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Contribution of rearranged actin structures to the spread of Ectromelia virus infection *in vitro*

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Summary. – We describe here a contribution of virus-induced actin tails and filopodia in transmission of Ectromelia virus (ECTV) infection in permissive cells detected by the immunofluorescence and confocal microscopy. Immunoblot analysis revealed profoundly decreased β-actin levels during ECTV replicative cycle in the infected cells 24 hrs post infection (p.i.). These results provided a basis for the further analysis of ECTV motion in the infected cells as well as for impact of ECTV infection on the cytoskeletal proteins.

Keywords: Ectromelia virus; cytoskeleton; actin tails

Introduction

The major virulence feature of every intracellular infectious agent (viruses, intracellular bacteria) is the way it moves from one host cell to another, what results in the spread of the infection. These pathogens have evolved a broad spectrum of mechanisms to exploit the host cell, among them a way to manipulate one of the three major cytoskeletal systems – actin microfilaments. Non-related invasive bacteria, e.g. *Listeria* sp., *Shigella* sp., *Rickettsia* sp., *Burkholderia* sp., and *Mycobacterium* sp. share the ability to infect non-phagocytic cells. They independently developed similar actin-based mechanisms named as actin tails or "comet tails" that are essential for inter-cellular propagation of the infection and avoidance of the innate and adaptive immune responses (Tilney and Portnoy, 1989; Cudmore *et al.*, 1996; Cossart and Sansonetti, 2004; Gouin *et al.*, 2005; Hybiske and Stephens, 2008). As obligate intracellular parasites, the viruses also evolved a variety of interactions with host cell actin upon entry, replication and egress. For example, Ebola virus requires the actin filaments for its uptake, and Influenza A virus nucleoprotein have been found to co-localize with the actin in cytoplasm of infected cell (Cudmore *et al.*, 1997; Döhner and Sodeik, 2004; Radtke *et al.*, 2006). Moreover, the microfilaments are used for a viral transcription by Human respiratory syncytial virus or may be incorporated in virions during morphogenesis of Rabies virus and Human immunodeficiency virus 1. Additionally, the latter virus needs actin for the export of unspliced genomic RNAs, budding and transmission to the uninfected cells (Cudmore *et al.*, 1997; Döhner and Sodeik, 2004; Radtke *et al.*, 2006). However, none of the described interactions is as special as Vaccinia virus (VACV) intracellular motion by means of the actin microfilaments (Highley and Way, 1997).

VACV is the best known prototype member of the genus *Orthopoxvirus,* the family *Poxviridae.* It is one of the largest and most complex dsDNA viruses used as a live attenuated vaccine against smallpox caused by Variola virus (Fenner *et al.*, 1989). During VACV replication cycle occurring entirely

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Abbreviations: CEV(s) = cell-associated enveloped virion(s); ECTV = Ectromelia virus; EV = extracellular virion; MV = mature virion; p.i. = post infection; TEM = transmission electron microscopy; VACV = Vaccinia virus; WV = wrapped virion

in the cytoplasm, several different morphological forms are produced. Among them two infectious particles can be distinguished**:** mature virion (MV) constituting the majority of viral progeny and extracellular virion (EV) released outside the cell (Smith *et al.*, 2002; Moss, 2006). Some EVs remain on the cell surface and are called cell-associated enveloped virions (CEVs). CEV plays a role in the spread of virions to the adjacent cells, while EV is responsible for the spread of virions to longer distances *in vitro* and probably also *in vivo* (Cudmore *et al.*, 1996; Smith *et al.*, 2002; Smith *et al.*, 2003).

First reports about VACV abilities to induce a formation of protrusions similar to microvilli tipped with viral particles were reported in 1970s (Stokes, 1976). Following research brought evidence that formation of the structures composed of actin and its cross-linking proteins is associated with the virus presence (Hiller *et al.*, 1979, 1981; Krempien *et al.*, 1981).

Present knowledge indicates that VACV exploits actin filaments during the late stage of its replicative cycle, while spreading directly from cell to cell (Radtke *et al.*, 2006). The fusion of the wrapped virion (WV) and the cell membrane leads to the release of structural viral proteins B5R and A36R that remain on the cell membrane underneath CEV. B5R protein activates Src-family kinases that phosphorylate tyrosine cytoplasmatic domains of A36R protein (Frischknecht *et al.,* 1999; Newsome *et al.*, 2004). This event results in the release of kinesin-1 from A36R protein and in the activation of actin polymerization enabling CEV transmission to the neighboring cells through the formation of actin tails (Smith *et al.*, 2003). It can be independently induced by Src- and Abl-family kinases and the latter induce also release of CEV from the infected cell (Reeves *et al.*, 2005; Radtke *et al.*, 2006).

