Antigens for the immunodiagnosis of *Echinococcus granulosus* infection: An update

David Carmena *, Aitziber Benito, Elena Eraso
Department of Immunology, Microbiology and Parasitology, Faculty of Pharmacy, University of the Basque Country, Vitoria, Spain

Received 8 November 2005; received in revised form 12 January 2006; accepted 7 February 2006
Available online 9 March 2006

Abstract

The taeniid tapeworm *Echinococcus granulosus* is the causative agent of the echinococcal disease, an important zoonosis with worldwide distribution. Accurate immunodiagnosis of the infection requires highly specific and sensitive antigens to be used in immunodiagnostic assays. The choice of an appropriate source of antigenic material is a crucial point in the improvement of the diagnostic features of tests, and must be based on the developmental stage of the parasite and the host. The most common antigenic sources used for the immunodiagnosis of echinococcal disease are hydatid cyst fluid, somatic extracts and excretory-secretory products from protoscoleces or adults of *E. granulosus*. Hydatid cyst fluid is the antigenic source of reference for immunodiagnosis of human hydatidosis, which is mainly based on the detection of antigens B and 5. Somatic extracts have been widely used in the serodiagnosis for *E. granulosus* infection in dogs and ruminant intermediate hosts, although in the last few years the detection of excretory-secretory products of the worm in faeces (coproantigens) have become the most reliable method for the detection of the parasite in the definitive host. This review emphasizes recent advances in the identification and characterization of novel antigens with potential for the immunodiagnosis of echinococcal disease. Progress in recombinant technologies and synthetic peptides are also discussed. The paper highlights the need to search for new antigenic components with high diagnostic sensitivity and specificity, a fact that remains a crucial task in the improvement of the immunodiagnosis of the disease.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *Echinococcus granulosus*; Echinococcal disease; Cystic echinococcosis; Intestinal dog echinococcosis; Immunodiagnosis

1. Introduction

Echinococcal disease is a zoonosis caused by the taeniid tapeworm *Echinococcus granulosus*. The adult worm lives in the small intestine of a carnivore (definitive host), and the intermediate larval stage can infect a wide range of mammal species – including humans – that acquire the infection through accidental ingestion of eggs. The domestic life cycle is maintained through dogs and ungulates, mainly sheep and cattle. The disease has a worldwide distribution, with a considerable impact in both human and animal health and causing important socio-economic consequences in endemic areas (Schantz et al., 1995).

In man, early detection of the infection can provide substantial improvements in the quality of the management and treatment of the disease. Diagnosis is currently based on identification of cyst structures by imaging techniques (ultrasoundography, computed tomography, X-rays) and confirmation by immunodiagnostic tests,
predominantly ELISA and immunoblotting (Eckert and Deplazes, 2004). Hydatid cyst fluid has been used as a main antigenic source for the primary immunodiagnosis of human cystic echinococcosis (CE) and also for the follow up of patients after surgical or pharmacological treatment (Ortona et al., 2003). However, this extract exhibits some problems, including lack of sensitivity, specificity, and difficulties with the standardization of its use. Particularly problematic is its cross-reactivity with sera from individuals infected by other helminths, mainly E. multilocularis and Taenia solium (Leggatt et al., 1992). It has been suggested that serodiagnosis of CE may be improved by use of recombinants proteins or by combining several defined antigens, including synthetic peptides (Zhang et al., 2003). Nonetheless, to date there is no standard, highly sensitive and specific test available for immunodiagnosis of human CE (Le et al., 2003).

Accurate diagnosis of the infection in the definitive host plays a central role in epidemiological studies and for surveillance of hydatid control programmes (WHO, 2001). Serum antibodies against the adult parasite were used to detect the intestinal stage of the infection in dogs. Somatic extracts of protoscoleces or adult worms have been the antigenic sources most commonly used for this purpose. However, ELISA results showed highly variable sensitivities, and cross-reactivity with other parasite species has been frequently reported (Jenkins et al., 1990). Currently, diagnosis of intestinal dog echinococcosis is mainly based on the detection of excretory-secretory products of the parasite in fecal samples (coproantigens) using ELISA. This technique considerably improves the sensitivity and specificity obtained with traditional serological tests, allowing the detection of the parasite even during the prepatent period (Fraser and Craig, 1997; Jenkins et al., 2000).

Serodiagnosis of CE in sheep, the main intermediate host of E. granulosus, has been carried out using hydatid cyst fluid and crude parasite antigens (Lightowlers and Gottstein, 1995; Kittelberger et al., 2002). However, poor antibody responses to infection and cross-reactions with other taeniid species (mainly Taenia hydatigena and Taenia ovis) are often obtained. Therefore, detection of specific serum antibodies in sheep is less useful than in human infection.

