Friche Passos, Lygia Maria
In vitro cultivation of Anaplasma marginale and A. phagocytophilum in tick cell lines: a review
Revista Brasileira de Parasitologia Veterinária, vol. 21, núm. 2, abril-junio, 2012, pp. 81-86
Colégio Brasileiro de Parasitologia Veterinária
Jaboticabal, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=397841484001
Introduction

Although more than 800 tick species have been identified worldwide (NAVA et al., 2009), relatively few species have yielded cell lines to date. The first continuous tick cell lines were established in 1975 (VARMA et al., 1975) and since then, the number of established cell lines has increased to over 50, derived from both ixodid and argasid species (Table 1).

Most tick cell lines were established from embryonic cells and grow three-dimensionally in specifically devised culture media under a normal atmosphere; incubation temperatures vary between 28 and 34 °C (BELL-SAKYI et al., 2007, 2009; MATTILA et al., 2007; MUNDERLOH et al., 1994). Normally more than one cell type is present in a culture; no tick cell clones have been reported thus far. Some cell lines grow relatively slowly and require less regular subcultures.
Table 1. Tick cell lines currently available.

<table>
<thead>
<tr>
<th>Tick species (ixodid)</th>
<th>No of cell lines</th>
<th>Source*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amblyomma americanum</td>
<td>2</td>
<td>UM</td>
<td>Kurtti et al. (2005)</td>
</tr>
<tr>
<td>Amblyomma variegatum</td>
<td>2</td>
<td>UE</td>
<td>Bell-Sakyi et al. (2000) and Bell-Sakyi (2004)</td>
</tr>
<tr>
<td>Rhipicephalus (Boophilus) decoloratus</td>
<td>3</td>
<td>UE</td>
<td>Bell-Sakyi (2004) and Lallinger et al. (2010)</td>
</tr>
<tr>
<td>Rhipicephalus (Boophilus) microplus</td>
<td>9</td>
<td>UE, UM,TAM</td>
<td>Bell-Sakyi (2004), Bell-Sakyi et al. (2007), Holman (1981) and Bell-Sakyi (personal communication)</td>
</tr>
<tr>
<td>Dermacentor albipictus</td>
<td>1</td>
<td>UM</td>
<td>Policastro et al. (1997)</td>
</tr>
<tr>
<td>Dermacentor andersoni</td>
<td>3</td>
<td>UM</td>
<td>Kurtti et al. (2005) and Simser et al. (2001)</td>
</tr>
<tr>
<td>Dermacentor (Anocentor) nitens</td>
<td>1</td>
<td>UM</td>
<td>Kurtti et al. (1983)</td>
</tr>
<tr>
<td>Dermacentor variabilis</td>
<td>1</td>
<td>UM</td>
<td>Kurtti et al. (2005) and Yunker et al. (1981)</td>
</tr>
<tr>
<td>Hyalomma anatolicum anatolicum</td>
<td>5</td>
<td>UE</td>
<td>Bell-Sakyi (1991)</td>
</tr>
<tr>
<td>Ixodes scapularis</td>
<td>7</td>
<td>UM</td>
<td>Munderloh et al. (1994)</td>
</tr>
<tr>
<td>Ixodes ricinus</td>
<td>4</td>
<td>UE, UM</td>
<td>Bell-Sakyi et al. (2007) and Simser et al. (2002)</td>
</tr>
<tr>
<td>Rhipicephalus appendiculatus</td>
<td>5</td>
<td>UE, UM, LSHTM</td>
<td>Bekker et al. (2002) and Bell-Sakyi (2004), Kurtti and Munderloh (1982) and Varma et al. (1975)</td>
</tr>
<tr>
<td>Rhipicephalus evertsi</td>
<td>2</td>
<td>UE</td>
<td>Bell-Sakyi (personal communication)</td>
</tr>
<tr>
<td>Rhipicephalus sanguineus</td>
<td>1</td>
<td>UM</td>
<td>Kurtti and Munderloh (1982)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tick species (argasid)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Carios capensis</td>
<td>4</td>
<td>UM</td>
<td>Mattila et al. (2007)</td>
</tr>
<tr>
<td>Ornithodoros moubata</td>
<td>6</td>
<td>UE</td>
<td>Bell-Sakyi et al. (2009)</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*UM = University of Minnesota (Kurtti and Munderloh). UE = University of Edinburgh (Bell-Sakyi). The Roslin Wellcome Trust Tick Cell Biobank http://tick-cells.roslin.ac.uk. TAM = Texas A&M University (Holman). LSHTM = London School of Hygiene and Tropical Medicine (VARMA et al., 1975).

Cryopreservation of tick cells in liquid nitrogen can be difficult; however, some cell lines can be preserved under refrigeration for up to one month (BASTOS et al., 2006; LALLINGER et al., 2010).