ECTV, the causative agent of mousepox, is closely related to VACV, but as opposed to VACV it has a very narrow host range (Buller and Palumbo, 1991). Only H-2^d, H-2^a, and H-2^k mice are sensitive to ECTV lethal infection, but not H-2^b mice (Buller *et al.*, 1986). Moreover, ECTV genome analysis showed its location on a separate branch of the phylogenetic tree in relation to other orthopoxviruses (Chen *et al.*, 2003; Gubser *et al.*, 2004). However, the terminal regions of the ECTV genome have higher degree of similarity to VACV than to other poxviruses (Ribas *et al.*, 2003).

The aim of this work was to document the changes induced by ECTV infection in the morphology and actin network of permissive cells of different origin. These findings may enable us to evaluate the involvement of ECTV in actin-based mechanisms for low-range spread.

Materials and Methods

Cell lines. HeLa cells and Vero cells were grown in DMEM (Invitrogen) supplemented with 7% FBS (Invitrogen). BALB/3T3 clone A31 cells were grown in DMEM supplemented with 4 mmol/l L-glutamine and 10% calf serum (ATCC). All cell lines were incubated at 37°C in 4.5% CO_2 humidified atmosphere.

Virus. A highly virulent Moscow strain of ECTV (ECTV-MOS; ATCC 1374) was propagated in Vero cells. Its infectivity was determined by plaque assay on Vero cells (Reeves *et al.*, 2005). Cell infection was carried out in DMEM with 1% of serum at MOI = 1. For mice experiments, 6-week-old BALB/c (H-2^d) mice were foot-pad infected with ECTV-MOS. At 12 days p.i. the spleens were aseptically removed and prepared for transmission electron microscopy (TEM) (Spohr de Faundez *et al.*, 1995). Ultrathin spleen sections were stained with uranyl acetate along with lead citrate and observed under a Jeol 100C TEM microscope (Japan).

Antiserum. Rabbit polyclonal antiserum was raised against complete ECTV-MOS particles. Briefly, after five injections of antigen, the rabbits were bled and the immune serum was obtained. Affinity chromatography purified polyclonal anti ECTV antibody was FITC-conjugated and used in immunofluorescence assays.

Immunofluorescence microscopy. Cell monolayers were grown on coverslips to reach 70–80% confluence and infected with ECTV as described above. At 19 hrs p.i. the infected cells were fixed with 4% paraformaldehyde (Sigma), permeabilized with 0.5% Triton-X and processed according to Mitchison Lab (Harvard Medical School) procedure (http://mitchison.med.harvard.edu/protocols/gen1.html). F-actin was stained with Alexa Fluor-633- (Invitrogen) or TRITC-phalloidin (Sigma). For visualization of DNA, Hoechst 33342 (Calbiochem) was used (1 μg/ml). Images were recorded using Olympus BX60 fluorescence microscope equipped with Color View III cooled CCD camera (100× immersion objective) or with Leica TCS SP2 confocal laser scanning microscope (63× immersion objective, digital zoom 2×). Subsequently, images were processed using the Cell^F software (Olympus) and Adobe Photoshop. The presented results were selected from triplicate experiments.

Immunoblot analysis. Vero cells were infected with ECTV at MOI = 1. At 8 or 24 hrs p.i. the cells were lysed and the proteins were extracted with Mammalian Cell Lysis Kit-1 (Sigma). The protein concentration of each sample was determined using QuantiPro™ BCA Assay Kit (Sigma). An equal amount of protein for each sample was loaded onto 12% polyacrylamide gel. After PAGE, the proteins were electroblotted onto a PVDF Westran Clear Signal (Whatman). To detect β-actin, blots were probed with mouse monoclonal anti-β-actin antibody (Sigma) followed by rabbit anti-mouse immunoglobulin conjugated with peroxidase (Sigma) and Chemiluminescent Peroxidase Substrate-1 (Sigma). The samples were analyzed by KODAK IMAGE STATION 4000MM (Kodak MI SE Program).

Results and Discussion

The cytoskeletal architecture plays a significant role in the spread of virus infection from one permissive cell to another. Moreover, viral cytoplasmic transport and their molecular interactions with cytoskeleton of the infected host are critical for effective innate and adaptive immune

responses. The aim of our study was to gain a better insight into ECTV interactions with the cytoskeleton of permissive cells of different origin: heterologous – human and monkey cells and homologous – cells derived from BALB/c mouse strain highly susceptible to ECTV. Duration of infection (19 hrs) was chosen on the basis of earlier observations of VACV, myxoma virus and ECTV replication cycle (Sodeik *et al.*, 1993; Niemialtowski *et al.*, 1994; Reeves *et al.*, 2005; Duteyrat *et al.*, 2006). At 19 hrs p.i., all viral morphological forms MVs, WVs, EVs, and CEVs should be present and changes in the cytoskeleton should be well-defined.

