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Special issue: Tick–host–pathogen interactions in the post-genomic era

Tick cell lines: tools for tick and tick-borne disease research

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Over 40 cell lines are currently available from 13 ixodid and one argasid tick species. The successful isolation and propagation of several economically important tick-borne pathogens in tick cell lines has created a useful model to study interactions between tick cells and these viral and bacterial disease agents. Tick cell lines have already proved to be a useful tool in helping to define the complex nature of the host–vector–pathogen relationship. With the availability of genomics tools, tick cell lines will become increasingly important as a complement to tick and tick-borne disease research *in vivo* once genetic transformation and gene silencing using RNA interference become routine.

Why tick cell lines?

Ticks are of huge economic significance worldwide, both as harmful parasites in their own right and as vectors of many pathogenic viruses, bacteria and protozoa of medical and veterinary importance [1]. *In vitro* culture systems, particularly continuous cell lines derived from vector and host tissues, have an invaluable and irreplaceable role in many aspects of tick and tick-borne pathogen research, including basic parasite biology, host–vector–pathogen relationships and disease control. In the three decades since the first ixodid tick cell lines were established [2], the scope of their use has broadened from a focus on propagation of tick-borne pathogens to include studies on tick biology, genomics, proteomics and genetic manipulation. The actual and potential range of applications for tick cell lines in tick and tick-borne disease research has never been wider.

Tick cell lines – what's available?

Attempts to cultivate tick cells date back over 50 years. Early studies resulted in primary cultures of tick cells and/or tissues capable of survival for up to 6 months; their use for propagation of viruses and bacteria has been comprehensively reviewed [3,4]. Improvements in methodology over two decades led to the establishment of the first continuous tick cell lines from *Rhipicephalus appendiculatus* [2]. Twelve years ago, when the last general review of the role of tick cell lines in vector–pathogen research was published [5], 20 cell lines were available from eight ixodid tick species. They had been used for propagation of *Borrelia burgdorferi*, *Rickettsia* spp., tick-borne spiroplasmas and various arboviruses [3,5,6]. There are now over 40 cell lines from 13 ixodid and, for the first time, one argasid tick species (Box 1, Table 1). The range of microorganisms, in particular prokaryotes, that can be propagated in tick cell lines has been extended and includes several bacterial pathogens of considerable medical and veterinary importance worldwide (Table 2).

Some tick species, such as *R. appendiculatus* and *Boophilus microplus*[*], have yielded several cell lines in different laboratories (Table 1), whereas cells of other species have proved difficult to culture continuously [4], despite relatively standardized protocols for setting up primary cultures. *Dermacentor andersoni*, one of the first tick species used in cell culture experiments [7], did not yield a cell line until more than 30 years later [8]. Similarly, although soft (argasid) ticks have received much less attention overall than hard (ixodid) ticks [3,7], it has taken ~30 years to establish the first argasid cell lines [9]. Perhaps the most important ingredients contributing to success in establishing tick cell lines are: (i) patience – continuous cultivation can take up to 5 years from the time the primary culture is initiated, and the overall success rate is very low [4]; and (ii) operator experience – of the 44

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[*] The genus *Boophilus* has been reclassified as a subgenus of *Rhipicephalus*; however, to avoid confusion over cell line nomenclature, *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *decoloratus* will be referred to hereafter as *B. microplus* and *B. decoloratus* respectively.

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Box 1. What are tick cell lines like?

Most of the currently available tick cell lines were established from embryonic cells, using simple methodology and making no attempt to select particular tissue types [3,18,57]. Primary cell cultures initiated from moulting nymphs after removal of the digestive and excretory system tissues [2] and cultures of whole moulting larval explants ([25,27]) have also yielded continuous cell lines. As a result, tick cell lines generally comprise two or more cell types [7] that can be present in varying proportions both at different times within a single culture and at different passage levels (Figure I). This mixture of cells seems to be essential for survival of the culture; attempts to clone tick cells have failed [18]. Moreover, individual cells within a tick cell line have a tendency to gain or lose chromosomes without affecting their survival [2,3,58].

