always resistant to all β-lactams without any exception.

The reason why NaCl increases resistance to vancomycin, as described previously [9], is not known, nor is the reason for the decrease in the MIC of teicoplanin, which to our knowledge has never been reported previously. Antagonism between β-lactams and vancomycin is very clear in the presence of NaCl 4% w/v (Fig. 1b), and this is particularly true for strain Mu3. For some other strains, the persistence of a few colonies within the β-lactam inhibition zone indicates that at least part of the population is not inhibited by high concentrations of β-lactams in the presence of vancomycin. Moreover, because of the short half-life of most β-lactams, it is anticipated that only low β-lactam levels are present to interact with glycopeptides for quite a long period. However, at high vancomycin concentrations, and for a very limited MIC range (0.5–1 mg/L), the interaction between vancomycin and β-lactams can be considered as synergistic.

In contrast to vancomycin, the interaction between teicoplanin and β-lactams is more often synergistic, as has been demonstrated previously for teicoplanin-resistant isolates of Staphylococcus epidermidis [11]; in the presence of NaCl there is enhanced synergy, depending on the precise concentration of the β-lactam (data not shown).

The precise clinical significance and reasons for these results are unknown, but it can be concluded that vancomycin should not be used in association with β-lactams for the treatment of GISA infections.

REFERENCES


RESEARCH NOTE

Use of quantitative and objective enzyme immunoassays to investigate the possible association between Chlamydia pneumoniae and Mycoplasma pneumoniae antibodies and asthma

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ABSTRACT

Sera from 150 consecutive patients with established asthma and 150 matched controls were examined for Chlamydia pneumoniae IgG and IgA with commercially available enzyme immunoassays (EIAs) detecting immune response solely to surface proteins of elementary bodies. The assays were also modified to measure combined immune response to surface proteins and family-specific lipopolysaccharide antigen. Mycoplasma pneumoniae IgG and IgA were measured with new commercial EIAs utilising P1-enriched protein fraction as an antigen. No statistically significant differences between the patient groups in terms of prevalence or levels of antibodies to either organism were found with these methods.

Keywords Asthma, Chlamydia pneumoniae, IgG and IgA class antibodies, Mycoplasma pneumoniae, sero-prevalence

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A possible association between Chlamydia pneumoniae infection and adult-onset asthma was first reported by Hahn et al. [1]. Some subsequent investigations confirmed the relationship between increased C. pneumoniae antibody titres and bronchial hyper-responsiveness and exacerbation of asthma in adults [2–5] or children [6], but results of other studies failed to confirm this association [7–9]. The possible association between Mycoplasma pneumoniae and asthma is also rather controversial [9–11]. Most such seroepidemiological studies have been performed using the microimmunofluorescence (MIF) method, but there has been a growing interest in other methodological approaches. Thus, Schumacher et al. [12] showed that the outcome and clinical conclusion regarding the association between C. pneumoniae and coronary heart disease was dependent on the method chosen. The present study aimed to investigate whether detection of combined C. pneumoniae antibody to both lipopolysaccharide (LPS) and surface proteins, compared to surface proteins only, would influence the results obtained. In addition, the possible association between M. pneumoniae and asthma was examined using new specific enzyme immunoassays (EIAs) [13].

During the period 1998–2000, 150 consecutive patients (60 men and 90 women; mean age 37.3 years) from Smolensk Clinical Hospital (Russia) with established asthma were included in the study on the basis of the following criteria: an increase of ≥15% in forced expiratory volume (FEV1) after inhalation of a bronchodilator (β2-agonist with a short action); and/or an increase of ≥20% in peak expiratory flow (PEF) after inhalation of a bronchodilator (β2-agonist with a short action); and/or variation in FEV1 (PEF) of ≥20% within a day on more than one occasion, with no radiological evidence of other pulmonary diseases during the previous 2 years. The adult onset of asthma was determined by questionnaire.

