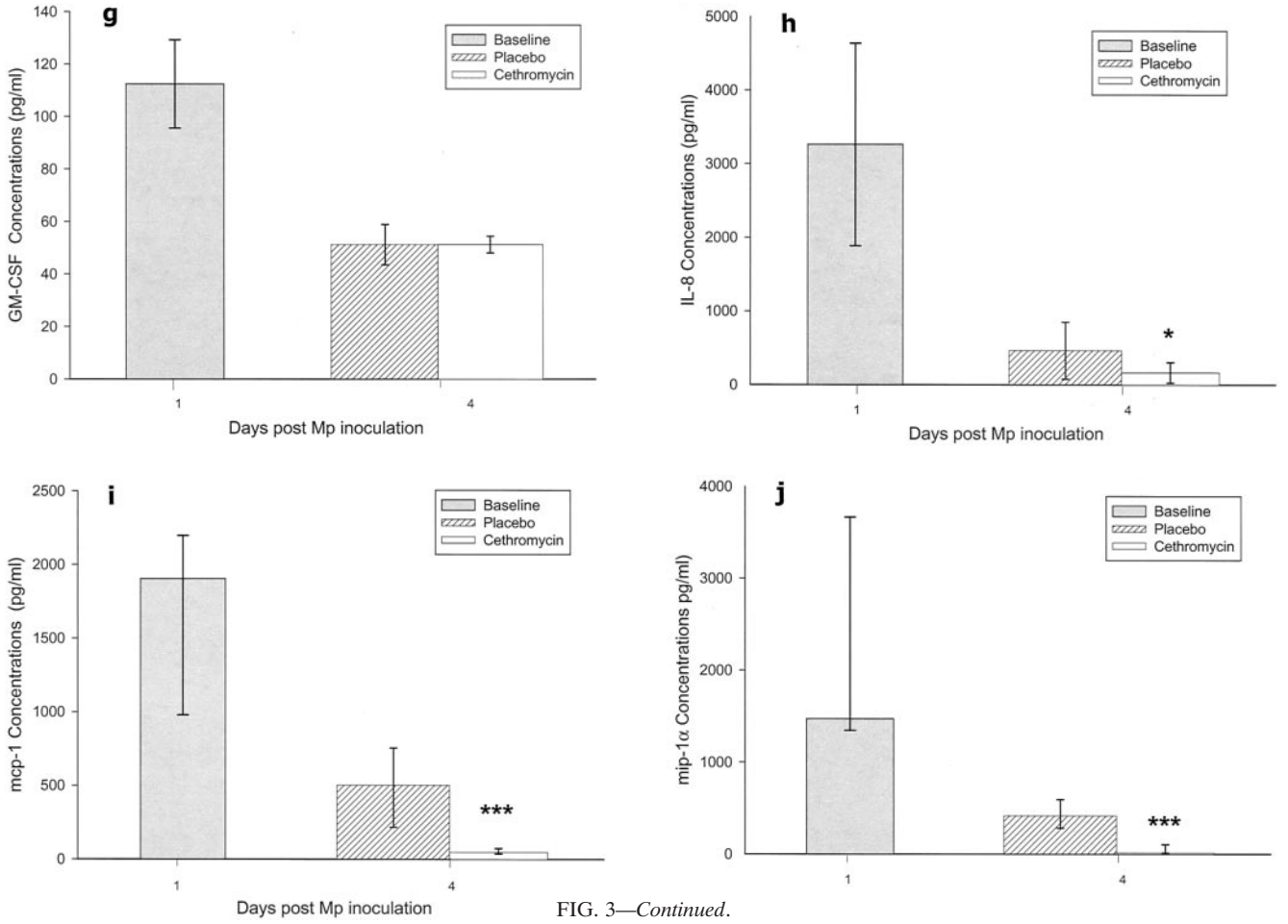

FIG. 3—*Continued*.

ously associated with disease severity in our laboratory's model (19) and an earlier study demonstrated that TNF-α released in response to *M. pneumoniae* could contribute to the enhancement of the cytotoxic activity of purified peritoneal macrophages and NK cells from BALB/c mice (2). Previous studies demonstrated that *M. pneumoniae* induced IL-1β gene expression following *M. pneumoniae* adherence to host target cells (52) or in the lungs of BALB/c mice acutely infected with *M. pneumoniae* (36). IL-1β and TNF-α share several activities, and in some situations, these two cytokines have been found to act synergistically (4, 39). In the present study, IL-1β and TNF-α concentrations were significantly correlated. IL-1β concentrations were strongly correlated with IL-2 concentrations, and both of these cytokines were in turn strongly correlated with histologic lung inflammation and abnormal pulmonary function tests. IL-1β is considered a multifunctional cytokine, and it activates T lymphocytes, enhancing the production of IL-2 and the expression of IL-2 receptors for lymphocyte stimulation (4). Lymphoid cell infiltration of the respiratory tract during mycoplasma infection suggests that lymphocyte activation is a key event in the progression of *M. pneumoniae* disease (10, 45).

