Virology 456-457 (2014) 39–42

Contents lists available at ScienceDirect

Virology

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

Brief Communication

Ultrastructural fingerprints of avian influenza A (H7N9) virus in infected human lung cells

Olivier Terrier [a,*], Coralie Carron [a], Gaelle Cartet [a], Aurélien Traversier [a], Thomas Julien [a], Martine Valette [b], Bruno Lina [a,b], Vincent Moules [a,c], Manuel Rosa-Calatrava [a,c]

[a] Virologie et Pathologie Humaine VirPath, EA 4610, Université Claude Bernard Lyon 1-Hospices Civils de Lyon, Lyon, France
[b] Centre national de référence des virus influenza (région Sud), Laboratoire de Virologie Est, Centre de Biologie et de Pathologie Est, Hospices Civils de Lyon, Bron, France
[c] VirNext, Faculté de Médecine RTH Laennec, Université Lyon 1, Lyon, France

ARTICLE INFO

Article history:
Received 23 February 2014
Returned to author for revisions
11 March 2014
Accepted 13 March 2014
Available online 28 March 2014

Keywords:
Viruses
Orthomyxoviridae
Influenza virus
H7N9
Host cellular ultrastructure
Infection fingerprints
Matrix protein
M1

ABSTRACT

In this study, we investigated the ultrastructural modifications induced by influenza A (H7N9) virus in human lung epithelial cells. One particular characteristic of H7N9 viral infection is the formation of numerous M1-associated striated tubular structures within the nucleus and the cytoplasm, which have only previously been observed for a limited number of influenza A viruses, notably the 2009 pandemic (H1N1) virus.

Introduction

In March 2013, a novel avian influenza A (H7N9) virus that infected humans was identified in China (H.-N. Gao et al., 2013; R. Gao et al., 2013). Infection of poultry with influenza A subtype H7 viruses occurs worldwide, but the introduction of this subtype into the human population, and the resulting fatal cases, has not been observed previously (Belser et al., 2013). The cases occurred in an initial wave ($n=133$) from February to May 2013, and since October 2013 a second wave of human cases has been occurring. As of 28 January 2014, the case fatality rate of all confirmed cases is 22%, but many cases are still hospitalized (WHO report, 28 January 2014). Over a very short period of time, intensive surveillance and research efforts have provided a first overview of this new influenza outbreak, notably in terms of clinical findings, epidemiology, pathogenicity and possible antiviral-resistance markers (Belser et al., 2013; Gao et al., 2013a, 2013b; Richard et al., 2013; Mok et al., 2013). The genotype of these H7N9 influenza viruses isolated from humans may have originated in China by reassortment of H9N2 viruses with duck viruses carrying H7 and N9 genes (D. Liu et al., 2013; Q. Liu et al., 2013; Van Ranst and Lemey, 2013). While there is a putative risk of H7N9 spread from person to person, with acquisition of several markers of adaptation to non-avian hosts or virulence in PB2, PB1-F2, M1 and NS1 viral proteins (D. Liu et al., 2013; Q. Liu et al., 2013), only a few studies have yet started to investigate the cellular biology of the H7N9 virus, to identify underlying mechanisms of adaptation.

Recently, we have revisited electron microscopy (EM) studies in infected cells (Anisimova et al., 1980; Ciampor, 1972; Terrier et al., 2012) and we have shown that influenza A viruses induce a major remodeling of the host cell ultrastructure and the formation of diverse viral structures depending on the subtype/strain and the genomic composition of the viruses (Terrier et al., 2012). Our data suggest that each influenza A virus strain could be associated with a specific cellular fingerprint, which possibly correlates with the functional properties of its viral components (Terrier et al., 2012).

