IgG and IgA response to Simkania negevensis in sera of patients with respiratory and gastrointestinal symptoms

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Simkania negevensis is an obligate intracellular Gram-negative bacterium belonging to the family of Simkaniaaceae in the order Chlamydiales (Everett et al., 1999), discovered as a contaminant in a variety of cell cultures (Kahane et al., 1993). S. negevensis is also able to replicate in various environmental free-living amoebae such as Acanthamoeba polyphaga and persists for long periods of time in amoebal cysts (Kahane et al., 2001).

Previous studies detected S. negevensis in water sources and showed that it is relatively resistant to chlorination procedures used for routine treatment of drinking water supplies (Kahane et al., 2004). Additional studies compared S. negevensis partial 16S rDNA sequences from clinical samples of children with pneumonia and domestic water samples from their homes, indicating a “common strain” of Simkania (Kahane et al., 2007a).

Epidemiologic studies reported widespread human exposure to this bacterium (Friedman et al., 2003) both in healthy subjects and in association with respiratory diseases in infants and adults (Lieberman et al., 1997; Kahane et al., 1998; Lieberman et al., 2002; Greenberg et al., 2003; Kumar et al., 2005; Fasoli et al., 2008). Few studies have been reported on the humoral and cell-mediated response in S. negevensis infections, unlike chlamydial infections (Meoni et al., 2009).

The seropositivity to S. negevensis in healthy population groups suggested the organism is a simple colonizer. However, in vitro studies have shown that S. negevensis can infect human cell cultures of various tissue origins, such as respiratory epithelial cells, gastrointestinal tract, genital tract and endothelial cells (Kahane et al., 2007b).

The aim of this study was to retrospectively evaluate the IgG and IgA response to S. negevensis in
sample sera in Italy from patients with respiratory and gastrointestinal symptoms in comparison to healthy controls. Serum antibodies were detected by the microimmunofluorescence (MIF) test. The possible cross-reactivity between S. negevensis and Chlamydia pneumoniae serology was evaluated.

A total of 326 sera, collected over a 24 month period in 2008-2010 for diagnostic investigations from patients admitted to S. Orsola Hospital (Bologna, Italy) were tested. In particular, 102 samples were from patients (aged 52.4±19.3 years) hospitalized with symptoms of lower respiratory tract infections and 224 from patients (aged 53.2±20.3 years) with gastrointestinal symptoms (diarrhoea, vomiting), fever and elevated inflammatory blood parameters (VES and neutrophils). The sera from the patients with enteric disorders were negative for antibody to enterovirus and Salmonella spp. As controls, 104 sera from blood donors (aged 45.6±12.4 years) were used.

S. negevensis Z (American Type Culture Collection VR-1471) and C. pneumoniae IOL-207 reference strains were grown in LLC-MK2 cells in six large well plates (Donati et al., 2003) and elementary bodies (EBs) were purified by use of sucrose gradients (Fukushi & Hirai, 1988).

The MIF test was performed according to the method of Wang and Grayston (1986). Sera were screened at 1:16 and 1:8 dilution in phosphate buffered saline (PBS) supplemented with 2% foetal calf serum, for IgG and IgA detection, respectively. FITC-conjugated goat antibody anti-human IgG diluted 1:40 or anti-IgA diluted 1:30 in PBS (Dako, Copenhagen, Denmark), were used. Positive sera were tested by serial dilutions and the reciprocal of the highest serum dilution considered positive represented the antibody titre of the sample. χ² test (p<0.05) was used in the statistical analysis of the data.

IgG and IgA response to S. negevensis detected by MIF test are shown in Table 1. The prevalence of IgG antibodies to S. negevensis was 50% (51/102) in sera of patients with lower respiratory tract infections, 68% (152/224) in sera of patients with gastrointestinal disorders and 35% (36/104) in sera of healthy controls. IgG positive sera showed titres ranging from 16 to 128.

