Ticks and Tick-borne Diseases 10 (2019) 207–212

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

# Inter- and intraspecies-specific adhesion of Lyme borreliae to human keratinocytes

Simon Bigelmayr, Arno Koenigs, Peter Kraiczy*

*Institute of Medical Microbiology and Infection Control, University Hospital of Frankfurt, Paul-Ehrlich-Str. 40, D-60596, Frankfurt, Germany*

ARTICLE INFO

*Keywords:*
Spirochetes
*Borrelia*
Keratinocyte
Adhesion
Host pathogen interaction

ABSTRACT

Spirochetes have developed sophisticated means to successfully colonize host tissues and survive in unfavorable environments. Attachment to human cells is thought to be a key step for the establishment of an infection that causes multiple clinical symptoms. Infection of host tissues largely depends on the ability of spirochetes to attach to different cell types. In this study, we examine the ability of spirochetes belonging to seven distinct genospecies (*Borrelia (B.) burgdorferi* sensu stricto (s.s.), *B. afzelii, B. garinii, B. spielmanii*, *B. bavariensis*, *B. lusitaniae,* and *B. valaisiana*) to adhere to human keratinocytes. Among the genospecies analyzed, *B. valaisiana* and *B. spielmanii* showed the strongest adherence while *B. bavariensis, B. garinii* and *B. afzelii* displayed moderate binding activity. By contrast, only a few cells of *B. burgdorferi* s.s. and *B. lusitaniae* bound to keratinocytes. Furthermore, intraspecies differences have also been observed among *B. garinii*, *B. bavariensis*, *B. afzelii*, and in particular *B. valaisiana*.

To further assess the role of infection-associated borrelial outer surface proteins for mediating interaction to human cells, a non-adherent and non-infectious *B. garinii* strains producing distinct complement regulator-acquiring surface proteins (CRASP) were employed. Interestingly, binding capacity to human keratinocytes increased up to four-fold in *B. garinii* cells producing ErpC but not CspA, CspZ or ErpP compared to wild-type *B. garinii* cells lacking CRASPs. Taken together, these data provide evidence that distinct borrelial genospecies differ in their ability to bind to human keratinocytes and, in addition, support a role of ErpC as a potential adhesin of spirochetes.

## 1. Introduction

Spirochetes causing Lyme disease/borreliosis have developed diverse strategies to successfully colonize host tissues and survive in an unfavorable, hostile environment (Brissette and Gaultney, 2014; Caine and Coburn, 2016; Coburn et al., 2013). Upon entry of the spirochetes into the human host through the bite of an infected tick, attachment to and colonization of different tissues is thought to be a central step for establishing an infection that may progresses, if left untreated, to multiple clinical symptoms including serious neurological (progressive encephalitis, chronic meningitis or encephalomyelitis), rheumatological as well as long-term dermatological manifestations (acrodermatitis chronica atrophicans) (Arvikar and Steere, 2015; Koedel et al., 2015; Steere et al., 2016).

Once transmitted to the human host, spirochetes establish infection by colonizing skin interface of the tick bite. In fact, skin is the key physical barrier and first line of defense against microbial pathogens. Despite its deceptively simple histology, the epidermis has a high diversity of resident immune or non-immune cells such dendritic cells, $CD4^+$ T helper cells ($T_h$), $\gamma\delta$ cells, natural killer cells, macrophages, mast cells, fibroblasts, and keratinocytes, most of which act as important immune sentinels (Nestle et al., 2009). Keratinocytes are known to function as central skin sentinels in order to prevent excessive inflammation and recognize foreign microorganisms by their pathogen-associated molecular pattern (PAMPs) and harmful substances by danger-associated molecular pattern (DAMPs). Although much efforts were undertaken to analyze the interaction of spirochetes with different cell types (Fischer et al., 2003; Galbe et al., 1993; Garcia-Monco et al., 1989; Guo et al., 1995; Klempner et al., 1995; Leong et al., 1998b; Parveen et al., 1999; Szczepanski et al., 1990; Thomas and Comstock, 1989), investigations aimed at elucidating the role of keratinocytes in sensing and mediating immune responses to tick-borne pathogens are in their infancy.

Spirochetes are capable of selectively adhering to various mammalian cell types *in vitro* including endothelial, epithelial or neuronal cells, fibroblasts, platelets as well as to components of the extracellular

* Corresponding author.
*E-mail address:* Kraiczy@em.uni-frankfurt.de (P. Kraiczy).

https://doi.org/10.1016/j.ttbdis.2018.10.006
Received 2 July 2018; Received in revised form 12 October 2018; Accepted 24 October 2018
Available online 28 October 2018
1877-959X/ 

