Immunoglobulin G subclass antibodies to rubella virus in chronic liver disease, acute rubella and healthy controls

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Abstract

Ten patients with chronic liver disease, seven healthy seropositive individuals with a remote history of rubella, and three patients with acute rubella were examined for serum levels of IgG subclasses and subclass antibodies against rubella virus structural proteins. One patient with AICAH had no detectable total or rubella specific IgG3 or IgG4. The liver disease patients were hypergammaglobulinemic and had greatly raised IgG1 levels. Patients with acute rubella lacked antibodies to the rubella virus E2 protein and showed no IgG4 antibody response. The liver disease patients showed a somewhat weaker IgG4 antibody response against the core (C) protein than healthy controls. However, differences are suggested within the subclasses in antibody reactivity against the individual rubella virus antigens. It is concluded that test systems that discriminate reactivities against individual antigens have to be used for characterization of viral antibody subclass profiles.

Keywords: Immunoglobulin; IgG-subclass; Rubella virus; Liver disease

1. Introduction

Autoimmune chronic active hepatitis (AICAH) is a chronic progressive liver disorder which primarily affects women in their teens and around menopause. In liver biopsies the characteristic findings are periportal infiltration of lymphocytes and plasma cells, piecemeal necrosis of the liver parenchyma and gradually developing fibrosis [1]. High titres of circulating autoantibodies, notably anti-nuclear and smooth muscle antibodies, and elevated serum IgG levels are regularly found [2]. No pathogen has consistently been implicated in the aetiology of the disease. When hepatitis C virus (HCV) was identified, high prevalence of antibodies to this virus was reported in patients with AICAH [3]. When more specific anti-HCV antibody tests became available later, the earlier findings could not be confirmed except for a small subgroup of patients (AICAH type 2) having antibody to a liver-kidney microsome fraction [4]. In one study a high proportion of AICAH patients from Italy was shown to be anti-HCV positive while patients from the United Kingdom were not [5], a finding which may reflect different prevalences of HCV infection or population differences in autoantibody response [6].
Very high titres of antibodies to rubella and measles virus antigens are found in most cases of AICAH [7,8]. The antibody response is specific and directed against most of the structural proteins [9,10]. Recently it has been shown that high rubella virus antibody titres may occasionally also be observed in some other chronic liver diseases [11]. The cause of this enhanced antibody response to measles and/or rubella virus is not known.

Reports of measles virus RNA in peripheral blood lymphocytes have been conflicting [12,13]. Attempts to isolate rubella virus from blood lymphocytes and to detect viral RNA in liver biopsy from AICAH patients have hitherto been unsuccessful [12,14].

The availability of monoclonal antibodies in the 1980s has made it possible to study IgG subclass profiles in a number of virus infections including rubella [15–17]. The rubella studies have mostly been performed using crude antigens in an ELISA system, and patients with AICAH and other chronic liver diseases have not been included. Using Western blot or radio-immunoprecipitation the antibody response against individual structural rubella proteins can now be studied [10,18–20].

There are several reports on a possible association between IgG subclass deficiencies and autoimmune diseases, for review see [21,22]. It is, however, unclear whether the subclass deficiencies contribute to the pathogenesis as the number of subjects examined is small.

The aim of the present study was to see if the IgG subclass profiles of the enhanced rubella antibody response in patients with chronic liver diseases differed from that of healthy seropositives and patients with acute rubella. We also followed one patient from the clinical onset and for eight months with repeated testing. The IgG subclass reactivity was monitored against individual antigens (the two envelope glycoproteins E1 and E2, and the core protein C) separated by Western blot.

2. Materials and methods

2.1. Sera

Sera were obtained from 6 patients with AICAH, 2 with cryptogenic liver cirrhosis and 2 with alcoholic liver cirrhosis. All ten patients had rubella haemagglutination inhibition (HI) titres of ≥ 640 (geometric mean titre 905). For comparison, sera from 7 healthy seropositive (geometric mean HI titre 88) individuals and from 3 cases of acute rubella (all having an HI titre of 320) were included. The 7 seropositive healthy individuals were adults above 30 years of age with a history of rubella in childhood. Their sera were IgM antibody negative. The sera from cases of acute rubella were collected about 20 days after clinical onset and were IgM antibody positive. In addition we have investigated the IgG subclass pattern in a patient (a 15-year-old girl) with AICAH from the clinical onset and for about 8 months. This patient had an HI titre of 20480 and a serum IgG level of 94.3 g/l at onset [14].