The mice experiments showed that virus preparation used in our research was able to induce the infectious mousepox in BALB/c (H-2^d) mice that displayed all the classical signs of the disease: hunched posture, rough hair coat, conjunctivitis, dermal pox lesions, swelling of the face and extremities (Fenner, 1981). Additionally, TEM observations of ultrathin sections prepared from infected spleens revealed the presence of viral particles with typical brick-shaped morphology (Fig. 1).

We observed that ECTV like VACV (Radtke *et al.*, 2006) induced alterations in the microfilament organization of the infected cells. These changes were best characterized by an abnormal morphology, e.g. shrunken, rounded and clumped appearance (Figs. 2, 4, 5) or by a formation of syncytia (Fig. 3). There was also a noticeable loss of stress fibers and increase in actin content of the cell cortex (Figs. 2, 3, 5) as compared to the neighboring non-infected cells. This may be the result of a shift of actin to the cell periphery, because additional actin is needed for the generation of protrusions. The infected cells produced either individual, long and thick projections similar to filopodia (Figs. 3, 4) or several kinds of small actin-rich microvilli resulting in the stelliform appearance of the cell (Fig. 2). Among them were included the specialized microvilli closely resembling VACV-associated actin tails (Cudmore *et al.*, 1996; Smith *et al.*, 2003; Radtke *et al.*, 2006). These structures are being formed in different cell lines. In our experiments we observed them in human HeLa cells (Fig. 2) and murine BALB/3T3 cells (Fig. 5). In the second case, the tails were well-defined, but not very abundant as compared to the numerous tails described for VACV (Zhang *et al.*, 2000). It appeared that they contributed to the cell-to-cell spread (Fig. 5). This mechanism of viral transmission may seem ineffective, when compared to the cell lysis that is believed to be the main mechanism of virus spread. However, the virus spread mechanism could be different *in vivo* as proven for neurotropic herpesviruses. The virus release by cell lysis was not required for their dissemination and pathology (Heffner *et al.*, 1993; Mulder *et al.*, 1996). Our study revealed for the first time the importance of actin polymerization in the spread of ECTV from cell to cell *in vitro*, what suggested that this type of infectious particles motion could be typical for orthopoxviruses. Among members of other virus families similar actin-rich microvilli have been observed on the surfaces of cells infected with Murine mammary tumor virus and Frog virus 3 (Cudmore *et al.*, 1997; Smith and Enquist, 2002). It is not known yet, if these protrusions also take part in the dynamic spread of these viruses, but they clearly play an important role in the budding of these viruses.

We noticed more prominent structures than actin tails in HeLa and Vero cell lines (Figs. 3, 4) containing numerous

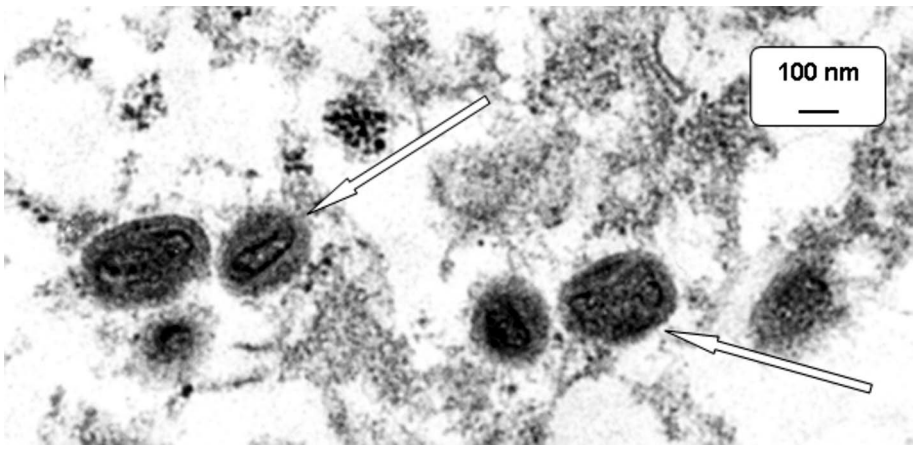

Fig. 1

ECTV particles (arrows) in the spleen of BALB/c (H-2^d) mice

TEM, day 12 p.i.

