ANTIBODY RESPONSES AGAINST EM18 AND EM16 
SERODIAGNOSTIC MARKERS IN ALVEOLAR AND CYSTIC 
ECHINOCOCCOSIS PATIENTS FROM NORTHWEST CHINA

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(Received December 19, 1996. Accepted January 17, 1997)

SUMMARY: Western blot analysis was carried out in order to evaluate new 
serodiagnostic markers, Em18 and Em16, for differentiation of alveolar 
echinococcosis (AE) from cystic echinococcosis (CE) using 36 serum samples from 
hydatid patients from Xinjiang, China, where AE and CE are both endemic and 
one double infection case has been reported. All AE cases except one (5/6) who ex-
hibited a calcified lesion and a single case of double infection showed antibody 
responses against Em18 and Em16. Some of CE patient sera (6/22) showed
antibody response against Em16 except one who showed that against Em18. Analyses of IgG subclass responses against Em18 and Em16 were carried out using all serum samples showing antibody responses against Em18 and/or Em16 (seven CE, five AE, and one AE + CE) and additional samples of three CE and 22 AE from Sichuan, China. IgG4 was the most predominant antibody subclass. Em18 and Em16 were recognized by both IgG4 and IgG1 (in most cases) or by either IgG4 or IgG1 (in minor cases) or by IgG3 (in very rare cases). Neither Em18 nor Em16 was recognized by IgG2 antibodies. The usefulness of Em18 and Em16 as potential new markers for serological differentiation of human AE and CE, respectively, is discussed.

INTRODUCTION

Alveolar echinococcosis (AE), caused by the fox tapeworm, *Echinococcus multilocularis* in the larval stage, is one of the most lethal helminthic infections of man and is often misdiagnosed as hepatic cancer. Recently, we reported that antibody responses against previously undescribed protein markers, designated Em18 and Em16, which were demonstrable by Western blotting, were good serologic markers for [1] differentiation of AE from other parasitoses including cystic echinococcosis (CE), caused by the dog tapeworm, *E. granulosus* in the larval stage, and [2] differentiation of active from inactive cases of AE (1,2). Other recent studies utilizing Western blotting have confirmed that Em18 is the most species-specific epitope for immunodiagnosis of human AE (3,4). In the present work, we tried to further evaluate the criterion for differentiation of AE from CE using 36 Chinese serum samples assayed blind with only information that some were from AE patients including one double infection case of AE and CE from Xinjiang, northwest China, where AE and CE occur sympatrically (5-7) and to analyze IgG subclass responses against Em18 and Em16 using AE and CE serum samples from Xinjiang (described above) and Sichuan, China (2). There are several reports stressing that IgG4 is the most predominant subclass in chronic helminthic infections including human CE and AE (3,8-11).

MATERIALS AND METHODS

*Serum samples:* For a blind test to differentiate serum samples of AE cases and/or an AE + CE double infection case from unknown samples, 36 Chinese serum samples, selected from those collected from clinical patients, confirmed in
Urumqi, Xinjiang, China, were shipped to Japan with the information that they included some AE cases and one case of confirmed double infection (12). All AE, CE and AE+CE patients were diagnosed by imaging analysis by ultrasonography and CT at first and all except one were confirmed by surgery and histopathology in Urumqi. There was no more clinical information on these patients except that some of them had calcified lesions. For analysis of IgG subclass, serum samples showing antibody responses against Em18 plus Em16 or Em18 only or Em16 only confirmed by Western blotting were examined: seven CE, five AE, and one AE+CE from 36 serum samples from Urumqi, Xinjiang (Table I), and three CE and 14 AE from Chongqing, Sichuan (2) and additional eight AE for follow-up study of prognosis from Chongqing, Sichuan (13).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting: SDS-PAGE and Western blotting were carried out in Japan, mainly at Gifu University, as described previously with commercially available precast isocratic 18% gels for two dimensions (#01-109, SDS-PAGE Mini, TEFCO, Tokyo) and a crude protoscolex extract of Echinococcus multilocularis originally obtained from one Japanese patient in Nagoya (1,2). Electrophoretic transfer of the antigens of lower molecules including Em18 and Em16 onto Immobilon (Millipore, Bedford, MA) membranes was carried out according to the previous work (2). Immobilon membranes with 6-cm width were each cut into 30 strips of 2-mm width and 2 cm length or shorter and used for Western blot analysis. Western blot analyses were carried out using 1/50 dilution serum samples, at least in duplicate. For detection of antibody responses against Em18 and Em16, Japanese and Chinese AE patients' pooled serum and a monoclonal antibody against Em16 were used as standard and positive controls, respectively (1,2). Antibody responses against Em18 and/or Em16 were assessed by using peroxidase-conjugated anti-human IgG (Cappel, Cochranville, PA) at 1/1,000 dilution. IgG subclass responses were analyzed using monoclonal antibodies against human IgG subclasses (G1, G2, G3, and G4, Zymed, San Francisco, CA) at 1/500 dilution (8). Peroxidase-conjugated anti-mouse IgG (Cappel) was used at 1/1,000 dilution for detection of monoclonal antibody against Em16 (1,2). Criterion of AE or CE diagnosis was based on the antibody responses against Em18 and Em16 by Western blotting: i.e., "+/+ or +/−" and "−/+" in "Em18/Em16" were judged as AE and CE, respectively (1).