In the last few years, an important effort has been made in order to obtain recombinant proteins, synthetic peptides or combinations of well-defined antigens that enhance diagnostic specificity (Zhang et al., 2003). However, the use of recombinant components still exhibit low diagnostic sensitivities in some cases (see below). Because of this, the characterization of new antigens and the standardisation of the techniques and antigen preparations currently available remain the most important tasks to be undertaken in order to improve the immunodiagnosis of CE.

2. Hydatid cyst fluid antigens

Hydatid cyst fluid (HCF) is a complex mixture of glyco and lipoproteins, carbohydrates and salts. Some of its components come from the host (mainly albumin and immunoglobulins), with the remaining products the result of the metabolic activity of the metacestode. HCF is considered the main antigenic source for the immunodiagnosis of human CE. For clinical practises crude HCF has a high sensitivity, ranging typically from 75% to 95% (Zhang et al., 2003). However, its specificity is often unsatisfactory and cross-reactivity with sera from patients infected with other cestode (89%), nematode (39%) and trematode (30%) species is commonly observed (Eckert and Deplazes, 2004). Because of this, crude HCF is specially recommended for mass serological screening (WHO, 2001), and it has now become more frequent to purely components such as the lipoproteins antigen B and antigen 5, the most relevant components of HCF for diagnostic purposes.

2.1. Antigen B (AgB)

AgB (Oriol et al., 1971) is a strongly immunogenic polymorphic protein of 120–160kDa that dissociates under reduced conditions into 8/12, 16, and 20/24kDa subunits, suggesting that it is composed of multimers of 8kDa subunits (Lightowlers et al., 1989). The role of AgB in the parasite biology is not completely elucidated, but it seems to be involved in the modulation of the host immune response. Thus, AgB has been reported as a protease inhibitor that inhibits neutrophil chemo- taxis (Shepherd et al., 1991), promotes a non-protective Th2 immune response (Riganò et al., 2001) and induces immune cells apoptosis in patients with active disease (Riganò et al., 2002). Recent investigations have suggested that AgB could also be involved in detoxifying mechanisms, sequestering and buffering xenobiotics (Chemal et al., 2005). Immunoblot detection of the smallest subunit (8kDa) of AgB has proved the most reliable tool in diagnostic studies (Ortona et al., 2000). Nonetheless, 18% of sera from CE patients do not produce detectable specific serum antibodies, and 39% of alveolar echinococcosis patients cross-react with this component (Maddison et al., 1989).