matrix (Fischer et al., 2003; Galbe et al., 1993; Garcia-Monco et al., 1989; Guo et al., 1995; Klempner et al., 1995; Leong et al., 1998a, b; Parveen et al., 1999; Szczepanski et al., 1990; Thomas and Comstock, 1989). Reflecting the attribute to exit the bloodstream following tissue adhesion and colonization, spirochetes produce distinct surface-exposed molecules exhibiting adhesive functions (Brissette and Gaultney, 2014; Caine and Coburn, 2016; Norris et al., 2010). At least 19 different determinants involved in tissue adhesion and interaction with extracellular matrix components have been described for *B. burgdorferi* sensu stricto (s.s.) so far, including well-characterized outer surface proteins such as DbpA, DbpB, BBK32, OspC, p66, RevA, RevB, Bgp, CspA, CspZ, BBA70, BB0347 and certain Erp proteins (for review see (Brissette and Gaultney, 2014; Coburn et al., 2013). Many of these proteins display multifunctional and redundant roles in the pathobiology of Lyme disease/borreliosis as well as in escaping innate immunity. Concerning adhesion to specific cell types, recognition of different proteoglycans, e.g. glycosaminoglycans (GAGs) is mediated by various outer surface proteins, e.g. DbpA, DbpB, and BBK32 (Coburn et al., 2005; Parveen et al., 1999). Variation in the expression of multiple GAG-binding adhesins among strains of the same genospecies might also account for the strain-specific attachment to different cell types (Leong et al., 1998a). It has been shown that certain CRASP proteins play a role in adhesion of borreliae as CspA and CspZ bind to diverse extracellular matrix components (Hallström et al., 2010). Assuming that borreliae come in direct contact with resident cells in the skin, we set out to determine whether spirochetes with confirmed (*Borrelia burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. spielmanii*, and *B. bavariensis*) and with uncertain pathogenicity (*B. lusitaniae* and *B. valaisiana*) differ in their ability to adhere to human keratinocytes. Although by no means complete, the data collected in this study provide evidence for an intra- and interspecies-specific difference among spirochetes belonging to the *B. burgdorferi* sensu lato (s.l.) complex. In addition, we also sought to elucidate the role of four surface-exposed proteins belonging to the CRASP protein family (CspA, CspZ, ErpC, and ErpP) in attachment of borreliae to human keratinocytes.

## 2. Material and methods

### 2.1. Bacterial strains, human cell line, and culture conditions

A panel of 12 isolates consisting of seven different genospecies belonging to the *Borrelia* (*B.*) *burgdorferi* sensu lato (s.l.) complex as well as four *B. garinii* strains ectopically producing CspA, CspZ, ErpC or ErpP was investigated. Unfortunately, the ErpA producing G1 strain was not available at the time of the study. The designations, biological, and geographical origins of each borrelial strain/isolate are summarized in Table 1. The generation of spirochetes ectopically producing distinct CRASP proteins in *B. garinii* G1 as a gain-of-function strain has been extensively described elsewhere (Hallström et al., 2013; Hammerschmidt et al., 2012, 2014; Siegel et al., 2010, 2008). Spirochetes were grown to mid-logarithmic phase ($1 \times 10^8$ cells/ml) at 33 °C in Barbour-Stoenner-Kelly (BSK) medium (Bio&Sell, Feucht, Germany) supplemented with 7% rabbit serum (Sigma-Aldrich) or in BSK supplemented with 50 µg/ml streptomycin (Siegel et al., 2008). Dark-field microscopy was used to check viability and motility of the bacterial cells before the cells density was determined using a Kova counting chamber (Hycor Biomedical, Garden Grove, CA) (Kraiczy et al., 2001). The human immortalized keratinocyte cell line, HaCaT, were cultured in Gibco™ DMEM (Fisher Scientific) supplemented with 10% heat-inactivated FCS (Sigma-Aldrich), 100 units/ml penicillin, and 100 µg/ml streptomycin at 37 °C and 5% $CO_2$ until they reached 90% confluency (Wittmann et al., 2012).

**Table 1**
*Borrelia* strains used in the study.

| Genospecies | Strain | Biological origin | Geographical origin |
|---|---|---|---|
| *B. burgdorferi* s.s. | B31 | Tick | United States |
| *B. burgdorferi* s.s. | 297 | CSF | United States |
| *B. afzelii* | FEM1-D15 | Skin | Germany |
| *B. afzelii* | MMS | Tick | Germany |
| *B. garinii* | G1 | CSF | Germany |
| *B. garinii* | PSma | Skin | Germany |
| *B. bavariensis* | PBi | CSF | Germany |
| *B. bavariensis* | PTrob | Skin | Germany |
| *B. spielmanii* | A14S | Skin | The Netherlands |
| *B. lusitaniae* | MT-M8 | Tick | Portugal |
| *B. valaisiana* | ZWU3 Ny3 | Tick | Germany |
| *B. valaisiana* | VS116 | Tick | Switzerland |

s.s., sensu stricto. CSF, cerebrospinal fluid.

### 2.2. Adhesion assays and immunofluorescence microscopy

Spirochetes grown in BSK medium with or without streptomycin (as selection for *B. garinii* cells ectopically producing CRASPs) were harvested at mid-logarithmic phase ($1 \times 10^8$ cells/ml) and washed twice with DMEM without antibiotics and FCS to remove BSA and additional serum constituents of the cultivating medium. Following counting (Kraiczy et al., 2001), spirochetes were pelleted at 5000 × g for 30 min at 4 °C and resuspended in 500 µl DMEM. Keratinocytes ($1.25 \times 10^5$) were seeded onto 24-well microtiter plates containing inlays with cover slides and incubated until confluent. Human cells were then exposed to highly viable and motile borrelial cells at a multiplicity of infection (MOI) of 50 for 2 h at 37 °C and washed thrice with PBS before being fixed for 10 min at 4 °C with 3.75% paraformaldehyde. Following two wash steps with PBS, cells were permeabilised by 0.2% (v/v) triton X-100 in PBS for 15 min at room temperature (RT). Cover slides were then blocked with 0.2% (w/v) BSA in PBS for 15 min at RT and after washing twice with PBS, a polyclonal anti-*Borrelia burgdorferi* antibody (Biozol Diagnostica) was added (dilution 1:1000). Following incubation for 60 min at RT, cover slides were washed twice with PBS and incubated with Alexa Fluor 488-conjugated anti-rabbit immunoglobulins (Molecular Probes) (1:1000) at RT. Following two wash steps with PBS, TRITC-conjugated phalloidin (1:1000) was added for 30 min at RT. Thereafter, cover slides were washed twice with PBS and cell nuclei were visualized by adding DAPI (1 µg/ml) for 10 min at 4 °C. Cells were mounted in Fluoprep (Biomérieux) before being examined under an Axio Imager M2 fluorescence microscope (Zeiss) equipped with a Spot RT3 camera (Visitron Systems).