The IgG seropositivity rate to S. negevensis showed a statistically significant difference in patients with respiratory infections (p=0.03, odds ratio 1.9, 95% confidence interval [CI]:1.08-3.31) and in patients with gastrointestinal problems (p<0.001, odds ratio 4, 95% CI: 2.26%-7.02) when compared with healthy controls. In patients with respiratory symptoms, IgG antibodies were increasingly prevalent with increasing age, starting from 18% in patients aged 30-40 years and increasing to 67% in patients aged over 65 years. The patients with gastrointestinal symptoms and the healthy controls did not show any age-related change in prevalence.

S. negevensis IgA were found in 13% (13/102) of the sera of patients with signs of respiratory infection, 18% (40/224) of the sera of patients with gastrointestinal disorders and 2% (2/104) of the healthy controls. IgA positive sera showed titres ranging from 8 to 32.

IgA positive sera to S. negevensis did not present IgA to C. pneumoniae. In relation to IgG positive sera to S. negevensis, 26 sera from patients with respiratory symptoms, 92 sera from patients with gastrointestinal signs and 21 sera from healthy controls reacted to C. pneumoniae at low titre. To evaluate cross-reacting antigens, a limited number of 20 sera reacting to S. negevensis and C. pneumoniae were absorbed with C. pneumoniae according to Yamaguschi et al. (2005) and then tested again by MIF. All these sera no longer reacted with C. pneumoniae and showed IgG titres to S. negevensis reduced within one dilution. The adsorption results showed that there was no substantial cross-reactivity between S. negevensis and C. pneumoniae antibodies, according to Yamaguschi et al. (2005) and Fasoli et al. (2008). Reports of exposure to S. negevensis are available.

### Table 1 - IgG and IgA antibodies to S. negevensis in adult patients by MIF technique

<table>
<thead>
<tr>
<th>Sera</th>
<th>No. of MIF positive sera/ No. of sera tested (%)</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
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<tr>
<td>Patients with respiratory infections</td>
<td>51/102 (50)</td>
</tr>
<tr>
<td>Patients with gastrointestinal signs</td>
<td>152/224 (68)</td>
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<tr>
<td>Blood donors</td>
<td>36/104 (35)</td>
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from various parts of the world, both in healthy subjects and in association with respiratory diseases in infants and adults. In Italy, the S. negevensis seroprevalence was first investigated in North Italian children with community-acquired pneumonia (CAP), showing that 20-30% of the children had measurable antibodies to S. negevensis (Fasoli et al., 2008). To our knowledge, no investigation has been performed on S. negevensis exposure in Italian adults to date. The present study investigated the IgG and IgA response to S. negevensis in two groups of adult Italian patients with respiratory and gastrointestinal problems by MIF test. The seropositivity IgG (50%) and IgA (13%) rates found in the subjects with respiratory signs were consistent with those previously published for adults (Friedman et al., 2006). According to other reports (Kumar et al., 2005; Yamasuchi et al., 2005; Friedman et al., 2006), the seropositivity rates increased with age. There are no comparative studies on seroprevalence to S. negevensis in patients with gastrointestinal disorders, although Lieberman et al. (1997) reported gastrointestinal symptoms in some CAP patients with serological evidence of acute S. negevensis infection. In subjects with gastrointestinal symptoms, we detected a significantly higher IgG (68%) and IgA (18%) seroprevalence than in the healthy controls. Organism-specific IgA may be an indication of current or recent infection (Friedman et al., 2006). The IgG and IgA response in subjects with gastrointestinal disorders, in association with the already described detection of S. negevensis in water sources, could support a possible oral route of infection other than droplets or close contact. Previous in vitro studies have shown that S. negevensis can infect the human gastrointestinal tract and is able to assume a persistent form of infection, which may lead to a prolonged inflammatory response (Kahane et al., 2007b). Furthermore, in an in vitro simulation model Kahane et al. (2008) suggested a role of monocyte/macrophages as vehicles of dissemination of S. negevensis to other body compartments. More extensive molecular studies and attempts to isolate the microorganism are needed to elucidate our observations on a possible association of S. negevensis with gastrointestinal infections and its pathogenic role.

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