Journal of Infection (2007) **55**, e13–e21

www.elsevierhealth.com/journals/jinf

Versatility of *Simkania negevensis* infection in vitro and induction of host cell inflammatory cytokine response

Simona Kahane, David Fruchter, Bella Dvoskin, Maureen G. Friedman*

Department of Virology and Developmental Genetics, Faculty of Health Sciences, Ben Gurion University of the Negev, P.O. Box 653, 84105-IL Beer Sheva, Israel

Accepted 11 March 2007
Available online 26 April 2007

KEYWORDS
Simkania negevensis;
Chlamydia;
Inflammatory cytokines;
Epithelial cells;
Endothelial cells;
Persistent infection

Summary *Objective: Simkania negevensis* (Sn) is an intracellular microorganism belonging to the family *Simkaniaceae* in the order *Chlamydiales* and has been associated with respiratory tract infections in infants and adults. The aim of this study was to analyze the outcome of Sn infection in different cell types.
Methods: The results of Sn infection were examined by infectivity assays, PCR and EM. The cellular response to infection was evaluated by following the synthesis of mRNA for inflammatory cytokines and cytokine secretion.
Results: Infections could be active, with production of progeny and cytopathic effects (CPE); persistent, induced by iron depletion or in minimally permissive cell types, with small numbers of infectious progeny; or cryptic, with no CPE or infectious progeny, but with Sn DNA detected. EM showed an abundance of EB and multiplying RB in active infection, small inclusions with mainly single RB particles in persistent infection, and aberrant inclusions in cryptic infection. We report reversion to active infection of iron-induced or spontaneous persistence; attempts to ''cure'' persistence with antibiotic treatment resulted in the absence of infectivity but not in the eradication of Sn DNA.
Conclusion: Sn infections are versatile and induce a host cell inflammatory response, which may be relevant to potential Sn pathologies in vivo.

Abbreviations: CPE, cytopathic effects; DFA, deferoxamine; EB, elementary bodies; INF-γ, interferon gamma; IPA, immunoperoxidase assay; MBC, minimum bactericidal concentration; m.o.i., multiplicity of infection; p.i., post infection; RB, reticulate bodies; Sn, *Simkania negevensis*.

* Corresponding author. Tel.:+972 8 647 9938; fax: +972 8 627 6215.
E-mail address: maureen@bgu.ac.il (M.G. Friedman).

0163-4453/$30
doi:10.1016/j.jinf.2007.03.002

Introduction

Simkania negevensis is an obligate intracellular microorganism belonging to the Simkaneaceae family in the order Chlamydiales.[1] Members of this order have extensive host biodiversity ranging from arthropods to animals and humans.[2] S. *negevensis* was discovered as a contaminant in a variety of cell cultures[3] and was shown to be able to grow also in various environmental free-living amoebae such as *Acanthamoeba polyphaga*.[4,5] Of all the Chlamydiales, infections of Chlamydiaceae microorganisms have been most intensively studied, both in vivo and in in vitro models. Forms of both active and persistent (chronic/latent/cryptic) infections have been described.[6,7] In active infections the microorganism proliferates and at the end of its growth cycle is able to spread the infection to neighboring cells. In contrast, in persistent infections, the microorganism survives in viable cultivable or uncultivable forms for long periods of time and eventually may become activated and spread the infection. Chlamydial in vitro models of persistent infection induced by interferon-γ (INF-γ) treatment, amino acid starvation, iron depletion, or antibiotic treatment,[8–11] have contributed to our understanding of the pathologies associated with these microorganisms. Persistent Chlamydiaceae fail to complete development from reticulate bodies (RB) into infectious elementary bodies (EB), but retain metabolic activity.[12] In persistent *Chlamydia trachomatis* or *Chlamydophila pneumoniae* infections, RB are enlarged and morphologically aberrant, and have reduced amounts of major outer membrane proteins, such as MOMP and omc B.[13]

Members of the novel families in the order Chlamydiales have been shown to infect humans;[2] our study is the first detailed documentation of persistent in vitro infection by any of these organisms. In examining a variety of cell culture models, we investigated conditions leading to the phenomenon of apparently persistent S. *negevensis* infections as well as conditions inducing reversion from persistent to active infection, and possible methods for curing the persistent state.