Molecular studies have demonstrated that AgB is encoded by a multigene family that is variably expressed, with at least five major gene clusters named EgAgB1...
by synthetic peptides (Barbieri et al., 1998; González-
ogenicities of AgB, efforts have been made to define
promising task that must be undertaken in the future.
serological profile for IgG subclass antibodies compared
the immunodiagnostic test used, as CE patients detected
in community screening studies may have a different
IgG4 responses occurred when cysts were degenerated
IgG1, IgG2, and
with cyst development, growth, and disease progression
EgAgB1 and EgAgB2 have been cloned, expressed as
human CE are pre-
EgAgB5 (Haag et al., 2004). However, recent phylogenic analyses failed to discriminate between iso-
forms EgAgB3 and EgAgB5 (Haag et al., in press). The
putative protein isoforms encoded by the five EgAgB
genes differ 44–81% in amino acid sequence, and the
switch from one isoform to another has been proposed as
a new mechanism to evade the host’s immune response
(Haag et al., 2004). Similarly, AgB is also expressed
in E. multilocularis, where five cDNAs encoding 8 kDa
subunit monomers of EmAgB (named as EmAgB8/1 to
EmAgB8/5) have been described (Manuti et al., 2004,
in press). EmAgB8/1-EmAgB8/4 showed more than 90% homology at both nucleotide and amino acid levels with
that of EgAgB1-EgAgB4, respectively, while the remain-
ing clone EmAgB8/5 represents a novel gene encoding
another 8 kDa subunit monomer of EmAgB. The fact
that these isoforms have been isolated from vesicles,
protozoa, and immature adult worms suggests a dif-
ferential expression throughout the developmental stages
of E. multilocularis (Manuti et al., 2006). To date, only
EgAgB1 and EgAgB2 have been cloned, expressed as
recombinant proteins and used for diagnosis. Of them,
EgAgB2 has shown the best features in terms of diag-
nostic efficiency, significantly higher than native AgB
(Virginio et al., 2003). Interestingly, a clear predomi-
nance of IgG4 response for both native and recombinant
AgB preparations have been observed in serum of CE
patients, suggesting that this may be the isotype of choice
to be assessed in serodiagnostic tests (McVie et al., 1997;
Virginio et al., 2003). In agreement with this, Daeki et
al. (2000) showed that an IgG4 response was associated
with cyst development, growth, and disease progression
cyst types I, II, and III), whereas the IgG1, IgG2, and
IgG3 responses occurred when cysts were degenerated or
Table 1.
In ruminants, but the
Diagnostic performances of the native and recombi-
nant AgB and the synthetic peptides derived from this
molecule in the immunodiagnosis of human CE are pre-
sented in Table 1. AgB has also been used in serological tests for
the detection of E. granulosus in ruminants, but the
results appear to be contradictory: Ibrahem et al. (1996)
obtained diagnostic sensitivities of 90% and 25% when
native and recombinant AgB were assayed in ELISA
with sheep sera, respectively. In a subsequent study
performed with a major number of serum samples,
Kittelberger et al. (2002) found very poor sensitivity
(11.2%) using native AgB. The authors indicated that
these differences may be related to the E. granulosus
strain involved, and considered that AgB is not a suit-
able antigen in the immunodetection of the parasite in
ruminants because of its low diagnostic sensitivity.
2.2. Antigen 5 (Ag5)
Ag5 (Capron et al., 1967) is a thermolabile and
highly immunogenic protein composed of 57 and 67 kDa
components (Di Felice et al., 1986) that under reduc-
ing conditions dissociates into 38 and 22–24 kDa sub-
units (Lightowers et al., 1989). The biological role of
this molecule is almost completely unknown, although
its elevated concentration in HCF suggests a relevant
the petide p176 has demonstrated highly valuable diag-
nostic features (González-Sapienza et al., 2000; Lorenzo
et al., 2005a). This peptide, derived from the sequence of
EgAgB1, has a 12-mer N-terminal fragment that appears
to be a major immunodominant epitope. The region
comprises the stretch EVYFERR (corresponding to
residues 17–24), and in particular Glu17, Lys19, Tyr20,
Glu21, and Arg26 have been found to be the most import-
(González-Sapienza and Cachau, 2003).
Recent studies have shown that the combination of
several well-defined antigens, including synthetic pep-
tides, may improve the performance of the immunoas-
says by reducing the number of false negative reactions
(Barbieri et al., 1998). In this way, González-Sapienza
et al. (2000) observed that a number of additional AgB
epitopes, with potential for increasing the diagnostic
sensitivity, appear to be discontinuous in nature. These
epitopes cannot be mimicked by linear peptides, and their
identification would be only possible by combinatorial
methods. This fact suggests that the same phenomenon
may occur in other well-known antigens, so the synthe-
sis and diagnostic evaluation of random peptides derived
from these molecules may be promising approaches that
must be taken into consideration in order to improve the
serodiagnosis of CE.
Diagnostic performances of the native and recombi-
nant AgB and the synthetic peptides derived from this
molecule in the immunodiagnosis of human CE are pre-
sented in Table 1. AgB has also been used in serological tests for
the detection of E. granulosus in ruminants, but the
results appear to be contradictory: Ibrahem et al. (1996)
obtained diagnostic sensitivities of 90% and 25% when
native and recombinant AgB were assayed in ELISA
with sheep sera, respectively. In a subsequent study
performed with a major number of serum samples,
Kittelberger et al. (2002) found very poor sensitivity
(11.2%) using native AgB. The authors indicated that
these differences may be related to the E. granulosus
strain involved, and considered that AgB is not a suit-
able antigen in the immunodetection of the parasite in
ruminants because of its low diagnostic sensitivity.
2.2. Antigen 5 (Ag5)
Ag5 (Capron et al., 1967) is a thermolabile and
highly immunogenic protein composed of 57 and 67 kDa
components (Di Felice et al., 1986) that under reduc-
ing conditions dissociates into 38 and 22–24 kDa sub-
units (Lightowers et al., 1989). The biological role of
this molecule is almost completely unknown, although
its elevated concentration in HCF suggests a relevant

