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CASE REPORT

Relapsing hepatitis due to cytomegalovirus?

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Summary

A case of agammaglobulinaemia with hyper IgM (the ‘hyper-IgM syndrome’) is described, in which a serological diagnosis of cytomegalovirus infection was made during repeated episodes of hepatitis. Three unrelated serological tests agreed with each other, but eventually it appeared that the reactions were non-specific. This shows the limitation of confirmation by tests which are used routinely.

Introduction

As a rule clinical virologists like their diagnoses to be based on two complementary approaches: on the one hand, the detection of virus by isolation, antigen detection or molecular probing, on the other hand, the demonstration of a serological response to the particular virus whose presence has been demonstrated. The value of this rule as a general one is illustrated by the following case report, which shows that serology has to be interpreted very cautiously in patients with some kind of humoral deficiency.

Case report

A 33-year-old woman, known in our hospital for many years to have a so-called ‘hyper IgM syndrome’ (high levels of IgM, virtual absence of IgA and IgG, but normal cellular immunity) presented with hepatitis. Nine months earlier, she had been admitted to another hospital because of jaundice and slight thrombocytopenia (100 x 10^9/l). Bilirubin was elevated (total, 512; direct, 174 µmol/l; normal, <10 µmol/l), as were alkaline phosphatase (276 U/l; normal, <120 U/l), ASAT (995 U/l; normal < 25 U/l) and gamma-GT (122 U/l; normal, < 35 U/l). Serology for hepatitis A, B and Epstein–Barr virus was negative, but IgM antibodies against cytomegalovirus (CMV) were detected. Mitochondrial, antinuclear, and smooth muscle antibodies were not found. The jaundice and liver abnormalities subsided spontaneously, but 4 months later a relapse occurred. Because IgM antibodies to CMV were again present in high concentration, she was considered to suffer from a relapsing CMV hepatitis. Urine cultures for CMV were negative. She was treated with Ganciclovir (250 mg b.d. for 35 days). Again there was improvement but this...
Table I Results of CMV serology

<table>
<thead>
<tr>
<th>Datum...</th>
<th>November 1988</th>
<th>March 1989</th>
<th>August 1989</th>
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<tr>
<td>Laboratory A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indirect ELISA*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Ig</td>
<td>≥ 3200</td>
<td>≥ 3200</td>
<td>—</td>
</tr>
<tr>
<td>Total IgM</td>
<td>≥ 1600</td>
<td>≥ 1600</td>
<td>—</td>
</tr>
<tr>
<td>Laboratory B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect immunofluorescence‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>—</td>
<td>—</td>
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<td>IgM</td>
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<tr>
<td>Laboratory C</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Indirect/direct ELISA‡</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IgG</td>
<td>—</td>
<td>—</td>
<td>Positive</td>
</tr>
<tr>
<td>IgM</td>
<td>—</td>
<td>—</td>
<td>Positive</td>
</tr>
<tr>
<td>IgA</td>
<td>—</td>
<td>—</td>
<td>Positive</td>
</tr>
<tr>
<td>Complement fixation test</td>
<td>—</td>
<td>—</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Virus neutralization test§</td>
<td>—</td>
<td>—</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

* Indirect ELISA, Organon Teknika, Boxtel, The Netherlands.
† Indirect immunofluorescence as described in reference 1.
‡ Indirect ELISA (IgG) and direct ELISA by antibody-capture technique.2
§ CMV strain AD169 was used and neutralization was scored as a reduction in fluorescent foci.3

Time liver function did not normalise (bilirubin: total, 386; direct, 270 μmol/l; alkaline phosphate, 328 U/l; ASAT, 408 U/l and gamma-GT, 75 U/l). For this reason the patient was transferred to our hospital, where we found a slightly enlarged liver, a firmly enlarged spleen and some ascites. Ultrasound showed a mobile stone in the gallbladder without evidence of obstruction or cholecystitis. Laboratory investigations showed bilirubin total, 16; direct 7 μmol/l; alkaline phosphate, 296 U/l; ASAT, 123 U/l and gamma-GT, 547 U/l. A liver biopsy showed marked cirrhosis and portal infiltration. Cultures from the liver biopsy, as well as repeated cultures from urine, remained negative for CMV. Serology again showed high levels of IgM, but also of IgA-antibodies against CMV (see Table I).1-3 Attempts were made to confirm the serology by immunoblotting. This showed a non-specific reaction pattern (Plate 1).4,5 Accordingly no antibody was detected by CMV-specific complement fixation or by virus neutralization.