Healthy volunteers (62 men and 88 women; mean age 34.5 years) with no clinical symptoms of asthma were frequency matched to asthma patients by age and sex, and included in a control group for comparison. The exclusion criteria for both groups were: a previous history of acute and chronic upper and lower respiratory tract diseases other than asthma; age > 55 years; serious underlying diseases (neoplasms, pulmonary diseases, chronic coronary heart disease); immunotherapy within the year before enrolment in the study; and antibiotic therapy during the current exacerbation of asthma. Signed informed consent was obtained from all subjects before enrolment. The research protocols were approved by the Ethical Committee of the Smolensk State Medical Academy.

After collection, sera were stored at −80 °C and sent in dry ice to the ThermoLabsystems (Helsinki, Finland) laboratory for analysis. All assays were performed blind by the same technician with the same batch of reagents. C. pneumoniae IgG and IgA antibodies were measured according to the instructions for the Chlamydia pneumoniae IgG and IgA EIA Kits (ThermoLabsystems). These assays measure antibodies to the surface protein antigens only. For the present study, the components of the kit were also optimised to allow the measurement of combined anti-protein and anti-LPS antibodies. The cut-offs for positivity were those recommended in the current kit instructions, namely > 45 EIUs (enzyme-immune units) for IgG and > 12 EIUs for IgA, irrespective of the conventional or modified assays being used.

M. pneumoniae antibodies were measured according to the instructions for the ThermoLabsystems Mycoplasma pneumoniae IgG and IgA EIA Kits. In these EIAs, antibody responses are meas-
Comparison of the levels of specific antibodies (expressed in enzyme-immune units) to Chlamydia pneumoniae and Mycoplasma pneumoniae in asthma patients and healthy controls

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Control (n = 150) Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Asthma (n = 150) Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Unadjusted*</th>
<th>Adjustedf</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pneumoniae IgA</td>
<td>12.4 ± 14.9</td>
<td>6.0</td>
<td>0–80</td>
<td>14.4 ± 16.9</td>
<td>9.0</td>
<td>1–86</td>
<td>0.279</td>
<td>0.566</td>
</tr>
<tr>
<td>C. pneumoniae IgA (LPS)</td>
<td>14.0 ± 14.6</td>
<td>9.0</td>
<td>0–73</td>
<td>16.5 ± 16.9</td>
<td>11.0</td>
<td>0–86</td>
<td>0.187</td>
<td>0.408</td>
</tr>
<tr>
<td>C. pneumoniae IgG</td>
<td>76.4 ± 84.5</td>
<td>47.0</td>
<td>2–445</td>
<td>80.3 ± 93.5</td>
<td>49.0</td>
<td>2–431</td>
<td>0.697</td>
<td>0.877</td>
</tr>
<tr>
<td>C. pneumoniae IgG (LPS)</td>
<td>85.0 ± 79.4</td>
<td>57.5</td>
<td>3–391</td>
<td>86.4 ± 83.1</td>
<td>61.5</td>
<td>1–376</td>
<td>0.719</td>
<td>0.902</td>
</tr>
<tr>
<td>M. pneumoniae IgA</td>
<td>17.2 ± 13.3</td>
<td>14.0</td>
<td>0–110</td>
<td>18.5 ± 12.9</td>
<td>14.5</td>
<td>4–91</td>
<td>0.412</td>
<td>0.311</td>
</tr>
<tr>
<td>M. pneumoniae IgG</td>
<td>124.3 ± 115.3</td>
<td>88.0</td>
<td>9–357</td>
<td>109.0 ± 94.6</td>
<td>79.0</td>
<td>6–432</td>
<td>0.212</td>
<td>0.869</td>
</tr>
</tbody>
</table>

*Student’s t-test for independent variables; t ANOVA model including: group (control, asthma), age, body-mass index, hypertension, smoking.

**Table 1.** Prevalence of seropositivity for Chlamydia pneumoniae- and Mycoplasma pneumoniae-specific antibodies

**Table 2.** Comparison of the levels of specific antibodies (expressed in enzyme-immune units) to Chlamydia pneumoniae and Mycoplasma pneumoniae in asthma patients and healthy controls

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REFERENCES