(76)
Table 1

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. of subjects tested</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cross-reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native AgB</td>
<td>210</td>
<td>IgG ELISA</td>
<td>63</td>
<td>81</td>
<td>AE, Cys., Schis.</td>
<td>McVie et al. (1997)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>90</td>
<td>IgG ELISA</td>
<td>77</td>
<td>86</td>
<td>AE</td>
<td>Barbieri et al. (1998)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>90</td>
<td>IgG ELISA</td>
<td>77</td>
<td>86</td>
<td>AE</td>
<td>González-Sapienza et al. (2000)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>204</td>
<td>IgG ELISA</td>
<td>73</td>
<td>100</td>
<td>None</td>
<td>Ortona et al. (2000)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>31</td>
<td>IgG ELISA</td>
<td>77</td>
<td>82</td>
<td>AE, Schis., T Ashton</td>
<td>Rott et al. (2000)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>129</td>
<td>IgG ELISA</td>
<td>60</td>
<td>93</td>
<td>Cys., T ox.</td>
<td>Virginio et al. (2003)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>59</td>
<td>IgG ELISA</td>
<td>80</td>
<td>77</td>
<td>AE, Cys.</td>
<td>Lorenzo et al. (2000)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>175</td>
<td>IgG IB</td>
<td>92</td>
<td>71</td>
<td>AE</td>
<td>Jin et al. (1999)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>87</td>
<td>IgG IB</td>
<td>66–73</td>
<td>98</td>
<td>AE, Cys.</td>
<td>Poretti et al. (1999)</td>
</tr>
<tr>
<td>Native AgB</td>
<td>204</td>
<td>IgG IB</td>
<td>73</td>
<td>100</td>
<td>None</td>
<td>Ortona et al. (2000)</td>
</tr>
<tr>
<td>rAgB8/1</td>
<td>210</td>
<td>IgG4 ELISA</td>
<td>65</td>
<td>91</td>
<td>AE</td>
<td>McVie et al. (1997)</td>
</tr>
<tr>
<td>rAgB8/1</td>
<td>31</td>
<td>IgG ELISA</td>
<td>84</td>
<td>91</td>
<td>Cys.</td>
<td>Virginio et al. (2003)</td>
</tr>
<tr>
<td>rAgB8/1</td>
<td>59</td>
<td>IgG ELISA</td>
<td>68</td>
<td>88</td>
<td>AE, Cys.</td>
<td>Lorenzo et al. (2000)</td>
</tr>
<tr>
<td>rAgB8/2</td>
<td>204</td>
<td>IgG IB</td>
<td>72</td>
<td>100</td>
<td>None</td>
<td>Ortona et al. (2000)</td>
</tr>
<tr>
<td>p176</td>
<td>31</td>
<td>IgG ELISA</td>
<td>84</td>
<td>98</td>
<td>Schis., T ox.</td>
<td>Rott et al. (2000)</td>
</tr>
<tr>
<td>p177</td>
<td>59</td>
<td>IgG ELISA</td>
<td>45</td>
<td>86</td>
<td>AE, Cys.</td>
<td>Lorenzo et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>44</td>
<td>96</td>
<td>AE, Schis., T ox.</td>
<td>Lorenzo et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>49</td>
<td>94</td>
<td>AE, Schis., T ox.</td>
<td>Gonzalez-Sapienza et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>80</td>
<td>93</td>
<td>AE, Schis., T ox.</td>
<td>Gonzalez-Sapienza et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>59</td>
<td>IgG ELISA</td>
<td>63</td>
<td>83</td>
<td>AE, Cys.</td>
<td>Lorenzo et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>38</td>
<td>92</td>
<td>AE, Schis., T ox.</td>
<td>Gonzalez-Sapienza et al. (2000)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>12–18</td>
<td>96–100</td>
<td>AE</td>
<td>Barbieri et al. (1998)</td>
</tr>
<tr>
<td>pGlu4</td>
<td>90</td>
<td>IgG ELISA</td>
<td>18</td>
<td>98</td>
<td>AE, Schis., T ox.</td>
<td>Gonzalez-Sapienza et al. (2000)</td>
</tr>
</tbody>
</table>

rAgB: recombinant AgB; p-: synthetic peptide; IB: immunoblotting; AE: alveolar echinococcosis; Cys.: cysticercosis; Onch.: onchocerciasis; Schis.: schistosomiasis; T ox.: toxocariasis.

Function in the development of the metacestode. The clonal sequence of the 38 kDa subunit indicates it is closely related to serine proteases of the trypsin family, but the catalytic Ser192 is substituted by Thr and no peptidase activity was found in in vitro assays. The 22 kDa subunit has heparan sulphate proteoglycans and calcium binding sites, suggesting that the molecule provides interactions with cell surfaces and the extracellular matrix (Lorenzo et al., 2003). Studies by sequencing of the N-terminal fraction of the 38 kDa subunit revealed a single amino acid sequence with alternative residues at some positions, demonstrating that Ag5 is present in different isoforms (Zhang and McManus, 1996). This fact suggests that Ag5, similar to AgB, may be encoded by a multigene family that is variably expressed, although this hypothesis must be confirmed with further molecular studies.

