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Variation in interferon sensitivity and induction between Usutu and West Nile (lineages 1 and 2) viruses

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ABSTRACT

Given the pivotal role of monocyte-derived dendritic cells (DCs) in determining the magnitude of the antiviral innate immune response, we sought to determine whether Usutu virus (USUV) and West Nile virus (WNV) lineages (L)1 and L2 can infect DCs and affect the rate of type I interferon (IFN) activation. The sensitivity of these viruses to types I and III IFNs was also compared. We found that USUV can infect DCs, induce higher antiviral activities, IFN alpha subtypes and the IFN stimulated gene (ISG)15 pathway, and is more sensitive to types I and III IFNs than WNVs. In contrast, we confirmed that IFN alpha/beta subtypes were more effective against WNV L2 than WNV L1. However, the replication kinetics, induction of IFN alpha subtypes and ISGs in DCs and the sensitivity to IFN lambda 1–3 did not differ between WNV L1 and L2.

Introduction

Usutu virus (USUV) and West Nile virus (WNV) belong to the family *Flaviviridae*. They are maintained in nature by bird–mosquito (mainly of the genus *Culex*) transmission cycles and incidental infections of mammals such as humans and horses, which are considered dead-end hosts (Hubálek et al., 2014). In Europe, the circulation of WNV has increased dramatically in recent years throughout the Mediterranean basin, central and eastern Europe. The emergence (Bakonyi et al., 2006) and quick dispersal (Valiakos et al., 2011; Bakonyi et al., 2013) of a lineage (L)2 WNV strain in large parts of Europe contributed significantly to this development. Furthermore, the emergence of USUV in Italy in or before 1996 (Weissenböck et al., 2013), in Austria in 2001 (Weissenböck et al., 2002), and its subsequent expansion to Hungary, Switzerland, Germany, the Czech Republic and Croatia has given additional cause for concern regarding the impact of mosquito-borne flaviviruses on human and animal health in Europe (Vittecoq et al., 2013). Recent evidence of the simultaneous occurrence of WNV- and USUV-associated human neuroinvasive disease emerged in Croatia during the 2013 WNV outbreak (Vilibic-Cavlek et al., 2014). While it is well recognized that WNV infection poses a serious threat for human health, the clinical importance of USUV is not yet fully understood due to its detection only in sporadic cases of human infection (Vazquez et al., 2011; Pierro et al., 2013). Although USUV dispersed quickly in central and southern Europe following its emergence, very little is known about its pathogenesis and activation of the innate immune response triggered by USUV infection (Beck et al., 2013; Blázquez et al., 2013).

Monocyte-derived dendritic cells (DCs) are one of the major producers of type I interferon (IFN) in response to flavivirus infections (Coccia, 2008), and they represent early targets of WNV infection following the bite of an infected mosquito (Qian et al., 2011), providing the virus with opportunities to manipulate DC functions as a means of

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evading host immunity. This manipulation of DC function is thought to be important in WNV pathogenesis, although most of the molecular mechanisms are currently unknown. While the importance of DCs in WNV pathogenesis has been investigated (Samuel and Diamond, 2005; Suthar et al., 2013), no such studies have been performed for USUV so far. Interestingly, our previous study found that USUV was highly sensitive to the antiviral actions of type I and III IFNs only when cells were treated prior to but not after viral infection, suggesting that an established USUV infection could overcome the antiviral effect of IFNs as previously determined for WNV (Scagnolari et al., 2013).

Given the pivotal role played by DCs in the successful induction of antiviral immune responses, we sought to determine whether USUV and WNVs (L1 and L2) could productively infect DCs and affect the expression of type I IFN. In particular, we determined the activation rate of the response of IFN alpha subtypes and IFN-stimulated genes (ISGs) in immature and mature DCs infected by USUV and WNVs (L1 and L2, Fig. 1). Lastly, to determine the potential role of type I and III IFNs in limiting USUV and WNV (L1 and L2) replication, we evaluated whether they differed in terms of sensitivity/resistance to IFN antiviral actions.

Results

Characterization of single-growth curve replication of USUV and WNVs (L1 and L2)

The growth kinetics of USUV and WNVs (L1 and L2) in immature DCs are shown in Fig. 2. Each dot represents the mean value of three separate experiments run in duplicate. As shown, all three viruses analyzed were able to grow in immature DCs. In particular, a WNV L1 titer of 8.7 Log RNA copies/ml was recorded in cellular fraction 24 h p.i., which peaked at approximately 9.6 Log RNA copies /ml between 48 and 72 h p.i. and decreased slowly after a further 12 and 24 h (Fig. 2). Likewise, the WNV L2 titer reached 8.7 Log RNA copies/ml in cellular fraction 24 h p.i. and peaked at approximately Log 9.5 RNA copies/ml 48 h p.i. The titer decreased slowly after another 24 and 48 h (Fig. 2). The WNV L2 titer was significantly different from that of WNV L1 only at 72 and 96 h p.i. (Fig. 2). In contrast, as far as the single-growth curve replication of USUV is concerned, we found that the USUV replication peak began earlier than that of the WNVs. In particular, USUV exhibited a titer of 4.7 Log RNA copies/ml at 12 h p.i. that rose rapidly to 5.5 Log RNA copies/ml after 24 h p.i.; over the next 24 and 48 h the USUV titer declined as observed for WNVs (Fig. 2). Further, the RNA copies of USUV measured on immature DCs at time of virus absorption (T0) and at 12, 24, 48, 72 and 96 h p.i. were significantly fewer than those observed for both WNVs (L1 and L2, Fig. 2).