Several bacterial genera, notably *Anaplasma*, *Ehrlichia*, *Borrelia* and *Rickettsia*, and numerous arboviruses have been propagated in tick cell cultures (BELL-SAKYI et al., 2007). And recently even the protozoan *Babesia bigemina* has been reported to infect and multiply in a tick cell line (RIBEIRO et al., 2009). Thus the increasing application of tick cell lines to study a range of infectious agents has a huge potential for provision of antigenic material from specific tick stages in large quantities and without the use of experimental animals.

### Culture Initiation and Propagation of *Anaplasma marginale*

Bovine anaplasmosis, caused by the intraerythrocytic rickettsia *Anaplasma marginale*, is a tick-transmitted disease characterized by anemia, weight loss, fever, abortion and death, leading to significant economic losses for dairy and beef producers (WANDURAGALA; RISTIC, 1993). After recovering from acute disease, animals develop a persistent infection characterized by low level, chronic rickettsemia (KIESER et al., 1990), acting as a source of infection for other animals within the herd or the geographic area.

First attempts to propagate *A. marginale* were limited to the erythrocytic stage, using a whole-blood culture system based on a method used for *Plasmodium* (KESSLER et al., 1979), and did not result in continuous propagation. Another attempt to cultivate *A. marginale* was based on infection of arthropod cell lines derived from mosquitoes, but again propagation did not occur (MAZZOLA et al., 1976). Later, the first attempt to infect a tick cell line derived from *Dermacentor variabilis* (RML-15) was reported (SAMISH et al., 1988), with numerous groups of *Anaplasma*-like particles being seen in the tick cell cytoplasm; however, after one passage a continuous culture was not established.

The major advance in cultivation of *A. marginale* was achieved in the mid-1990s with the tick cell line IDE8, derived from embryonic *Ixodes scapularis* ticks (MUNDERLOH et al., 1994), and since then this system has been successfully used to propagate isolates of *A. marginale* in the United States (MUNDERLOH et al., 1996; BLOUIN; KOCAN, 1998; BLOUIN et al., 2000) and South Africa (ZWEYGARTH et al., 2006). Recently, a Brazilian isolate of *A. marginale* (UFMG1, GenBank accession number EU676176), morphologically distinct from other cultured isolates due to the presence of an inclusion appendage (RIBEIRO et al., 1997), has been successfully established in IDE8 cells (BASTOS et al., 2009), although this isolate was shown not to infect *Rhipicephalus (Boophilus) microplus* ticks (RIUE et al., 2005). Interestingly, the same isolate infected a cell line derived from *R. (B.) microplus* ticks (ESTEVES et al., 2009).

*A. marginale* cultures can be initiated from blood obtained from infected calves during ascending rickettsemia (BLOUIN et al., 2000; BASTOS et al., 2009). Blood samples are collected with anticoagulant, and usually the blood cells are washed and subsequently cryopreserved in liquid nitrogen using DMSO as cryoprotectant. IDE8 cells are cultured in L-15B medium supplemented with various additives, following standard procedures (MUNDERLOH et al., 1994). Culture flasks containing growing layers of IDE8 cells can be infected with *A. marginale*
cryostabilates. After defrosting the blood stabilates by immersion in a 37 °C water bath, the content of the cryovials is centrifuged (10,000 × g, for 20 minutes) and the pelleted erythrocytes are resuspended in a complete culture medium which is suitable for the propagation of A. marginale (MUNDERLOH et al., 1996). Inoculated flasks are incubated at 34 °C and the first medium change can be carried out 24 hours after inoculation, whereas subsequent medium changes are done weekly. The cultures should be monitored by direct examination under an inverted microscope and/or by microscopic examination of Giemsa-stained cytocentrifuge smears (Figure 1).

Initially, well defined parasitophorous vacuoles containing compact colonies are formed; subsequently, approximately two weeks later, large colonies are observed and their contents are released into the culture medium after disruption of the vacuole membrane. A. marginale-infected cells can be propagated continuously, and infection rates can reach up to 80%.

A. marginale bacteria can be quantified and used as antigenic material for diagnostic tests or for immunization trials (GARCIA-GARCIA et al., 2004; BASTOS et al., 2010). And almost importantly, it has been shown that cultured A. marginale strains retain their antigenic properties and infectivity for cattle after successive passages (MUNDERLOH et al., 1996; BLOUIN; KOCAN, 1998; BARBET et al., 1999).

This in vitro culture system has opened a new window for studies of pathogen–host cell interactions under controlled conditions, allowing comparative in vitro and in vivo studies on cell attachment, invasion and intracellular development of A. marginale, as well as the development of infection-inhibition and ELISA-based screening assays for evaluation of bacteria growth and infection levels (BLOUIN et al., 2003).