2.2. Virus antigen

The virus antigen used in Western blot was semi-purified extracellular virions of the Therian strain of rubella virus kindly provided by Prof. A. Salmi, Turku, Finland.

2.3. Haemagglutination inhibition (HI)

The test was performed essentially as described [23] including preabsorption of sera with kaolin (Flow Laboratories, Irvine, UK) at pH 8.5, the use of trypsin treated human 0 erythrocytes and 4 haemagglutination units of antigen (Rubella Antigen for HIT, Behringwerke AG, Marburg, Germany). Gammaglobulin 16.5% (Kabi Pharmacia AB, Uppsala, Sweden) served as a positive control. The rubella HI titre of the gammaglobulin preparation was 1280.

2.4. Total IgG subclass test

Total IgG subclass determination was performed by single radial immunodiffusion using Human IgG subclass RID Combi-Kit from CLB (Central Laboratorium van de Bloedtransfusiedienst van het Neder-landse Rode Kruis. Amsterdam, The Netherlands).

One serum showed no IgG3 or IgG4. In order to verify these results the serum was examined by an ELISA test using monoclonal antibodies (NycoMed, Oslo).
2.5. SDS-PAGE

SDS-PAGE was done as described by Laemmli [24]. The viral antigen was denatured and reduced by boiling in 4% SDS and 5% 2-mercaptoethanol in the sample buffer. The stacking gel contained 4% acrylamide and the separating gel 12.5% acrylamide. The gels were run for 1 h in a mini-PROTEAN II apparatus (BioRad, California) at 30 mA constant current.

2.6. Dot-blot analysis of the HRP-conjugated anti-subclass antibodies for estimation of proper dilutions for immunoblotting

A modification of the antigen spot test was used [25]. Purified IgG1-IgG4 in two-fold dilutions were spotted onto PVDF membranes (1600 ng–12.5 ng) in PBS with 0.001% BSA. The strips were then incubated for 1 h in DS and washed 3 × 10 min with PBS-Tween 20. The HRP-conjugated monoclonal anti-human IgG subclass reagents were added in different dilutions. IgG1 (clone MH161-1, batch ME3(62.138)): 1/100, IgG2 (CDC clone HP6014, batch MH162-1-ME3): 1/50, IgG3 (clone MH163-1, batch ME41): 1/25 and IgG4 (clone mh164-4, batch ME4): 1/50. At these dilutions a minimum of 25 ng of the subclass antibodies could be detected.

2.7. Immunoblotting for detection of IgG subclass rubella antibodies

After electrophoresis the proteins in the SDS-PAGE gel were transferred to PVDF (poly-vinylidine difluoride)-membranes (Millipore Corporation, Bedford) in an electroblot apparatus (BioRad) at 30 V overnight in a carbonate buffer (10 mM NaHCO₃, 3 mM Na₂CO₃ and 20% methanol, pH 9.9). The membranes were cut into 4 mm wide strips and blocked for 1 h at room temperature in 5 × Denhardt solution (DS) [26] supplemented with 0.1% Nonidet P-40, 1.5% BSA (bovine serum albumin), 1 × borate-buffered saline (pH 8.2), 0.05% gelatin [27] and rabbit serum 1:40 (A. M. Szilvay, Bergen, personal communication). Each strip was incubated overnight without rocking in patient serum diluted 1:100 in DS without rabbit serum. After three washes with PBS containing 0.05% Tween 20, the strips were immersed in horseradish peroxidase (HRP)-conjugated monoclonal mouse anti-human IgG subclass reagents (Janssen Biochimica, Belgium) and incubated for 1 h at room temperature with rocking. The strips were then washed two times in PBS-Tween 20 and then two times in PBS. The bands were visualized by the addition of Enhanced Chemiluminescence (ECL) Western blotting reagents (Amersham, UK) and exposed to ECL film. Rubella antibody negative sera gave no bands by immunoblotting.

2.8. Quantitation of immunocomplexes by densitometric analysis

The developed ECL films after Western blotting were scanned by using Hoefer GS-300 Scanning Densitometer, and the data were analysed by the GS 365 data system. The peaks were integrated with Gaussian curve fit.

3. Results

3.1. Total serum IgG subclass contents

The single immunodiffusion method was used to quantitate total IgG subclass. All patients with liver disease had increased serum levels of total IgG1 (Table 1), the mean value being 4.3 times that of the healthy controls. The IgG2 level was slightly raised in the non-AICAH liver disease group while the IgG3 and IgG4 contents were within the normal range. One AICAH patient had no detectable total IgG3 or IgG4. This result was verified by an ELISA method.