In this study, we examined the ultrastructural modifications induced by influenza A (H7N9) virus in cultured human lung epithelial cells. With the help of immunogold-labeling EM and confocal microscopy (CM), we identified an abundant accumulation of M1-associated

* Correspondence to: Laboratoire de Virologie et Pathologie Humaine EA 4610, Faculté de Médecine RTH Laennec, Université Lyon 1, 11 Rue Guillaume Paradin, 69372 Lyon, France.
E-mail addresses: olivier.terrier@univ-lyon1.fr (O. Terrier), manuel.rosa-calatrava@univ-lyon1.fr (M. Rosa-Calatrava).

http://dx.doi.org/10.1016/j.virol.2014.03.013
0042-6822/

striated tubular structures in the nucleus and the cytoplasm, that have only been observed with a limited number of influenza strains, notably the 2009 pandemic (H1N1) virus (Goldsmith et al., 2011; Terrier et al., 2012).

Results and discussion

Human lung epithelial A549 cells were mock-infected or infected with influenza virus strain A/Anhui/1/2013 (H7N9) at a multiplicity of infection of 1 and incubated for 24 h. Cells were then fixed and embedded for standard EM or anti-M1 immunogold labeling EM, in Epon or Lowicryl resin, respectively, as previously described (Terrier et al., 2012).

EM of the ultrathin sections revealed numerous zones of viral budding, with the accumulation of electron dense material near to the plasma membrane (Fig. 1A and B). The morphology of the released viral particles was heterogeneous with spheroidal and filamentous shapes. The average size and density of the surface glycoprotein spikes (data not shown) were in agreement with those previously measured for several other influenza A viruses (Moulès et al., 2011).

We then investigated the host cell ultrastructure modifications induced by H7N9 infection, and compared with mock-infected cells (Fig. 1D versus 1C). One of the most noticeable observations for the H7N9 infected cells was the extensive remodeling of the nucleolar compartment, which we have previously reported as a common feature for influenza A viruses (Terrier et al., 2012).

