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Tissue-specific transcription profile of cytokine and chemokine genes associated with flavivirus control and non-lethal neuropathogenesis in rabbits

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ABSTRACT

We previously showed that New Zealand White (NZWRs) and cottontail rabbits (CTRs) are a suitable model for studying immune mechanisms behind virus control and non-lethal neuropathogenesis associated with West Nile virus (WNV) and Murray Valley encephalitis virus (MVEV) infections. In the current study, we observed that MVEV infection induced high IFNα, TNFα, IL6, and CXCL10 transcript levels in the brains of weanling NZWRs, unlike infection with the less virulent $WNV_{NSW2011}$. These transcript levels also correlated with encephalitis severity. Widespread STAT1 protein expression in brain with moderate neuropathology suggests that IFN-I signaling is crucial for limiting neural infection and mediating non-lethal neuropathogenesis. Unlike NZWRs, CTRs limit neuroinvasion without upregulation of many cytokine/chemokine transcripts, suggesting a species-dependent virus control mechanism. However, the common IFNγ, TNFα and IL6 transcript upregulation in specific lymphoid organs suggest some conserved elements in the response against flaviviruses, unique to all rabbits.

1. Background

Over 90% of West Nile virus (WNV) infections in humans and horses are non-lethal (Mostashari et al., 2001; Lindsey et al., 2010; Nielsen et al., 2008). Infections can range from asymptomatic infections to self-resolving febrile disease to more severe, but mostly non-lethal neuroinvasive disease (Mostashari et al., 2001; Lindsey et al., 2010; Nielsen et al., 2008). We have recently established a non-lethal WNV infection model in rabbits (Suen et al., 2015). None of the New Zealand white (NZWR) and cottontail (CTR) rabbits infected with the Australian WNV strain of intermediate virulence, $WNV_{NSW2011}$, succumbed to the virus challenge, regardless of age or species of the rabbits (Suen et al., 2015). We had chosen NZWRs and CTRs for the comparison, since we were interested in the susceptibility profile of rabbits from different genetic and environmental background to infection with this recently isolated outbreak strain (Suen et al., 2015). When challenged with the highly virulent and closely related Murray Valley encephalitis virus (MVEV) or the North American WNV strain, WNV_{TX8667}, a similar resistant phenotype was observed in all infected NZWRs and CTRs, respectively (Suen et al., 2015). While all rabbits were asymptomatic following virus challenge, we observed varying degrees of neuropathology and virus dissemination to lymphoid tissues (Suen et al., 2015). The preliminary assessment of type I and II interferon (IFN-I/II) gene transcription in the draining popliteal lymph node (dPLN) and brain of $WNV_{NSW2011}$-and MVEV-infected weanling NZWRs (wNZWRs) suggested a different transcription pattern of cytokine genes between

Abbreviations: WNV, West Nile virus; MVEV, Murray valley encephalitis virus; NZWRs, New Zealand White rabbits; wNZWRs, weanling NZWRs; aNZWRs, adult NZWRs; CTRs, cottontail rabbits; pi, post-infection; dPLN, draining popliteal lymph node; ndPLN, non-draining popliteal lymph node; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; CXCL, C-X-C motif ligand; NF-kB, nuclear factor kappaB; STAT, signal transducers and activators of transcription; qPCR, quantitative PCR; ANOVA, analysis of variance

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these two groups, which may explain the different kinetics of neutralizing antibody production, the extent of systemic virus dissemination and neuropathology observed (Suen et al., 2015).

The present study further investigated the transcription profile of important cytokine and chemokine genes in rabbits post-flavivirus infection. We characterized the transcription kinetics of selected pivotal inflammatory genes in the dPLN, non-draining popliteal lymph node (ndPLN), spleen, and brain of $WNV_{NSW2011}$-infected NZWRs and CTRs from the previous study (Suen et al., 2015). Comparative transcription profiles were characterized in MVEV-infected NZWRs and WNV_{TX8667}-infected CTRs. Genes profiled included tumor necrosis factor-alpha (TNFα), interleukin 6 (IL6), interleukin 4 (IL4), chemokine (C-X-C motif) ligand 10 (CXCL10) and interleukin 10 (IL10). In addition, the transcription profiles of IFN-I/II were also expanded to the ndPLN and spleen in the current study. These genes have been selected, in order to cover the spectrum of antiviral (IFNα/β and γ), pro-inflammatory (TNFα, IL6, IL4), chemotactic (CXCL10) and anti-inflammatory (IL10) response in infected rabbits. Some of these cytokines can also have the opposite function under specific conditions (e.g. TNFα and IL6) (Zakharova and Ziegler, 2005; Scheller et al., 2011). These cytokines/chemokine have also been reported to be important determinants of the disease outcomes after WNV infections in humans (Venter et al., 2009; Cheeran et al., 2005), horses (Bourgeois et al., 2011; Pusterla et al., 2006) and the mouse (Shirato et al., 2004; Shrestha et al., 2008, 2006; Venter et al., 2005). Since the existing mouse models do not consistently recapitulate non-lethal disease associated with virulent WNV infections in humans and horses, as already discussed in length in (Suen et al., 2015; Suen et al., 2014), we were interested in the relevance of these genes in the phenotypically resistant rabbit model. Via this investigation, we also aimed to characterize the association between cytokine/chemokine response in the brain and non-lethal neuropathogenesis. To this end, using immunohistochemical (IHC) labeling, we characterized the expression of pivotal transcription factors responsible for mediating inflammation, such as nuclear factor kappaB (NF-kB) (p100/p52), signal transducers and activators of transcription (STAT)−1 and STAT3, in brains with high cytokine/chemokine transcript levels.