Tick cell lines share several characteristics with the arthropods from which they were derived. As befits haematophagous parasites, they grow in mammalian culture media supplemented with mammalian serum, at incubation temperatures between 28 °C and 34 °C, although some lines will also grow at 37 °C. Some tick cell lines thrive in acidic conditions (pH 6.5–6.8), similar to the environment of proliferating cells within developing larvae, nymphs and adults [5], whereas others are propagated at neutral to alkaline pH, enabling growth of acid-sensitive pathogens [16]. Tick cells do not exhibit contact inhibition and most will grow readily in three dimensions; tick cells are generally not strongly adherent, growing as a combination of incomplete monolayer and suspension culture. They divide relatively slowly, can be maintained at high cell densities (10^6–10^7 cells ml^{-1}), and many lines do not require regular subculture, making them particularly suitable for isolation of slow-growing microorganisms. Short-term storage at 12 °C [3] or even 4 °C [59] is often preferable to cryopreservation, as frozen tick cells can be difficult to resuscitate reliably [3,57]; however, cells cryopreserved in liquid nitrogen can survive for at least 12 years [18]. Individual cultures of some tick cell lines can be extremely long-lived, surviving for several years with regular medium changes and occasional subcultures, reflecting the ability of ixodid ticks to exist for extremely long periods of time between blood meals in nature.

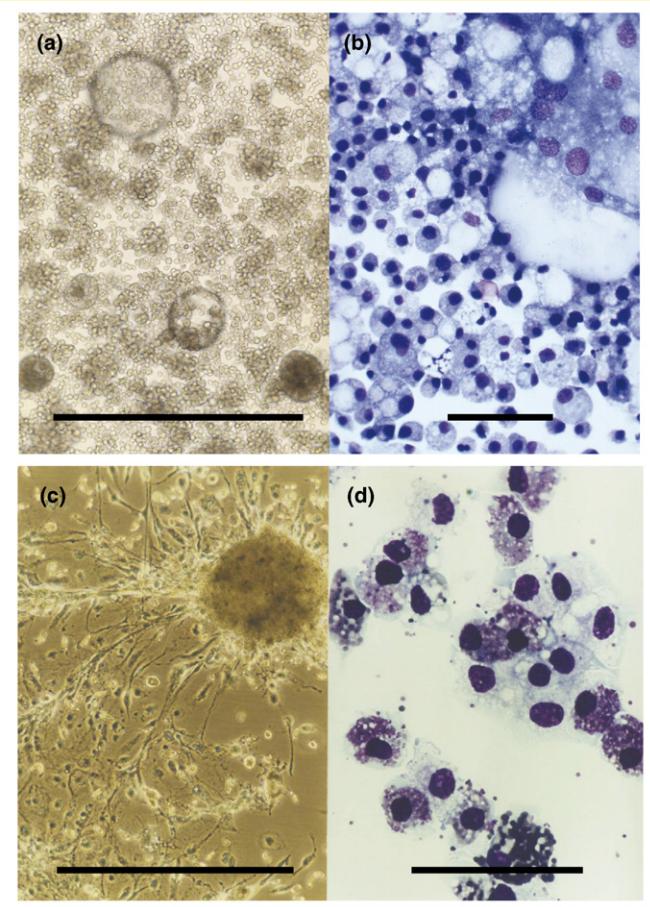

Figure I. **(a,b)** *Ixodes ricinus* cell line IRE/CTVM18; **(c,d)** *Amblyomma variegatum* cell line AVL/CTVM17. (a,c) Live phase contrast; the scale bar represents 500 μm; (b,d) cytocentrifuge smears stained with Giemsa; the scale bar represents 50 μm.

