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Brief Communication

West Nile Virus infection triggering autoimmune encephalitis: Pathophysiological and therapeutic implications

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ABSTRACT

Background: A contributing factor in triggering autoimmune phenomena is pathogen infections. Here we describe a case that expands the spectrum of infection-associated autoimmune encephalitis and discuss plausible pathogenetic mechanisms.
Design: Case report and in silico analysis.
Results: A patient with West Nile Virus infection developed autoimmune encephalitis with positive anti-glycine receptor antibodies. Combination therapy with corticosteroids and intravenous immunoglobulin resulted in the resolution of encephalitis signs and symptoms. An in silico analysis unveiled certain sequence similarities between viral antigens and receptor sequence fragments suggesting a molecular mimicry autoimmunization process.
Conclusions: Our case indicates that West Nile Virus infections can trigger autoimmune encephalitis. Our finding expands the spectrum of autoimmune conditions that can develop following an infection. Whether the autoimmunization process is due to molecular mimicry or due to the expansion of natural autoantibody clones merits further investigation.

1. Introduction

Clinical observations indicate that pathogen infections (either bacterial, viral or protozoan) can trigger autoimmunity. Typical examples include Guillen Barre syndromes (GBS), where the development of neurological symptoms follow a gastrointestinal infection by *Campylobacter jejuni* [1] and Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) where a subset of children present with rapid onset of obsessive-compulsive disorder (OCD) or tic disorders and these symptoms are caused by group A beta-hemolytic streptococcal (GABHS) infections [2]. Other autoimmune conditions, are possibly triggered by latent viral infections e.g. multiple sclerosis where Epstein Bar virus (EBV) sequences inserted into the genome may act as trigger for the autoinflammatory cascade [3]. Recently, similar observations have been made in a group of diseases called autoimmune encephalopathies; the best studied example is autoimmununne encephalitis, most often harboring anti-NMDAR antibodies, triggered by a *Herpes simplex 1* infection in both adult and children populations [4]. Pathophysiologicaly, these phenomena can be explained by either molecular mimicry e.g. in the GBS cases where the same structural epitope exists in the bacterium and in the cell membrane ganliosides or by the augmentation of natural autoimmunity where pre-existing B-cell clones are expanded and end up producing harmful autoantibodies.

West Nile Virus (WNV) is a mosquito-borne single strand RNA flavivirus that infects humans, causing symptoms ranging from fever and minor symptoms (myalgias, arthralgias) to severe encephalitis, flaccid paralysis, and death. Reports of WNV patients who subsequently developed myasthenia gravis, a disease caused by autoantibody-mediated disruption of signaling at the neuromuscular junction, indicate a possible -yet disputable- link between WNV infection and autoimmunity [5]. We present the case of a WNV infected patient who developed autoimmune encephalitis positive for autoantibodies against Glycine receptor (GlyR) and responded to immunosuppresive therapy. The possible pathogenetic links between infection and autoimmunity are discussed.

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2. Results

2.1. Clinical description

An 84-year-old man was admitted with acute encephalitis and flaccid paralysis. Two days before hospitalization, during an acute febrile illness, he had experienced severe muscle weakness, chills, headache, neck pain, abdominal pain, vomiting and diarrhea followed by drowsiness, transient obtundation, and confusion. On admission, the patient was aware, with dysarthria, nuchal rigidity and Lasec's sign. He had flaccid and symmetrical tetraparesis and abolished myotonic reflexes. The electroencephalogram showed diffuse brady-arrhythmias without any epileptic discharges. Brain and spinal cord MRI was normal. Cerebrospinal fluid (CSF) was clear, with 55 cells, protein 99 mg/dL and glucose 71 mg/dL. CSF PCR was negative for infectious agents including *Escherichia coli* K1, *Haemophilus influenza, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae, Streptococcus pneumonia,* Cytomegalovirus, Enterovirus, HSV-1, HSV-2, HHV-6, Parechovirus, *Varicella zoster* virus, *Cryptococcus neoformans/gattii* and *Mycobacterium tuberculosis*. High titers of serum WNV IgM antibodies were detected with ELISA, and WNV PCR was also positive.

The clinical picture of the patient worsened over the next days in terms of the level of consciousness, reaching stupor with no response to verbal or motor stimuli. The patient received only supportive treatment and over a 10-day period showed progressive significant improvement of his neurological condition, with the exception of episodes of psychomotor agitation. Clinical improvement was confirmed with a second CSF analysis (6 cells, protein 48 mg/dL, glucose 79 mg/dL). At this point of the disease course, the patient experienced a second phase of worsening of the level of consciousness with de novo drowsiness, limitation of verbal answers and simple task performance. Bilateral extrapyramidal signs and bilateral Babinski sign were noted.