### 2.3. Statistical analysis

Raw data were analyzed by unpaired student's *t*-test (GraphPad Prism 5.03, GraphPad Software, San Diego, CA). All assays were conducted at least as in duplicates in three independent experiments.

## 3. Results and discussion

Keratinocytes which constitute 90% of the epidermal cells gain in importance as relevant sensors of the 'dermal immune system' that alert this versatile/complex organ to the entry of foreign pathogens by activation of tissue-resident cells and perpetuation of the inflammasome machinery. Moreover, keratinocytes also play a role in recruiting host immune cells as well as in modulating cytokine production (Nestle et al., 2009). Certainly, the mammalian skin represents a truly insurmountable obstacle for spirochetes per se, however, borreliae overcome this important protective physical barrier by the route of transmission via the bite of an infected tick. Once transmitted to the human host, spirochetes disseminate promptly from the site of infection, entering different host tissues including the skin interface. As an early dermal-induced inflammatory response an erythema chronicum migrans appears as the most common manifestation of Lyme disease/borreliosis pointing towards a direct interaction with the tissue-resident

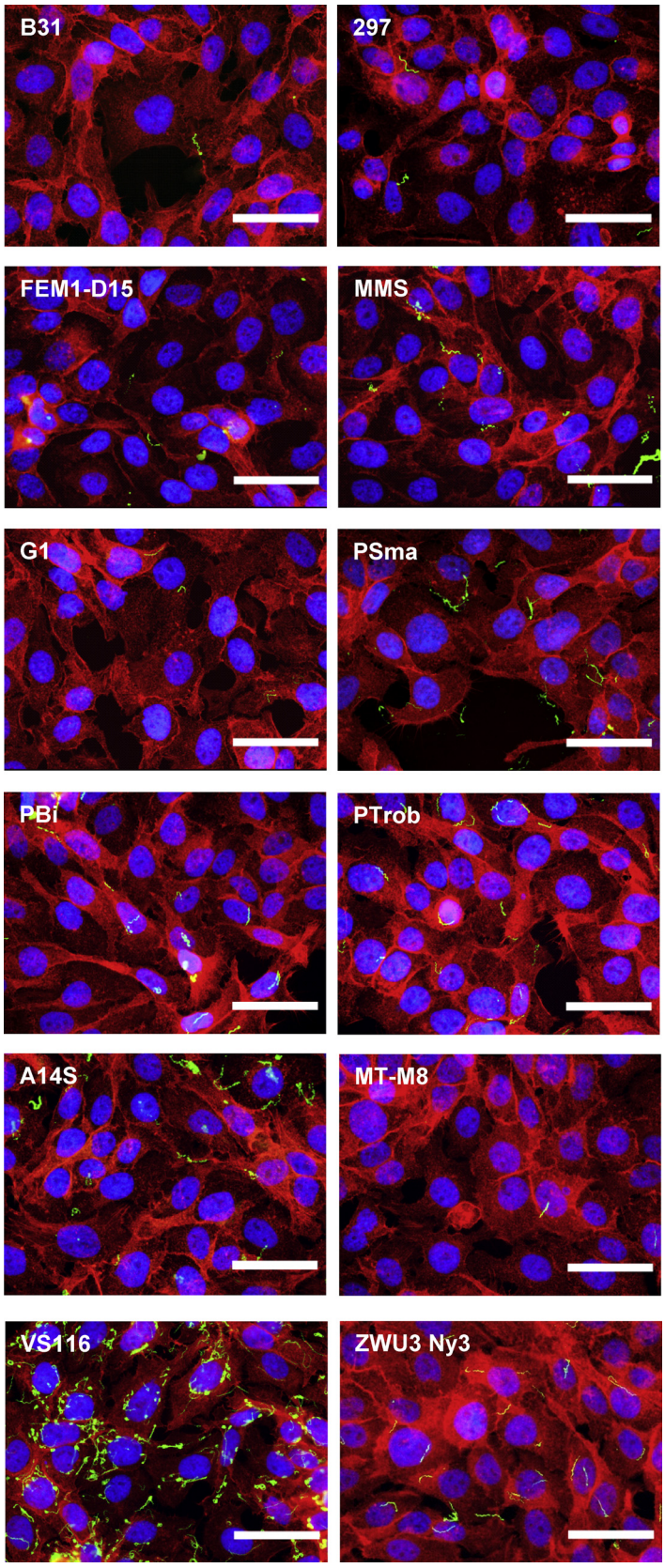


**Fig. 1.** Adhesion of spirochetes to human keratinocytes.
Keratinocytes ($2.5 \times 10^5$) were seeded in microtiter plates, grown to confluency and infected with different borrelial isolates (MOI of 50). After fixation, spirochetes bound to the cells were visualized with a polyclonal anti-*Borrelia* antibody (green) and the actin filaments in the cytoskeleton as well as the nuclei of mouse cells were stained by using TRITC-conjugated phalloidin (red) and DAPI (blue), respectively. Shown are representative data from three independent experiments each performed at least in duplicate. The scale bar in each panel represents 50 µm. The spirochetes were observed at a magnification of 1000. The data were recorded with an Axio Imager M2 fluorescence microscope (Zeiss) equipped with a Spot RT3 camera (Visitron Systems). Each panel shown is representative of at least 100 keratinocytes. Cells were counted and the numbers of adhered spirochetes were determined. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cells of the skin including keratinocytes. Furthermore, keratinocytes appears to be a physiological relevant cell type to investigate adhesive properties of borreliae. In an initial attempt, we investigated the interaction of *Borrelia* with keratinocytes adhesion of spirochetes belonging to the five pathogenic genospecies (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. spielmanii*, and *B. bavariensis*) and two genospecies with disputed pathogenic potential (*B. lusitaniae* and *B. valaisiana*) (Table 1) by employing adhesion assays followed up by