Table 1. Tick cell lines that are currently available[a]

Tick species	Instar	Cell lines	Refs
Amblyomma americanum	Embryo	AAE2, 12	[9,49]
Amblyomma variegatum	Larva	AVL/CTVM13, 17	[25,27]
Boophilus decoloratus[b]	Embryo	BDE/CTVM16	[25]
Boophilus microplus[b]	Embryo	*B. microplus* IX, VII-SCC BME26 BME/CTVM2, 4, 5, 6	[3,13,25,62][c]
Carios capensis	Embryo	CCE1, 2, 3, 5	[9][d]
Dermacentor albipictus	Embryo	DALBE3	[9,16,42]
Dermacentor andersoni	Embryo	DAE3, 15, 100	[8,9]
Dermacentor (Anocentor) nitens	Embryo	ANE58	[3]
Dermacentor variabilis	Embryo	DVE1	[9]
Hyalomma anatolicum anatolicum	Embryo	HAE/CTVM7, 8, 9, 10, 11	[57]
Ixodes scapularis	Embryo	IDE2, 8, 12 ISE5, 6, 18, 25	[18,63]
Ixodes ricinus	Embryo	IRE11 IRE/CTVM18, 19, 20	[10,25,64][c]
Rhipicephalus appendiculatus	Embryo	RAE25 RAE/CTVM1	[3,25]
	Nymph	RA243, 257 RAN/CTVM3	[2,26]
Rhipicephalus sanguineus	Embryo	RSE8	[3]

[a]Additional tick cell lines mentioned in previous reviews [3,7] are, as far as the authors can ascertain, no longer available.
[b]The genus *Boophilus* has been reclassified as a subgenus of *Rhipicephalus*; however, to avoid confusion over cell line nomenclature, *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *decoloratus* are referred to herein as *B. microplus* and *B. decoloratus* respectively.
[c]L.B-S., unpublished.
[d]T.J. Kurtti, unpublished (U.G. Munderloh, pers. commun.).

Table 2. Microorganisms propagated in tick cell lines since 1995

Microorganism species	Mammalian host (disease caused)	Tick cell line(s) used	Refs
Anaplasma marginale	Cattle (anaplasmosis)	IDE8, ISE6, IRE/CTVM18	[16,17,29–31, 44,45,61]
Anaplasma phagocytophilum	Domestic ruminants (tick-borne fever), horses (equine granulocytic ehrlichiosis), humans (human granulocytic anaplasmosis)	IDE8, ISE6	[20–23,32,37,46, 47,52]
Anaplasma ovis	Sheep (anaplasmosis)	IDE8	[29]
Anaplasma sp.	White-tailed deer	ISE6	[33]
Anaplasma sp. (Omatjenne)	Cattle	IDE8	[30]
Borrelia burgdorferi	Rodents, dogs, humans (Lyme disease)	ISE6, IDE8	[40,41]
Borrelia lonestari	Humans (southern tick-associated rash illness)	ISE6	[34]
Ehrlichia canis	Dogs (canine ehrlichiosis/tropical canine pancytopaenia)	IDE8, ISE6, IRE/CTVM18	[19,49][a]
Ehrlichia chaffeensis	Humans (human monocytic ehrlichiosis)	ISE6	[49,50]
Ehrlichia ruminantium	Cattle, sheep, goats, wild ruminants (heartwater, cowdriosis)	AVL/CTVM13, 17, BDE/CTVM16, BME/CTVM2, 6, IDE8, IRE/CTVM18, RAE25 RAE/CTVM1, RAN/CTVM3,	[24–28,48]
Rickettsia rickettsii	Humans (Rocky Mountain spotted fever)	IDE2, DALBE3, ISE6, IDE8	[38,42,64,65]
Rickettsia peacockii		DAE100, ISE6, BME26, DVE1, DAE3, DAE15, IDE12, IDE2, IDE8, IRE11, CCE3	[8,9,65,66]
Rickettsia monacensis	?	ISE6, IRE11, DAE100, IDE8	[51,64,66]
Rickettsia helvetica	Humans (fever, perimyocarditis?)	IRE11	[64]
Rickettsia montanensis	Various small mammals	IDE2, DALBE3	[38]
Rickettsia sp. (spotted fever group)	?	RAE25, IDE2, IDE8	[35]
Rickettsia felis	Humans (flea-associated spotted fever)	ISE6	[36]
Wolbachia persica		DALBE3	[38]
Tick-borne encephalitis virus	Humans (tick-borne encephalitis)	RA257, ISE6, RAE/CTVM1, AVL/CTVM17, IRE/CTVM18,	[10,11,60,67]
West Nile virus	Horses, humans (West Nile fever)	ISE6, RAE/CTVM1, AVL/CTVM17, IRE/CTVM18	[10]
Powassan virus	Humans (Powassan fever/encephalitis)	ISE6, RAE/CTVM1, AVL/CTVM17, IRE/CTVM18	[10]
Langat virus	Rodents, experimentally infected humans (Langat encephalitis)	ISE6, RAE/CTVM1, AVL/CTVM17, IRE/CTVM18	[10]
Louping ill virus	Sheep, other domestic/companion animals, grouse (Louping ill encephalitis)	ISE6, RAE/CTVM1, AVL/CTVM17, IRE/CTVM18	[10]
Venezuelan equine encephalitis virus	Horses, humans (encephalitis)	RAE/CTVM1	[10]
Dugbe virus	Humans (fever), domestic ruminants	ISE6	[12]
Hazara virus	Humans (no disease but related to Crimean-Congo haemorrhagic fever virus)	ISE6	[12]
Thogoto virus	Humans (encephalitis), domestic ruminants	RAE/CTVM1, BME/CTVM6, HAE/CTVM9	[b]