Of the cytokines and chemokines evaluated, MCP-1 and MIP-1α concentrations in BAL fluid had the strongest inverse

TABLE 1. Correlations among outcome variables at day 4

Variable	Correlation with:											
	HPS			AO[a]			AHR[b]			Cethromycin therapy		
	r	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
M. pneumoniae culture	0.2	0.3	19	0.4	0.1	18	0.1	0.6	18	−0.2	0.3	19
HPS				0.5	0.03	20	0.5	0.04	20	−0.6	<0.001	23
AO							0.7	<0.001	20	−0.6	0.007	20
AHR										−0.4	0.08	20

[a] AO, airway obstruction.
[b] AHR, airway hyperreactivity.

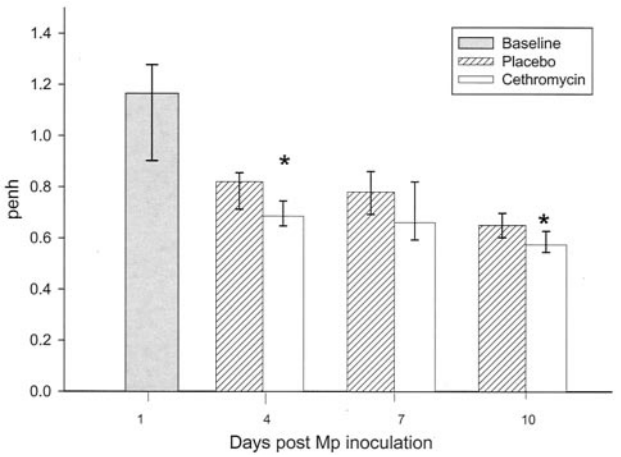

FIG. 4. Median P_{enh} of mice infected with *M. pneumoniae* (Mp) and treated with either cethromycin or placebo. Error bars represent 75th to 25th percentiles. *, $P < 0.05$. $n = 5$ to 10 mice per cethromycin treatment group and 5 to 15 mice per placebo group.

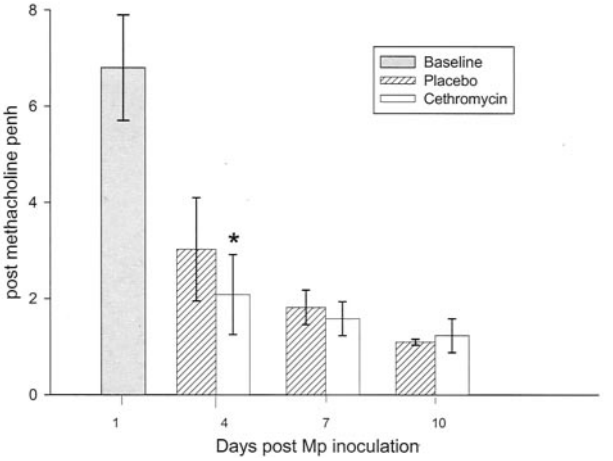

FIG. 5. Mean post-methacholine exposure P_{enh} of mice infected with *M. pneumoniae* (Mp) and treated with either cethromycin or placebo. Error bars represent standard deviations. *, $P < 0.05$. $n = 5$ to 10 mice per cethromycin treatment group and 5 to 15 mice per placebo group.

correlation with cethromycin therapy. These chemokines significantly correlated with lung inflammation and abnormal pulmonary function tests, as has been previously demonstrated in this model (19). The production of β-chemokines has been described with other mycoplasma respiratory infections in mice and is likely responsible in part for the mononuclear cell recruitment in the lungs, which seems to be a key event in the pathogenesis of mycoplasma respiratory disease and in the later development of chronic inflammation (42).

The reduction of TH1 cytokine concentrations observed with cethromycin therapy suggests that these cytokines play an important role in the inflammatory response in the lung infected with mycoplasma (24, 32, 35). The importance of the TH1 cytokines in response to acute *M. pneumoniae* respiratory infection, particularly that of IFN-γ, was previously demonstrated (19). IFN-γ has also been postulated to be an important mediator of lung inflammation in mice infected with *M. pulmonis* (34). In addition to IFN-γ, the present study also evaluated the concentrations of IL-12. There was a significant correlation between IFN-γ and IL-12 concentrations, and both cytokines were associated with lung inflammation and abnormal pulmonary function tests, particularly airway obstruction.