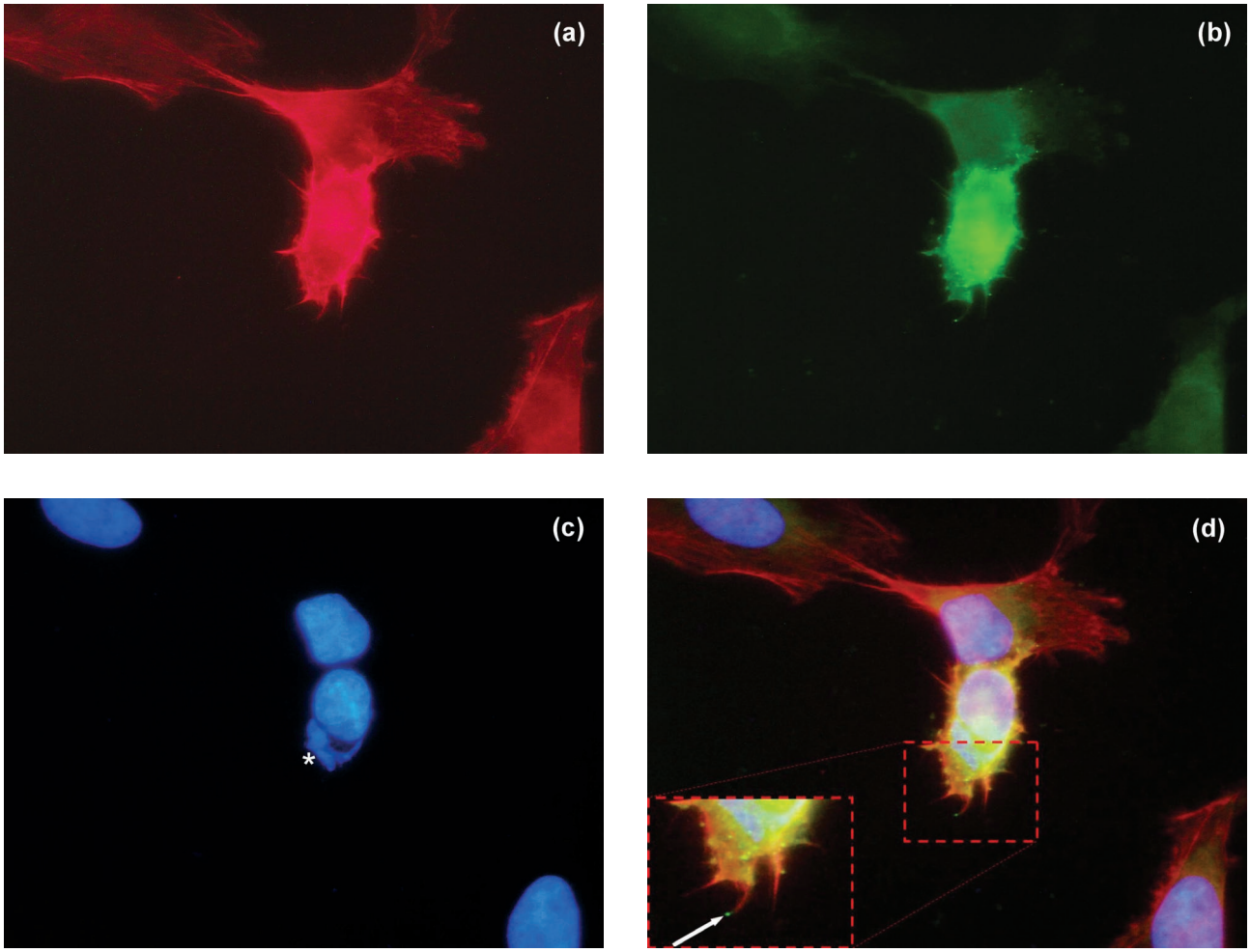

Fig. 2

Immunofluorescence of ECTV-infected HeLa cells

Staining for actin (a), ECTV (b), and cellular nuclei and viral DNA – extranuclear replication centers are indicated by an asterisk (c). Merged images – red box is shown in expanded scale. White arrow indicates ECTV virion on the tip of actin tail (d).

virions in contrast to a tail that propelled only one virus particle to its tip. These structures resembled projections that have been described as filopodia for VACV, strain MVA or as "cytoplasmic corridors" for myxoma virus (Gallego-Gomez *et al.*, 2003; Duteyrat *et al.*, 2006). Similar actin-containing projections have been also found to be induced by several herpesviruses (Favoreel *et al.*, 2007). Above-mentioned structures seemed to contribute to the direct spread of virions into adjacent cells without being exposed to the external microenvironment. This mechanism along with macropinocytosis and apoptotic mimicry that VACV uses to enter different cell types (Mercer and Helenius, 2008), could be another example of the poxviruses strategy for an effective escape from cellular and molecular components of the immune system.

To investigate whether all the abovementioned prominent changes in the actin cytoskeleton affected the total level of β-actin in cells during ECTV replicative cycle, cell lysates of mock-infected and ECTV-infected (8 and 24 hrs p.i.) Vero cells were prepared and used in immunoblot analysis. Actin-specific bands of 43 K were detectable during the entire observation period for both infected and mock-infected cells (Fig. 6a) The amount of actin in infected cells was heavily reduced at 24 hrs p.i., what was quantified by densitometry (Fig. 6b) showing an over 5-fold decrease compared to the mock-infected cells. This could indicate degradation of actin after being heavily rearranged by the virus or general degradation of the cellular proteins due to the late stage of infection. This finding is quite unusual as actin is considered to be a housekeeping gene and protein and is widely used as