3.2. Immunoblot analysis of IgG subclass antibodies to rubella virus proteins

3.2.1. Healthy seropositive individuals

When the amount of antibody was quantitated densitometrically the major relative differences were seen in the IgG4 antibody reactivities (Table 3). The IgG subclass antibody pattern of the healthy seropositive individuals differed from that of liver disease patients only by a relatively strong IgG4 anti C response and a weak anti-E1 response, opposite to the pattern found in the liver disease patients (Table 3). The most conspicuous differences from acute
rubella were the presence of anti-E2 in all healthy individuals, the stronger IgG1 anti-E1 and weaker anti-C response, and detectable anti-IgG4 reactivity.

3.2.2. Acute rubella patients

The three patients with acute rubella showed a low IgG1 anti-E1 reactivity, none of them had detectable IgG antibody against E2, and none of them showed IgG4 activity. All had IgG3 specific for the E1 and C rubella proteins.

3.2.3. Liver disease patients

All individuals with liver disease had IgG antibodies against the rubella virus antigens E1 and C (Table 2). About 60% had IgG2 antibodies against E1 and C, 30% against E2, and 80% had IgG3 antibodies against E1 and C, 70% against E2. IgG4 anti-E1 was absent in 3, anti-E2 in 9 and anti-C in 3 of the 10 patients examined. The relative IgG4 reactivities against E2 and C (Table 3) were weak in non AICAH patients, and no IgG4 anti-E2 was seen in the AICAH patients. The AICAH patient without detectable total IgG3 and IgG4, showed no IgG3 or IgG4 reactivity against rubella virus specific proteins. Otherwise all liver disease patients had detectable antibody subclass IgG.

We followed the IgG subclass antibody response of one patient from the clinical onset of AICAH and for about 8 months. At onset there was no IgG2 antibody against the three rubella virus structural proteins (Fig. 1A) but a strong IgG1 response against

<table>
<thead>
<tr>
<th>Category (no.)</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (7)</td>
<td>5.8 (2.9-7.5)</td>
<td>4.6 (1.8-8.7)</td>
<td>0.3 (0.2-0.3)</td>
<td>1.0 (0.2-1.4)</td>
</tr>
<tr>
<td>AICAH b (6)</td>
<td>22.6 (16.0-29.3)</td>
<td>3.1 (1.2-5.1)</td>
<td>0.5 (0.2-1.8)</td>
<td>0.6 (0.2-1.1)</td>
</tr>
<tr>
<td>Other liver diseases: c (4)</td>
<td>28.1 (17.3-10.5)</td>
<td>7.2 (3.5-11.3)</td>
<td>0.5 (0.2-0.9)</td>
<td>0.8 (0.2-2.1)</td>
</tr>
</tbody>
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\[^a\] Normal range (RID subclass kit, CLB; see Materials and methods).

\[^b\] AICAH = Autoimmune chronic active hepatitis.

\[^c\] Two patients with alcoholic liver disease and two patients with cryptogenic liver cirrhosis.
E1. After 14 days a weak IgG2 response against E1 showed up but could not be detected later. During the 8-month observation period there was a switch to an increased IgG4 and a decreased IgG3 anti-E1 response, while there was an increase in the IgG3 anti-C response. Antibodies to E2 were seen throughout, mainly in IgG1.

In summary, the main findings were high levels of total IgG1 in the hypergammaglobulinemic liver disease patients and lack of antibody response to the rubella virus E2 and absence of IgG4 rubella antibodies in acute rubella. The liver disease patients had somewhat lower level of IgG4 antibodies, and one patient with AICAH had no detectable total or specific IgG3 and IgG4.

4. Discussion

Immune deficiencies may be primary or they may be acquired due to immunosuppressive therapy, malnutrition or viral infection. Deficiencies of IgG result in frequent and serious infections, whilst deficiencies of individual IgG subclasses may lead to less florid infections. Isolated IgG2 deficiency is associated with recurrent respiratory infections in children [28] and poor antibody response to polysaccharide antigens [29,30]. Isolated IgG3 deficiency is the most common of the IgG subclass deficiencies (50–60%) and is found in adults and children with recurrent respiratory infections [28] and may be associated with IgG1, IgG2, IgA and C2 deficiency [31–33].