[a]E.Z., unpublished.
[b]K. Hagmeier, G. Kochs, pers. commun.

cell lines listed in Table 1, 23 were established by T.J. Kurtti and U.G. Munderloh and 17 by L.B-S.

What can you do with tick cell lines?

Virologists (Box 2) have been using *R. appendiculatus* cell lines for propagation and study of arboviruses for over 30 years [2,4,6]; recently lines from several other tick species have also been applied successfully for this purpose [10–12] (Table 2). Although tick cell lines have not yet been widely used in studies on tick physiology [3], the generation of organophosphate-resistant strains from the *B. microplus* cell line VII-SCC [13] provided an opportunity to study development *in vitro* of acaricide resistance in this species [14]. Immune-responsive c-type lysozymes were recently identified and characterized at the molecular level in the *D. andersoni* DAE100 cell line [15]. Whereas protozoologists have in the past found tick organ cultures to be a more suitable environment than cell cultures for *in vitro* development of tick-borne protozoa [3], bacteriologists have been the most comprehensive exploiters of tick cell lines during the past decade, aided by concurrent developments in molecular genomics and proteomics.

Propagation of Ehrlichia *and* Anaplasma

The most significant recent developments in pathogen propagation in tick cell lines concern *Ehrlichia* and *Anaplasma*. Several members of these genera of obligately intracellular bacterial pathogens have been established in tick cell lines since 1995 (Table 2), among which *Anaplasma marginale* [16,17] has been most extensively exploited (Box 3). Continuous cultivation of these pathogens has been achieved in cell lines derived from various tick species, predominantly *Ixodes scapularis* [18]. *Ehrlichia canis*, the causative agent of canine ehrlichiosis, was successfully propagated in IDE8 cells [19] and, more recently, in *Ixodes ricinus* IRE/CTVM18 cells (E.Z., unpublished) (Figure 1a,b).

Box 2. Virology and tick cell lines

Tick cell lines have been used to study a variety of subjects relating to virus pathogenesis and virus evolution. For example, when arthropod-borne viruses (arboviruses) infect ticks and/or tick cell lines, they do not induce noticeable pathologic or cytopathic changes [6]. Moreover, tick-borne arboviruses readily establish persistent infections in tick cell lines, which can be subcultured indefinitely and remain infected throughout the period of subculture [6]. This contrasts with mammalian hosts *in vivo* and *in vitro*, for which arbovirus infection often results in death. The precise molecular basis for this difference in host response to virus infection has never been satisfactorily explained. However, in a recent publication [60] that compared the effects of tick-borne encephalitis virus on tick and mammalian cell lines, the virus maturation pathway was followed using immunoelectron microscopy. There were marked differences in the site of appearance of the individual structural virus proteins and also in their pattern of movement and dispersal through the infected cells. Cellular compartments remained almost completely intact in the tick cells, whereas in the mammalian cells, ultrastructural changes were marked and the cells died within 50 h of virus infection.