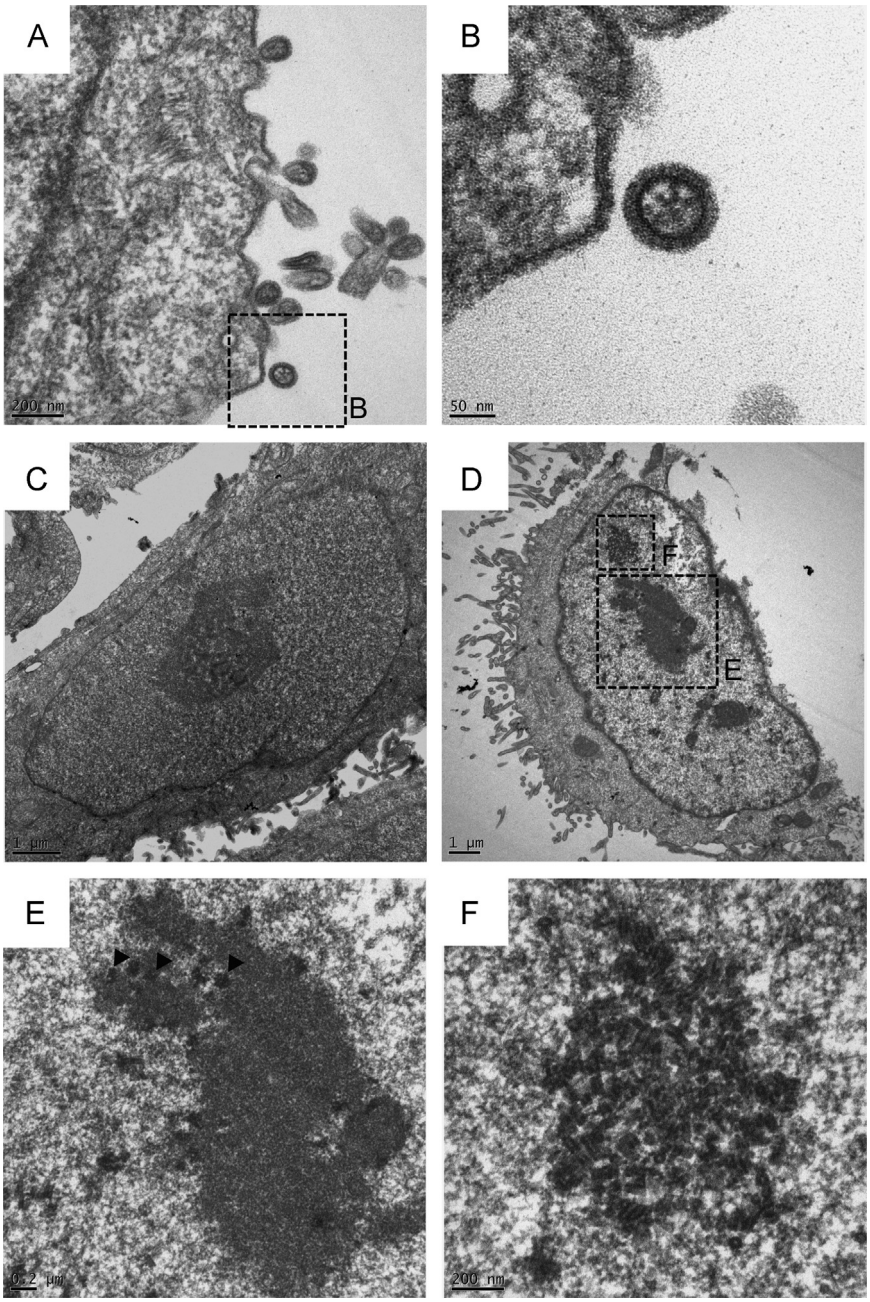

Fig. 1. Ultrathin EM section of A549 human lung cells infected with avian influenza A (H7N9) virus. (A) Pleomorphic viral particles visible at budding regions. (B) Glycoprotein spikes and viral genome segments. (C) Non-infected cell. (D) Influenza A H7N9 infected cell. The disruption of nucleolar compartments and the formation of virally induced structures are clearly visible. (E) Detailed view of H7N9-induced disruption of the nucleolar compartments. Numerous electron dense round-shaped bodies were observed inside or in the proximity of the nucleoli (black arrowheads). (F) Detailed view of virally-induced striated structures in the nucleus.

Indeed, the regular components of the nucleoli, such as the fibrillar centers (FCs) and dense fibrillar components (DFC), were easily distinguishable in non-infected cells (Fig. 1C), and had totally disappeared at 24 h post-infection (Fig. 1D). Concomitantly, numerous electron dense round-shaped bodies were observed inside or in the proximity of the nucleoli (Fig. 1D and E, black arrows). Another striking observation was the strong accumulation of striated tubular structures within the nucleoplasm (Fig. 1D and F). To further investigate these remarkable rod-like structures, we made use of favorable ultrathin sections (generally above the nucleolus plane), where they accumulate in large nuclear areas, as illustrated in Fig. 2A. The lengths of these structures were heterogeneous (100–300 nm) with an average diameter of 43.5+/−6.0 nm (mean+/−SD, $n=20$). In some cross sections, it was possible to measure an inter-striae distance of 8.0+/−0.6 nm (mean+/−SD, $n=10$) (Fig. 2A, panel b and B). These structures were mainly located in the nucleoplasm, but also within the cytoplasm at 24 h post-infection (hpi) (Fig. 2D and E). The characteristics of these H7N9 structures were very similar to other striated tubular structures previously described in only a limited number of other influenza viruses, such as contemporary H3N2 human lineages, the pandemic and WSN H1N1 strains and A/chicken/Netherland/2003 A (H7N7) virus (Terrier et al., 2012; Ciampor, 1972; Anisimova et al., 1980; Goldsmith et al., 2011). Of note, the wide abundance of striated tubular structures within the nucleus of H7N9 infected cells is a specific signature only shared with the 2009 pandemic H1N1 virus (Terrier et al., 2012).

For H3N2 viruses, we have shown that these structures are associated with the viral matrix protein (M1) (6). To further characterize those induced by H7N9, we performed immunogold-labeling EM using a goat polyclonal primary antibody raised against M1 (PA1-85626, Thermo-scientific) and a donkey anti-goat