Evaluation of IFN induction in DC cultures after USUV and WNVs (L1 and L2) infection

Having observed that USUV and WNVs (L1 and L2) titers peaked in immature DCs between 12 and 48 h p.i., we determined the time course and amount of IFN antiviral activity and IFN alpha subtypes at different time points (12, 24, 48 h) p.i. The analysis was performed at a MOI of 1–0.1–0.01 $TCID_{50}$/cell.

The results showed that a significant induction of IFN activity can be recorded in immature DCs infected by USUV or WNVs at most of the time points analyzed (Fig. 3). The only exception was the lack of measurable IFN activity at 24 and 48 h p.i. in immature DCs infected by both WNVs at a MOI of 1 $TCID_{50}$/cell (Fig.3 Panel A). We also observed that IFN activity in USUV-infected immature DCs is greater than that induced by both WNVs (L1 and L2) at all the MOI analyzed. Furthermore, WNV L2 induced a higher amount of IFN activity than WNV L1 at 48 h p.i. (MOI = 0.1 $TCID_{50}$/cell) while the IFN activity in immature DCs did not differ between WNV L1 and WNV L2 in all other conditions analyzed (Fig. 3Panel A).

In addition, as shown in Fig. 3 (Panel A), reducing the MOI from 1 to 0.01 of $TCID_{50}$/cell resulted in a greater induction of IFN activity in USUV-infected immature DCs both at 24 and 48 h p.i. To a lesser extent, the antiviral activity induced by WNVs also increased with decreasing MOI.

Then we evaluated IFN activity in DCs induced to mature with LPS 1 h p.i. with USUV and WNVs (L1 and L2) (Fig. 3 Panel B). We found that LPS alone induced measurable IFN activity only at 12 h

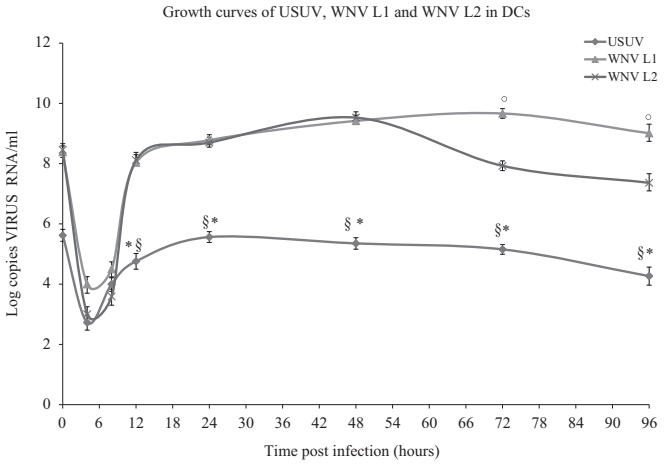

Fig. 2. Growth curves of Usutu virus (USUV), West Nile virus (WNV) lineage (L)1 and West Nile virus L2 in immature DCs. Initial infections were done at a MOI of 1 $TCID_{50}$/cell. Aliquots of the cells (6×10^5) were collected at the time of virus adsorption (i.e. 90 min post infection [p.i.], time point 0) and at 4, 8, 12, 24, 48, 72, and 96 h (h) p.i. Virus titers in the cellular fraction at each collection time were determined using a TaqMan-based real-time RT-PCR technique for viral RNA quantification. Data are expressed as number of viral RNA copies/ml. Each dot represents the mean ± standard deviations of three separate experiments run in duplicate. Mean values were compared using Student's *t*-test. The replication kinetics of USUV was significantly different from that of WNVs (L1 and L2) where indicated. Symbols for statistical comparison: *USUV vs WNV L1 $p < 0.05$; §USUV vs WNV L2 $p < 0.05$; °WNV L1 vs WNV L2 $p < 0.05$.

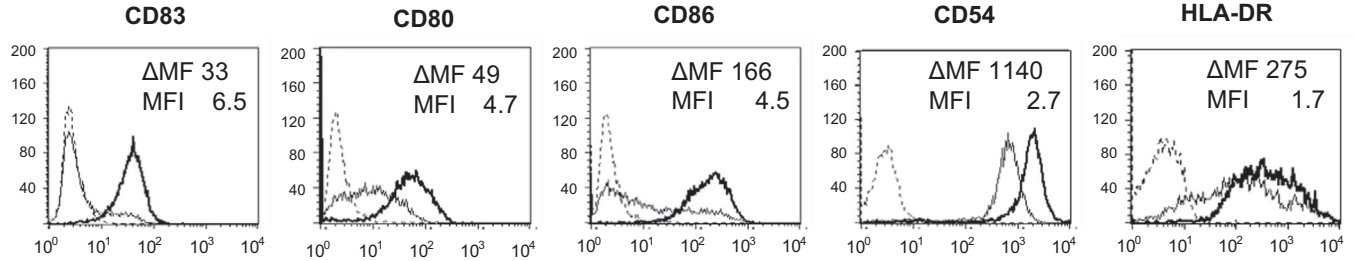

Fig. 1. Immature monocyte-derived dendritic cells (DCs) and those induced to mature with LPS stimulation. DCs were generated from peripheral blood $CD14^+$ cells cultured with GM-CSF and IL-4. On day 5 the resulting cells were > 95% $CD1a^+$ and $CD14^-$. Immature DCs were left untreated, or stimulated with LPS (1 μg/ml). After 24 h incubation DCs were stained with isotype control mAbs (dotted line) or with mAbs directed against indicated surface molecules. Thin and bold lines indicate fluorescence distribution of immature and mature DCs, respectively.