A second feature of tick cell lines has often been exploited to isolate tick-borne viruses from field material. It is well known that the virus present in field material might not readily infect laboratory-maintained mammalian cell lines. On the other hand, tick cell lines are often susceptible to infection by the virus contained in field material. Thus, tick cell lines can be used successfully to isolate wild-type tick-borne viruses [3,6]. In addition, many mosquito-borne viruses will readily infect tick cells, whereas few tick-borne viruses will grow in mosquito cells [3,6,10]. This provides an additional diagnostic aid when isolating viruses from field material.

It has also been observed that tick-borne viruses evolve more slowly than mosquito-borne viruses, and this can be partly explained by the slower turnover of ticks (including virus-infected ticks), which can remain dormant for many months. However, the results of molecular studies of tick-borne viruses replicating in tick cells suggest that they do not undergo mutational changes at the same high frequency often seen in mammalian cells. Thus, in evolutionary terms, tick-borne arboviruses often characteristically evolve gradually (clinally).

Organisms derived from IDE8 cells remained infective, causing clinical ehrlichiosis in dogs; a cell line derived from the natural vector, *Rhipicephalus sanguineus*, did not become infected *in vitro* [19]. *Ehrlichia equi*, the human granulocytic ehrlichiosis agent, and *Ehrlichia phagocytophila*, all recently re-classified as *Anaplasma phagocytophilum*, have been successfully propagated in the *I. scapularis* cell lines IDE8 or ISE6 or both [20–22]. Both the equine [20] and ovine [22] variants derived from tick cell cultures remained infective for their respective mammalian hosts, and antigen prepared from *A. phagocytophilum*-infected IDE8 cells was as sensitive and specific in ELISA as infected ovine granulocyte antigen [23]. The first continuous propagation of *Ehrlichia ruminantium* was carried out in IDE8 cells [24] and subsequently in cell lines from other non-vector species *R. appendiculatus*, *Boophilus decoloratus**, *B. microplus* and *I. ricinus* and the vector species *Amblyomma variegatum* (Figure 1c,d) [25–27]. After prolonged maintenance of *E. ruminantium* in tick cell cultures, some isolates could be re-established in bovine endothelial cell cultures [26]. Certain combinations of *E. ruminantium* and tick cell line were shown to have potential as a vaccine in sheep [28]. However, in subsequent trials the immunogenicity was lost for reasons that remain unclear and require further investigation (L.B-S., PhD thesis, Utrecht University, 2004). *Anaplasma ovis*, a pathogen of sheep and goats, was cultured in IDE8 cells and used to investigate the phenomenon of infection exclusion [29]. Recently a previously uncharacterized *Anaplasma* species, *Anaplasma* sp. (Omatjenne) from South Africa, was propagated continuously in IDE8 cells [30]. In the same study, an Israeli strain of *A. marginale* grew in both IDE8 and IRE/CTVM18 cells, the first report of continuous cultures of