To the contrary, we have found that the TH2 response is not as significant in the acute *M. pneumoniae* respiratory infection model (19). Regarding IL-4, we have not documented significant differences between infected and noninfected mice when measuring concentrations in BAL fluid within the first 7 days after inoculation. More recently, an experiment was conducted which compared *M. pneumoniae*-infected BALB/c and C57BL/6 mouse strains with their respective uninfected controls. No statistically significant differences were demonstrated in the concentrations of IL-2, IL-4, IL-5, and GM-CSF in the BAL specimens (M. Fonseca-Aten, A. M. Rios, A. Mejias, S. Chavez, K. Katz, A. M. Gomez, G. H. McCracken, and R. D. Hardy, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr B-1671, 2003).

IL-4 concentrations were not associated with markers of disease severity, and they were not affected by cethromycin therapy. The role of IL-4 in the innate immune system has not

TABLE 2. Correlations of cytokines and chemokines in BAL samples at day 4

Cytokine or chemokine	Correlation with:														
	M. pneumoniae cultures			HPS			AO[a]			AHR[b]			Cethromycin therapy		
	r	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
IL-1β	0.2	0.4	15	0.5	0.05	19	0.3	0.2	16	0.6	0.02	16	−0.5	0.03	19
IL-2	0.3	0.3	15	0.5	0.01	19	0.4	0.2	16	0.4	0.1	16	−0.4	0.09	19
TNF-α	0.2	0.3	19	0.7	<0.001	23	0.4	0.09	20	0.3	0.2	20	−0.6	0.005	23
IL-12	0.5	0.06	15	0.5	0.02	19	0.6	0.01	16	0.4	0.2	16	−0.7	0.002	19
IFN-γ	0.7	0.004	15	0.7	<0.001	19	0.7	<0.001	16	0.6	0.01	16	−0.6	0.008	19
IL-4	0.03	0.9	15	0.4	0.1	19	0.3	0.2	16	0.4	0.1	16	−0.1	0.5	19
GM-CSF	0.02	0.9	15	0.3	0.1	19	0.08	0.7	16	0.3	0.2	16	0.05	0.8	19
IL-8	0.2	0.4	19	0.3	0.1	23	0.4	0.1	20	0.4	0.04	20	−0.4	0.003	23
MCP-1	0.3	0.3	19	0.6	0.001	23	0.6	0.005	20	0.4	0.07	20	−0.8	<0.001	23
MIP-1α	0.2	0.3	19	0.6	0.007	22	0.5	0.05	19	0.2	0.3	19	−0.8	<0.001	22

[a] AO, airway obstruction.
[b] AHR, airway hyperreactivity.

TABLE 3. Correlations of cytokines and chemokines in BAL samples at day 4

Cytokine or chemokine	Correlation with:														
	IL-1β			IL-2			TNF-α			IL-12			INF-γ		
	r	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
IL-1β				0.7	0.002	19	0.4	0.06	19	0.4	0.06	19	0.5	0.01	19
IL-2							0.5	0.04	19	0.5	0.01	19	0.5	0.02	19
TNF-α										0.2	0.3	19	0.5	0.01	19
IL-12													0.7	0.001	19
MCP-1	0.07	0.7	19	0.3	0.1	19	0.5	0.01	19	0.8	<0.001	19	0.8	<0.001	19
MIP-1α	0.3	0.2	19	0.3	0.2	19	0.5	0.02	19	0.7	<0.001	19	0.6	0.01	19

been well defined, and its contribution for first-time infections may be minimal (16). GM-CSF, which has been implicated in eliciting an allergic immune response with IL-5 and IL-3 (4), was not an important determinant of disease severity in this study, and its concentrations were not affected by cethromycin therapy. Human studies have revealed both TH1 and TH2 pulmonary host responses to be significant in *M. pneumoniae* pneumonia. The relative contributions of each are under investigation (9, 25, 27).

The discrepancy observed between the antimicrobial and anti-inflammatory effects of cethromycin therapy, also observed for clarithromycin therapy (20), demonstrates the importance of the host immune response, as opposed to the invading microbe, in the pathogenesis of *M. pneumoniae* infection (5, 23, 46). In this particular study, *M. pneumoniae* cultures were not associated with lung histopathology on day 4, whereas some of the cytokines and chemokines in the BAL fluid were strongly correlated with lung histopathology, supporting the immunopathological nature of *M. pneumoniae* infection.

Innate immunity provides the main mechanisms of defense after the initial encounter with *M. pneumoniae* in this model (5, 19, 21), and this response in turn will likely influence the type of adaptive immune response in the later stages of infection as well as in subsequent encounters with this microorganism (21). The impact of antibiotics, such as clarithromycin and cethromycin, appears to be beneficial to the host, despite their inability to achieve complete eradication of *M. pneumoniae* from the airway. Their effects on the acutely released cytokines and chemokines may not only hasten the resolution of the acute illness but may also contribute to improved long-term clinical outcomes, such as decreased recurrent reactive airway disease associated with chronic *M. pneumoniae* disease, even though *M. pneumoniae* is not completely eradicated from the airway.

ACKNOWLEDGMENT

This work was supported in part by a research grant from Abbott Laboratories.

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