RESULTS

Immunoblot Evaluation of Em18 and Em16

Based on the clinical information, the 36 serum samples included seven AE, 21 CE, one AE plus CE [double infection with E. multilocularis and E. granulosus
and seven others (not AE nor CE). The results of 36 serum samples' antibody responses against Em18/Em16 and the confirmed clinical diagnoses with some modification due to Japanese group's inquiries (see Discussion) are summarized in Table I: five of the seven AE sera showing antibody responses against Em18 and Em16 were judged as AE but the remaining two showing no antibody response against Em18 were judged as other than AE or inactive AE with calcified lesion, i.e., one had a calcified lesion and the other was not AE but CE (Table I). One case with confirmed double infection of *E. multilocularis* and *E. granulosus* (12) showed antibody responses against both Em18 and Em16 as typical AE cases. One of CE sera was misjudged as AE with antibody response against Em18. Other six CE patient sera showed antibody response against Em16 only. The remaining 14 (finally 15) CE sera showed no antibody response against Em18 or Em16 as was the case for the seven others.

*IgG Subclass Responses against Em18 or Em16*

Sera from five AE cases, one double infected case and seven CE cases from Urumqi, Xinjiang who showed antibody responses against Em18 and/or Em16 (Table I) and 22 AE and three CE cases from Chongqing, Sichuan (2) (see Materials and Methods) were examined to determine which IgG subclass responded against Em18 and/or Em16. A summary is shown in Table II. In the 10 CE cases, only three responded against Em18, whereas nine responded against Em16. One CE case from Chongqing, which showed antibody responses against Em18 and Em16 exceptionally and misdiagnosed as AE (2), recognized Em18 by IgG1 but recognized Em16 by IgG4. One CE case from Urumqi responded against Em16 by IgG3. In contrast, most of AE cases responded against both Em18 and Em16 by IgG4 and IgG1. One AE case from Chongqing (2) responded against both Em18 and Em16 by IgG3 plus IgG1. Another AE case from Chongqing (2) responded against Em18 by IgG1 plus IgG4 but simultaneously responded against Em16 by IgG4, IgG1 plus IgG3.
Table I. Antibody responses against Em18 and/or Em16 and confirmed clinical diagnoses of 36 serum samples from Urumqi, Xinjiang, China

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Antibody responses against Em18/Em16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>6 AE</td>
<td>5</td>
</tr>
<tr>
<td>1 AE+CE</td>
<td>1</td>
</tr>
<tr>
<td>22 CE</td>
<td>0</td>
</tr>
<tr>
<td>7 others</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>: with almost calcified lesion, <sup>b</sup>: misjudged as AE, <sup>c</sup>: one CE was included in AE at first and later confirmed.

