Introduction
As well as its antimicrobial actions, erythromycin may have immunomodulating effects, such as attenuation of neutrophil chemotaxis, inhibition of the production of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-6, and inhibition of the synthesis of IL-8 and other chemokines. Thus erythromycin may have therapeutic value for various infectious and inflammatory disorders independent of its antimicrobial properties.

Erythromycin has been reported to be effective in the treatment of chronic inflammatory conditions of the lower respiratory tract, such as diffuse panbronchiolitis. Diffuse panbronchiolitis is characterized by chronic inflammation in the respiratory bronchioles with infiltration of plasma cells and lymphocytes. The chronic inflammatory lesions often extend to the more proximal bronchioles, leading to secondary bronchiectasis in the more advanced stages. Early in the course of the disease sputum cultures are often positive for Haemophilus influenzae, while in the advanced stage this microorganism is replaced by Pseudomonas aeruginosa. The effectiveness of erythromycin seems to be due to mechanisms other than its antimicrobial action, since this macrolide has no activity towards P. aeruginosa. Interestingly, bacteria are not always eradicated from sputum in responsive patients.

Inflammation during pneumonia is orchestrated by locally produced pro-inflammatory cytokines [e.g. TNF-α, IL-6, interferon-γ (IFN-γ) and IL-8] and anti-inflammatory cytokines (e.g. IL-10). Local modulation of the cytokine network may serve as an important addition to antibiotic therapy. The effect of erythromycin on cytokine synthesis induced by P. aeruginosa is unknown. Therefore, in the present study we sought to determine the effect of erythromycin on cytokine production in whole blood stimulated with heat-killed P. aeruginosa (HKPA) in vitro. Furthermore we determined the effect of other antibiotics with anti-Pseudomonas activity on cytokine release.

Materials and methods
Reagents and bacteria
Erythromycin was purchased from Abbott (Amstelveen, The Netherlands). The antibiotics with anti-Pseudomonas activity were purchased from: imipenem (Merck Sharp and Dohme, Haarlem, The Netherlands); ceftazidime (Glaxo, Glaxo, UK); gentamicin (Sanochemia, Breda, The Netherlands); and ciprofloxacin (Novartis, Houten, The Netherlands).

Brief reports
Erythromycin inhibits Pseudomonas aeruginosa-induced tumour necrosis factor-α production in human whole blood
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Erythromycin has been shown to be beneficial for panbronchiolitis, a disorder linked to infection with Pseudomonas aeruginosa. Erythromycin, but not the anti-Pseudomonas antibiotics imipenem, ceftazidime, gentamicin and ciprofloxacin, caused a dose-dependent decrease in the production of tumour necrosis factor (TNF)-α by whole blood stimulated with heat-killed P. aeruginosa. The release of interleukin (IL)-10, IL-6, interferon-γ and IL-8 was inhibited only at the highest erythromycin concentration. Inhibition of TNF-α production by erythromycin may, at least in part, explain the efficacy of this macrolide during panbronchiolitis despite its lack of activity for P. aeruginosa.
Zeist, The Netherlands); gentamicin (Centrafarm, Etten-Leur, The Netherlands); and ciprofloxacin (Bayer, Mijdrecht, The Netherlands). Only clinically relevant concentrations were used in whole blood stimulations (erythromycin, imipenem and ceftazidime 10⁻⁵–10⁻³ M; gentamicin 10⁻⁶–10⁻⁴ M; ciprofloxacin 10⁻⁷–10⁻⁵ M).

HKPA was used as a stimulus. *P. aeruginosa* (serotype PA103) was obtained from a clinical isolate. The bacteria were cultured overnight in 1 L Luria broth at 37°C, harvested by centrifugation, washed twice in pyrogen-free 0.9% NaCl, resuspended in 10 mL 0.9% NaCl and heat-inactivated for 60 min at 80°C.