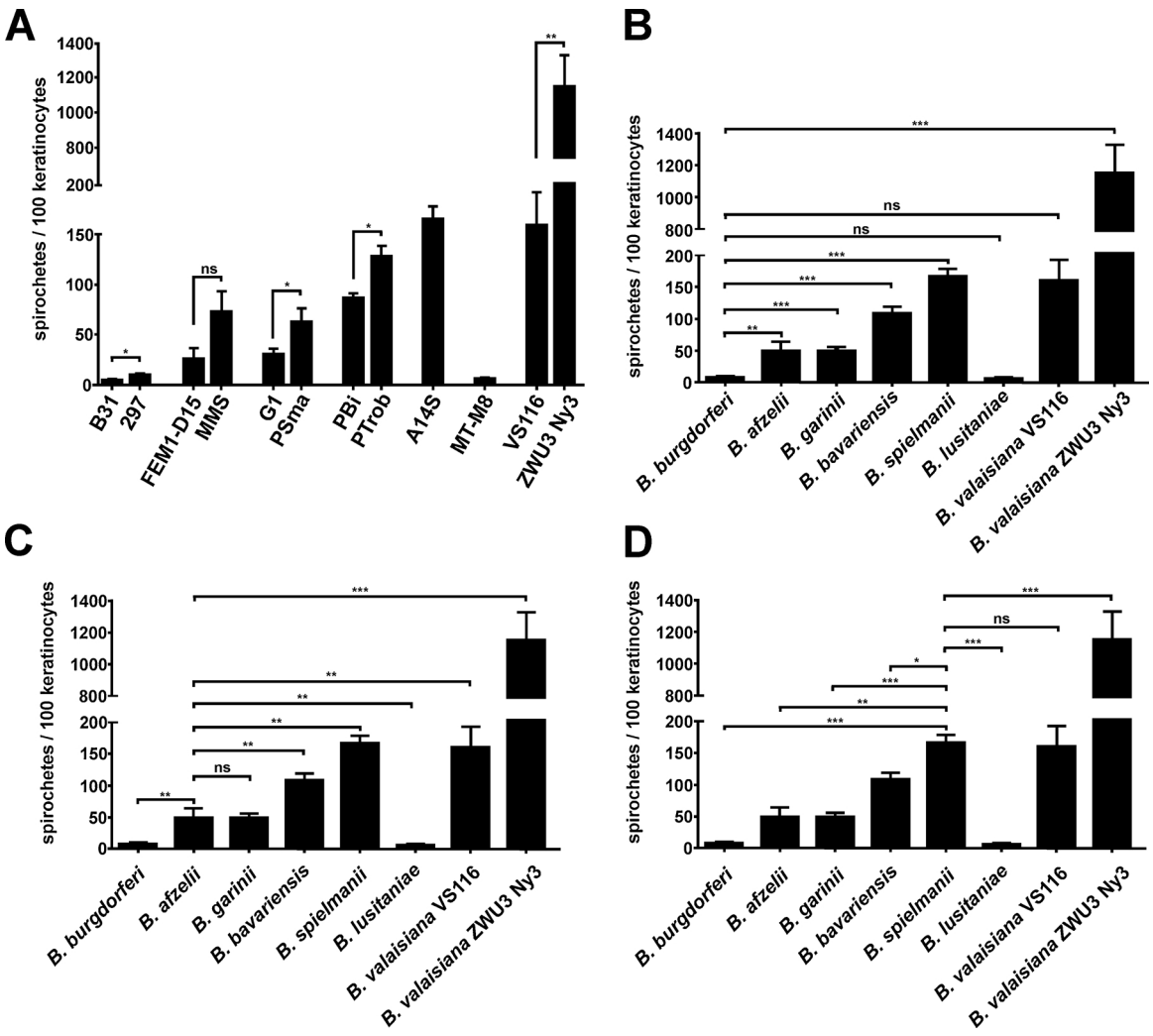


**Fig. 2.** Genospecies-specific differences of spirochetes in adhesion to human keratinocytes.
Statistical analysis of data presented in Fig. 1. For the statistical analysis, the mean of the number of spirochetes per 100 keratinocytes have been used for the calculation. Error bars correspond to SD. A, differences between all strains analyzed. B, genospecies-specific differences between *B. burgdorferi* and all other genospecies. C, genospecies-specific differences between *B. afzelii* and all other genospecies. D, genospecies-specific differences between *B. spielmanii* and all other genospecies. ns, not significant; *, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$ (two-tailed, unpaired student´s *t*-test).

immunofluorescence microscopy. Human-derived HaCaT cells ($1.25 \times 10^5$) were grown to confluency and infected with spirochetes at a MOI of 50. The cells were then washed to remove unbound bacteria and fixed in paraformaldehyde. Adherent spirochetes were stained with a polyclonal anti-*Borrelia burgdorferi* antibody and visualized with an appropriate secondary antibody. The actin cytoskeleton was stained with TRITC-conjugated phalloidin and cell nuclei were counterstained with DAPI.

Among the seven genospecies analyzed, *B. valaisiana* and *B. spielmanii* showed the strongest attachment (up to 1200 spirochetes per 100 keratinocytes) while *B. bavariensis*, *B. garinii* and *B. afzelii* displayed moderate binding capacity (up to 120 spirochetes per 100 keratinocytes) suggesting a possible interaction of these five genospecies with resident cells in the human skin (Fig. 1). By contrast, only a few cells of *B. burgdorferi* s.s. and *B. lusitaniae* bound to this cell type. Furthermore, intra-species differences have also been observed among *B. garinii*, *B. bavariensis*, *B. afzelii*, and in particular *B. valaisiana* (Fig. 2B-D).

