

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Venter M, Burt FJ, Blumberg L, Fickl H, Paweska J, Swanepoel R. Cytokine induction after laboratory-acquired West Nile virus infection. *N Engl J Med* 2009;360:1260-2.

Supplementary Appendix

Laboratory confirmation

When the accident occurred it was immediately reported to the occupational health nurse who took a baseline blood sample shortly after the incident (day 0). Blood was also taken on days 8, 9, 10, 11, 13, 16 and 26 post infection (p.i.) and serum stored at -70°C for subsequent analysis. WNV and flavivirus specific reverse transcription polymerase chain reaction (RT-PCR) on the patient's acute serum were strong positive on day 8 p.i.^{1,2}. The patient was seronegative for WNV antibodies at the time of the accident and remained seronegative from days 0 to 11 p.i. by IgM capture ELISA. By day 16 (day 8 of symptoms) an IgM antibody titer of 1:64 was demonstrable which increased to 1:2048 on day 26. The patient had received the Aventis Pasteur Yellow Fever virus vaccine approximately 6 months earlier and seroconversion was confirmed by yellow fever Hemagglutination inhibition assay (HIA) 6 weeks post-immunization with a titre of 1:40.

Strain characterisation

Neuropathogenesis experiments by intraperitoneal and intracerebral inoculation using a dilution series of strain SPU93/01 were carried out in 3-4 week old mice as described previously^{3,4}. SPU93/01 was originally isolated from an immunocompetent female hospitalized with fever, rash, myalgia, and non fatal encephalitis in the Gauteng province, South Africa⁵ and confirmed as a lineage II strain by full genome sequencing⁶.

Five mice, inoculated intraperitoneally at the time of the accident with 20ul of tissue culture supernatant containing 50TCID₅₀ U/ul (1000 TCID₅₀U total), the same inoculum that had caused the accident, started dieing by day 6 p.i. and were all dead by day 7 p.i.

Intraperitoneal and intracerebral mouse neuroinvasive experiments carried out as described in⁴ confirmed this strain to be highly neuroinvasive with a lethal dose (LD)₅₀ value of 4.9. This is comparable to highly neuroinvasive lineage I (NY385/99) (LD₅₀=7.9) and II (H442 (LD₅₀=3.2) and SPU116/89 (LD₅₀=2.0)) WNV strains used in comparative experiments⁴.

Serum cytokine measurement

A Bioplex bead-based suspension array assay (Biorad, Hercules California) was used to measure the levels of 15 different cytokines simultaneously in acute serum according to the manufactures' recommendation. Cytokines frequently associated with pathogenesis and protection in various diseases (Th1/2) were selected. These included Interleukin (IL)4, IL5, IL6, IL8, IL9, IL10, IL13, IL15, eotaxin (CCL11), interferon- γ (IFN γ), interferon-inducible protein-10 (IP10)(CXCL10), macrophage chemoattractant protein1 (MCP1)(CCL2), macrophage inflammatory protein(MIP1 α)(CCL3), MIP1 β (CCL4), RANTES (regulated upon activation, normal T cell expressed and secreted)(CCL5) and tumour necrosis factor alpha (TNF α). Healthy control serum was taken from the patient 5 years after the incident and used for background subtraction. Reactions were analyzed with Bioplex Manager software version 4.1 and run on a Luminex instrument against a 8 point standard curve generated for each cytokine, in the same serum standard diluent used for specimens. IFN α levels were measured with the human interferon alpha ELISA kit (PBL Biomedical Laboratories, New Brunswick, New Jersey USA) according to the manufacturer's recommendation using the same serum and control samples described above.

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