The patient received intravenous immunoglobulin 1 g/kg body weight daily for three days as well as 1 g pulse of methyl prednisone with significant clinical improvement. The two-phase disease and therapeutic response to immunotherapy prompted the physicians to test the patient serum for autoimmune encephalitis autoantibodies. Cell-based assay revealed antibodies only against GlyR. He received 1 g pulses of methyl prednisone for 4 more days with excellent response. He progressively regained higher functions and was discharged from the hospital after 15 days walking with assistance. The patient received tapered corticosteroid treatment for 2 months following discharge. Positive serum anti-GlyR antibodies were detected in testing performed one and three months after hospitalization. In the last clinical examination, the patient was in pristine clinical condition, with no neurological signs.

2.2. In silico analysis

An in silico analysis of possible epitope similarities between the WNV proteins and GlyR was performed. Sequence comparison of each of the glycine receptor subunits (α1-α4 and β) with the WNV polyprotein using the BLAST tool revealed several fragments exhibiting significant similarity (Fig. 1). Most of the similar fragments also contained antigenic peptides, suggesting that they are candidate epitopes [6]. Antigenicity prediction was performed by a tool that uses amino-acid physicochemical properties, namely hydrophobicity, accessibility and flexibility, as well as their frequency of occurrence in experimentally known epitopes. Among the sequences exhibiting significant alignment, a fragment of the predicted transmembrane region of the GlyR α4 isoform 2 displayed the longest stretch of consecutive identical amino acids with a WNV polyprotein sequence within the helicase domain of the non-structural protein 3. This fragment is also predicted to be antigenic on both the viral and the human protein. Nevertheless, antibodies against GlyR are predominantly directed against the α1 subunit [7]. Experimental confirmation of the immunogenic potential of the specific viral epitopes and their potential to cause encephalitis via epitope spreading against the receptor is needed to test whether molecular mimicry and intramolecular spreading is the culprit in this case.

3. Discussion

This is the first report of a patient with glycine receptor autoantibodies showing complete response to IVIg and corticosteroid treatment following WNV infection. Glycine receptor antibodies have been associated with Progressive encephalomyelitis with rigidity and myoclonus (PERM) and Stiff Person Syndrome variants [8]. These autoantibodies are presumed to disrupt normal function of the glycine receptor, which forms a ligand-gated chloride channel, generating inhibitory currents.

Post-viral infectious autoimmune encephalitis has been previously documented, primarily in multiple cases of NMDAR encephalitis following HSV-1 infections [9]. Similarly to our case, autoimmune attack often follows resolution of the infection, as evidenced from viral titer diminution, and responds to prompt immunotherapy [10]. In these anti-NMDAR cases there was no evidence of molecular mimicry, rather autoimmunisation is thought to occur from the release of blood brain barrier protected antigens to the circulation. Post-infectious GlyR autoimmunity has also been reported, in a patient who developed typical PERM following brucellosis [11]. This patient was treated for his autoimmune condition using the anti-B cell monoclonal antibody rituximab, resulting in anti-GlyR antibody titer diminution in the CSF. In another individual, Stiff person syndrome and anti-Glutamate decarboxylase (GAD) antibodies developed following WNV infection [12].

A question arising from our case is whether the two seemingly separate events i.e. the viral encephalitis and autoimmune encephalitis are etiologically connected. Several mechanisms have been proposed. Augmentation of naturally occurring auto-reactive B cells [13] in genetically predisposed individuals could result from tissue destruction, following brain parenchyma infection, which releases autoantigens to draining lymph nodes. Moreover, interference of the WNV proteins with the nuclear factor (NF)–κB pathway could alter the physiological response mediated by this master immune regulator, leading to aberrant reactivity and autoimmunity. Another possible mechanism is molecular mimicry, according to which, sequence or structural similarities between microbial and autoantigen epitopes cause the immunological reaction to evolve to autoimmunity. The latter, was presented in the HSV-SPS case where a stretch of homology was detected between WNV and GAD65 [12]. Similarly, neurological complications following Zika virus infection (another flavivirus) have also been attributed to potential mimicry between gangliosides and surface molecules of the infectious agent [14]. This further supports that selection of unique immunogenic peptide sequences of the WNV polyprotein is important in the design of diagnostic tools and vaccines [15]. It would be of interest testing a large cohort of WNV encephalitis patients for the presence of autoantibodies, not only against GlyR antibodies but also for the presence of an array of antibodies known to be associated with autoimmune encephalitis. Finally, our case confirms previous observations that immunotherapy is necessary to combat autoimmune disorders following any anti-viral or anti-microbial initial therapy.

Declaration of Competing Interest

None.

