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References

1. Ergonul O. Crimean–Congo hemorrhagic fever virus: new outbreaks, new discoveries. Curr Opin Virol. 2012;2:215–20. http://dx.doi.org/10.1016/j.coviro.2012.03.001
2. Whitehouse CA. Crimean–Congo hemorrhagic fever. Antiviral Res. 2004;64:145–60. http://dx.doi.org/10.1016/j.antiviral.2004.08.001
3. David-West TS, Cooke AR, David-West AS. Seroepidemiology of Congo virus (related to the virus of Crimean haemorrhagic fever) in Nigeria. Bull World Health Organ. 1974;51:543–6.
4. Gonzalez JP, LeGuenno B, Guillaud M, Wilson ML. A fatal case of Crimean–Congo haemorrhagic fever in Mauritania: virological and serological evidence suggesting epidemic transmission. Trans R Soc Trop Med Hyg. 1990;84:573–6. http://dx.doi.org/10.1016/0035-9203(90)90045-G
5. Wilson ML, LeGuenno B, Guillaud M, Desoutter D, Gonzalez JP, Camicas JL. Distribution of Crimean–Congo hemorrhagic fever viral antibody in Senegal: environmental and vectorial correlates. Am J Trop Med Hyg. 1990;43:557–66.
6. Nabeth P, Cheikh DO, Lo B, Faye O, Vall IO, Niang M, et al. Crimean–Congo hemorrhagic fever, Mauritania. Emerg Infect Dis. 2004;10:2143–9. http://dx.doi.org/10.3201/eid1012.040535
7. Tall A, Sall AA, Faye O, Diatta B, Sylla R, Faye J, et al. Two cases of Crimean–Congo haemorrhagic fever (CCHF) in two tourists in Senegal in 2004. Bull Soc Pathol Exot. 2009;102:159–61.
8. Rees DJ, Dioli M, Kirkendall LR. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae). Mol Phylogenet Evol. 2003;27:131–42. http://dx.doi.org/10.1016/S1055-7903(02)00374-3
9. Barker SC. Distinguishing species and populations of rhipicephaline ticks with its 2 ribosomal RNA. J Parasitol. 1998;84:887–92. http://dx.doi.org/10.2307/3284614
10. Deyde VM, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Crimean–Congo hemorrhagic fever virus genomics and global diversity. J Virol. 2006;80:8834–42. http://dx.doi.org/10.1128/JVI.00752-06

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Letters

Coxsackievirus A16 Encephalitis during Obinutuzumab Therapy, Belgium, 2013

To the Editor: Enterovirus infections are associated with many clinical manifestations, and specific virus groups or serotypes are associated with specific manifestations. Coxsackievirus A16, a common cause of hand, foot and mouth disease, rarely causes encephalitis. Although most enterovirus infections are cleared by cellular immune responses, invasive enterovirus disease is prevented or controlled by neutralizing antibodies (1). Thus, patients with humoral immunodeficiencies are susceptible to serious enterovirus infections.

Nine cases of enteroviral encephalitis (1 caused by echovirus 13, 1 caused by coxsackievirus A16, 2 caused by enterovirus 71, and 5 caused by unknown enteroviruses) have been reported after therapy with rituximab, a monoclonal antibody (MAb) that causes secondary hypogammaglobulinemia (2). We describe coxsackievirus A16 encephalitis in a patient who was receiving treatment with the MAb obinutuzumab.

A 67-year-old woman with non-Hodgkin lymphoma showed complete remission after 6 cycles of treatment with bendamustine and obinutuzumab. Induction immunochemotherapy was followed by obinutuzumab maintenance therapy. At admission, she had received 7 of 12 scheduled treatments.

The patient was hospitalized because of a history of high-grade fever that did not respond to antimicrobial drugs, confusion, general weakness, and urinary incontinence. She had a neutrophil count of 3.1×10^9 cells/L but had severe lymphocytopenia (0.3 $\times 10^9$ cells/L and an absolute CD4 cell count of 0.082×10^9 cells/L) and low

serum immunoglobulin levels (IgG 3.86 g/L [reference range 7.51–15.6 g/L], IgA 0.07 g/L [reference range 0.82–4.53 g/L], and IgM 0.13 g/L [reference range 0.46–3.04 g/L]). Cerebrospinal fluid (CSF) samples were collected on days 1, 4, and 6. CSF leukocyte counts increased from 14 cells/mm^3 (day 1) to 60 cells/mm^3 (day 4) (35% and 27% lymphocytes, respectively). Cytologic and immunophenotypic analyses showed no cerebromeningeal lymphoma infiltration. Total protein levels in CSF increased from 561 mg/L on day 1 to 771 mg/L on days 4 and 6.

