Ticks and Tick-borne Diseases 5 (2014) 415–422

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Coinfection of tick cell lines has variable effects on replication of intracellular bacterial and viral pathogens

Anna Moniuszko[a,b,1], Claudia Rückert[a,1,2], M. Pilar Alberdi[a,3], Gerald Barry[a,4], Brian Stevenson[c], John K. Fazakerley[a,d], Alain Kohl[a,5], Lesley Bell-Sakyi[a,*,2]

[a] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, Scotland, UK
[b] Department of Infectious Diseases and Neuroinfections, Medical University in Białystok, Żurawia 14, 15-540 Białystok, Poland
[c] Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, MS421 Chandler Medical Center, 800 Rose Street, Lexington, KY 40536-0298, USA
[d] The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK

ARTICLE INFO

Article history:
Received 6 August 2013
Received in revised form 10 January 2014
Accepted 29 January 2014
Available online 29 March 2014

Keywords:
Tick cell line
Coinfection
Ehrlichia
Borrelia
Semliki Forest virus
Ixodes spp.

ABSTRACT

Ticks transmit various human and animal microbial pathogens and may harbour more than one pathogen simultaneously. Both viruses and bacteria can trigger, and may subsequently suppress, vertebrate host and arthropod vector anti-microbial responses. Microbial coinfection of ticks could lead to an advantage or disadvantage for one or more of the microorganisms. In this preliminary study, cell lines derived from the ticks *Ixodes scapularis* and *Ixodes ricinus* were infected sequentially with 2 arthropod-borne pathogens, *Borrelia burgdorferi* s.s., *Ehrlichia ruminantium*, or Semliki Forest virus (SFV), and the effect of coinfection on the replication of these pathogens was measured. Prior infection of tick cell cultures with the spirochaete *B. burgdorferi* enhanced subsequent replication of the rickettsial pathogen *E. ruminantium* whereas addition of spirochaetes to cells infected with *E. ruminantium* had no effect on growth of the latter. Both prior and subsequent presence of *B. burgdorferi* also had a positive effect on SFV replication. Presence of *E. ruminantium* or SFV had no measurable effect on *B. burgdorferi* growth. In tick cells infected first with *E. ruminantium* and then with SFV, virus replication was significantly higher across all time points measured (24, 48, 72 h post infection), while presence of the virus had no detectable effect on bacterial growth. When cells were infected first with SFV and then with *E. ruminantium*, there was no effect on replication of either pathogen. The results of this preliminary study indicate that interplay does occur between different pathogens during infection of tick cells. Further study is needed to determine if this results from direct pathogen–pathogen interaction or from effects on host cell defences, and to determine if these observations also apply in vivo in ticks. If presence of one pathogen in the tick vector results in increased replication of another, this could have implications for disease transmission and incidence.

Introduction

Tick-borne viral and bacterial pathogens are major threats to human and animal health worldwide (Jongejan and Uilenberg, 2004). In Europe, changes in climate, population density, leisure activities, and agricultural practices are increasing the threat from tick-borne diseases (Gray et al., 2009; Jaenson et al., 2009; Danielova et al., 2010; Godfrey and Randolph, 2011). Understanding the interactions between pathogen and vector, and transmission from arthropod to vertebrate, may lead to novel interventions to prevent these diseases.

Ixodes ricinus, commonly known as the sheep tick or castor bean tick, feeds on a wide range of warm-blooded vertebrate

* Corresponding author. Tel.: +44 0 1483 231396; fax: +44 0 1483 233024.
E-mail address: lesley.sakyi@pirbright.ac.uk (L. Bell-Sakyi).
[1] These authors contributed equally to this work.
[2] Present address: The Pirbright Institute, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK.
[3] Present address: Instituto de Investigación en Recursos Cinegéticos, Ronda de Toledo s/n., 13071 Ciudad Real, Spain.
[4] Present address: MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Garscube Estate, Glasgow G61 1QH, UK.
[5] Present address: MRC-University of Glasgow Centre for Virus Research 8, Church Street, Glasgow G11 5JR, UK.

