Identification of a Kunjin/West Nile-like flavivirus in brains of patients with New York encephalitis

Thomas Briese, Xi-Yu Jia, Cinnia Huang, Leo J Grady, W Ian Lipkin

Molecular analysis of brains from patients of the recent New York City encephalitis outbreak reveals the presence of a flavivirus not previously described in the Americas.

In late August, 1999, health officials reported an outbreak of encephalitis accompanied by severe weakness and axonal neuropathy in Queens, New York, USA. Serological analysis of five of six patients for the presence of antibodies to North American arboviruses yielded results consistent with infection with St Louis encephalitis virus, which prompted establishment of a mosquito eradication programme by the State and City of New York. Concurrently, wildlife observers noted increased mortality of avian species including free-ranging crows and exotic birds housed in the Bronx Zoo. Histological analysis of birds revealed meningoencephalitis and myocardiitis. Avian mortality is not characteristic of St Louis Encephalitis virus; therefore, efforts were made to identify other pathogenic arboviruses.

Samples of brain material from five patients with encephalitis were selected for molecular analysis. Based on findings in one brain consistent with the presence of flavivirus antigen (Centers for Disease Control and Prevention, Atlanta, GA, USA; unpublished data), oligonucleotide primers were designed to hybridise to conserved regions within the NS3 and NS5 genes of a wide variety of flaviviruses.

Several degenerate primer sets were designed for use in reverse transcription (RT) PCR (figure 1, table 1). One primer set (NS3-1), which encompassed conserved motifs in the serine protease and nucleotide triphosphatase or RNA helicase domains of NS3 sequences (EDL/FlaU5004, 5’-GGA ACD TCM GGH TCN CCH AT and Fla-L5457, 5’- GTG AAR TGD GCY TCR TCC AT) was based on the primer sequences published by Chow and colleagues. Various nested primer sets towards the 5’ end of NS5 sequences, including conserved aminoacid motifs AKG SRA IWXM W LGARRX LFE AEL GFLN X HW, DDTAGWDT, and QRSGQXVXY were selected for use in domain-specific differential display. This method enables detection of differences in nucleic-acid populations. The cloned and sequenced amplification products were generated with primer sets NS5-1.1 (EDL/Fla-U9093, 5’- AGY MGR GCH ATH TGG TWY ATG TGG and EDL/Fla-L9279, 5’- TCC CAV CDD GCK GTR TCA TC), NS5-1.2 (EDL/Lp146, 5’- TGG AAA AGC YAA AGG NAG and EDL/Lp 154, 5’- GTG TCC CAN CDD GCD GTR TC) and NS5-1.3 (EDL/Lp151, 5’- GAY ACH GCH GGN TGG GAC AC and EDL/Lp153, 5’- GCA TAD GTS)

Table 1: Positions of primers and amplification products in prototypic flavivirus genome

<table>
<thead>
<tr>
<th>Region</th>
<th>Kunjin virus</th>
<th>West Nile virus</th>
<th>St Louis virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS3-1</td>
<td>BB (96)</td>
<td>B2 (94)</td>
<td>NAS</td>
</tr>
<tr>
<td>NS5-2</td>
<td>87 (99)</td>
<td>80 (95)</td>
<td>70 (76)</td>
</tr>
<tr>
<td>NS5-3</td>
<td>88 (100)</td>
<td>80 (96)</td>
<td>80 (89)</td>
</tr>
</tbody>
</table>

Percentage identity of nucleotide sequence for three regions of the New York isolate genome with Kunjin and West Nile viruses. Percentage identity of deduced aminoacid sequences shown in parentheses. NAS=no available sequence.

Table 2: Sequence-analysis data for New York isolate genome with Kunjin and West Nile viruses

ACM ACY TGN CC). Primer set NS5-3 (EDL/Fla-U10098, 5’- TGG ATG CAN ACD GAA GAY ATG CT and EDL/Fla-L10750, 5’- GGG GTC TCC TMT AAC CWC TAG) spanning the 3’ end of NS5 and untranslated sequence was based on the universal primers of Tanaka3 and Pierre and colleagues. Primer set NS5-2 used an inverted version of the universal forward primer (EDL/Fla-L10098 5’- TGG ATG CAN ACD GAA GAY ATG CT) in conjunction with primer EDL/Fla-U9954 (5’- GSS AAA KCH TAY GCN CAV ATG TGG).