Bacterial and fungal cultures were negative. Cryptococcal antigen was not detected in CSF. Serologic test results were negative for *Borrelia* spp., *Listeria* spp., parvovirus B19, measles virus, and galactomannan. PCR results for CSF were repeatedly negative for herpes simplex virus, varicella zoster virus, cytomegalovirus, *Toxoplasma gondii*, JC polyomavirus, human herpesvirus 6, Epstein-Barr virus, and mumps virus. PCR results and culture were negative for *Mycobacterium* spp. Serum samples were negative for antibodies against neuronal nuclear (Hu, Ri, and Yo) antigens. However, enterovirus RNA was detected by reverse transcription PCR in all CSF samples. Sequencing of the virion protein 1 gene obtained directly from RNA extracted from CSF identified the virus as coxsackievirus A16 (*3*).

Computed tomography scan of the brain on day 2 showed no abnormalities. However, brain magnetic resonance imaging scans on the third and fourth days showed bilateral, multiple, hyperintense white matter lesions in the periventricular region and cerebral hemispheres. Treatment was started empirically with broad-spectrum antimicrobial drugs and acyclovir; the acyclovir was stopped after infection with herpes simplex virus was excluded.

On day 4, imaging indicated development of aphasia and right hemiparesis without new lesions. The patient was transferred for mechanical ventilation after a grand mal seizure. Treatment with intravenous immune globulin (IVIG, 400 mg/kg) was started on day 4 and given for 5 consecutive days, which resulted in marked and continued neurologic improvement. Monthly doses of IVIG (500 mg/kg) resulted in normal serum IgG levels. Four months after initial examination, the virus has been cleared but the patient still has intermittent confusion and language defects. Treatment with IVIG will be continued for an additional 6 months.

Use of MAbs against CD20 B-cell antigen has become standard treatment for B-cell lymphomas and an increasing number of autoimmune disorders (*4,5*). However, resulting hypogammaglobulinemia predisposes patients to opportunistic infections, including progressive multifocal leukoencephalopathy and enterovirus infections (*2,6*). MAbs with enhanced activity against CD20 (e.g., obinutuzumab) have been developed. Obinutuzumab has been approved by the US Food and Drug Administration for treatment of chronic lymphocytic leukemia. Studies regarding the use of obinutuzumab for other B-cell malignancies are ongoing (*7*).

We anticipate that more cases of enteroviral encephalitis might develop, given the increasingly frequent use of MAbs against CD20 and widespread occurrence of enteroviruses. However, in view of the few reported cases (*2*), we also suspect that many cases remain undiagnosed despite availability of several pan-enterovirus diagnostic kits, which can detect low viral loads. Thus, clinicians should be suspicious of severe enterovirus infections in patients receiving MAbs.

Any patient receiving MAbs against CD20 who has neurologic symptoms should be screened for infection with enterovirus RNA. In contrast to JC polyomavirus–associated progressive multifocal leukoencephalopathy, enteroviral encephalitis can be successfully treated by early administration of IVIG, which might contain neutralizing antibodies, albeit in variable amounts (*8*). In the absence of double-blinded, placebo-controlled clinical studies of treatment for severe enterovirus infections, no specific antiviral therapy has been approved. However, 3 capsid inhibitors (pleconaril, pocapavir [V-073], and the pirodavir analog BTA-798) that show activity against enteroviruses are being developed (*9*). Pocapavir has potent activity against poliovirus and appears to be safe and well tolerated (*10*). In the United States, this drug is available by special request from the Food and Drug Administration.

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References

1. Mao Q, Wang Y, Yao X, Bian L, Wu X, Xu M, et al. Coxsackievirus A16: epidemiology, diagnosis, and vaccine. Hum Vaccin Immunother. 2013;10. [Epub ahead of print].
2. Kassab S, Saghi T, Boyer A, Lafon ME, Gruson D, Lina B, et al. Fatal case of enterovirus 71 infection and rituximab therapy, France, 2012. Emerg Infect Dis. 2013;19:1345–7. http://dx.doi.org/10.3201/eid1908.130202
3. Thoelen I, Moës E, Lemey P, Mostmans S, Wollants E, Lindberg AM, et al. Analysis of the serotype and genotype correlation of VP1 and the 5′ noncoding region in an epidemiological survey of the human enterovirus B species. J Clin Microbiol. 2004;42:963–71. http://dx.doi.org/10.1128/JCM.42.3.963-971.2004
4. Griffin MM, Morley N. Rituximab in the treatment of non-Hodgkin's lymphoma: a critical evaluation of randomized controlled trials. Expert Opin Biol Ther. 2013;13:803–11. http://dx.doi.org/10.1517/14712598.2013.786698
5. Rosman Z, Shoenfeld Y, Zandman-Goddard G. Biologic therapy for

autoimmune diseases: an update. BMC Med. 2013;11:88 http://dx.doi.org/10.1186/1741-7015-11-88.