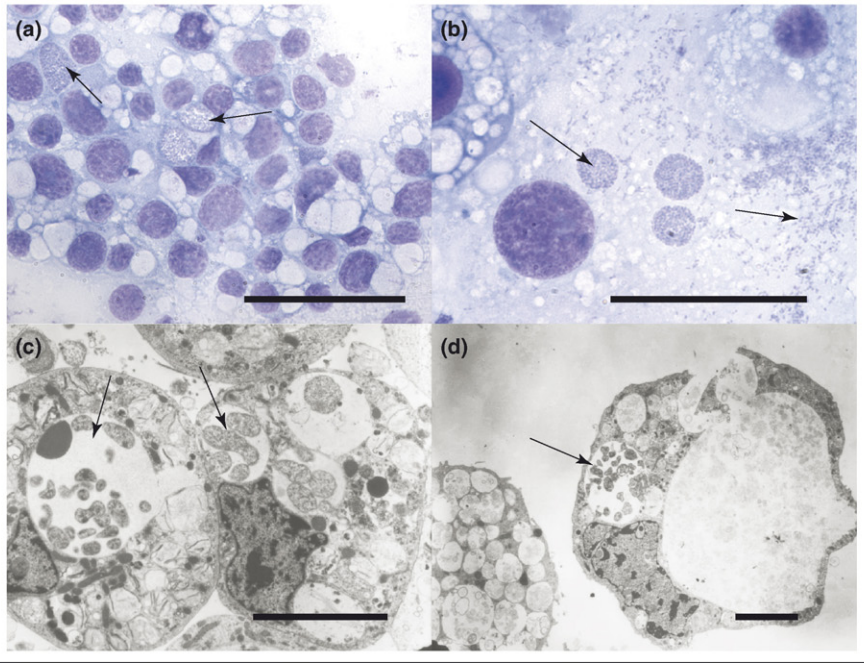

Figure 1. *Ehrlichia* spp. propagated in tick cell lines. (a) *Ehrlichia canis* in IDE8 cells; (b) *E. canis* in IRE/CTVM18 cells; (c) *Ehrlichia ruminantium* in IDE8 cells; (d) *E. ruminantium* in AVL/CTVM13 cell. (a,b) Cytocentrifuge smears stained with Giemsa; the scale bar represents 50 μm; (c,d) transmission electron micrographs; the scale bar represents 5 μm. Arrows indicate bacterial colonies.

Box 3. *Anaplasma marginale* in tick cell culture

The first continuous *in vitro* culture system for the pathogen *A. marginale* was established in the *I. scapularis* cell line IDE8 from infected bovine erythrocytes [16]. Cultured *A. marginale* are infective for cattle and are potent antigens for use in vaccine preparations and serological tests. This model culture system for studying pathogen–host cell interactions in a controlled environment [17] has provided corroboration for *in vivo* studies, including:

- Infection and development of *A. marginale* in tick cells (Figure I): adhesion of the dense (infective) stage to the host cell membrane initiates an endocytotic process. After transformation to the reticulated stage and multiplication by binary fission, large colonies are formed. Colony membranes fuse with the host cell membrane and dense forms are released by exocytosis.
- Evaluation of the binding potential of two major surface proteins thought to be involved in adhesion to host cells (MSP1a and MSP1b): recombinant *E. coli* expressing the surface polypeptides was allowed to react with IDE8 cells. Adhesion assays indicated that MSP1a was an adhesin for tick cells, whereas MSP1b was not, suggesting that the role of the MSP1 complex varies between vertebrate and invertebrate hosts.
- Infection inhibition assay: antisera from naturally infected cattle or cattle immunized with erythrocytic *A. marginale* or with recombinant MSP1 complex did not inhibit infection of tick cells by *A. marginale*, whereas antisera from cattle or rabbits immunized with individual MSP1a and MSP1b significantly reduced infections [61].
- *In vitro* ELISA-based screening assay: evaluating the effects of tetracycline on parasite growth, enabling accurate quantification of infection levels in treated and untreated cultures, and demonstrating the infection-enhancing effect of phospholipase A2 from tick saliva in the cultured cells.
- Different geographic isolates established in culture have retained their unique MSP1a identity. Several isolates that are not infective for ticks were also not infective for IDE8 cells. Cultures infected with one isolate could not be subsequently infected with a second isolate demonstrating an infection exclusion phenomenon [29].