http://dx.doi.org/10.1016/j.ttbdis.2014.01.010
1877-959X

hosts and transmits the flavivirus tick-borne encephalitis virus (TBEV), the spirochaete *Borrelia burgdorferi* sensu lato (s.l.), the obligate intracellular rickettsiae *Anaplasma phagocytophilum*, *Candidatus* Neoehrlichia mikurensis, and *Rickettsia helvetica*, and the protozoa *Babesia divergens* and *Babesia microti*. Of these zoonotic pathogens, TBEV and *B. burgdorferi* s.l. are increasingly recognised as causing serious disease in significant numbers of human patients in areas of high *I. ricinus* prevalence (Fulop and Poggensee, 2008; Sumilo et al., 2007; Czupryna et al., 2011). Surveys in Central and Eastern Europe have shown that individual ticks may be simultaneously infected with more than one of these pathogens (Alekseev et al., 2001; Reye et al., 2010; Tomanovic et al., 2010; Gern et al., 2010), but nothing is known about the effect of coinfection on pathogen replication or infectivity at the cellular level. In the closely related tick species *Ixodes persulcatus*, Alekseev et al. (2003) found a high incidence of multiple pathogen infections and suggested that *B. microti* can only survive in these ticks in the presence of coinfecting *Borrelia* spp. Popov et al. (2007) detected over 40% of unfed adult *I. persulcatus* ticks coinfected with multiple pathogens by PCR; transmission electron microscopic examination revealed cytopathic changes in salivary gland cells infected with *Ehrlichia muris* or a flavivirus, although coinfection of the same cell or organ was not observed.

Even individually, little is known about the interactions of pathogenic bacteria and viruses with ticks. Manipulation of the tick midgut and salivary gland environments in vivo by *B. burgdorferi* (Hovius et al., 2007; Schuijt et al., 2008) and *A. phagocytophilum* (Pedra et al., 2010; Sukumaran et al., 2006; Sultana et al., 2010; Ayllon et al., 2013), and of tick cells in vitro by *A. phagocytophilum* (Pedra et al., 2010; Sultana et al., 2010; Ayllon et al., 2013) have been reported. Presence of tick cells affects in vitro expression of *B. burgdorferi* s.s. outer surface proteins (Obonyo et al., 1999) and genes associated with the starvation-associated stringent response, which is usually triggered by nutritional stress such as amino acid starvation (Bugrysheva et al., 2002). Infection of *Ixodes scapularis* cell lines with the intracellular bacteria *A. phagocytophilum* or *Anaplasma marginale* causes changes in transcription levels of some host cell genes (de la Fuente et al., 2007, 2008; Zivkovic et al., 2009; Villar et al., 2010; Ayllon et al., 2013). It has also been shown that *A. phagocytophilum* coopts ubiquitin during infection in ticks and ISE6 cells (Huang et al., 2012), which may influence cell cycle, cell viability, or replication of a second intracellular pathogen.

Much less is known about the interaction between arboviruses and tick cells in vitro. Arboviruses normally cause a persistent, low-level infection of long duration in tick cells, which is in contrast to their rapid induction of a cytopathic effect in most mammalian cells (Pudney, 1987). The maturation process of TBEV in a tick cell line was found to differ from that seen in a mammalian cell line (Senigl et al., 2006). In a recent ultrastructural study of infection of tick cells with the closely-related flavivirus Langat virus (LGTV), round vesicles and tubular structures of unknown function were associated with endoplasmic reticulum in, respectively, acute and persistent infection (Offerdahl et al., 2012).