**Whole blood stimulation**

Whole blood stimulation was performed as described previously. Briefly, blood was collected aseptically from healthy subjects using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson, Rutherford, NJ, USA). Anticoagulation was achieved using endotoxin-free heparin (Heparine Leo Pharmaceutical Products B.V, Weesp, The Netherlands) (10 U/mL blood, final concentration). Whole blood, diluted 1:1 in sterile RPMI-1640 (Gibco-BRL, Life Technologies Inc., Paisley, Scotland, UK), was stimulated for 4–24 h at 37°C with HKPA (amounts equivalent to 10⁶ cfu/mL, final concentration) in sterile polypropylene tubes (Becton Dickinson). For these experiments, polypropylene tubes were prefilled with 0.75 mL of RPMI with or without the appropriate concentrations of HKPA and antibiotics, after which 0.75 mL of heparinized blood was added. For each experiment, blood was taken from six different volunteers. Tubes were then mixed gently and placed in the incubator. After the incubation, plasma was prepared by centrifugation and stored at −20°C until assays were performed.

**Assays**

Concentrations of cytokines were measured by specific ELISAs according to the manufacturer’s instructions. Erythromycin has no bactericidal or bacteriostatic activity against *P. aeruginosa*. We have demonstrated that erythromycin dose-dependently inhibited HKPA-induced synthesis of TNF-α, IL-10, IL-6, IFN-γ and IL-8 (Table I). Maximum inhibition was achieved at a concentration of 10⁻³ M (*P < 0.05*). None of the other antibiotics demonstrated an inhibitory effect on cytokine synthesis induced by HKPA (data shown for TNF-α in Table II).

**Discussion**

Erythromycin has no bactericidal or bacteriostatic activity against *P. aeruginosa*. We have demonstrated that erythromycin dose-dependently inhibited HKPA-induced synthesis of TNF-α, IL-10, IL-6, IFN-γ and IL-8.

**Statistical analysis**

All values are given as mean ± standard error (s.e.). Two sample comparisons were performed using the Wilcoxon test for matched samples. *P < 0.05* was considered to represent a statistically significant difference.

**Results**

Incubation of whole blood without HKPA did not result in detectable levels of TNF-α, IL-10, IL-6, IFN-γ or IL-8, and none of the antibiotics used in the experiments described led to production of cytokines in whole blood (data not shown). Incubation of whole blood with HKPA (10⁶ cfu/mL) resulted in a time-dependent release of all cytokines measured. TNF-α, IL-10, IL-6, IFN-γ and IL-8 were detectable after 4 h incubation, and high concentrations were reached after 16 h incubation (TNF-α: 17.9 ± 2.6 ng/mL; IL-10: 4.7 ± 1.5 ng/mL; IL-6: 116.3 ± 6.3 ng/mL; IFN-γ: 2.3 ± 0.5 ng/mL; IL-8: 42.5 ± 4.9 ng/mL). Therefore, a 16 h incubation was used for subsequent investigations. Erythromycin dose-dependently inhibited HKPA-induced synthesis of TNF-α, IL-10, IL-6, IFN-γ and IL-8 (Table I). Maximum inhibition was achieved at a concentration of 10⁻³ M (*P < 0.05*). None of the other antibiotics demonstrated an inhibitory effect on cytokine synthesis induced by HKPA (data shown for TNF-α in Table II).

**Table I.** Effect of erythromycin on the synthesis of TNF-α, IL-10, IL-6, IFN-γ and IL-8 in whole blood stimulated with *P. aeruginosa*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Concentration (ng/mL) at erythromycin concentration of</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 M</td>
</tr>
<tr>
<td>TNF-α</td>
<td>18.4 ± 3.6</td>
</tr>
<tr>
<td>IL-10</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>IL-6</td>
<td>277.6 ± 113.0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>7.1 ± 2.1</td>
</tr>
<tr>
<td>IL-8</td>
<td>52.2 ± 4.9</td>
</tr>
</tbody>
</table>

Whole blood, diluted 1:1 in RPMI 1640 was stimulated with 10⁶ cfu/mL HKPA in the presence of different concentrations of erythromycin for 16 h at 37°C. Data are mean ± s.e. of blood from six healthy volunteers.

*a* *P < 0.05* compared with no erythromycin.

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Erythromycin inhibits TNF-α production

Table II. Effect of antimicrobial agents with anti-pseudomonas activity on the synthesis of TNF-α in whole blood stimulated with *P. aeruginosa*

<table>
<thead>
<tr>
<th>Agent</th>
<th>TNF-α (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>10.8 ± 0.7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>15.5 ± 3.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10.6 ± 1.1</td>
</tr>
</tbody>
</table>

Whole blood, diluted 1:1 in RPMI 1640 was stimulated with 10⁵ cfu/mL HKPA in the presence of different concentrations of imipenem, ceftazidime, gentamicin and ciprofloxacin for 16 h at 37°C. Data are means ± s.e. of blood from six healthy volunteers.