Ag5 has been widely used in the serodiagnosis of human CE, particularly by means of the identification of a precipitation line (arc 5) in immunoelectrophore-
sis assays. However, 10–40% of surgically confirmed hydatid patients have no detectable anti-Ag5 antibodies in ELISA assays (Schantz and Gottstein, 1986). In addition, the antigen has been reported to cross-react with antibodies in sera from patients with other cestode, trematode, and nematode infections (Yarzabal et al., 1977; Di Felice et al., 1986). Part of this cross-reactivity was associated with the presence of phospholipidic choline bound to the 38 kDa subunit (Shepherd and McManus, 1987; Lightowlers et al., 1989). In recent studies using ELISA, native Ag5 has evidenced diagnostic sensitivities and specificities ranging between 50–54% and 89–92%, respectively (Barbieri et al., 1998; González et al., 2000). These authors also identified and cloned a metacestode-specific novel component (named P29) immunologically related to, but distinct from, Ag5. This finding would imply that much of the information derived from studies carried out using antibodies to Ag5 could be equivocal as a result of the cross reactivity between both Ag5 and P29. All these data demonstrate that Ag5 is less useful than AgB for diagnostic purposes.

The full-length gene of Ag5 has been recently cloned using sequence information derived from internal fragments of the antigen (Lorenzo et al., 2003). Two recombinant forms corresponding to the unprocessed polypeptide chain of the antigen (rAg5) and its 38 kDa subunit (rAg5-38s) have been evaluated in serological tests, showing a markedly decreased diagnostic performance than that of the parent protein (Lorenzo et al., 2005b). In addition, Chamekh et al. (1992) reported the use of the synthetic peptide 89–122 in serology, as it mimicks an epitope of Ag5. The authors found a diagnostic sensitivity of 85%, and cross-reactivity with sera from patients with alveolar echinococcosis only. Nevertheless, the same synthetic peptide provided two- to three-fold lower sensitivities in later studies (Barbieri et al., 1998; González et al., 2000), indicating that results can vary enormously depending on the panel of patients sera used. This fact highlights the need to use the same panel of sera to effectively compare the diagnostic value of the different antigens available, as recommended by Lightowlers and Gottstein (1995).

Diagnostic performances of the native and recombinant Ag5 and Ag5-derived synthetic peptides in the immunodiagnosis of human CE are presented in Table 2.

### 2.3. Other antigens from HCF

Although AgB and Ag5 are the best characterised antigens for diagnostic purposes, other components from HCF have been evaluated in serological tests by different research groups. Al Yaman and Knobloch (1989) identified a protein of 48 kDa (Eg48) that provided a diagnostic sensitivity and specificity of 100% and 82%, respectively. In a further study, Kanwar et al. (1992) reported the recognition by immunobloting of a 116 kDa component with high diagnostic performances that, under reducing conditions, dissociates into 75, 66, and 45 kDa subunits. The circulating form of the antigen (free or

---

### Table 2

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. of subjects tested</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cross-reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE: CE patients; Patients with other diseases; Healthy subjects.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native Ag5</td>
<td>90</td>
<td>88</td>
<td>28</td>
<td>IgG ELISA</td>
<td>50</td>
<td>92</td>
</tr>
<tr>
<td>Native Ag5</td>
<td>87</td>
<td>289</td>
<td>200</td>
<td>Arc5</td>
<td>58–63</td>
<td>97</td>
</tr>
<tr>
<td>Native Ag5</td>
<td>39</td>
<td>51</td>
<td>29</td>
<td>IgG ELISA</td>
<td>54</td>
<td>89</td>
</tr>
<tr>
<td>rAg5</td>
<td>34</td>
<td>36</td>
<td>18</td>
<td>IgG ELISA</td>
<td>65</td>
<td>89</td>
</tr>
<tr>
<td>rAg5-38s</td>
<td>34</td>
<td>36</td>
<td>18</td>
<td>IgG ELISA</td>
<td>21</td>
<td>97</td>
</tr>
<tr>
<td>pAg5-122</td>
<td>40</td>
<td>52</td>
<td>10</td>
<td>IgG, A, M ELISA</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>pAg5-122</td>
<td>90</td>
<td>88</td>
<td>28</td>
<td>IgG ELISA</td>
<td>16–23</td>
<td>77–100</td>
</tr>
<tr>
<td>pAg5-122</td>
<td>39</td>
<td>51</td>
<td>29</td>
<td>IgG ELISA</td>
<td>44</td>
<td>100</td>
</tr>
</tbody>
</table>