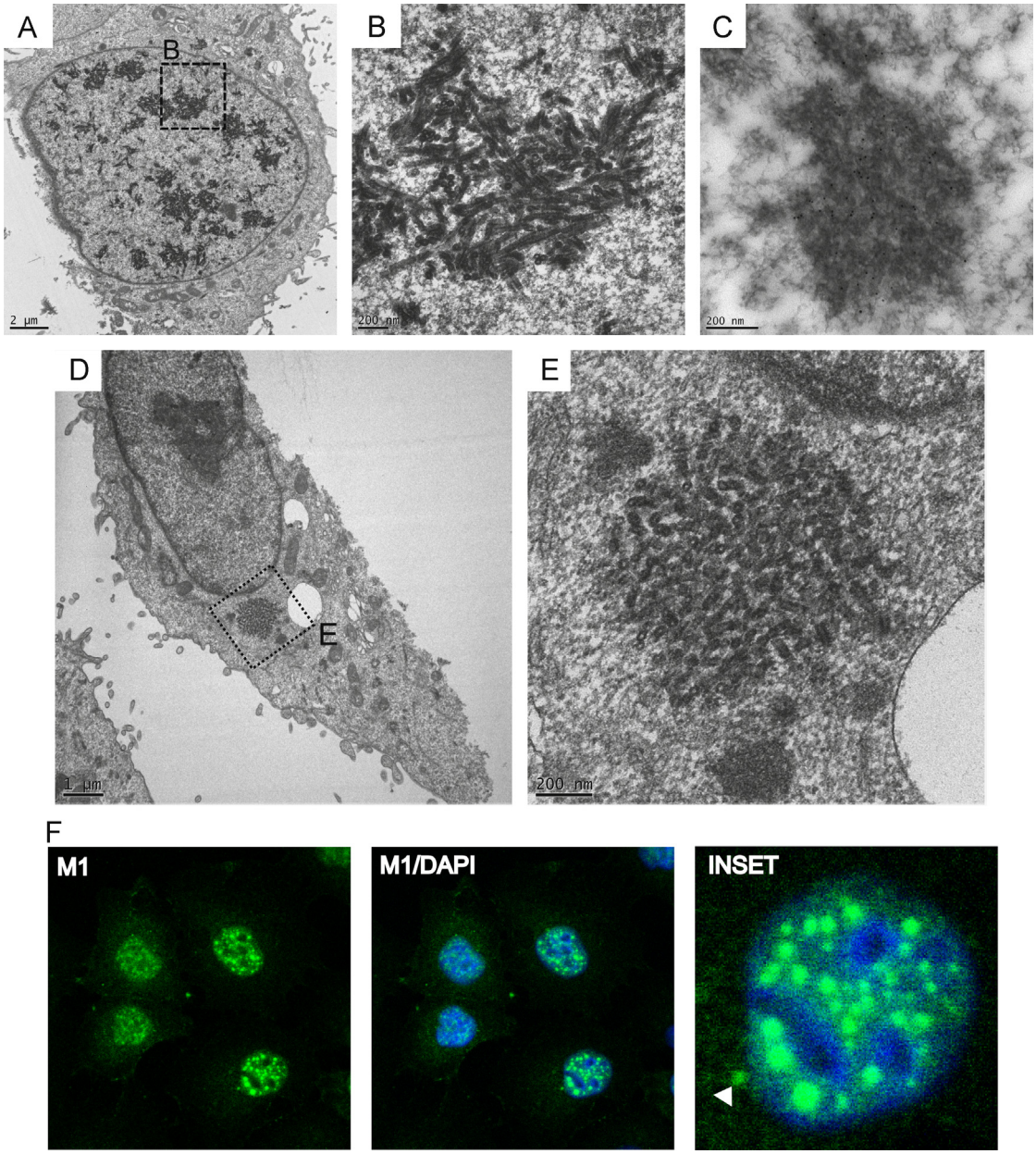

Fig. 2. H7N9 M1-associated striated tubular structures in the nucleus and cytoplasm observed using EM and CM. (A) Numerous striated tubular structures were quite visible using ultrathin sections. (B) Detailed view of virally-induced striated tubular structures. (C) Immunogold labeling EM indicating that M1 is associated with the striated tubular structures. (D and E) Presence of striated tubular structures visible in the cytoplasm of ultrathin sections. (F) EM results correlated with the subcellular localization of M1 observed by CM, with the formation of large M1 spots in the nucleoplasm and cytoplasm, to a lesser extent (white arrowhead).

secondary antibody conjugated to gold particles (10 nm diameter, BB international). At 24 hpi, the gold particles were almost exclusively associated with the areas of accumulation of striated tubular structures (Fig. 2C). This result also correlated with the subcellular localization of M1 observed by CM, with the formation of large M1-labeled areas within the nucleoplasm (Fig. 2F) and cytoplasm to a lesser extent (Fig. 2F). These observations confirm that M1 protein is associated with the striated tubular structures induced during H7N9 infection.