Erythromycin inhibits cytokine production induced by *P. aeruginosa*. Conceivably, this and other immunomodulatory effects may at least in part, explain the beneficial effects of erythromycin during panbronchiolitis.

Previous studies focusing on the effects of erythromycin and other macrolides on inflammatory responses *in vitro* and *in vivo* have yielded similar results. Tamaoki et al. recently showed the effect of erythromycin on cytokine production by IgG immune complex-stimulated rat alveolar macrophages *in vitro*, demonstrating a reduction in TNF and IL-1β production when macrophages were co-incubated with erythromycin; *in vivo* rats pre-treated with erythromycin had fewer neutrophils in the alveoli than control rats after intratracheal administration of rabbit IgG. Recently, the role of cytokines was investigated in a mouse model of chronic *P. aeruginosa* infection mimicking diffuse panbronchiolitis. Concentrations of TNF-α, IL-1β, IL-2, IL-4, IL-5 and IFN-γ were measured serially in the lungs until 60 days after inoculation, demonstrating significantly higher levels during the course of the disease compared with baseline. Clarithromycin, another macrolide, significantly inhibited the production of TNF-α and IL-1β in the lung. Treatment with anti-TNF-α antibodies significantly reduced the number of lymphocytes in the lung, but did not change the number of viable bacteria. These data are in line with the suggestion that macrolides modulate the production of cytokines, and thereby influence the inflammatory response and the clinical outcome.

Erythromycin most potently attenuated the production of TNF-α, which is in line with the effect of this antibiotic on cytokine production induced by *Streptococcus pneumoniae*. The role of TNF-α in respiratory tract infection may vary depending on the pathogen involved. Indeed, in murine pneumonia caused by *S. pneumoniae* or *Klebsiella pneumoniae*, endogenous TNF-α was found to be important for the clearance of bacteria from the pulmonary compartment. In contrast, endogenous TNF-α impairs host defence during murine pneumonia caused by *P. aeruginosa*. It is possible that in pneumonias such as those caused by *S. pneumoniae* and *K. pneumoniae* a certain pro-inflammatory cytokine response within the pulmonary compartment is required to combat the invading microorganism, while in chronic pneumonias such as diffuse panbronchiolitis, in which an ongoing inflammatory response contributes to an adverse outcome, attenuation of the inflammatory response might be helpful in the therapy.

IL-8 is thought to be the key neutrophil chemotactic cytokine in patients with diffuse panbronchiolitis. Indeed, significant reductions in bronchoalveolar lavage fluid (BALF) neutrophil percentages and in neutrophil chemotactic activity were observed in post-erythromycin treatment BALF of patients with diffuse panbronchiolitis. Significantly higher pre-treatment levels of IL-8 were found in BALF of patients with diffuse panbronchiolitis than in the BALF of control subjects. Treatment for 1–24 months significantly reduced BALF levels of IL-8 in diffuse panbronchiolitis patients, in parallel with a reduction in BALF neutrophils. Our data on inhibition of IL-8 production by erythromycin *in vitro* extend these findings.

Erythromycin only reduced the production of IL-10, IL-6, IFN-γ and IL-8 at the highest concentrations tested. In this respect it is important to note that this inhibitory effect is not related to a cytotoxic effect of erythromycin on cytokine producing cells, since we established previously that this antibiotic does not influence cell viability at this concentration. Furthermore, although concentrations of erythromycin are much lower in airways, intracellular accumulation of erythromycin is probably necessary to accomplish the effects in lungs *in vivo*.

Pneumonia is associated with local production of cytokines. We demonstrated that erythromycin, but not antibiotics with antimicrobial activity against *Pseudomonas* spp., inhibits TNF-α production in whole blood stimulated with *P. aeruginosa* *in vitro*. Together with other reported anti-inflammatory effects of macrolides, these data suggest that during the treatment of pneumonia the immunomodulatory actions of this group of antibiotics may offer a potential advantage during chronic respiratory infections such as panbronchiolitis.
References


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