6. Casulo C, Maragulia J, Zelenetz AD. Incidence of hypogammaglobulinemia in patients receiving Rituximab and the use of intravenous immunoglobulin for recurrent infections. Clin Lymphoma Myeloma Leuk. 2013;13:106–11. http://dx.doi.org/10.1016/j.clml.2012.11.011

7. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med. 2014. [Epub ahead of print]. http://dx.doi.org/10.1056/NEJMoa1313984

8. Wildenbeest JG, van den Broek PJ, Benschop KS, Koen G, Wierenga PC, Vossen AC, et al. Pleconaril revisited: clinical course of chronic enteroviral meningoencephalitis after treatment correlates with in vitro susceptibility. Antivir Ther. 2012;17:459–66. http://dx.doi.org/10.3851/IMP1936

9. Abzug MJ. The Enteroviruses: problems in need of treatments. J Infect. 2014;68:S108–14. http://dx.doi.org/10.1016/j.jinf.2013.09.020

10. Hincks JR, Collet MS. Safety and pharmacokinetics of pocapavir, an oral antiviral candidate against poliovirus. In: Abstracts of the 53th Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, Colorado, Sep 10–13, 2013. Washington (DC): American Society for Microbiology; 2013. Abstract no. A-017d.

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Another Dimension

Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Serologic Evidence of Influenza A(H1N1) pdm09 Virus Infection in Northern Sea Otters

To the Editor: Sporadic epizootics of pneumonia among marine mammals have been associated with multiple animal-origin influenza A virus subtypes (*1–6*); seals are the only known nonhuman host for influenza B viruses (*7*). Recently, we reported serologic evidence of influenza A virus infection in free-ranging northern sea otters (*Enhydra lutris kenyoni*) captured off the coast of Washington, USA, in August 2011 (*8*). To investigate further which influenza A virus subtype infected these otters, we tested serum samples from these otters by ELISA for antibody-binding activity against 12 recombinant hemagglutinins (rHAs) from 7 influenza A hemagglutinin (HA) subtypes and 2 lineages of influenza B virus (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/20/5/13-1890-Techapp1.pdf). Estimated ages for the otters were 2–19 years (online Technical Appendix Table 2); we also tested archived serum samples from sea otters of similar ages collected from a study conducted during 2001–2002 along the Washington coast (*9*).

Of the 30 sea otter serum samples collected during 2011, a total of 21 (70%) had detectable IgG (≥200) for rHA of influenza A(H1N1)pdm09 virus (pH1N1) strain A/Texas/05/2009. Four of 7 serum samples that showed IgG ≥6,400 against pH1N1 rHA also showed low cross-reactivity (IgG 200) against rHA of A/Brisbane/59/2007, a previous seasonal influenza A(H1N1) virus (Figure, panel A; online Technical Appendix Table 1). No IgG was detected in any samples for any of the other 11 rHAs tested (IgG ≤100), and the sea otter serum samples collected during 2001–2002 did not react with any of the rHAs tested, including pH1N1 (IgG ≤100; Figure, panel A).

Next, we tested serum samples by using a hemagglutination inhibition (HI) assay with whole influenza virus to detect strain-specific antibodies that inhibit receptor binding. Of the 30 samples collected during 2011, a total of 22 (73%) showed HI antibody titers of ≥40 against pH1N1 virus. Titers against all other human and avian viruses tested were ≤10 for all samples by HI assay using turkey red blood cells (RBCs) (Figure, panel B; online Technical Appendix Table 3). No influenza A or B virus–specific HI antibodies were detected in the samples collected during 2001–2002 (data not shown). Although nasal swab specimens were collected from sea otters in the 2011 study, all specimens were negative for influenza virus by testing in embryonated eggs and by real-time PCR for detection of influenza A viral RNA (data not shown). These results suggest that sea otters were infected with influenza A virus sometime before the August 2011 sample collection date.

Although none of the 2011 samples showed HI titers to influenza A/duck/New York/96 (H1N1) virus (dk/NY/96) by testing using turkey RBCs (online Technical Appendix Table 2), titers against this strain were detected when using horse RBCs, which is a more sensitive means for the detection of mammalian antibodies against some avian influenza subtypes (*10*). Of the 22 samples that had HI titers ≥40 to pH1N1 virus, 16 also had HI titers ≥40 against dk/NY/96 by horse RBC HI assay (online Technical Appendix Table 2). However, titers against this strain were on average ≈4–8-fold lower than those for the pH1N1 virus strain, which suggests that that the titers against dk/NY/96 were the result of serologic cross-reactivity with avian- and swine-origin pH1N1 viruses.

To further test for cross-reactivity, 4 sea otter serum samples were