Table II. Summary of IgG subclass responses in immunoblot against Em18 and Em16 in 10 CE, 27 AE and one AE + CE cases in northwest China

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>IgG subclasses responding against Em18</th>
<th>IgG subclasses responding against Em16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10 CE</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>27 AE</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1 AE+CE</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>: from Xinjiang, <sup>b</sup>: from Sichuan, <sup>c</sup>: including one case from Sichuan showing antibodies of IgG<sub>1</sub> + G<sub>4</sub> + G<sub>3</sub>. 
DISCUSSION

The present results strongly support the observation that Em18 is a good marker for immunoblot differentiation of human AE from CE (2-4). Two AE cases showing no response against Em18 were speculated by the Japanese group that these two were probably not active AE but due to AE with calcified lesions only (= inactive AE) or possibly CE infection. Re-evaluation of the clinical background information in UK revealed that one was AE with almost calcified lesion, which could still have some viable parasite tissues, whereas the other was CE but misclassified and informed to the Japanese group as AE by the same family name who had AE. Previous observations with Alaskan AE cases only with calcified lesions did not exhibit antibody responses against Em18 or Em16 (8), whereas all AE cases with active lesions from Japan and China so far examined and most of the Alaskan AE cases with active lesions showed antibody responses at least against Em18 (1,2,8). The patient with confirmed double infection of *E. multilocularis* and *E. granulosus* (12) showed serum antibody responses against Em18 plus Em16 similarly to other typical cases of AE. This was not so curious since most CE cases showed no antibody response against Em18 or Em16 (1,2). However, one case of CE showed antibody response against Em18 only. If this patient had no AE lesion(s), this is the first CE case showing antibody response against Em18 without response against Em16. Recent testing of confirmed CE patient sera from Uruguay or Libya, where no AE has been reported, did not find any indication of anti-Em18 responses (3,4). Therefore, perhaps this Chinese CE case which showed a positive antibody response against Em18 might have been exposed to *E. multilocularis* as well as *E. granulosus*. Further evaluation is necessary for such CE patients whose serological characteristics indicate antibody response against Em18.

Information on the IgG subclass antibody responses (Table II) revealed that IgG1 and IgG4 were the most predominant subclasses responding to Em18 and Em16 as previously reported (3,4,8). The IgG subclasses in most CE cases, which responded against Em16, were either IgG1 or IgG4 or both IgG1 and IgG4, whereas those in most of the AE patient sera were IgG1 with IgG4. One CE case from Urumqi showed an IgG3 response against Em16 exclusively, whereas one AE case from Chongqing (2) showed strong IgG3 as well as IgG1 responses against both Em18 and Em16. There was no AE nor CE case showing IgG2 response against Em18 or Em16. As Em18 and Em16 appear not to include polysaccharide epitopes (Ito et al., unpublished data), it may be rational that IgG2 does not re-
spond against Em18 or Em16 (9). Further analysis of IgG subclass responses against Em18 and/or Em16 in AE and CE is necessary to evaluate the correlation between the IgG subclass responses and the clinical features. Most recent reports (3,4) on Chinese (Xinjiang), French, Japanese, Uruguayan and Libyan sera revealed that IgG4 and IgG1 were the most dominant in AE and CE but no case exhibited IgG3 or IgG2 responses in Western blotting. Therefore, the fact that one AE and one CE from Sichuan, China, showed IgG3 responses against *Echinococcus* antigens appeared to be interesting.

Based on the present results and other reports on Em18 (1-4,8), we conclude that Em18 is the most specific native antigen of *E. multilocularis* in Western blot analysis and detection of antibody response against Em18 is highly useful for immunodiagnosis of AE. In contrast, Em16 is shared between *E. multilocularis* and *E. granulosus* and antibody response against Em16, in the absence of response against Em18, appear unique to CE, as we previously speculated (2). Therefore, differentiation between AE and CE may be achieved in the majority of cases through immunoblot analysis of differential antibody binding to Em18 and Em16 in *E. multilocularis* protoscolex extracts. Em18 and Em16 are, therefore, expected to be good candidate markers for establishing simple and sensitive means for serodiagnosis of AE and CE, respectively.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ohyama Health Foundation, the Uehara Memorial Foundation, the Nissan Science Foundation and Grants-in-aid (06044089, 07044243) for Science Research (International Collaboration Project) from the Ministry of Education, Science, Sports and Culture, Japan (Monbusho) to A. Ito. We are also grateful to the Wellcome Trust (UK), the European Commission (STD2-TS20056) and the British Council's Academic Link's in the China Scheme for their support in part to H. Wen and P. S. Craig.
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