In addition, in vitro cultivation of A. marginale in tick cells has been used in comparative studies with infected bovine erythrocytes for differential bacterial gene transcriptional analyses and expression profile of outer membrane proteins to search for potential A. marginale vaccine candidates (BRAYTON et al., 2006; GARCIA-GARCIA et al., 2004; NOH et al., 2006), and in RNA interference approaches for functional studies to discover genes/proteins that are differentially expressed in tick cells in response to infection with A. marginale (DE LA FUENTE et al., 2007; KOCAN et al., 2009). These studies indicate that A. marginale affects the expression of tick genes important for tick survival and pathogen multiplication; thus, a molecular mechanism occurs by which tick cell gene expression mediates the development of the pathogen through ticks.

**Culture Initiation and Propagation of Anaplasma phagocytophilum**

Anaplasma phagocytophilum, a tick-transmitted gram-negative bacterium, is an emerging zoonotic infection, transmitted by ixodid ticks (PAROLA et al., 2005). Since its first discovery in Scotland in 1932, the microorganism has gained more and more attention in veterinary medicine as the agent of tick-borne fever in ruminants and granulocytic anaplasmosis in a wide variety of domestic animals, such as dogs, cats, and horses (WOLDEHIWET, 2010). Humans are accidental hosts (PAROLA et al., 2005; CARRADE et al., 2009) and the severity of the so-called human granulocytic anaplasmosis (HGA) ranges from mild to more serious infections and in rare cases even death. The enzootic cycle includes rodents (BROWN et al., 2003) and possibly birds (BJOERSDORFF et al., 2001) as reservoir hosts.

Successful initiation and maintenance of A. phagocytophilum in the I. scapularis tick cell lines IDE8 and/or ISE6 has been reported (MUNDERLOH et al., 1996; WOLDEHIWET et al., 2002). Although tick cell lines are not routinely used for direct isolation of A. phagocytophilum, it was shown that organisms derived from infected HL60 cells can invade and grow in tick cell cultures (MUNDERLOH et al., 1999).

Very recently several European isolates of A. phagocytophilum were established in IDE8 tick cells (ZWEYGARTH et al., 2010, 2011); these include isolates derived from horses, dogs and one isolate derived from roe deer, which has been preliminary characterized (SILAGHI et al., 2011) (Table 2). Cultures are initiated by adding infected granulocytes from venous blood of infected animals into tick cell cultures. Whole blood (WOLDEHIWET et al., 2002), washed buffy coat cells (MUNDERLOH et al., 1999) or white blood cells after hypotonic lysis of the erythrocytes (MUNDERLOH et al., 1996; ZWEYGARTH et al., 2010) can be used for initiation. Culture propagation is basically the same as with A. marginale, including culture medium and incubation temperature.

**In vitro cultivation of A. phagocytophilum** in tick cells has been used for a wide range of studies and applications, from morphological ultrastructural analyses of geographical variant strains to investigations on susceptibility of strains to antibiotics and neutralizing effects of immune serum (WOLDEHIWET, 2010). For example, recently the ISE6 cell line has been used for isolation of variants of A. phagocytophilum directly from ticks (MASSUNG et al., 2007), representing a new tool for studies on genetics, proteomics and biological differences amongst strains differing in infectivity for mice and humans. This in vitro system has also been useful for the comparisons of genome transcriptional and protein expression from vertebrate and tick cell cultures. Whole blood (WOLDEHIWET et al., 2002), washed buffy coat cells (MUNDERLOH et al., 1999) or white blood cells after hypotonic lysis of the erythrocytes (MUNDERLOH et al., 1996; ZWEYGARTH et al., 2010) can be used for initiation. Culture propagation is basically the same as with A. marginale, including culture medium and incubation temperature.
cells (JAURON et al., 2001), and the generation of the whole genome transcript profiling for *A. phagocytophilum* by tiling array assays (NELSON et al., 2008).

A recent study comparing tick cell gene expression profiles in response to *A. phagocytophilum* and *A. marginale* infection by microarray and real-time RT-PCR analyses indicated differential gene expression, possibly related to differences in the life cycle of the two pathogens in ticks (ZIVKOVIC et al., 2009). However, despite the tremendous impact of cultivation of tick cells on tick research, differences in expression patterns indicate that *in vitro* studies should be corroborated with *in vivo* studies (DE LA FUENTE et al., 2010).

**Final Remarks**

Tick cell *in vitro* systems constitute a good source of tick-stage antigenic material, with a huge potential for development of improved vaccines and diagnostic assays, particularly in tropical areas where *Anaplasma* infections are endemic and the maintenance of animal donors free from hemoparasites is difficult and expensive. In addition, they provide a useful model system for studies such as molecular analysis, gene transcription, cellular and molecular relationships among pathogens and vectors, and comparative *in vitro/in vivo* studies.

**Acknowledgements**

The author thanks Dr Erich Zweygarth and Dr Lesley Bell-Sakyi for critically revising the manuscript and Ms H. Schöl for photographic technical support.

**References**


Ribeiro MFB, Passos LMF, Guimarães AM. Ultrastructure of Anaplasma marginale with an inclusion appendage, isolated in Minas Gerais State,


