An unusual case of anti-\textit{Borrelia burgdorferi} immunoglobulin G seroconversion caused by administration of intravenous gammaglobulins

\textit{V. Luyasu}, S. Mullier, O. Baurain, and M. Dupuis

\textsuperscript{1}Service de Biopathologie et laboratoire de référence Borreliose de Lyme, \textsuperscript{2}Service de Pédiatrie and \textsuperscript{3}Service de Neurologie, Clinique Saint-Pierre, Groupe de Recherche et d’Information sur la maladie de Lyme (RILY), Avenue Reine Fabiola 9, Ottignies 1340, Belgium

\textsuperscript{*}Tel: +032/10 437160  Fax: +032/10 437188  E-mail: v.luyasu@intervweb.be

Administration of gammaglobulins to individuals without specific anti-\textit{Borrelia burgdorferi} antibodies may lead to immunoglobulin G (IgG) conversion as detected by enzyme-linked immunosorbent assay (ELISA). In some cases however, complementary techniques such as Western blot or avidity will be of prime importance in distinguishing the start of an infection from the passive immunization induced by the gammaglobulins. In all cases, the key element before reaching conclusions in relation to any of these investigations remains the confrontation between the clinical context and the biological findings. This is the scenario that has been followed in our observation.

\textit{Clin Microbiol Infect} 2001; 7: 697–699

\section{CLINICAL CONTEXT}

A 5-year-old child was admitted to the hospital presumptive with a diagnosis of meningitis. Over the previous 10 days, he had been suffering from pain in the legs, and during the last 48 h he had been vomiting, had experienced slight photophobia, had shown difficulty in walking, and had been in pain but not febrile. The parents reported no diarrhea and no gastroenteritis. Clinical examination revealed painful mobility of the neck. Seventy-two hours after admission, the child lost the osteo-tendinous reflex and developed speech difficulty. Lyme neuro-borreliosis was suspected even though there was neither history of a tick bite nor erythema migrans over the previous months.

The overall investigations for blood tests did not present anything particular. Prior to lumbar puncture, the brain and full spine magnetic resonance imaging with gadolinium was normal. The cerebrospinal fluid (CSF) results were as follows: leukocytes, 5/mm\textsuperscript{3}; erythrocytes, 1/mm\textsuperscript{3}; bacterial culture, sterile. Testing for anti-\textit{Borrelia burgdorferi} IgG and IgM antibodies in the CSF was negative with both ELISA and Western blot. However, ELISA for IgM antibody was positive in the first serum sample (Table 1). A search for \textit{B. burgdorferi} DNA in the CSF using the polymerase chain reaction (PCR) was negative. The CSF had an increased total protein of 227 mg/dL (normal value 0–45 mg/dL) and an increase in quantitative IgG and IgM proteins at 27 mg/dL (normal value 0–4 mg/dL), and 4.80 mg/dL (normal value 0–0.80 mg/dL), respectively. The glucose was slightly increased to 70 mg/dL (normal value 45–65 mg/dL) and the lactic acid was normal at 2.1 mmol/L (normal value 1.1–2.4 mmol/L).

To identify the potential cross-reactions, which may cause false-positive IgM with ELISA, we undertook in-depth study for syphilis and brucellosis and for infection with Epstein–Barr virus and \textit{Chlamydia}. All these investigations were negative.

Agarose electrophoresis of CSF performed simultaneously with the serum sample did not reveal any oligoclonal IgG pattern suggestive of intrathecal synthesis of IgG antibody.

Electromyography (EMG) showed that the nerve conduction velocities in the legs were clearly slowed down (32–37 m/s; normal value >45 m/s) while the latencies of both external popliteal sciatic nerves were strongly increased (8.9 and 10.5 ms; normal value <5 ms). This indicated an acute inflammatory demyelinating polyneuropathy.

In addition to these EMG abnormalities and the motor weakness, the increased total CSF protein, and an albuminocytological dissociation with few cells, were characteristic of Guillain–Barre syndrome. Until this diagnosis was established, no antibiotic treatment had been given for a presumptive neuroborreliosis. The patient was then treated for 5 days, from the 5th day following admission, with intravenous gammaglobulins (Sandoglobuline) at a dose of 400 mg/kg. This treatment was successful and the child improved within a few days. He recovered his mobility completely but remained under observation for several months. So far, after 2 years of follow-up, no relapse of the disease has been observed.
DISCUSSION

In our case, the diagnosis of neuroborreliosis was questionable since tests for the intrathecal synthesis of Borrelia burgdorferi-specific IgG and IgM antibody, and for borrelial DNA in the CSF by PCR were negative. In addition, the tests for specific IgG and IgM antibodies using Western blot were negative in the first serum sample. This created the need for sequential serum samples which could demonstrate a seroconversion after a lag phase. We now know that some new and promising immunoassays improve the reliability of antibody detection in the early phase [4–7]. None of these, however, were available to our laboratory.