2. Methods

2.1. Virus

An Australian equine pathogenic WNV strain ($WNV_{NSW2011}$), a North American outbreak WNV strain (WNV_{TX8667}) and the virulent MVEV prototype strain ($MVE_{1\text{-}51}$) were used for the *in vivo* challenges. The justification for the use of MVEV and WNV_{TX8667} as control strains has been discussed previously (Suen et al., 2015). $WNV_{NSW2011}$ was originally isolated from the brain of an infected horse during the 2011 Australian outbreak of WNV in horses (Frost et al., 2012). $WNV_{NSW2011}$ was passaged initially on *Aedes albopictus* C6/36 cells, followed by one passage in African green monkey (Vero) cells, and an additional passage in C6/36 cells prior to use. $MVE_{1\text{-}51}$ was originally isolated during the 1951 Australian human outbreak (Dalgarno et al., 1986; Lobigs et al., 1986). It has since been passaged multiple times in suckling mouse brains, as well as in Vero cells. A final passage in C6/36 cells was performed prior to use of $MVE_{1\text{-}51}$. WNV_{TX8667} was isolated from a bluejay during the 2012 human WNV outbreak in Texas, and was kindly provided by Prof. Robert Tesh, University of Texas Medical Branch (Galveston, TX, USA). WNV_{TX8667} was passaged once in Vero cells prior to use (Suen et al., 2015). All virus stocks were titrated in Vero cells, with titers expressed as either fifty percent endpoint of tissue culture infective dose ($TCID_{50}$) for $WNV_{NSW2011}$ and MVEV, or plaque forming units (PFU) for $WNV_{NSW2011}$ and WNV_{TX8667} (Suen et al., 2015). The $TCID_{50}$ and PFU titer for the $WNV_{NSW2011}$ stock varied by less than 1 $\log_{10}$ ($10^{9.7}$ $TCID_{50}$/mL and $10^{8.8}$ PFU/mL).

2.2. Animals and experimental design

The *in vivo* challenge of NZWRs and CTRs has been previously described in detail (Suen et al., 2015). Briefly, a total of 63 rabbits were inoculated with 10^5 $TCID_{50}$ for NZWRs or PFU for CTRs, intradermally in the left hind footpad. Three to nine rabbits were selected at random for scheduled euthanasia at time-points, day 3 and 7 post-infection (pi) for CTRs, day 3, 7, 12 and 18 pi for weanling NZWRs, and day 7 pi only for adult NZWRs (aNZWRs). A total of six NZWRs and two CTRs were sham-inoculated with cell culture media. All animals were monitored daily by a registered veterinarian with measurements of rectal/body temperature, weight and clinical scores recorded (Suen et al., 2015). Experimental infections were conducted in Physical Containment Level 2 (for NZWRs) and Biosafety Level 3 (for CTRs) animal holding facilities at the University of Queensland (UQ) and Colorado State University (CSU), respectively, as described in (Suen et al., 2015). Ethical approval had been granted by the respective animal ethics committee (UQ Animal Ethics Committee (SVS/369/12/ARC); CSU Institutional Animal Care and Use Committee (#14-5170)). In order to assess the differences in the transcription profiles of the cytokine/chemokine genes, we selected three rabbits per each of the available time-points for the comparisons (Table 1). The specific group comparisons for assessing virus, species and age-dependent transcription profiles in this study are outlined in Table 1.

Table 1
Outline of comparisons in this study.

Comparisons	Weanling NZWRs		Adult NZWRs		CTRs	
	$WNV_{NSW2011}$ ($n=12$)	MVEV ($n=12$)	$WNV_{NSW2011}$ ($n=3$)	MVEV ($n=3$)	$WNV_{NSW2011}$ ($n=6$)	WNV_{TX8667} ($n=6$)
Virus-dependent profile (in NZWRs)	X	X				
Virus-dependent profile (in CTRs)					X	X
Species-dependent profile against $WNV_{NSW2011}$	X				X	
Species-dependent profile against virulent control strains		X				X
Age-dependent profile in NZWRs against $WNV_{NSW2011}$	X		X			
Age-dependent profile in NZWRs against MVEV		X		X		

X, comparison of the groups; virulent control strains refer to MVEV for NZWRs and WNV_{TX8667} for CTRs. Note that 6 sham-inoculated wNZWRs and 2 sham-inoculated CTRs were used as calibrators. However, due to the discovery of an abscess in the right hock of one of the CTR controls, we have excluded this animal from analysis, since the active inflammatory process may have biased the transcription profile for this animal.