Detection of Simkania negevensis in cell culture by using a monoclonal antibody

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Simkania negevensis, belonging to the family Simkaniaceae in the order Chlamydiales, is associated with bronchiolitis in infants (Kahane et al., 1998), community-acquired pneumonia in adults (Lieberman et al., 1997) and acute exacerbation of chronic obstructive pulmonary disease (COPD) (Lieberman et al., 2002).

S. negevensis is sensitive to tetracyclines and macrolides, similar to chlamydiae; a quinolone resistance is present in S. negevensis (Casson and Greub, 2006) whereas no tetracycline resistance has been shown in this bacterium to date, unlike the presence of many C. suis tetracycline-resistant strains in animal chlamydial infections (Di Francesco et al., 2008).

Few data on the immune response in S. negevensis infection can be found in the literature, while several studies on chlamydial immune response have been published (Meoni et al., 2009). Although only few reports on the prevalence of S. negevensis in human infections are present, owing to the unavailability of commercial diagnostic tests, a high seroprevalence of antibodies to S. negevensis in healthy adults from different part of the world has been reported (Friedman et al., 1999; Friedman et al., 2000; Friedman et al., 2006).

Evidence of infection with S. negevensis has been supported by culture, polymerase chain reaction (PCR) and in-house serological tests (Friedman et al., 2006). Culture needs polyclonal hyper-immune animal sera raised against S. negevensis (Casson and Greub, 2006) whereas no tetracycline resistance has been shown in this bacterium to date, unlike the presence of many C. suis tetracycline-resistant strains in animal chlamydial infections (Di Francesco et al., 2008).

In the present study, a monoclonal antibody (mAb), D5-14, raised in our laboratory against Chlamydia trachomatis LGV2 serotype, stained Simkania negevensis inclusions in S. negevensis-infected cells by using the immunofluorescence test. D5-14 mAb, reacting in immunoblot with an approximately 64-66-kDa protein of C. trachomatis LGV2 serotype, recognized a protein with the same molecular mass when tested with S. negevensis elementary bodies.

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tested 20 mAbs, raised in our laboratory against Chlamydia trachomatis LGV2 serotype, against S. negevensis infected cells. The mAb D5-14 was the only one which stained S. negevensis inclusions in S. negevensis-infected cells by using the immunofluorescence test. D5-14 mAb reacted also against C. pneumoniae and C. psittaci-infected cells.

S. negevensis and C. trachomatis LGV2 serotype were grown in monolayer cultures of LLC-MK2 cells (a continuous cell line prepared from Rhesus monkey kidney). Murine mAbs directed against C. trachomatis LGV2 serotype were produced as previously described (Cevenini et al., 1987).

In order to evaluate the time of appearance of positive immunofluorescence in infected cell cultures, D5-14 mAb was tested by an indirect immunofluorescence assay in which S. negevensis or C. trachomatis-infected LLC-MK2 cells were used as antigen. At various time intervals after infection with S. negevensis or C. trachomatis, the cells were fixed with methanol. Uninfected LLC-MK2 cells were used as control. Bound mAb was detected with fluorescein-conjugated anti-mouse immunoglobulins (Dako, Copenhagen, Denmark).

For immunoblotting identification of the protein recognized by D5-14 mAb, S. negevensis elementary bodies (EBs) were purified by sucrose density-gradient (Fukushi & Hirai, 1988). C. trachomatis LGV2 serotype EBs were purified in the same way. The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) procedure of Laemmlli (1970) was followed using a 4-12% (w/v) precast gel (Life Technologies). The molecular weight marker (Invitrogen) was used as a molecular mass marker. After protein electrophoretic transfer from gels into nitrocellulose sheets (Comanducci et al., 1994), the nitrocellulose paper was stained with Ponceau-S (0.1% in 1% acetic acid) to ensure the transfer of proteins. The strips were then blocked with 3% albumin in sodium phosphate, 0.15M NaCl, 0.05% (v/v) Tween 20 (PBST). The nitrocellulose paper was then cut into strips which were incubated with the mAb overnight at room temperature under gentle agitation. The strips were washed 3 times with PBST, followed by incubation for 2 h at room temperature with peroxidase-labelled rabbit anti-mouse immunoglobulins (Dako) diluted 1:1000 in PBST. The strips, washed 3 times, were then transferred to distilled water and the immunoblots developed by using 4-chloro-naphtol (Bio-Rad) and then rinsed with distilled water.

In order to characterize the immunoglobulin subclass of D5-14 mAb, the IsoQuick Strips for mouse monoclonal isotyping (Sigma-Aldrich, St. Louis, MO USA) was used. The D5-14 mAb, reacting in immunoblot with a 64-66-kDa protein of C. trachomatis (LGV2 serotype), recognized a protein with the same molecular mass when tested with S. negevensis EBs (Figure 1).

The results of the immunofluorescence test of S. negevensis-infected cell cultures at various times of infection revealed that D5-14 mAb, reacting positively as early as 8 h post-infection with

**FIGURE 1 - Western blot analysis of mAbD5-14 with C. trachomatis (lane 1) and Simkania negevensis (lane 2).**
C. trachomatis inclusions, reacted 16 h post-infection with S. negevensis inclusions. In addition, the intensity of the fluorescence and the dimension of the detected inclusions of C. trachomatis and S. negevensis, peaked at 48 h and 72 h p.i., respectively (Figure 2). IgG1 K chain was the isotype class identified in D5-14 mAb.

S. negevensis from clinical samples can be isolated in cell line monolayers and polyclonal hyper-immune animal sera are used for its detection. The D5-14 mAb described in the present study was able to detect S. negevensis in cell monolayers by immunofluorescence as early as 16 h p.i. with a more intense reactivity 72 h p.i., in comparison to the reactivity against C. trachomatis LGV2 serotype detectable as early as 8 h p.i. with a high intensity of fluorescence 48 h p.i. In immunoblot D5-14 mAb recognized a protein very close to the 64 kDa protein of the molecular weight marker in both C. trachomatis and S. negevensis EBs. Previous studies performed on antigens and the membrane structure of S. negevensis showed a polypeptide pattern significantly different from that of other Chlamydiales members. Monoclonal antibodies against chlamydial OMP1 and OMP2 proteins did not react with any corresponding polypeptides of S. negevensis, while reactivity with polyclonal, monospecific antibodies to the 60 kDa chlamydial heat shock pro-

\begin{figure}
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\caption{Chlamydia trachomatis inclusions detected by monoclonal antibody D5-14 after 8 h (a) and 48 h (b) post infection; Simkania negevensis inclusions detected by monoclonal antibody D5-14 after 16 h (c) and 72 h (d) post infection.}
\end{figure}
tein was observed (Kahane et al., 1993). Additional immunoblot analysis of S. negevensis using hyperimmune murine sera (Friedman et al., 2003) or microimmunofluorescence–positive human sera (Yamaguchi et al., 2005) showed a strong immunoreactivity to a 37–42 kDa set of three bands and a 64 kDa antigen. Our results demonstrate the reactivity of a monoclonal antibody against a protein showing the same molecular weight of the protein recognized as a major target of the humoral immune response to S. negevensis. Therefore, D5-14 mAb could be used, as a confirmatory test, in the diagnosis of S. negevensis infection by the immunofluorescence test.

REFERENCES