Whilst the ELISA detected the IgM antibody in all three serum samples, yielding a strong suggestion of early disseminated borrelia infection [8], the Western blot on a second step, did not confirm the specificity of the antibody.

This strategy, based on Western blot as a second-tier test, is one recommended by the Centers for Disease Control (Rockville, USA) to resolve any positive or equivocal result found with a sensitive first-line ELISA assay [1]. In our endemic area, serological determinations of IgM-specific antibody with ELISA may be hampered by cross-reactions, which tend to decrease the positive predictive value of the test [9]. Cross-reactions are less frequent with Western blot because each antigen isolated from the spectrum of B. burgdorferi reacts individually, as opposed to the antigen mixture used in the ELISA [10]. Such cross-reactions emerge in clinical conditions when epitopes of B. burgdorferi show homology with virus or bacterial epitopes in the host. There may eventually be a stimulation of B lymphocytes by Epstein–Barr virus infection, leading to a polyclonal hyperactivity of B cells. Cross-reactions have also been reported from individuals living in tropical countries at a rate of 98% for ELISA and 57% for Western blot [11].

In our patient, the evidence for an IgG seroconversion was supported by the presence of IgG antibody in the second sample (day 9), as compared with the negative results of the first day after admission. Although on the one hand Western blot confirmed this seroconversion based on the IgG antibody, on the other hand only antigens indicative of old immunization, i.e. P100, P39 and P18, were detected. Indeed, any antigen like OspC, P41 or their combination with P18.5 and P18, which are specific of early localized or early disseminated disease, were missing [12]. In addition, our in-house technique of avidity demonstrated a maturation rate of IgG at 75 and 79%, on samplings of day 9 and 30, respectively.

To assess the extent to which the intravenous gammaglobulins were involved in the seroconversion, we investigated the corresponding concentration of anti-B. burgdorferi with ELISA and Western blot. The primary solution of gammaglobulins was diluted into a negative serum sample to 10 mg/mL; from this, the working solutions were 1:231 for IgG antibody and 1:42 for IgM antibody. As expected, IgG anti-B. burgdorferi was positive both with ELISA and Western blot and showed a characteristic high avidity value. Overall, data from intravenous gammaglobulins as well as from the sequential serum samples were the proof of past immunity relative to B. burgdorferi.

To evaluate the potential role of Campylobacter jejuni in the occurrence of the Guillain–Barré syndrome [13,14] – up to 36% of patients present significantly high titers of specific antibody – a complement fixation technique was requested from the National Reference Laboratory of the University Hospital, St. Pierre in Brussels (Professor J. P. Butzler, Dr. D. Van Beers and M. Duys). The antibody titers were low at 1/32, 1/32 and

Table 1: Serology of the serum samples and commercial gammaglobulins

<table>
<thead>
<tr>
<th>Tests</th>
<th>Cut off Units</th>
<th>Units in serum samples</th>
<th>Gammmoglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Admission</td>
<td>Day 9</td>
</tr>
<tr>
<td>IgG EIA*</td>
<td>&lt;6 UI/mL</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>IgM EIA*</td>
<td>&lt;0.34</td>
<td>0.66 (Pos)</td>
<td>0.62 (Pos)</td>
</tr>
<tr>
<td>IgG Western blotb</td>
<td>&lt;Score 6</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P18</td>
</tr>
<tr>
<td>IgM Western blotb</td>
<td>&lt;Score 6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgG avidityc</td>
<td>NA</td>
<td>75%</td>
<td>79%</td>
</tr>
</tbody>
</table>

*a Enzygnost Borreliosis, Dade Behring; *b Recomblot Borrelia IgG/IgM, Mikrogen [1,2]; *c House technique with Dade Behring avidity reagent on BEPIII [3].

NA, non-applicable; NT, not tested.
1/64 on days 1, 9 and 30, respectively. They were indicative of past immunity since no significant change was detected. The detection of HLA-B35, which is a potential marker for predisposition to a Guillain–Barre syndrome [15], was negative (Professor M. De Bruyère, Immunohematology Department, Faculty of Medicine, Catholic University of Louvain, UCL, Brussels). Stool culture with a view to detecting Campylobacter bacteria was not performed.

B. burgdorferi has been suspected to be associated with Guillain–Barre syndrome [16,17]. However, in our case diagnosis of Lyme borreliosis was definitely ruled out because the seroconversion of the specific IgG antibody was demonstrated as being due to passive immunization by the gammaglobulins. Such a serological pitfall has been more frequently observed in toxoplasmosis, from which one case reported in the literature involved a pregnant woman [18].

We should remember that Lyme borreliosis is a clinical diagnosis with a serological test for antibody to be used as just one piece of information in a complex picture. Also, this observation is an opportunity to emphasize the crucial integration of the clinical background with the serological profile in order to optimize the interpretation of all investigations.

REFERENCES