Moreover, strain *B. valaisiana* ZWU3 Ny3 showed the highest capability to adhere to HaCaT cells followed by *B. spielmanii* strain A14S, *B. bavariensis* PTrob and PBi as well as *B. afzelii* MMS (Fig. 2A). Both analyzed *B. burgdorferi* s.s. strains exhibited background level of binding as did *B. lusitaniae* MT-M8 (Fig. 2B). Both, *B. afzelii* FEM1-D15 and *B. garinii* G1 bound at very low levels (< 30 cells) to human keratinocytes (Fig. 2A). To what extent the binding of borrelial cells to keratinocytes has advantages for their survival remain unclear. Thus, it is tempting to speculate that spirochetes immune evasion is facilitated by migration to deeper tissues, thereby colonizing a niche in which the innate immune system is less active. Conversely, a massive infection, such as that observed by *B. valaisiana* ZWU3 Ny3 (Fig. 1) might cause a strong immune response and the release of high levels of chemokines and cytokines (Kern et al., 2015). This leads to the recruitment of macrophages and other cells of the immune system. As a result, spirochetes would have to deal more intensively with the skin immune system in order not to be killed. Thus, too much stickiness to human cells may have detrimental effects on the survival of these pathogens in the vertebrate host. In addition to the spirochetes' ability to attach to host cells, the infectivity of a given spirochete species also depends on other factors, such as the capability for these bacteria to be transmitted from ticks to hosts or evade the host immune clearance at the initial time point of infection. Thus, our observation that *B. spielmanii* and *B. valaisiana* displayed a stronger affinity to keratinocytes may not be necessarily correlated with their ability to infect the human host.

For pathogens that cause systematic infection, recruitment of plasminogen plays a critical role to promote their dissemination from the

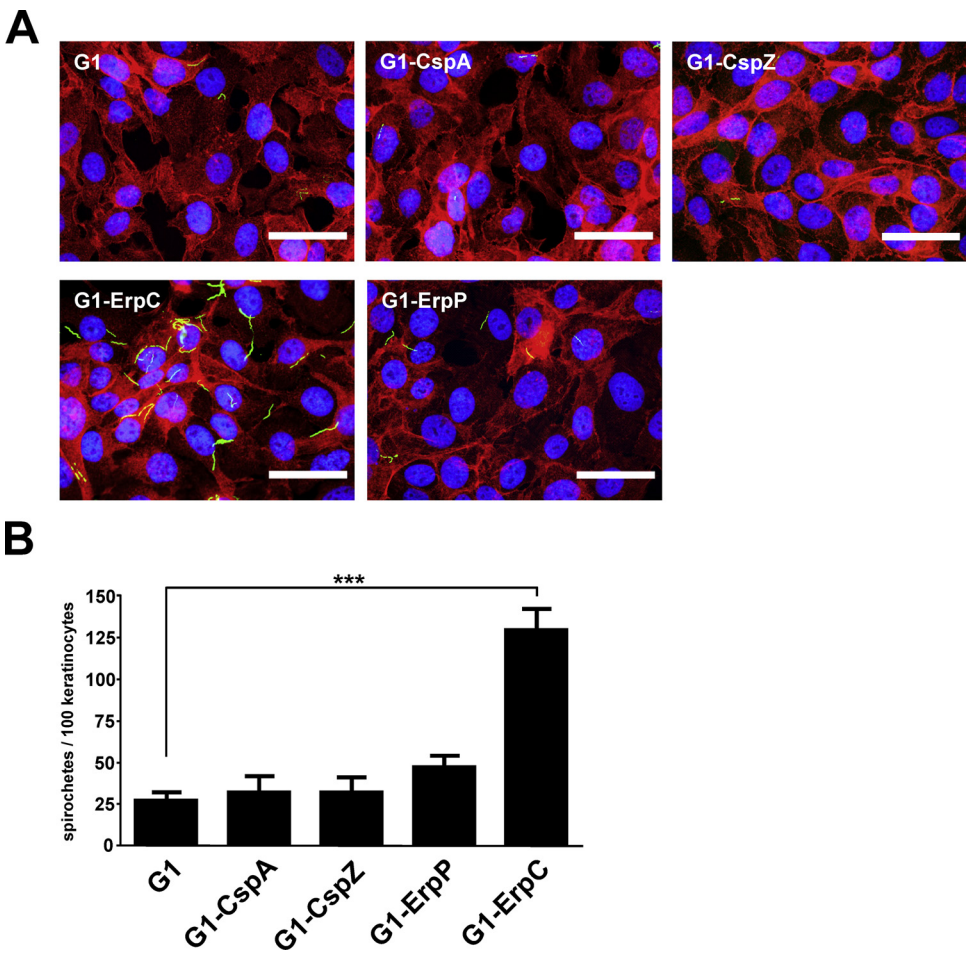


**Fig. 3.** Adhesion of CRASP-producing spirochetes to human keratinocytes.
A, keratinocytes ($2.5 \times 10^5$) were seeded in microtiter plates, grown to confluency and infected with different borrelial isolates (MOI of 50). After fixation, spirochetes bound to the cells were visualized with a polyclonal anti-*Borrelia* antibody (green) and the actin filaments in the cytoskeleton as well as the nuclei of mouse cells were stained by using TRITC-conjugated phalloidin (red) and DAPI (blue), respectively. Shown are representative data from three independent experiments each performed at least in duplicate. The scale bar in each panel represents 50 µm. The spirochetes were observed at a magnification of 1000. The data were recorded with an Axio Imager M2 fluorescence microscope (Zeiss) equipped with a Spot RT3 camera (Visitron Systems). Each panel shown is representative of at least 100 keratinocytes. Cells were counted and the numbers of adhered spirochetes were determined.
B, statistical analysis of data presented in Fig. 3A. The numbers represent spirochetes counted from 100 HaCaT cells in three independent experiments. For the statistical analysis, the mean of the number of spirochetes per 100 keratinocytes have been used for the calculation. Error bars correspond to SD ***, $p < 0{,}001$ (two-tailed, unpaired student´s *t*-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