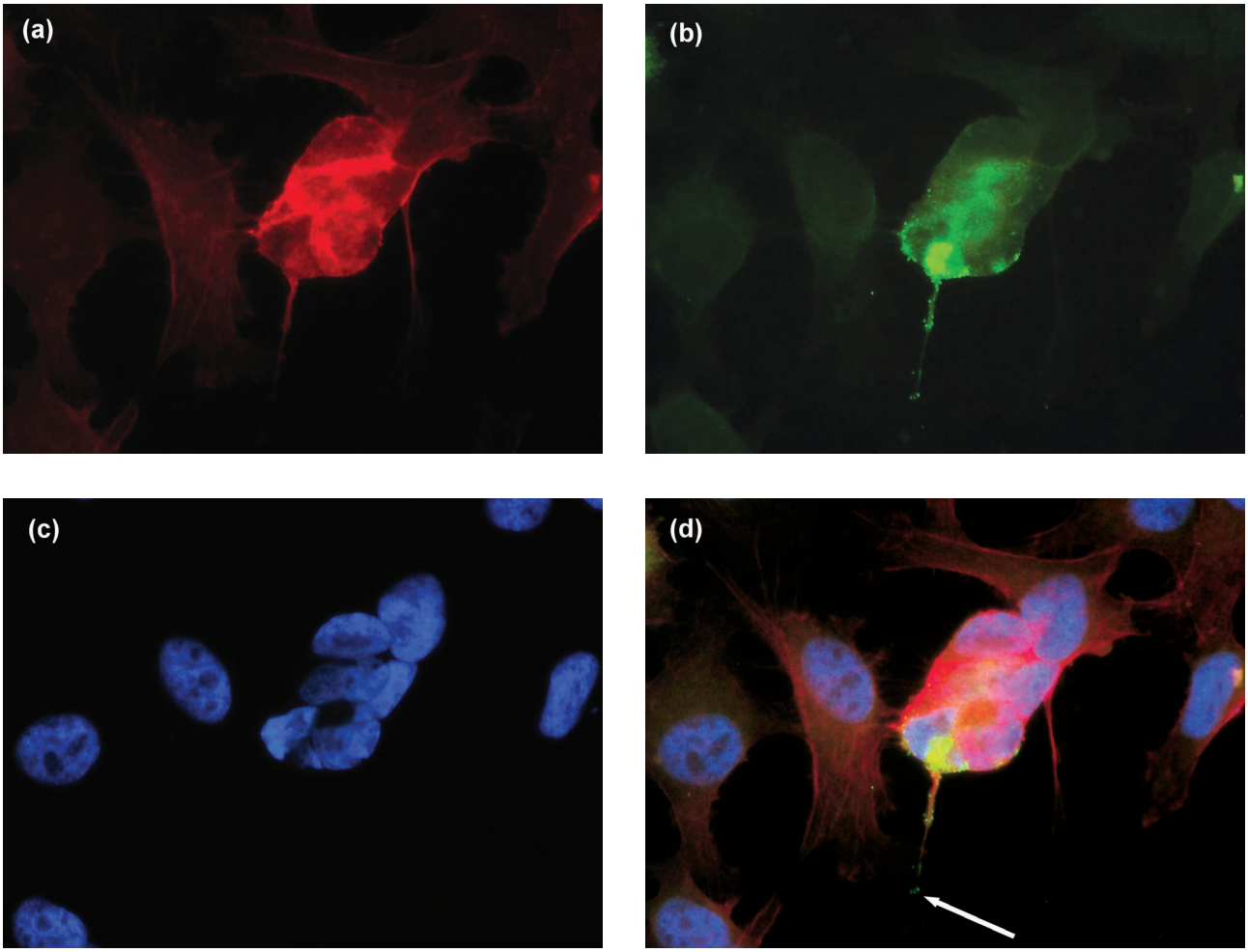

Fig. 3

Immunofluorescence of ECTV-infected HeLa cells

Staining for actin (a), ECTV (b), and cellular nuclei and viral DNA (c). Merged image – white arrow indicates actin-rich projection containing several virions (d).

an immunoblot loading control (Guerra *et al.*, 2003; Kuhn *et al.*, 2005). However, further investigation is needed, but these results suggested reconsideration of actin as a reference protein/gene for the experiments with ECTV.