Materials and methods

Media and growth of microorganisms

Cell cultures obtained from ATCC included: NCI-H292 human lung, mucoepidermoid (CRL 18148); BEAS-2B bronchial epithelium (CRL-9609); HT-29 colorectal adenocarcinoma cell line (HTB-38); HUV-EC-C vascular endothelium (CRL 1730); HEC-1A endometrial adenocarcinoma cell line (HTB-112), and were grown in culture as recommended by ATCC. Stocks of *S. negevensis* (ATCC VR1471^T) or *C. trachomatis* L2 were cultivated on Vero cells grown in RPMI medium as previously described.[14] Infectivity of Simkania in various cultures was measured on Vero cells by an immunoperoxidase assay (IPA) using rabbit polyclonal Sn-specific antibodies.[14] Unless specified otherwise, *S. negevensis* infection in the various experiments was carried out at a multiplicity of infection (m.o.i.) of between 1 and 3.

Iron depletion was induced with deferoxamine (DFA) (Sigma) at various concentrations in cultures grown in the presence of 5% fetal bovine serum (rather than the usual 15%). The iron content of the resulting growth medium was 1.3 μM. DFA treatment of the cells was initiated 24 h prior to infection with *S. negevensis*.

Electron microscopy (EM)

Infected cultures were fixed, embedded in araldite and stained for electron microscopy as described by Biberfeld.[15]

Cytokine detection

Human inflammatory cytokine detection multiplex PCR kits (Inflammatory Cytokine Set and Th1/Th2 Cytokine Set 2, Biosource, USA) were used to detect synthesis of mRNA for various cytokines. The protocol consisted of isolation of total cellular RNA (RNeasy, Qiagen), cDNA synthesis (Sensiscript, Qiagen) and the use of cDNA as a template for multiplex primers, followed by analysis of the products vs. reference cytokine amplicons. For each model culture, Sn infected and uninfected controls were examined at 5 or more time points after infection (early and late in the infection). The PCR assay is not quantitative, however, testing at various times post infection provided support for the consistency of the results. In addition, specific ELISA assays (Biosource, USA) were used to test for secretion of cytokines IL-8, IL-6, IL-1β and IL-4, using the manufacturer's protocols.

Results

Persistent infection induced by iron depletion

Since *S. negevensis* is resistant to penicillin and to IFN-γ treatment and can grow without tryptophan,[16] in order to induce persistence, the organism was grown under conditions of iron depletion, using various concentrations of DFA. Iron-depleted and non-depleted Vero cells were infected with *S. negevensis* at various m.o.i., and the growth or survival of the bacteria was followed by IPA. Fig. 1 shows Sn infectivity found relative to control untreated cells, as a function of concentration of DFA, at different times post infection and at two different m.o.i. In its normal developmental cycle in Vero cells, 3 days post infection (p.i.) is the end of the exponential growth and the beginning of a plateau stage.[17] As expected, in the presence of high concentrations of DFA survival of *S. negevensis* was greater at 3 days than in later stages of infection (days 7–10).

In addition, increasing concentrations of DFA led to cytopathic changes in infected cells as seen by light microscopy and Giemsa staining, such as distortion of cells and some loss of contact between them (data not shown). A higher multiplicity of infection resulted in a paradoxical increased yield of *S. negevensis*, as shown in Fig. 1 (right panel, a representative experiment of 4 carried out). The increase, although small, was found consistently and was surprising because with more organisms competing for any iron present, less relative growth of the bacteria at the higher m.o.i. would have been expected. At present we have no explanation for the phenomenon observed.

The morphology of bacterial particles in a state of apparent persistent infection induced by iron deprivation