initial infection site to multiple tissues (Bhattacharya et al., 2012) because activated plasmin(ogen) as an unspecific serine protease can eliminate attached bacteria from the host cells (Singh et al., 2012). In fact, the presence of plasmin(ogen) promotes dissemination of spirochetes in the mammalian host (Coleman et al., 1997). Moreover, Ixonnexin, an *Ixodes scapularis* plasminogen-binding protein from tick saliva is able to convert plasminogen to plasmin (Assumpcao et al., 2018) raising the possibility that salivary proteins promote migration and penetration of *Borrelia* at early time points of infection. Further studies of salivary proteins in facilitating spirochetes dissemination from biting site of skin to distal tissues and also detachment from keratinocytes are warranted.

Recently, it has been shown that CRASP proteins differ in their capacity to bind to various components of the extracellular matrix (Hallström et al., 2010) suggesting that these molecules might also be responsible for binding of spirochetes to other cells types including cells residing in the human skin. To further assess the role of these infection-associated borrelial outer surface proteins for mediating interaction to human keratinocytes under more physiologic conditions, *B. garinii* cells producing distinct CRASPs originally derived from *B. burgdorferi* s.s. B31 were employed (Hallström et al., 2013; Hammerschmidt et al., 2012, 2014; Siegel et al., 2010, 2008). Adhesion assays following immunofluorescence microscopy were conducted as described above by infection of HaCaT cells with spirochetes at a MOI of 50. As depicted in Fig. 3, spirochetes producing ErpC showed the highest capability to adhere to HaCaT cells while cells positive for CspA, CspZ, and ErpP bind to the same extent as wild-type *B. garinii* G1 (< 50 cells) (Fig. 3A and B). The comparative analyses of the three dimensional structures of both Erp proteins revealed that, in contrast to ErpP, an extended loop region between β-strands β3 and β4 exist in ErpC that is orientated outwardly of the molecule (Brangulis et al., 2015; Caesar et al., 2013). Such a loop structure containing additional charged residues (Asp and Lys) might promote attachment of this particular strain to human keratinocytes. Taken together, these data support a role of ErpC in particular as a potential adhesin of spirochetes.

Recently, it has been shown that the skin with its different cell types is an important gateway for the multiplication, dissemination, and adaptation of spirochetes (Kern et al., 2015). In this particular study, the authors emphasized that specific bacterial as well as host-derived factors account for the development of an infection in the vertebrate host. Of note, besides OspC and RST (16 S–23 S rRNA intergenic spacer type), two well-known pathogenic markers (Jones et al., 2006; Liveris et al., 1996; Seinost et al., 1999; Wormser et al., 2008), additional factors are required to promote dissemination of the spirochetes following transmission. In fact, the heterogeneity of the population producing a large mixture of diverse proteins and variants thereof on the spirochetal surface must also to be taken into account in the considerations of a multilayered process of infection (Rego et al., 2014). This process also includes tissue-specificity of adhesins. Concerning the involvement of CRASP proteins, it has been shown that these molecules are produced at different time points during the mammalian-tick infection cycle (Bykowski et al., 2007; Hart et al., 2018; Miller and Stevenson, 2006; Miller et al., 2003; von Lackum et al., 2005). Among the five CRASP proteins analyzed so far, CspA is produced at 4 days post infection (Bykowski et al., 2007) implying that this protein may play a role when spirochetes initiate the infection. However, we have shown that a *cspA* deficient mutant colonizes skin at similar levels as wild type strain via needle infection (Hart et al., 2018) suggesting that CspA possesses no adhesive properties on skin-derived cells. The data with the gain-of-function strain producing CspA presented in this study

underlines our *in vivo* findings. Taken together, further studies are warranted to delineate the specific role of ErpC in skin colonization of *Borrelia*.

## 4. Conclusions

Attachment of spirochetes to host cells is a crucial factor for the colonization of host tissue, the subsequent penetration of the bacteria into deeper tissues and for dissemination. This study provides evidence that spirochetes differ in their ability to attach to cells of dermal origin, in particular human keratinocytes on an intra-species level. Moreover, differences could also be observed between certain isolates of the same genospecies (inter-species level). In addition, by employing distinct strains producing different CRASPs, we showed that ErpC mediates adhesion of spirochetes to human keratinocytes.

## Declarations of interest

None.

## Acknowledgements

We thank Jessica Günnewig for skillful and expert technical advice. We thank Yi-Pin Lin for fruitful discussion and critical reading of the manuscript. This work forms part of the diploma thesis of SB.

