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Auto-antibodies neutralizing type I and III IFNs in patients with WNV infection

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Introduction

Mosquito-borne West Nile virus (WNV) infection represents a leading cause of neuroinvasive diseases in humans. While older age is the strongest known predictor of severe WNV infection, the role of antiviral immune determinants remains still elusive. Emerging evidence has described that circulating auto-antibodies neutralizing type I Interferon (IFN-I), a sort of autoimmunity, can underlie life-threatening COVID-19. Therefore, we aimed to evaluate the presence of auto-antibodies in critically-ill WNV patients by investigating the serum prevalence of auto-antibodies to a cytoplasmic sensor of WNV RNA, melanoma differentiation antigen 5 (MDA-5), as well as binding (BAB) and neutralizing (NAB) antibodies to type I and III IFNs. The relationship between anti-IFN NAB and IFN signature was also characterized.

Methods

Serum samples collected from WNV positive patients (n=35) were examined in our study. The analysis of anti IFN-alpha BAB as well as the quantitative detection of anti-MDA5 auto-antibodies was performed using ELISA assays. Investigation of anti IFN-I (IFN-alpha, IFN-beta, IFN-omega, and IFN-epsilon) and anti IFN-III (IFN-lambda 1-3) NABs were performed by a bioassay based on IFN-induced inhibition of encephalomyocarditis virus (EMCV) cytopathic effect on human lung carcinoma epithelial cells (A549). Transcript levels of IFN-stimulated gene 56 (ISG56) were analyzed through RT/Real Time PCR

Results

Anti IFN-alpha BAB were found in 10 out of 35 WNV patients (28.6%). Among BAB positive patients, 9 WNV infected individuals (90%) had heterogenous levels of anti IFN-alpha2 NAB. All serum samples positive to anti IFN-alpha2 NAB showed cross reactivity to IFN-omega. A positive correlation was found between the levels of anti-IFN BAB and anti IFN-alpha2 NAB (p=0.0019; r=0.8768) as well as between anti IFN-alpha2 and anti IFN-omega NAB titers (p=0.050; r=0.660). In a subgroup of anti IFN-I NAB positive WNV patients (n=6), we found that the levels of anti IFN-alpha2 NAB titers negatively correlated with ISG56 gene expression (p=0.0167; r=-0.9411). Anti IFN-I NAB were associated with neuroinvasive disease (85.71%) rather febrile illness symptoms (14.28%) (p=0.0325). The only patient with febrile illness symptoms had NAB against IFN-lambda1-3 but not to IFN-lambda2. None of the WNV patients had anti IFN-beta/-epsilon NAB and auto-antibodies against MDA5.

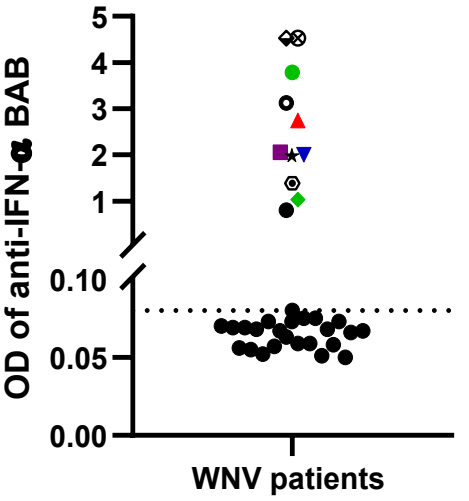

Figure 1. Levels of binding auto-antibodies (BAB) to IFN-α in WNV patients (n=35). Positive results were defined as an optical density (OD) > 0.08, chosen as median signal value (dotted red line) obtain from serum samples of healthy donors.

Demographic and clinical characteristics of WNV positive patients

	WNV patients	WNV female patients	WNV male patients	Anti-IFN-α BAB patients	Anti-IFN-α2 NAB patients	Anti-IFN-ω NAB patients	Anti-IFN-β NAB patients	Anti-IFN-ε NAB patients	Anti-IFN-λ1 NAB patients	Anti-IFN-λ2 NAB patients	Anti-IFN-λ3 NAB patients	Anti-MDA5-Abs patients
Total patients**	35	13/35 (37)	22/35 (63)	10/35 (28.5)	9/35 (25.7)	9/35 (25.7)	0/35 (0)	0/35 (0)	1/35 (2.85)	0 (0)	1/35 (2.85)	0 (0)
Age*	63 (12 – 94)	65 (12 – 80)	56 (20 – 94)	71 (63 – 78)	69 (63 – 78)	69 (63 – 78)	NA	NA	63	NA	63	NA
Gender (M/F)	22/13	NA	NA	7/3[Δ]	6/3[▲]	6/3[▲]	NA	NA	1/0	NA	1/0	NA
Clinical manifestation**												
Febrile illness symptoms	1/26 (3.85)	NA	1/15 (6.7)	1/7 (14.3)	1/7 (14.3)	1/7 (14.3)	NA	NA	1/1 (100)	NA	1/1 (100)	NA
Neuroinvasive disease	25/26 (96.15)	11/11 (100)	14/15 (96.1)	6/7 (96.1)	6/7 (96.1)[^]	6/7 (96.1)	NA	NA	NA	NA	NA	NA

*Data are expressed as median (Min-Max); ** Data are expressed as total number (%) of WNV patients. Clinical data were available for 26 out of 35 WNV patients. Statistical analysis was performed using Yates Chi-square or Fisher's exact tests. WNV=West Nile virus. [Δ]p=0.580 for anti-IFN-α BAB of male patients vs for anti-IFN-α BAB of female patients; [▲]p=0.783 for anti-IFN-α/-ω NAB of male patients vs for anti-IFN-α/-ω NAB of female patients; [^] p=0.032 for neurological disease vs febrile illness symptoms of WNV patients positive to anti-IFN-α/-ω NAB.