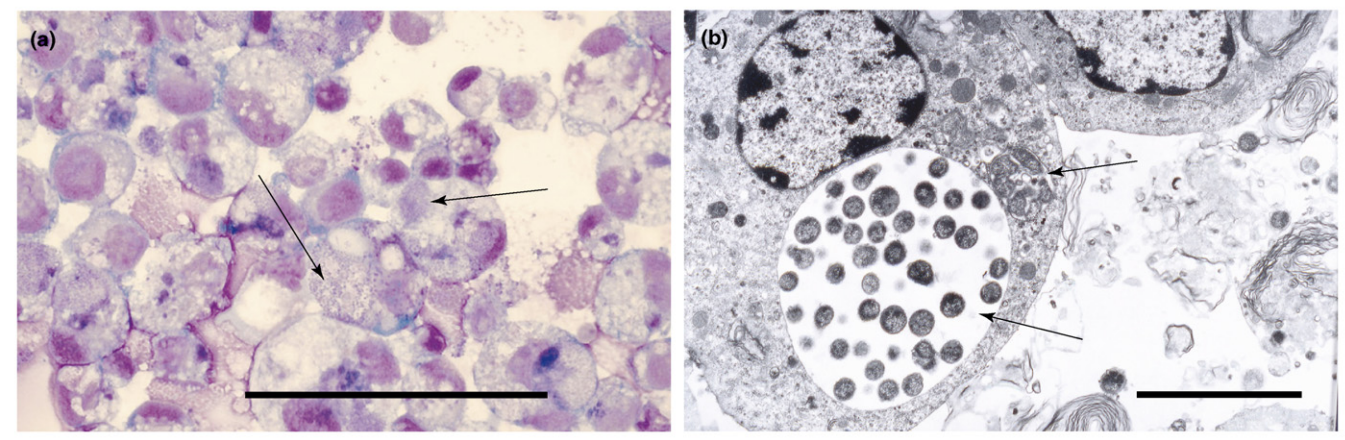

Figure I. *Anaplasma marginale* in IDE8 cells. **(a)** Cytocentrifuge smear stained with Diff-Quick; the scale bar represents 50 μm; **(b)** transmission electron micrograph; the scale bar represents 5 μm. Arrows indicate bacterial colonies.

this pathogen in cells other than *I. scapularis*. Adaptation of *A. marginale* and *A. phagocytophilum* to growth in tick cells facilitated infection *in vitro* of mammalian endothelial cells, a host cell type not previously known to be invaded by these pathogens *in vivo* [31].

Isolation of pathogens

In addition to being useful for propagation of pathogens, some of which cannot be grown *in vitro* in any other culture system, tick cell lines have application in isolation of previously uncharacterized tick-associated microorganisms from nature [3,6]. These actual and potential functions are facilitated by the range of tick species from which cell lines are now available, and the well-documented ability of some of these lines to support growth of microorganisms that are not transmitted by the parent tick, or even, in the case of some arboviruses, not transmitted by ticks at all [6,10,25]. The first isolations of *A. phagocytophilum* from human blood were made using tick (IDE8) and human (HL60) cells [32]; although growth was initially much faster in the HL60 cells, the principle of isolating unknown pathogens using tick cells was established. Subsequently, a previously uncultivable *Anaplasma* sp. was isolated into ISE6 cells from the blood of white-tailed deer [33], and the same cell line was used for the first isolation of the aetiological agent of southern tick-associated rash illness in humans, *Borrelia lonestari*, by co-cultivation with tissues of the vector tick *Amblyomma americanum* [34]. Similarly, the *R. appendiculatus* cell line RAE25 was used to isolate a *Rickettsia* sp. of the spotted fever group by co-cultivation with midgut tissues from an *A. americanum* tick [35]. Very recently, the ISE6 cell line was used to isolate previously uncultivated strains of *Rickettsia felis* from cat fleas [36] and *A. phagocytophilum* from *I. scapularis* ticks [37].

Tick symbionts

Many ticks in the field have been found to harbour bacterial symbionts, which in some cases are closely related to known pathogens [38]. *Rickettsia peacockii*, an endosymbiont of *D. andersoni* that seems to interfere with transmission of *R. rickettsii*, was found to cause a chronic infection of the *D. andersoni* cell line DAE100 [8]. Several symbiotic and pathogenic *Rickettsia* species have been propagated in tick cell lines, facilitating characterization and investigation of their relationships with their host cells (Table 2). The *I. scapularis* cell line IDE2 was found to be chronically infected with a virus that has no detectable cytopathic effect on the tick cells and is presumably transmitted transovarially (vertically, from one generation to the next through the

eggs) because IDE2 was embryo-derived [39]. This virus is also present in IDE8, but is absent from ISE6 (U.G. Munderloh, pers. commun.).