## References

Arvikar, S.L., Steere, A.C., 2015. Diagnosis and treatment of Lyme arthritis. Infect. Dis. Clin. North Am. 29, 269–280.
Assumpcao, T.C., Mizurini, D.M., Ma, D., Monteiro, R.Q., Ahlstedt, S., Reyes, M., Kotsyfakis, M., Mather, T.N., Andersen, J.F., Lukszo, J., Ribeiro, J.M.C., Francischetti, I.M.B., 2018. Ixonnexin from tick saliva promotes fibrinolysis by interacting with plasminogen and tissue-type plasminogen activator, and prevents arterial thrombosis. Sci. Rep. 8, 4806.
Bhattacharya, S., Ploplis, V.A., Castellino, F.J., 2012. Bacterial plasminogen receptors utilize host plasminogen system for effective invasion and dissemination. J. Biomed. Biotechnol. 2012, 482096.
Brangulis, K., Petrovskis, I., Kazaks, A., Akopjana, I., Tars, K., 2015. Crystal structures of the Erp protein family members ErpP and ErpC from *Borrelia burgdorferi* reveal the reason for different affinities for complement regulator factor H. Biochim. Biophys. Acta 1854, 349–355.
Brissette, C.A., Gaultney, R.A., 2014. That's my story, and I'm sticking to it-an update on *B. burgdorferi* adhesins. Front. Cell. Infect. Microbiol. 4, 41.
Bykowski, T., Woodman, M.E., Cooley, A.E., Brissette, C.A., Brade, V., Wallich, R., Kraiczy, P., Stevenson, B., 2007. Coordinated expression of *Borrelia burgdorferi* complement regulator-acquiring surface proteins during the Lyme disease spirochete's mammal-tick infection cycle. Infect. Immun. 75, 4227–4236.
Caesar, J.J., Johnson, S., Kraiczy, P., Lea, S.M., 2013. ErpC, a member of the complement regulator-acquiring family of surface proteins from *Borrelia burgdorferi*, possesses an architecture previously unseen in this protein family. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 69, 624–628.
Caine, J.A., Coburn, J., 2016. Multifunctional and redundant roles of *Borrelia burgdorferi* outer surface proteins in tissue adhesion, colonization, and complement evasion. Front. Immunol. 7, 442.
Coburn, J., Fischer, J.R., Leong, J.M., 2005. Solving a sticky problem: new genetic approaches to host cell adhesion by the Lyme disease spirochete. Mol. Microbiol. 57, 1182–1195.
Coburn, J., Leong, J., Chaconas, G., 2013. Illuminating the roles of the *Borrelia burgdorferi* adhesins. Trends Microbiol. 21, 372–379.
Coleman, J.L., Gebbia, J.A., Piesman, J., Degen, J.L., Bugge, T.H., Benach, J.L., 1997. Plasminogen is required for efficient dissemination of *Borrelia burgdorferi* in ticks and for enhancement of spirochetemia in mice. Cell 89, 1111–1119.
Fischer, J.R., Parveen, N., Magoun, L., Leong, J.M., 2003. Decorin-binding proteins A and B confer distinct mammalian cell type-specific attachment by *Borrelia burgdorferi*, the Lyme disease spirochete. Proc. Natl. Acad. Sci. U. S. A. 100, 7307–7312.
Galbe, J.L., Guy, E., Zapatero, J.M., Peerschke, E.I., Benach, J.L., 1993. Vascular clearance of *Borrelia burgdorferi* in rats. Microb. Pathog. 14, 187–201.
Garcia-Monco, J.C., Fernandez-Villar, B., Benach, J.L., 1989. Adherence of the Lyme disease spirochete to glial cells and cells of glial origin. J. Infect. Dis. 160, 497–506.
Guo, B.P., Norris, S.J., Rosenberg, L.C., Hook, M., 1995. Adherence of *Borrelia burgdorferi* to the proteoglycan decorin. Infect. Immun. 63, 3467–3472.
Hallström, T., Haupt, K., Kraiczy, P., Hortschansky, P., Wallich, R., Skerka, C., Zipfel, P.F., 2010. Complement regulator-acquiring surface protein 1 of *Borrelia burgdorferi* binds to human bone morphogenic protein 2, several extracellular matrix proteins, and plasminogen. J. Infect. Dis. 202, 490–498.
Hallström, T., Siegel, C., Morgelin, M., Kraiczy, P., Skerka, C., Zipfel, P.F., 2013. CspA from *Borrelia burgdorferi* inhibits the terminal complement pathway. mBio 4.
Hammerschmidt, C., Hallstrom, T., Skerka, C., Wallich, R., Stevenson, B., Zipfel, P.F., Kraiczy, P., 2012. Contribution of the infection-associated complement regulator-acquiring surface protein 4 (ErpC) to complement resistance of Borrelia burgdorferi. Clin. Dev. Immunol., 349657.
Hammerschmidt, C., Koenigs, A., Siegel, C., Hallstrom, T., Skerka, C., Wallich, R., Zipfel, P.F., Kraiczy, P., 2014. Versatile roles of CspA orthologs in complement inactivation of serum-resistant Lyme disease spirochetes. Infect. Immun. 82, 380–392.
Hart, T., Nguyen, N.T.T., Nowak, N.A., Zhang, F., Linhardt, R.J., Diuk-Wasser, M., Ram, S., Kraiczy, P., Lin, Y.P., 2018. Polymorphic factor H-binding activity of CspA protects Lyme borreliae from the host complement in feeding ticks to facilitate tick-to-host transmission. PLoS Pathog. 14, e1007106.
Jones, K.L., Glickstein, L.J., Damle, N., Sikand, V.K., McHugh, G., Steere, A.C., 2006. *Borrelia burgdorferi* genetic markers and disseminated disease in patients with early Lyme disease. J. Clin. Microbiol. 44, 4407–4413.
Kern, A., Schnell, G., Bernard, Q., Boeuf, A., Jaulhac, B., Collin, E., Barthel, C., Ehret-Sabatier, L., Boulanger, N., 2015. Heterogeneity of *Borrelia burgdorferi* sensu stricto population and its involvement in *Borrelia* pathogenicity: study on murine model with specific emphasis on the skin interface. PLoS One 10, e0133195.