*Ag5*: recombinant Ag5; *p*—synthetic peptide; *Arc5*: immunoprecipitation of the arc5; *AE*: alveolar echinococcosis; *Cys.*: cysticercosis.
immune-complexed) was also identified in 85% of the sera from CE patients tested (Kanwar and Vinayak, 1992). Antigenic components of 100 and 130 kDa, probably related to the 116 kDa antigen described by Kanwar et al. (1992), were also identified in camel HCF by Shambesh et al. (1995). These molecules showed a diagnostic sensitivity of 94%, although cross-reactivity with sera from alveolar echinococcosis and cysticercosis patients were found. However, the low number of sera analyzed in these studies does not allow the obtaining of decisive results.

3. Protoscolex and adult somatic antigens

Initial immunological responses to *E. granulosus* in the definitive host are directed against infective protoscoleces and later, against the adult parasite. Because of this, somatic extracts from protoscoleces and adults have been the most suitable source of antigens in the immunodetection of the infection in dogs and other canids. However, performances of the serological tests when using these antigenic preparations have been hampered by several methodological problems. ELISA results showed highly variable sensitivities, ranging from 40 to 90% (Jenkins et al., 1990; Gasser et al., 1994). These studies also demonstrated that 25–60% of the sera from dogs infected with *E. granulosus* did not show significant levels of specific antibody, and revealed cross-reactivity with other parasite species (Gasser et al., 1988). In addition, antigenic differences have been found in protoscoleces from different species of intermediate hosts (Rafiei and Craig, 2002). Several causes have been proposed to explain the low levels of specific serum antibodies, like sequestration of antibodies and formation of circulating immunocomplexes (Gasser et al., 1993), parasite’s immune evasion mechanisms (Gasser et al., 1994), low immune response of the host (Gasser et al., 1988, 1993) or host nutritional status (Gasser et al., 1992). To help address these issues, simultaneous detection of serum antibodies and circulating antigens has been suggested as an alternative to improve the diagnostic sensitivity of the assays (Spinelli et al., 1996).

Previous studies demonstrated that antigenic components of 27 and 94 kDa from crude protoscolex extracts were specifically identified by 95% and 62% of dog sera experimentally infected with *E. granulosus*, respectively (Gasser et al., 1989, 1992). Other polypeptides with molecular masses of 43, 35, 20, and 14 kDa demonstrated different levels of cross-reactivity with sera from dogs infected with other cestodes or nematodes. However, these results have not been corroborated in further studies using a larger number of sera.

Due to the lack of specificity and sensitivity of the antigens currently available, serological tests are considered unreliable for the detection of *E. granulosus* in the definitive host. In an attempt to improve this situation, the secreted protein 14-3-3 (Siles-Lucas et al., 2000), the fatty acid-binding protein EgDf1 (Chabalgoity et al., 2000), and the fibrillar protein EgA31 (Saboulard et al., 2003), all antigens derived from the *E. granulosus* adult worm, have been expressed. These recombinant proteins exhibited strong immunogenic properties in dogs experimentally infected with the parasite. The evaluation of their potential use in developing new diagnostic tools and candidate vaccines against the echinococcal disease in the definitive host are currently under study.

Diagnostic performances of the crude extracts and the recombinant proteins of protoscoleces in the serodiagnosis of the echinococcal infection in the definitive host are presented in Table 3.

In the last few years, a considerable amount of work has been done in order to evaluate the potential of

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. of dogs tested</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cross-reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPx</td>
<td>30</td>
<td>IgG ELISA</td>
<td>97</td>
<td>100</td>
<td>None</td>
<td>Gasser et al. (1990)</td>
</tr>
<tr>
<td>SPx</td>
<td>50</td>
<td>IgG ELISA</td>
<td>40</td>
<td>70</td>
<td>-</td>
<td>Jenkins et al. (1990)</td>
</tr>
<tr>
<td>SPx</td>
<td>78</td>
<td>IgG ELISA</td>
<td>76</td>
<td>98</td>
<td>T. hyd., T. vul</td>
<td>Gasser et al. (1992)</td>
</tr>
<tr>
<td>SPx</td>
<td>228</td>
<td>IgG, A, E ELISA</td>
<td>73–84</td>
<td>97–100</td>
<td>–</td>
<td>Gasser et al. (1993)</td>
</tr>
<tr>
<td>SPx</td>
<td>75</td>
<td>IgG, A, E ELISA</td>
<td>61</td>
<td>97</td>
<td>–</td>
<td>Gasser et al. (1994)</td>
</tr>
<tr>
<td>SPx</td>
<td>36</td>
<td>IgG ELISA</td>
<td>80</td>
<td>92</td>
<td>Taenia spp.</td>
<td>Benito et al. (2001)</td>
</tr>
<tr>
<td>r10P1</td>
<td>30</td>
<td>IgG ELISA</td>
<td>20</td>
<td>100</td>
<td>None</td>
<td>Gasser et al. (1990)</td>
</tr>
</tbody>
</table>