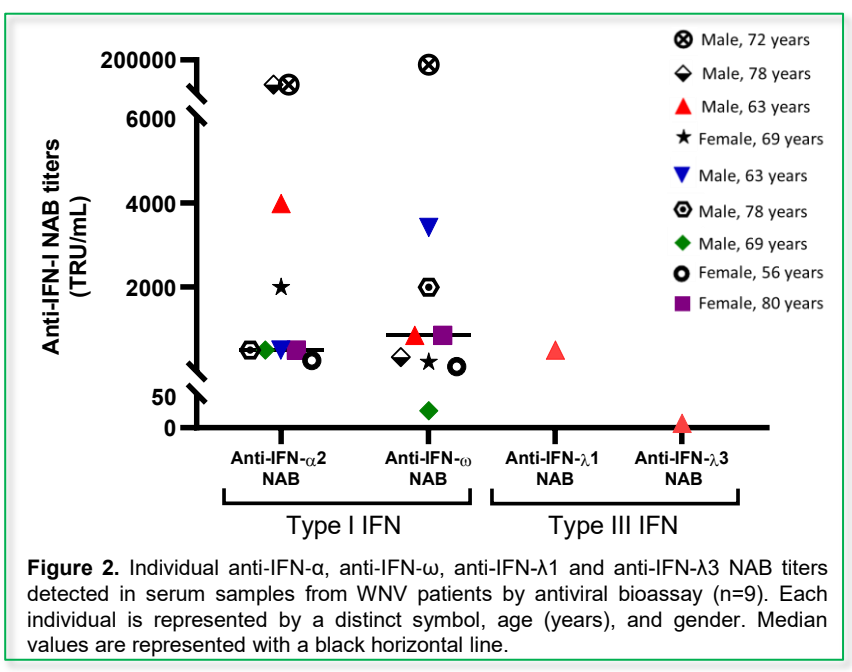

Figure 2. Individual anti-IFN-α, anti-IFN-ω, anti-IFN-λ1 and anti-IFN-λ3 NAB titers detected in serum samples from WNV patients by antiviral bioassay (n=9). Each individual is represented by a distinct symbol, age (years), and gender. Median values are represented with a black horizontal line.

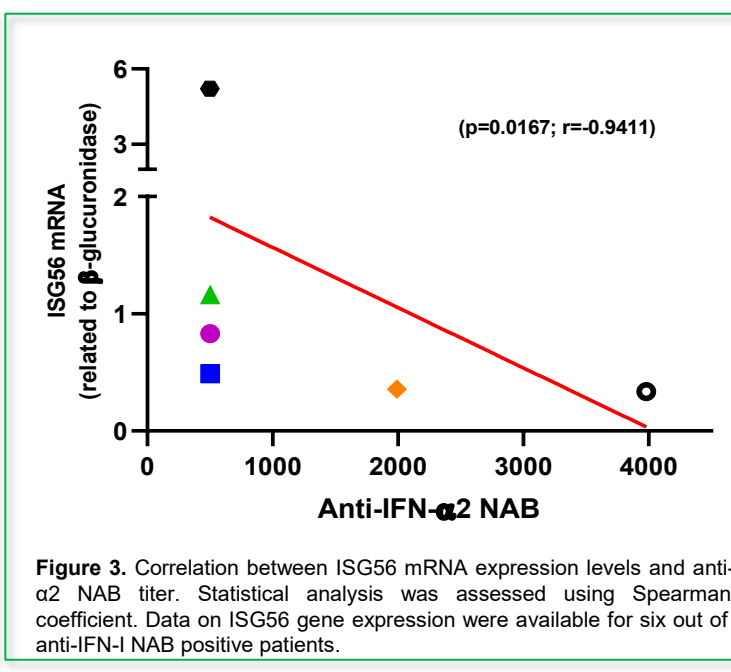

Figure 3. Correlation between ISG56 mRNA expression levels and anti-IFN-α2 NAB titer. Statistical analysis was assessed using Spearman's ρ coefficient. Data on ISG56 gene expression were available for six out of nine anti-IFN-I NAB positive patients.

Conclusion

Our findings demonstrate that NAB positivity to type I/III IFNs but not auto-antibodies to MDA5 distinguishes WNV patients and suggest that the development of anti-IFNs NAB might imply in a dysregulation of IFN downstream signaling pathways leading to severe WNV infection.