In conclusion, we showed that the actin network of cells contributed to the plasticity of cells in non-infected or infected state. Our experiments demonstrated for the first time that ECTV infection induced actin rearrangements and production of dynamic protrusions in the cell lines of

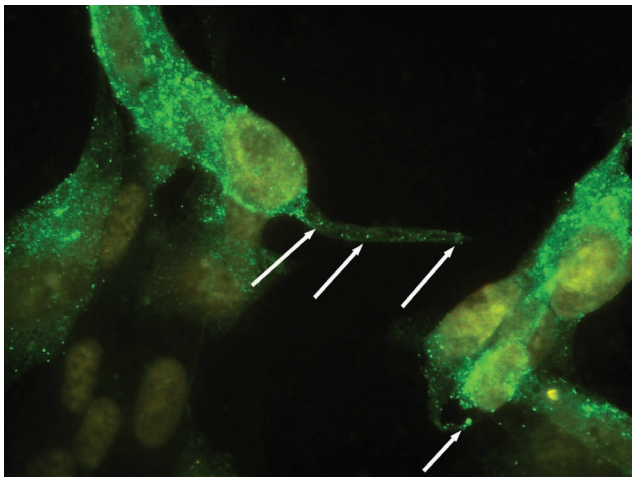

Fig. 4

Immunofluorescence of ECTV-infected Vero cells

Staining for ECTV, white arrows indicate filopodia containing several ECTV virions.

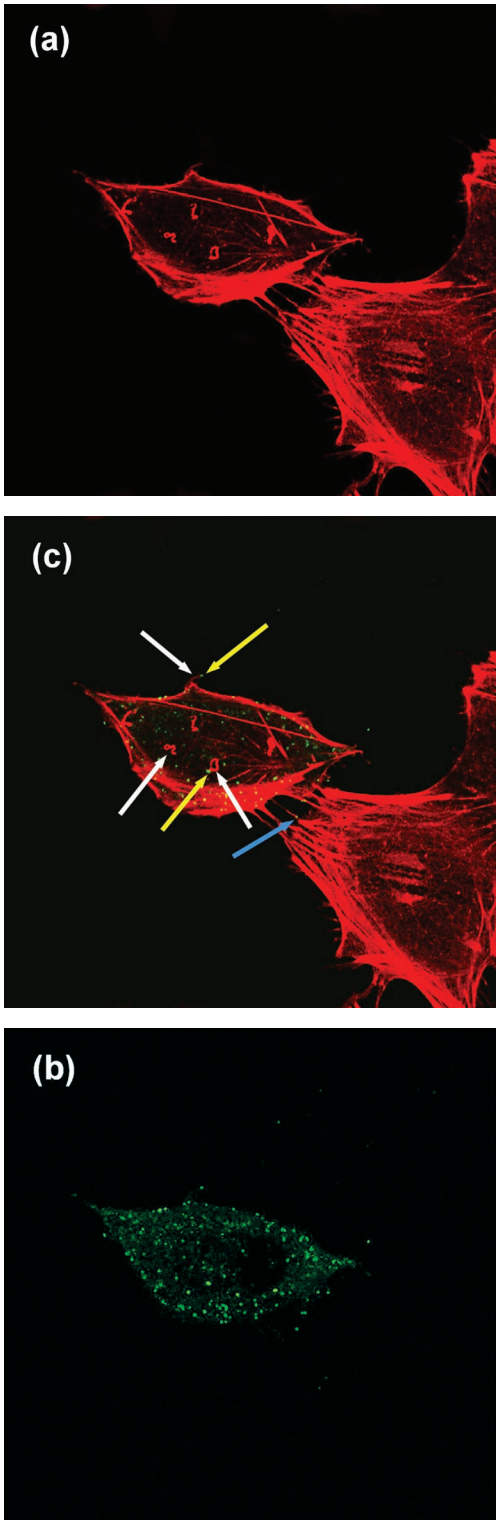

Fig. 5

Confocal microscopy of ECTV-infected BALB/3T3 cells

Staining for actin (a) and ECTV (b). Merged images – actin tails on cell surface (white arrows), virions (yellow arrows) and a viral particle delivered *via* actin tail to neighboring cell (blue arrow) (c).