Pathogen genomics and proteomics

Tick cells provided an important environment for studies on stage-specific gene transcription and protein expression in *B. burgdorferi*; co-cultivation of spirochaetes with IDE8 and ISE6 cells influenced temperature-dependent outer surface protein expression associated with increased infectivity of spirochaetes for the mammalian host [40] and modulated transcription of genes involved with the starvation-associated stringent response [41]. Similarly, temperature-dependent protein expression was observed in *R. rickettsii* propagated in IDE2 and *Dermacentor albipictus* DALBE3 cells at both 28 °C and 34 °C [42]; the antigen profile in tick cells at 34 °C was similar to that seen in mammalian cells, whereas several proteins of unknown function present at the higher temperature were apparently not expressed at 28 °C.

In the search for *A. marginale* vaccine candidates, *I. scapularis* cell lines have had an essential role in many studies of differential gene transcription and outer membrane protein expression [43]. For example, levels of expression of the major surface proteins MSP1a and 1b in tick cells and bovine erythrocytes correlated with their roles as adhesins for the different host cells [44], and levels of expression of outer membrane proteins encoded by the *msp2* gene superfamily differed markedly between tick and bovine cells [45]. In *A. phagocytophilum,* the immunodominant p44 antigen predominated in human cells but not in tick cells and might be involved in regulatory changes that mediate survival of the pathogen by immune modulation after tick transmission [46]. The *p44* gene expression site was found to be polymorphic in human and tick cells, with sequence changes in p44 variants being influenced by host cell type and culture conditions [47]. Although all 16 members of the *E. ruminantium* major antigenic protein 1 (*map1*) multigene family were transcribed *in vitro* in mammalian cells, between 4 and 11 paralogs were transcribed in different tick cell lines [48]. Differential macrophage and tick cell-specific protein expression from the p28/p30 outer membrane protein multigene locus in *Ehrlichia chaffeensis* and *E. canis* has been described [49]. Using proteomic approaches it was shown that proteins expressed in infected macrophages are the products of genes that differ from those expressed in infected tick cells [50].

The future – what else can be done with tick cell lines?

Tick cell lines have had a role in studies involving genetic manipulation of pathogens; *I. scapularis* cells infected with transformants of *R. monacencis* were found to be a useful system for studying interactions between rickettsiae and host cells [51], and induction of pathogen-derived resistance in ISE6 cells through silencing of tick-borne nairoviruses via RNA interference (RNAi) was described recently [12].

Genetic manipulation of tick cell lines *per se* has also been reported. Stable transfection, or transformation, of the ISE6 cell line to express a fluorescent protein was described [52,53]; the transformed tick cells supported growth of transformed *A. phagocytophilum*. Moreover, expression of the fluorescent protein by the transformed ISE6 cells was successfully silenced temporarily by RNAi [54]. Although there have been no published reports as yet of silencing of native tick genes in tick cell lines, RNAi has been shown to be functional in ticks and isolated tick tissues and is therefore an effective tool with which to study gene function at the tick–host–pathogen interface [55]. Silencing of genes *in vitro* in tick cell lines will create additional opportunities to investigate the functions of tick proteins at the cellular level. Moreover, in the search for previously unidentified, pharmacologically active proteins for anti-tick vaccines [56], it will be particularly useful to couple RNAi in ticks to high-throughput analysis in tick cells.

Although one must always be cautious when extrapolating from *in vitro* systems to whole ticks *in vivo*, further application of genomics tools such as RNAi and transfection (Box 4) to uninfected and pathogen-infected culture systems will increase the importance of the complementary role of tick cell lines in tick and tick-borne disease research.

Box 4. Outstanding questions – what can be done to advance the use of tick cell lines as research tools?

- Is there a need for more cell lines from ixodid tick species?
- Is there a need for more cell lines from argasid tick species?
- How can tick cell culture technology be made more accessible to the research community? Could this be achieved through a global repository for tick cell lines and training of a new generation of dedicated researchers in establishment and care of tick cell lines?
- How and why do individual cells in tick cell lines survive with aneuploid chromosome complements, and could this be exploited in determination of the function of individual genes?
- Can silencing of tick genes by RNAi be carried out on tick cell lines?
- Can additional tick cell lines be transfected and/or stably transformed and, if so, how can this be exploited?
- What role can tick cell lines play in development of anti-tick vaccines for domestic animals?

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