The aim of this preliminary study was to analyse the kinetics of pathogen replication in a model system, namely tick cell cultures infected with an extracellular bacterium followed by an intracellular bacterium or a virus, and vice versa. We used a strain of the extracellular bacterium *B. burgdorferi* sensu stricto (s.s.), KS20, that is transformed with a plasmid encoding green fluorescent protein (GFP) under a highly expressed promoter (Babb et al., 2004), enabling us to easily visualise the presence of spirochaetes by fluorescence microscopy in live tick cell cultures. We used the obligately intracellular tick-borne bacterium *Ehrlichia ruminantium*, causative agent of heartwater disease in ruminants, which grows in a wide range of cell lines from different ixodid tick species (Bell-Sakyi, 2004) and can be easily visualised in Giemsa-stained cytocentrifuge smears prepared from infected cell cultures. We chose to use the mosquito-borne arbovirus Semliki Forest virus (SFV) (Togaviridae; Alphavirus) because it has been shown previously to replicate well in tick cell lines over a short 1–3-day timescale (Pudney et al., 1979; Garcia et al., 2005; Barry et al., 2013) and because there are a number of useful virus constructs available containing reporter genes such as eGFP and *Renilla* luciferase (*RLuc*) (Fragkoudis et al., 2007; Kiiver et al., 2008) allowing quick and simple monitoring of virus replication. Here, we present data on pathogen replication (relative increase or decrease) in tick cell lines derived from the *B. burgdorferi* vector species *I. scapularis* and *I. ricinus* infected sequentially with 2 of the 3 pathogens *B. burgdorferi* s.s., *E. ruminantium*, and SFV.

Table 1
Experimental design. Tick cell lines ISE6 and IRE/CTVM19 were inoculated sequentially with 2 of the arthropod-borne pathogens *B. burgdorferi* s.s., *E. ruminantium*, and Semliki Forest virus (SFV) and sampled for quantification of pathogen replication at 3–5 time points as shown. Each experiment was carried out twice, with 3 or 6 replicate wells for each treatment.

Tick cell line	First pathogen (time of inoculation)	Second pathogen (time of inoculation)	Sample time points after addition of second pathogen
ISE6	*B. burgdorferi* (0 h)	*E. ruminantium* (24 h)	24, 48, 72 h
ISE6	*E. ruminantium* (0 h)	*B. burgdorferi* (168 h)	24, 48, 72 h
ISE6	*B. burgdorferi* (0 h)	SFV (24 h)	12, 24, 48, 54, 72 h
IRE/CTVM19	*B. burgdorferi* (0 h)	SFV (24 h)	12, 24, 48, 54, 72 h
ISE6	SFV (0 h)	*B. burgdorferi* (48 h)	0, 6, 24 h
IRE/CTVM19	SFV (0 h)	*B. burgdorferi* (48 h)	0, 6, 24 h
ISE6	*E. ruminantium* (0 h)	SFV (168 h)	24, 48, 72 h
ISE6	SFV (0 h)	*E. ruminantium* (48 h)	24, 48, 72 h

Materials and methods

Tick cell lines

All culture media and supplements were obtained from Sigma Aldrich unless otherwise indicated. The *I. scapularis* embryo-derived cell line ISE6 (Kurtti et al., 1996) was maintained at 32 °C in L-15B300 medium (Munderloh et al., 1999) supplemented with 10% tryptose phosphate broth (TPB), 5% foetal calf serum (FCS, Invitrogen), 2 mM L-glutamine (L-glut), and 0.1% bovine lipoprotein concentrate (MP Biomedicals). The *I. ricinus* embryo-derived cell line IRE/CTVM19 (Bell-Sakyi et al., 2007; Pedra et al., 2010) was maintained at 28 °C in L-15 (Leibovitz) medium supplemented with 10% TPB, 20% FCS, and L-glut. Culture media were supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin except in experiments involving *B. burgdorferi*. Both cell lines were grown in 2-ml volumes in flat-sided cell culture tubes (Nunc). When required for experiments, cells of either tick cell line were seeded into wells of 24-well plates (Nunc) in 1-ml volumes of appropriate complete culture medium at a density of $6–10 \times 10^5$ cells per ml and incubated overnight to allow the cells to attach. All experiments were carried out twice, with 3 or 6 replicate wells per treatment; sample time points were chosen to precede earliest expected detectable increase in *B. burgdorferi* numbers (wild-type population doubling time of 12 h, Kurtti et al., 1988) and to include expected peak [24–48 h post infection (p.i.)] and subsequent decrease of SFV replication (Pudney et al., 1979) (Table 1).