Protein sequence alignments have shown that the M1 from influenza H7N9 and other viruses able to induce striated tubular structures (Terrier et al., 2012) do not harbor any specific amino-acid changes, with respect to M1 from viruses unable to induce these structures (data not shown). The apparent ordered structuring of these M1-associated structures and their abundance suggest a putative function in influenza-host interactions and interplay with key host cell factors. M1 is involved in the formation of viral particles late in infection, as a matrix protein on the inner side of the cellular membrane. During the early stages of infection, M1 is also involved in the trafficking of viral ribonucleoproteins (Bui et al., 2000; Ma et al., 2001). The nuclear localization of the striated tubular structures may be directly or indirectly connected to M1-related functions. Future studies are required to further characterize the kinetic of formation, composition and biological significance of these M1-associated structures.

Conclusions

In conclusion, we examined the ultrastructural modifications induced by influenza A H7N9 virus in cultured human lung epithelial cells and identified abundant M1-associated striated tubular structures within the nucleus and the cytoplasm, a cellular signature shared with the 2009 pandemic (H1N1) virus. The role of these structures in viral replication and pathogenesis will need to be further investigated, notably by attempting to correlate these virally induced structures with biological functions. This short study highlights the importance of EM and CM techniques in the understanding of host-pathogen interactions, notably in the case of emerging and re-emerging pathogens.

Funding

This work was supported by University Claude Bernard of Lyon and the Civil Hospitals of Lyon. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

The authors would like to thank Rongbao Gao, from the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, China, for the A/Anhui/1/2013 (H7N9) strain used in this study.

References

Anisimova, E., Ghendon, Y., Markushin, S., 1980. Ultrastructural changes in cells induced by temperature-sensitive mutants of fowl plague virus at permissive and non-permissive temperature. J. Gen. Virol. 47, 11–18.

Belser, J.A., Gustin, K.M., Pearce, M.B., Maines, T.R., Zeng, H., Pappas, C., Sun, X., Carney, P.J., Villanueva, J.M., Stevens, J., Katz, J.M., Tumpey, T.M., 2013. Pathogenesis and transmission of avian influenza A (H7N9) virus in ferrets and mice. Nature 501, 556–559.

Bui, M., Wills, E.G., Helenius, A., Whittaker, G.R., 2000. Role of the influenza virus M1 protein in nuclear export of viral ribonucleoproteins. J. Virol. 74, 1781–1786.

Ciampor, F., 1972. Electron microscopy of tissue culture cells infected with myxoviruses. I. Nucleo-cytoplasmic changes in A0-WSN influenza virus-infected chick embryo cells. Acta Virol. 16, 9–16.

Gao, H.-N., Lu, H.-Z., Cao, B., Du, B., Shang, H., Gan, J.-H., Lu, S.-H., Yang, Y.-D., Fang, Q., Shen, Y.-Z., Xi, X.-M., Gu, Q., Zhou, X.-M., Qu, H.-P., Yan, Z., Li, F.-M., Zhao, W., Gao, Z.-C., Wang, G.-F., Ruan, L.-X., Wang, W.-H., Ye, J., Cao, H.-F., Li, X.-W., Zhang, W.-H., Fang, X.-C., He, J., Liang, W.-F., Xie, J., Zeng, M., Wu, X.-Z., Li, J., Xia, Q., Jin, Z.-C., Chen, Q., Tang, C., Zhang, Z.-Y., Hou, B.-M., Feng, Z.-X., Sheng, J.-F., Zhong, N.-S., Li, L.-J., 2013. Clinical findings in 111 cases of Influenza A (H7N9) virus infection. N. Engl. J. Med. 368, 22772285.