Fig. 1 IgG subclass antibody response to rubella virus structural proteins (E1, E2 and C) in one patient with AICAH from the clinical onset of the disease and for about 8 months. The bars represent the amount of antibodies expressed as arbitrary units after densitometrical recording of the Western blot strips.
Isolated IgG4 deficiency is associated with recurrent respiratory tract infections and may be associated with other Ig deficiencies, particularly IgG2 [34,35]. In the present work we have studied IgG subclass levels and rubella antibody reactivity in ten patients with chronic liver disease, six AICAH patients and two each with alcoholic and cryptogenic liver cirrhosis. They all showed high rubella antibody titres in the rubella haemagglutination inhibition (HI) test, about ten times that of healthy seropositives. Patients suffering from primary biliary cirrhosis (PBC) were not included since enhanced rubella and/or measles antibody responses have never been seen in this disorder [11]. All but one patient had subclass IgGs at levels above the minimal normal value (Table 1) and showed subclass antibody reactivity.

The most conspicuous finding was the very high IgG1 levels which accounted for the high total IgG seen in AICAH patients and in the presently examined non-AICAH cirrhosis patients. One patient with alcoholic liver cirrhosis had a somewhat elevated IgG2 level (11.3 g/l). Previously it has been reported that AICAH is associated with increase in IgG1 antibodies and a reduction in IgG4[36]. All AICAH patients in this study had high levels of IgG1 antibodies. One of these patients had no detectable total or rubella virus specific IgG3/IgG4 antibodies. IgG3 deficiency has been associated with increase in tendency of infection while lack of IgG4 antibodies may also be seen in healthy individuals [21].

No consistent changes of IgG subclass profiles have been described in chronic liver disease, but some patients with alcoholic liver disease may show an increase in all subclasses [37]. The cause of the hypergammaglobulinemias seen in various chronic liver diseases (raised IgG in AICAH and certain cirrhoses, IgM in PBC and IgA in alcoholic liver disease [38]) is not known, but T-lymphocyte dysfunction of B-lymphocyte regulation has been suggested [7,36,39,40]. IgG anti-viral antibodies are in general somewhat restricted to IgG1 and IgG3 [41–43]. Antibodies of the IgG4 subclass are less often found whilst those of IgG2 (characteristic of anti-polysaccharide antibodies) are rare [32,41]. Antibodies appearing during natural infections may be different from those seen after vaccination with the same virus, probably due to different doses and routes of administration and duration of exposure [34,44]. In one study of acute measles, specific IgG3 antibodies were not detected, a rather unusual finding in virus infections [45]. Also, healthy seropositive controls lacked specific IgG3 while IgG1 and IgG4 reactivities were seen.

In rubella virus infections responses have been demonstrated in IgG1 and IgG3 subclasses in an ELISA test (using whole rubella virus as antigen) [17]. Following both acute rubella infections and vaccination with live rubella virus [46] the IgG2 and IgG4 responses are reported to be weak. In the present study we also found significant IgG1 and IgG3 responses and no IgG4 response in the patients with acute rubella, but in addition there was a strong IgG2 reactivity against both E1 and C in one patient. No IgG antibody to E2 was found in the acute rubella sera. This was expected since the E2 antigen is the coating antigen in the passive haemagglutination test [47] which does not become positive until 2–3 weeks after the appearance of the rash and HI (anti-E1) antibodies [48].

In the sera from seropositive healthy individuals IgG4 reactivities were found against E1 and in particular against C indicating a switch to IgG4 when compared to acute rubella where IgG4 antibodies were not seen. While anti-C dominated the acute rubella response, comparable reactivities against E1 and C were seen in the healthy seropositives. These findings will, however, have to be corroborated as part of a larger scale investigation.

In the none-AICAH liver disease patients there was a low IgG4 antibody response against C compared to healthy controls and acute rubella patients (Tables 2 and 3). None of the liver disease patients exhibited the pattern of acute rubella with a lack of anti-E2. Also the patient which we followed for 8 months from clinical onset had E2 antibodies from the clinical onset. There was no other indication of a recent primary infection as the rubella IgM antibody test was negative [14]. The patient was apparently in a hyperimmunoreactive state with an extremely high serum IgG content (94.3 g/l) at the time of clinical onset. This patient showed a fairly good IgG4 anti-C response throughout the observation period in contrast to the other liver disease patients.

Obviously the patterns of antibody reactivity is not quite uniform in liver disease patients and may
depend on the clinical stage of the disease and treatment with immunosuppressive drugs. The antibody profiles obtained in the present study using Western blot show a complex reactivity pattern. Extended patient materials as well as longitudinal studies are required to allow more definite conclusions about the relation between subclass reactivity and liver disease. However, our results clearly show the necessity of studying IgG subclass antibody reactivities by means of methods that discriminate antibody specificities. Significant differences in specific reactivities may otherwise be masked.

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References