Klempner, M.S., Noring, R., Epstein, M.P., McCloud, B., Hu, R., Limentani, S.A., Rogers, R.A., 1995. Binding of human plasminogen and urokinase-type plasminogen activator to the Lyme disease spirochete, Borrelia burgdorferi. J. Infect. Dis. 171, 1258–1265.
Koedel, U., Fingerle, V., Pfister, H.W., 2015. Lyme neuroborreliosis-epidemiology, diagnosis and management. Nat. Rev. Neurol. 11, 446–456.
Kraiczy, P., Skerka, C., Brade, V., Zipfel, P.F., 2001. Further characterization of complement regulator-acquiring surface proteins of Borrelia burgdorferi. Infect. Immun. 69, 7800–7809.
Leong, J.M., Robbins, D., Rosenfeld, L., Lahiri, B., Parveen, N., 1998a. Structural requirements for glycosaminoglycan recognition by the Lyme disease spirochete, Borrelia burgdorferi. Infect. Immun. 66, 6045–6048.
Leong, J.M., Wang, H., Magoun, L., Field, J.A., Morrissey, P.E., Robbins, D., Tatro, J.B., Coburn, J., Parveen, N., 1998b. Different classes of proteoglycans contribute to the attachment of *Borrelia burgdorferi* to cultured endothelial and brain cells. Infect. Immun. 66, 994–999.
Liveris, D., Wormser, G.P., Nowakowski, J., Nadelman, R., Bittker, S., Cooper, D., Varde, S., Moy, F.H., Forseter, G., Pavia, C.S., Schwartz, I., 1996. Molecular typing of *Borrelia burgdorferi* from Lyme disease patients by PCR-restriction fragment length polymorphism analysis. J. Clin. Microbiol. 34, 1306–1309.
Miller, J.C., Stevenson, B., 2006. *Borrelia burgdorferi erp* genes are expressed at different levels within tissues of chronically infected mammalian hosts. Int. J. Med. Microbiol. 296 (Suppl. 40), 185–194.
Miller, J.C., von Lackum, K., Babb, K., McAlister, J.D., Stevenson, B., 2003. Temporal analysis of *Borrelia burgdorferi* Erp protein expression throughout the mammal-tick infectious cycle. Infect. Immun. 71, 6943–6952.
Nestle, F.O., Di Meglio, P., Qin, J.Z., Nickoloff, B.J., 2009. Skin immune sentinels in health and disease. Nat. Rev. Immunol. 9, 679–691.
Norris, S.J., Coburn, J., Leong, J.M., Hu, L.T., Höök, M., 2010. Pathobiology of Lyme disease *Borrelia*. In: Samuels, D.S., Radolf, J.D. (Eds.), *Borrelia*: Molecular Biology, Host Interaction and Pathogenesis. Caister Academic Press, pp. 299–331.
Parveen, N., Robbins, D., Leong, J.M., 1999. Strain variation in glycosaminoglycan recognition influences cell-type-specific binding by lyme disease spirochetes. Infect. Immun. 67, 1743–1749.
Rego, R.O., Bestor, A., Stefka, J., Rosa, P.A., 2014. Population bottlenecks during the infectious cycle of the Lyme disease spirochete Borrelia burgdorferi. PLoS One 9, e101009.
Seinost, G., Dykhuizen, D.E., Dattwyler, R.J., Golde, W.T., Dunn, J.J., Wang, I.N., Wormser, G.P., Schriefer, M.E., Luft, B.J., 1999. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect. Immun. 67, 3518–3524.
Siegel, C., Hallström, T., Skerka, C., Eberhardt, H., Uzonyi, B., Beckhaus, T., Karas, M., Wallich, R., Stevenson, B., Zipfel, P.F., Kraiczy, P., 2010. Complement factor H-related proteins CFHR2 and CFHR5 represent novel ligands for the infection-associated CRASP proteins of Borrelia burgdorferi. PLoS One 5, e13519.
Siegel, C., Schreiber, J., Haupt, K., Skerka, C., Brade, V., Simon, M.M., Stevenson, B., Wallich, R., Zipfel, P.F., Kraiczy, P., 2008. Deciphering the ligand-binding sites in the *Borrelia burgdorferi* complement regulator-acquiring surface protein 2 required for interactions with the human immune regulators factor H and factor H-like protein 1. J. Biol. Chem. 283, 34855–34863.
Singh, B., Fleury, C., Jalalvand, F., Riesbeck, K., 2012. Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host. FEMS Microbiol. Rev. 36, 1122–1180.
Steere, A.C., Strle, F., Wormser, G.P., Hu, L.T., Branda, J.A., Hovius, J.W., Li, X., Mead, P.S., 2016. Lyme borreliosis. Nat. Rev. Dis. Primers 2, 16090.
Szczepanski, A., Furie, M.B., Benach, J.L., Lane, B.P., Fleit, H.B., 1990. Interaction between *Borrelia burgdorferi* and endothelium in vitro. J. Clin. Invest. 85, 1637–1647.
Thomas, D.D., Comstock, L.E., 1989. Interaction of Lyme disease spirochetes with cultured eucaryotic cells. Infect. Immun. 57, 1324–1326.
von Lackum, K., Miller, J.C., Bykowski, T., Riley, S.P., Woodman, M.E., Brade, V., Kraiczy, P., Stevenson, B., Wallich, R., 2005. *Borrelia burgdorferi* regulates expression of complement regulator-acquiring surface protein 1 during the mammal-tick infection cycle. Infect. Immun. 73, 7398–7405.
Wittmann, M., Doble, R., Bachmann, M., Pfeilschifter, J., Werfel, T., Mühl, H., 2012. IL-27 Regulates IL-18 binding protein in skin resident cells. PLoS One 7, e38751.
Wormser, G.P., Brisson, D., Liveris, D., Hanincova, K., Sandigursky, S., Nowakowski, J., Nadelman, R.B., Ludin, S., Schwartz, I., 2008. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. J. Infect. Dis. 198, 1358–1364.