SPx: crude protoscolex extract; r-: recombinant protein; E.g.: *Echinococcus granulosus*; T. hyd.: *Taenia hydatigena*; T. vul.: *Taenia vulpis*.

* Dogs without *E. granulosus* infection.
Table 4

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. of subjects tested</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cross-reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CE patients</td>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAd</td>
<td>137 28 11</td>
<td>IgG ELISA</td>
<td>82</td>
<td>65</td>
<td>Taenia spp., Cys.</td>
<td>Ersfeld et al. (1997)</td>
</tr>
<tr>
<td>SPx</td>
<td>137 28 11</td>
<td>IgG ELISA</td>
<td>90</td>
<td>48</td>
<td>Taenia spp., Cys.</td>
<td>Ersfeld et al. (1997)</td>
</tr>
<tr>
<td>rAg4</td>
<td>28 29 28</td>
<td>IgG ELISA</td>
<td>90</td>
<td>57</td>
<td>AE, Try.</td>
<td>Rafiei and Craig, 2002</td>
</tr>
<tr>
<td>rEgcMDH</td>
<td>129 65 203</td>
<td>IgG ELISA</td>
<td>90</td>
<td>95</td>
<td>Cys.</td>
<td>Virginio et al. (2003)</td>
</tr>
<tr>
<td>rEgcCalP2</td>
<td>129 65 203</td>
<td>IgG ELISA</td>
<td>84</td>
<td>97</td>
<td>Cys., Try.</td>
<td>Virginio et al. (2003)</td>
</tr>
<tr>
<td>rEpAFFP</td>
<td>129 65 203</td>
<td>IgG ELISA</td>
<td>59</td>
<td>96</td>
<td>Cys., Try.</td>
<td>Virginio et al. (2003)</td>
</tr>
<tr>
<td>rEpC1</td>
<td>324 502 70</td>
<td>IgG IB</td>
<td>92</td>
<td>96</td>
<td>AE, Cys.</td>
<td>Li et al. (2003)</td>
</tr>
<tr>
<td>rTPElg</td>
<td>100 218 20</td>
<td>IgG IB</td>
<td>39</td>
<td>69</td>
<td>AE, Cys.</td>
<td>Li et al. (2004)</td>
</tr>
</tbody>
</table>

SAd: crude adult worm extract; SPx: crude protoescolex extract; r-: recombinant protein; AE: alveolar echinococcosis; Cys.: cysticercosis; Fil.: filariasis; Schis.: schistosomiasis; Try.: trypanosomiasis.

Protoscolex or adult-derived antigens in the serodiagnosis of human CE (Table 4). Although crude extracts exhibit poor specificity (Rafiei and Craig, 2002), several recombinant proteins have evidenced high diagnostic performances, particularly rEpC1 (Li et al., 2003) and rEgcMDH (Virginio et al., 2003). rEpC1 is a truncated sequence from *E. granulosus* protoscoleces that encodes a 8.4 kDa polypeptide, and rEgcMDH is a cestode cytosolic malate dehydrogenase homologue. Both recombinant antigens were tested using a large panel of serum samples and showed high levels of sensitivity and specificity. The evaluation of the diagnostic potential of these molecules for the detection of *E. granulosus* in the definitive and other intermediate hosts is still to be determined.

In contrast with human and dogs, very little research has been done to date to apply protoscoleces-derived antigens for the serodiagnosis of *E. granulosus* infection in ruminants, despite the fact that protoscoleces are part of the maturing hydatid cysts. Specific serum antibodies levels in naturally infected sheep are often low, probably because the immunological responses are inadequate in many animals or they are not maintained throughout the course of the infection (Lichtowlers and Gottstein, 1995). Additionally, serological cross-reactions between *E. granulosus* and other cestodes (mainly *T. hydatigena* and *T. ovis*) limit the specific diagnosis of the infection (Yong et al., 1984). In a study using a large panel of 1261 sheep sera, Kitchberger et al. (2002) found that crude protoscoleces extract exhibits a diagnostic sensitivity and specificity of 65% and 98%, respectively, which is considerably more effective than AgB and the recombinant ECG95 oncosphere protein. The authors indicated that this antigenic preparation should be useful for the detection of the presence of infected sheep on a flock basis, but not for identification of individual animals infected with *E. granulosus*. To date, no antigenic extract has exhibited satisfactory sensitivity and specificity for routine ovine serodiagnostic use.