Four of the five brain samples contained flavivirus sequences. Amplification products from two patients were submitted for dideoxy DNA sequencing (figure 1). The sequences obtained from these two patients were virtually identical and alignments of sequences from from both with other flavivirus sequences showed the presence of a Kunjin/West Nile-like virus (figure 2, table 2).

Kunjin virus and West Nile virus are closely related members of the Japanese encephalitis virus serogroup; the former has been reported in Australia, and the latter in Africa, Asia, and Europe. Kunjin virus has not been associated with mortality in human beings. A West Nile virus outbreak in Romania in 1996 had a death rate of 4–8%; the ratio of apparent to inapparent infections was estimated to be about 1:325. Neither Kunjin virus nor West Nile virus infections have been described in the Americas.

Figure 1: Positions of primers and amplification products in prototypic flavivirus genome
Retinoic acid for treatment of multicentric Castleman’s disease

Philipppe Rieu, Dominique Droz, Antoine Gessain, Jean-Pierre Grünfeld, Olivier Hermine

Multicentric Castleman’s disease is an aggressive course with poor prognosis, and its treatment remains uncertain. We report a woman with multicentric Castleman’s disease that was successfully treated with prednisone and retinoic acid. A 35-year-old woman had been in excellent health until 1 month before admission, when she developed progressive weakness, fever in the evening, and oedema with weight gain. Physical examination on admission showed normal blood pressure, multiple enlarged small peripheral lymph nodes, hepatosplenomegaly, pleural effusion, and marked peripheral oedema. No neuropathy or skin changes were noted. Investigation showed mild renal insufficiency (creatinine=140 μmol/L) with proteinuria (0.7 g/d) and microscopic haematuria. Blood count showed a normocytic anaemia (Hb=10 g/dL; MCV=87 fl) without laboratory signs of haemolysis. Leucocyte and platelet counts were normal. CRP-reactive protein was 53 mg/L. Plasma albumin and globulin concentrations, serum immunoelectrophoresis, fibrinogen, antinuclear antigen, serum complement, antiphospholipid antibodies, blood culture, and viral serological tests (HBV, HCV, HIV-1) were normal or negative. Biopsy of an auxiliary lymph node showed angiofolliclary lymphoid hyperplasia, consistent with Castleman’s disease of the hyaline vascular type. PCR analysis for HHV-8 sequences on lymph-node biopsy material was negative. Renal biopsy showed lesions similar to those of thrombotic microangiography. No immune deposits were identified by immunofluorescence. In hospital, hypertension developed and was gradually controlled with the use of three antihypertensive agents. Once blood pressure was controlled, oral prednisone (0.5 mg/kg day) was started. After 2 weeks, fever, weakness, oedema, peripheral lymphadenopathy, and splenomegaly persisted. All-trans-retinoic acid treatment (45 mg/m² daily orally) was then added to prednisone. This resulted in marked improvements in her symptoms within a week. After 1 month of treatment, physical examination and laboratory tests were normal. Prednisone was gradually reduced and completely discontinued after 12 months. Retinoic acid was initially given for 6 weeks, and thereafter for 14 days every 2 weeks for 9 months. After 1 year off treatment, she remains without symptoms and with normal laboratory tests.

Retinoic acid have been recognised as an important immunoregulating agents. It has potential therapeutic effects in diseases associated with overproduction of interleukin-6 (IL-6) and endothelial injury such as Kaposi’s sarcoma and POEMS syndrome.1,2 two diseases that have been reported in the course of multicentric Castleman’s disease. Recently, Raife et al reported a patient with thrombotic thrombocytopenia purpura (TTP), refractory to conventional therapy, who entered into remission after 13-cis retinoic acid treatment.1 It is noteworthy that IL6 is raised in serum of children with TTP and that IL6 levels vary with disease activity.2 IL6 plays a central part in the pathophysiology of Castleman’s disease and vascular lesions are important features. The response to retinoic acid and prednisone seen in this patient may therefore result from modulation of IL-6/IL-6R autocrine/paracrine loop or a beneficial effect on endothelial injury.

3 Raife TJ, M Arthur, Peters C, Kider CT, Lenzt SR. Remission after