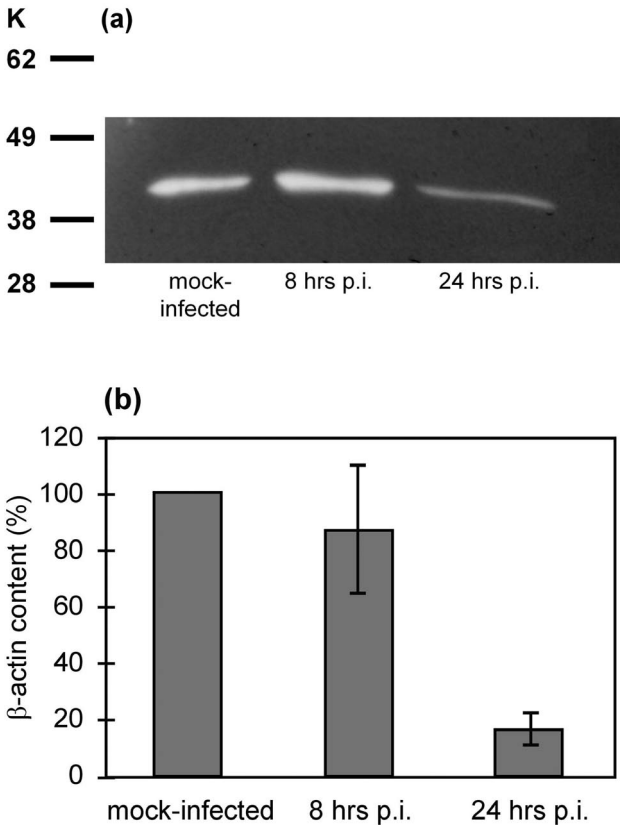

Fig. 6

Effect of ECTV infection on β-actin content in Vero cells

Immunoblot analysis (a) and its quantitative evaluation (b). The positions and size of marker proteins are indicated on the left (a). The bars indicate standard deviation (b).

different origin, but the effect was cell-line dependent, e.g. virus-containing filopodia in Vero in contrast to the actin tails in BALB/3T3 cells. These results suggest that the kind of developed protrusion is connected with some unique host factors that remain to be identified. We hope that the results presented here will set a base for future experimental studies exploring not only the pathologic manifestation of ECTV infection, but also the regulation of in-cell signaling.

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References

Buller RM, Palumbo GJ (1991): Poxvirus pathogenesis. Microbiol. Rev. 55, 80–122.

Buller RM, Potter M, Wallace GD (1986): Variable resistance to ectromelia (mousepox) virus among genera of Mus. Curr. Top. Microbiol. Immunol. 127, 319–322.

Chen N, Danila MI, Feng Z, Buller RML, Wang C, Han X, Lefkowitz EJ, Upton C (2003): The genomic sequence of ectromelia virus, the causative agent of mousepox. Virology 317, 165–186. doi:10.1016/S0042-6822(03)00520-8

Cossart P, Sansonetti PJ (2004): Bacterial invasion: the paradigms of enteroinvasive pathogens. Science 304, 242–248. doi:10.1126/science.1090124

Cudmore S, Reckmann I, Griffiths G, Way M (1996): Vaccinia virus: a model system for actin-membrane interactions. J. Cell Sci. 109, 1739-l747.

Cudmore S, Reckmann I, Way M (1997): Viral manipulations of the actin cytoskeleton. Trends Microbiol. 5, 142–148. doi:10.1016/S0966-842X(97)01011-1

Döhner K, Sodeik B (2004): The Role of the Cytoskeleton During Viral Infection. CTMI 285, 67–108.

Duteyrat JL, Gelfi J, Bertagnoli S (2006): Ultrastructural study of myxoma virus morphogenesis. Arch. Virol. 151, 2161–2180. doi:10.1007/s00705-006-0791-2

Favoreel HW, Enquist LW, Feierbach B (2007): Actin and Rho GTPases in herpesvirus biology. Trends Microbiol. 9, 426–433. doi:10.1016/j.tim.2007.08.003

Fenner F (1981): Mousepox (infectious ectromelia): past, present, and future. Lab. Animal Sci. 31, 553–559.

Fenner F, Wittek R, Dumbell KR (1989): The Orthopoxviruses. Academic Press, San Diego, pp. 10–13.

Frischknecht F, Moreau V, Röttger S, Gonfloni S, Reckmann I, Superti-Furga G, Way M (1999): Actin based motility of vaccinia mimics receptor tyrosine kinase signalling. Nature 401, 926–929. doi:10.1038/44860

Gallego-Gomez JC, Risco C, Rodriguez D, Cabezas P, Guerra S, Carrascosa JL, Esteban M (2003): Differences in virus-induced cell morphology and in virus maturation between MVA and other strains (WR, Ankara, and NYCBH) of vaccinia virus in infected human cells. J. Virol. 77, 10606–10622. doi:10.1128/JVI.77.19.10606-10622.2003

Gouin E, Welch MD, Cossart P (2005): Actin-based motility of intracellular pathogens. Curr. Opin. Microbiol. 8, 35–45. doi:10.1016/j.mib.2004.12.013

Gubser C, Hué S, Kellam P, Smith GL (2004): Poxvirus genomes: a phylogenetic analysis. J. Gen. Virol. 85, 105–117. doi:10.1099/vir.0.19565-0

Guerra S, Lopez-Fernandez LA, Pascual-Montano A, Munoz M, Harshman K, Esteban, M (2003): Cellular gene expression survey of Vaccinia virus infection of human HeLa cells. J. Virol. 77, 6493–6506. doi:10.1128/JVI.77.11.6493-6506.2003

Heffner S, Kovacs F, Klupp BG, Mettenleiter TC (1993): Glycoprotein gp50-negative pseudorabies virus: a novel approach toward a nonspreading live herpesvirus vaccine. J. Virol. 67, 1529–1537.