Gao, R., Cao, B., Hu, Y., Feng, Z., Wang, D., Hu, W., Chen, J., Jie, Z., Qiu, H., Xu, K., Xu, X., Lu, H., Zhu, W., Gao, Z., Xiang, N., Shen, Y., He, Z., Gu, Y., Zhang, Z., Yang, Y., Zhao, X., Zhou, L., Li, X., Zou, S., Zhang, Y., Li, X., Yang, L., Guo, J., Dong, J., Li, Q., Dong, L., Zhu, Y., Bai, T., Wang, S., Hao, P., Yang, W., Zhang, Y., Han, J., Yu, H., Li, D., Gao, G.F., Wu, G., Wang, Y., Yuan, Z., Shu, Y., 2013. Human infection with a novel avian-origin influenza A (H7N9) virus. N. Engl. J. Med. 368, 1888–1897.

Goldsmith, C.S., Metcalfe, M.G., Rollin, D.C., Shieh, W.-J., Paddock, C.D., Xu, X., Zaki, S.R., 2011. Ultrastructural characterization of pandemic (H1N1) 2009 virus. Emerg. Infect. Dis. 17, 2056–2059.

Liu, D., Shi, W., Shi, Y., Wang, D., Xiao, H., Li, W., Bi, Y., Wu, Y., Li, X., Yan, J., Liu, W., Zhao, G., Yang, W., Wang, Y., Ma, J., Shu, Y., Lei, F., Gao, G.F., 2013. Origin and diversity of novel avian influenza A H7N9 viruses causing human infection: phylogenetic, structural, and coalescent analyses. Lancet 381, 1926–1932.

Liu, Q., Lu, L., Sun, Z., Chen, G.-W., Wen, Y., Jiang, S., 2013. Genomic signature and protein sequence analysis of a novel influenza A (H7N9) virus that causes an outbreak in humans in China. Microbes Infect. Inst. Pasteur 15, 432–439.

Ma, K., Roy, A.M., Whittaker, G.R., 2001. Nuclear export of influenza virus ribonucleoproteins: identification of an export intermediate at the nuclear periphery. Virology 282, 215–220.

Mok, C.-K., Chang, S.-C., Chen, G.-W., Lo, Y.-L., Chen, S.-J., Wu, H.-S., Liu, M.-T., Chang, F.-Y., Lin, T.-Y., Shih, S.-R., 2013. Pyrosequencing reveals an oseltamivir-resistant marker in the quasispecies of avian influenza A (H7N9) virus. J. Microbiol. Immunol. Infect. 13, 201–206.

Moulès, V., Terrier, O., Yver, M., Riteau, B., Moriscot, C., Ferraris, O., Julien, T., Giudice, E., Rolland, J.-P., Erny, A., Bouscambert-Duchamp, M., Frobert, E., Rosa-Calatrava, M., Pu Lin, Y., Hay, A., Thomas, D., Schoehn, G., Lina, B., 2011. Importance of viral genomic composition in modulating glycoprotein content on the surface of influenza virus particles. Virology 414, 51–62.

Richard, M., Schrauwen, E.J.A., de Graaf, M., Bestebroer, T.M., Spronken, M.I.J., van Boheemen, S., de Meulder, D., Lexmond, P., Linster, M., Herfst, S., Smith, D.J., van den Brand, J.M., Burke, D.F., Kuiken, T., Rimmelzwaan, G.F., Osterhaus, A.D.M.E., Fouchier, R.A.M., 2013. Limited airborne transmission of H7N9 influenza A virus between ferrets. Nature 501, 560–563.

Terrier, O., Moules, V., Carron, C., Cartet, G., Frobert, E., Yver, M., Traversier, A., Wolff, T., Riteau, B., Naffakh, N., Lina, B., Diaz, J.-J., Rosa-Calatrava, M., 2012. The influenza fingerprints: NS1 and M1 proteins contribute to specific host cell ultrastructure signatures upon infection by different influenza A viruses. Virology 432, 204–218.

Van Ranst, M., Lemey, P., 2013. Genesis of avian-origin H7N9 influenza A viruses. Lancet 381, 1883–1885.