Discussion

For a number of reasons we doubted whether these antibodies reflected CMV infection. Firstly, such an unusual course of CMV-hepatitis is not likely to occur in humoral immunodeficiency.6,7 Secondly, cultures for CMV were negative and the liver biopsy showed no histological evidence of CMV infection.

The decision to treat the patient with Ganciclovir was made after the results
Plate i. Immunoblotting was performed with the AD 169 strain of CMV, which was purified by sucrose gradient centrifugation. Electrophoresis was essentially as described by Laemmli\(^4\) and blotting as described by Dunn\(^5\). Strips were incubated overnight with sera in a 1:50 dilution. After washing, the strips were incubated for 2 h with alkaline phosphatase-labelled antisera to human IgG, IgM, IgA and IgE. Details of this procedure will be described in Henk P. Janssen, 'Rapid diagnosis of CMV infections in transplant patients', thesis, Nijmegen, the Netherlands, in preparation. C\(1\) and C\(2\), positive control sera (renal transplant patients). S\(1\) and S\(2\), sera from patient with hyper IgM syndrome. Lanes G, M, A, E, IgG-, IgM-, IgA- and IgE-specific antibodies.
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of laboratories A and B (see Table I) were obtained. The 16-fold increase in titre of IgM antibodies was taken as evidence of an active CMV infection. A false positive result was at that time considered unlikely. Firstly, rheumatoid factors, if present, were routinely removed by absorption. Secondly, IgG antibody was not present and could not interfere, and finally the indirect immunofluorescence test was in accord with the initial ELISA results.8

In our hospital it became clear that the serology was non-specific: it was a surprise to find completely non-specific patterns on immunoblotting (Plate 1). both ELISA and Western blot in our laboratory were performed with gradient-purified CMV as antigen. Further evidence for the non-specific character of the serology came from complement fixation and virus neutralization tests, both of which were negative.

Additional examination for IgM antibody against a whole series of viruses other than CMV were negative, and rheumatoid factor was not demonstrable.

In patients with hyper-IgM syndrome the high level of IgM is mainly the result of a non-specific polyclonal B-cell response. Some of the patients (including ours) show in addition a primary IgM response.6

It is not easy to explain the non-specific reactions. Rheumatoid factor, which in the presence of IgG antibodies can cause a false positive reaction in IgM tests, was not involved.8 Some kind of undefined 'stickiness' as has been reported for African sera which have also increased levels of IgM, might be the cause.9 However, it remains to be explained why the non-specific response was restricted to CMV.

This case demonstrates that serology has only limited value in the diagnosis of infection in agammaglobulinaemia. Although obvious, this is sometimes forgotten. Most patients have no antibodies of their own, but have antibodies from treatment with immunoglobulin injections.6,7 Because of this, other means should be sought to diagnose infection. This problem has rarely been discussed: a computer search over the last five years (National Library of Medicine, Medline) revealed only a single example of an agammaglobulinaemic patient on whom the diagnosis of an HIV infection was made by the polymerase chain reaction (PCR).10 Similarly, our patient was tested by PCR for hepatitis C infection and was found negative. There are several more conditions in which adequate formation of antibody fails or is strongly delayed, e.g. AIDS and bone marrow transplantation. Here the same problems will be encountered.

Usually a positive result in an indirect IgM test will be confirmed in a direct test, preferably an antibody capture assay because of its specificity.8 This procedure turned out to be of no use in this patient. Only a classical complement fixation assay and immunoblotting gave clear results. Thus routine serological tests cannot be used for confirmation.

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References