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WNV polyprotein AAV54504.1  150  TAAGKNLCIVRAMDVGYMCDDTITYECPVLSAGNDPEDI  188
                                 T   K L I R  +V Y    T+T  CP +   N P D+                    Score: 20.8, Positives: 18/39 (46%)
GlyR alpha 1 AAH74980.1     140  TTDNKLLRISRNGNVLYSIRITLTLACP-MDLKNFPMDV  177

WNV polyprotein AAV54504.1  150  TAAGKNLCIVRAMDVGYMCDDTITYECPVLSAGNDPEDIDCWCT  193
                                 T   K L I +   V Y    T+T  CP +   N P D+   CT              Score: 18.9, Positives: 19/44 (43%)
GlyR alpha 2 AAH32864.1     146  TTDNKLLRISKNGKVLYSIRLTLTLSCP-MDLKNFPMDVQT-CT  187

WNV polyprotein AAV54504.1  150  TAAGKNLCIVRAMDVGYMCDDTITYECPVLSAGNDPEDI  188
                                 T   K L I +  +V Y    T+T  CP +   N P D+                    Score: 20.4, Positives: 18/39 (46%)
GlyR alpha 3 AAC39919.1     145  TTDNKLLRIFKNGNVLYSIRLTLTLSCP-MDLKNFPMDV  182

WNV polyprotein AAV54504.1 1774  RLMSPHRVPNYNLFVM  1789
                                 R ++ HRVP++    M                                           Score: 17.7, Positives: 10/16 (62%)
GlyR α4 is.2 NP_001165756.1 317  RELACHRVPHFPFIPM  332

WNV polyprotein AAV54504.1  199  IRYGRCTKTRHSRRSRRSLTVQTHGESTLANKKGAWMDSTKATRYLVKTESWI  251
                                 I YG CTK          + V      TL  + G +M    A   L+   SW+       Score: 20.0, Positives: 20/53 (37%)
GlyR β AAC71034.1           238  IEYGNCTKYYKGTGYYTCVEVIF----TLRRQVGFYMMGVYAPTLLIVVLSWL  286
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Fig. 1. Sequence alignment of the regions of the WNV polyprotein and GlyR subunits producing the highest similarity scores. Bold font indicates predicted antigenic peptides.

References

[1] H.J. Willison, B.C. Jacobs, P.A. van Doorn, Guillain-Barre syndrome, Lancet (London, England). 388 (10045) (2016) 717–727.

[2] T. Cutforth, M.M. DeMille, I. Agalliu, D. Agalliu, CNS autoimmune disease after streptococcus pyogenes infections: animal models, cellular mechanisms and genetic factors, Future Neurol. 11 (1) (2016) 63–76.

[3] M. Laurence, J. Benito-Leon, Epstein-Barr virus and multiple sclerosis: updating Pender's hypothesis, Mult. Scler. Relat. Disord. 16 (2017) 8–14.

[4] T. Armangue, M. Spatola, A. Vlagea, et al., Frequency, symptoms, risk factors, and outcomes of autoimmune encephalitis after herpes simplex encephalitis: a prospective observational study and retrospective analysis, Lancet Neurol. 17 (9) (2018) 760–772.

[5] A.A. Leis, G. Szatmary, M.A. Ross, D.S. Stokic, West nile virus infection and myasthenia gravis, Muscle Nerve 49 (1) (2014) 26–29.

[6] A.S. Kolaskar, P.C. Tongaonkar, A semi-empirical method for prediction of antigenic determinants on protein antigens, FEBS Lett. 276 (1–2) (1990) 172–174.

[7] A. Carvajal-Gonzalez, M.I. Leite, P. Waters, et al., Glycine receptor antibodies in PERM and related syndromes: characteristics, clinical features and outcomes, Brain. 137 (Pt 8) (2014) 2178–2192.

[8] A. Swayne, L. Tjoa, S. Broadley, et al., Antiglycine receptor antibody related disease: a case series and literature review, Eur. J. Neurol. 25 (10) (2018) 1290–1298.

[9] J. Galli, S.L. Clardy, A.L. Piquet, NMDAR encephalitis following herpes simplex virus encephalitis, Curr. Infect. Dis. Rep. 19 (1) (2017) 1.

[10] H. Alexopoulos, S. Akrivou, S. Mastroyanni, et al., Postherpes simplex encephalitis: a case series of viral-triggered autoimmunity, synaptic autoantibodies and response to therapy, Ther. Adv. Neurol. Disord. 11 (2018) (1756286418768778).

[11] E.E. Magira, H. Alexopoulos, E. Charitatos, D. Michas, M.C. Dalakas, Progressive encephalomyelitis with rigidity and myoclonus (PERM): brucellosis as a possible triggering factor and long-term follow-up therapy with rituximab, Ther. Adv. Neurol. Disord. 9 (1) (2016) 69–73.

[12] S. Hassin-Baer, E.D. Kirson, L. Shulman, et al., Stiff-person syndrome following West Nile fever, Arch. Neurol. 61 (6) (2004) 938–941.

[13] S. Avrameas, H. Alexopoulos, H.M. Moutsopoulos, Natural autoantibodies: an Undersugn Hero of the immune system and autoimmune disorders-a point of view, Front. Immunol. 9 (2018) 1320.

[14] J.M. Anaya, C. Ramirez-Santana, I. Salgado-Castaneda, C. Chang, A. Ansari, M.E. Gershwin, Zika virus and neurologic autoimmunity: the putative role of gangliosides, BMC Med. 14 (2016) 49.

[15] G. Capone, G. Lucchese, M. Calabro, D. Kanduc, West Nile virus diagnosis and vaccination: using unique viral peptide sequences to evoke specific immune responses, Immunopharmacol. Immunotoxicol. 35 (1) (2013) 64–70.