Higley S, Way M (1997): Actin and cell pathogenesis. Curr. Opin. Cell. Biol. 9, 62–69. doi:10.1016/S0955-0674(97)80153-6

Hiller G, Jungwirth C, Weber K (1981): Fluorescence microscopical analysis of the life cycle of vaccinia virus in the chick embryo fibroblasts. Exp. Cell. Res. 132, 81–87. doi:10.1016/0014-4827(81)90085-9

Hiller G, Weber K, Schneider L, Parajsz C, Jungwirth C (1979): Interaction of assembled progeny pox viruses with the cellular cytoskeleton. Virology 98, 142–153. doi:10.1016/0042-6822(79)90533-6

Hybiske K, Stephens R (2008): Exit strategies of intracellular pathogens. Nat. Rev. Microbiol. 6, 99–110. doi:10.1038/nrmicro1821

Krempien U, Schneider L, Hiller G, Weber K, Katz E, Jungwirth C (1981): Conditions for pox virus-specific microvilli formation studied during synchronized virus assembly. Virology 113, 556–564. doi:10.1016/0042-6822(81)90183-5

Kuhn M, Desloges N, Rahaus M, Wolff MH (2005): Varicella-Zoster Virus Infection Influences Expression and Organization of Actin and alpha-Tubulin but Does Not Affect Lamin A and Vimentin. Intervirology 48, 312–320. doi:10.1159/000085100

Mercer J, Helenius A (2008): Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. Science 320, 531–535. doi:10.1126/science.1155164

Moss B (2006): Poxvirus entry and membrane fusion. Virology 344, 48–54. doi:10.1016/j.virol.2005.09.037

Mulder W, Pol J, Kimman T, Kok G, Priem J, Peeters B (1996): Glycoprotein D-negative pseudorabies virus can spread transneuronally via direct neuron-to-neuron transmission in its natural host, the pig, but not after additional inactivation of gE and gI. J. Virol. 70, 2191–2200.

Newsome TP, Scaplehorn N, Way M (2004): Src mediates a switch from microtubule- to actin-based motility of vaccinia virus. Science 306, 124–129. doi:10.1126/science.1101509

Niemialtowski MG, Spohr de Faundez I, Gierynska M, Malicka E, Toka FN, Schollenberger A, Popis A (1994): The inflammatory and immune response to mousepox (infectious ectromelia) virus. Acta Virol. 38, 299–307.

Radtke K, Döhner K, Sodeik B (2006): Viral interactions with the cytoskeleton: a hitchhiker's guide to the cell. Cell. Microbiol. 8, 387–400. doi:10.1111/j.1462-5822.2005.00679.x

Reeves PM, Bommarius B, Lebeis S, McNulty S, Christensen J, Swimm A, Chahroudi A, Chavan R, Feinberg MB, Veach D, Bornmann W, Sherman M, Kalman D (2005): Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. Nat. Med. 11, 731–739. doi:10.1038/nm1265

Ribas G, Rivera J, Saraiva M, Campbell RD, Alcami, A (2003): Genetic variability of immunomodulatory genes in ectromelia virus isolates detected by denaturing high-performance liquid chromatography. J. Virol. 77, 10139–10146. doi:10.1128/JVI.77.18.10139-10146.2003

Smith GA, Enquist LW (2002): Break ins and break outs: viral interactions with the cytoskeleton of mammalian cells. Annu. Rev. Cell. Dev. Biol. 18, 135–161. doi:10.1146/annurev.cellbio.18.012502.105920

Smith GL, Murphy BJ, Law M (2003): Vaccinia virus motility. Annu. Rev. Microbiol. 57, 323–342. doi:10.1146/annurev.micro.57.030502.091037

Smith GL, Vanderplasschen A, Law M (2002): The formation and function of extracellular enveloped vaccinia virus. J. Gen. Virol. 83, 2915–2931.

Sodeik B, Doms RW, Ericsson M, Hiller G, Machamer CE, van' t Hof W, Van Meer G, Moss B, Griffiths G (1993): Assembly of vaccinia virus: Role of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks. J. Cell. Biol. 121, 521–541. doi:10.1083/jcb.121.3.521

Spohr de Faundez I, Gierynska M, Niemialtowski MG, Malicka E, Popis A (1995): Ectromelia virus establishes a persistent infection in spleen dendritic cells and macrophages of BALB/c mice following the acute disease. Adv. Exp. Med. Biol. 378, 257–261.

Stokes GV (1976): High-voltage electron microscope study of the release of vaccinia virus from whole cells. J. Virol. 18, 636–643.

Tilney LG, Portnoy DA (1989): Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, Listeria monocytogenes. J. Cell. Biol. 109, 1597–1608. doi:10.1083/jcb.109.4.1597

Zhang WH, Wilcock D, Smith GL (2000): Vaccinia Virus F12L Protein Is Required for Actin Tail Formation, Normal Plaque Size, and Virulence. J. Virol. 74, 11654–11662. doi:10.1128/JVI.74.24.11654-11662.2000