### 4. Protoscolex and adult excretory-secretory products

Very little research has been directed towards the description of excretory-secretory products (ESP) from *E. granulosus* protoscoleces and adult worms. Recently, Carmena et al. (2004) carried out the characterization of the protoscoleces ESP, identifying 20 major protein components by SDS-PAGE. The extract showed phosphatase, lipase, and glucosidase enzymatic activities, but no protease activity could be detected. Two new *E. granulosus* antigens with molecular masses of 89 and 74 kDa were specifically recognized by sera from patients with hydatidosis, and are potential candidate diagnostic antigens in the immunodiagnosis of human CE. Cross-reactivity studies using immunoblot-inhibition and ELISA-inhibition assays have shown that protoscoleces ESP share a high proportion of antigenic components with protoscoleces somatic extracts, and to a lesser extend with hydatid cyst fluid (Carmena et al., 2005b).

Protoscolex and adult ESP have also been assayed as antigenic sources in the serodiagnosis of intestinal dog echinococcosis using ELISA and immunoblotting.
When adult ESP was tested with sera from dogs naturally infected with *E. granulosus*, three components larger than 94 kDa, two triples of 68/94 and 39/43 kDa, and seven proteins less than 30 kDa in size were identified (Gasser et al., 1992). Using protoscolex ESP, Carmena et al. (2005a) found seven antigenic components ranging in size from 46 to 130 kDa, with the 89 and 50 kDa polypeptides showing promising features as diagnostic antigens. However, these data must be corroborated in further studies with a larger panel of dog sera.

During the last two decades, the detection of *E. granulosus* in the definitive host has been mainly based on the detection of parasite ESP in faecal samples (coproantigens) by ELISA using antibodies against adult somatic antigens (Allan et al., 1992), and excretory-secretory products from proglottids (Deplazes et al., 1992) or protoscolecies (Benito and Carmena, 2005). This technique has demonstrated to be the most reliable tool currently available for epidemiological purposes, improving considerably the diagnostic features of the traditional serological tests (Craig et al., 1995; Fraser and Craig, 1997). Additionally, the test is capable of detecting prepatent period as early as 7 days p.i. in experimental infections (Ahmad and Nizami, 1998), and ELISA results correlate well with the worm burden in the dog intestine (Craig et al. 1995; Ahmad and Nizami, 1998). ELISA values decrease to negative levels 2–4 days after the elimination of the parasite, allowing the accurate determination of the current status of infection in the dog (Jenkins et al., 2000).

Table 5 summarizes the diagnostic performances of protoscolex and adult ESP in the immunodiagnosis of intestinal dog echinococcosis.

### 5. Oncosphere antigens

Oncospheres are the infective stage of *E. granulosus* in the intermediate host. Therefore, an elevated immune response against oncosphere-derived antigens is expected in the early stages of the infection. Gasser et al. (1991) identified three major components in oncosphere extracts using sera from dogs experimentally infected with *E. granulosus*. These polypeptides (37, 30, and 22 kDa) were apparently oncosphere-specific and the authors proposed their use for serological discrimination between prepatent and patent infection in dogs. In the same way, Rogan et al. (1992) found that some infected and uninfected people from hydatid endemic areas had a higher anti-oncospheral antibody level than people from non-endemic regions, suggesting the possibility of using this antigenic extract in epidemiological surveys. Later work demonstrated strong antibody responses against decrease to negative levels 2–4 days after the elimination of the parasite, allowing the accurate determination of the current status of infection in the dog (Jenkins et al., 2000).

<table>
<thead>
<tr>
<th>Antigen antigenum</th>
<th>No. of dogs tested</th>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cross-reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With E.g.</td>
<td>Dogs with other infections</td>
<td>Helminth-free dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES Ad.</td>
<td>78</td>
<td>51</td>
<td>95</td>
<td>IgG ELISA 81</td>
<td>94</td>
<td><em>T. hyd.</em></td>
</tr>
<tr>
<td>Anti-S Ad.</td>
<td>15</td>
<td>27</td>
<td>30</td>
<td>CpaAg-ELISA 87</td>
<td>96</td>
<td><em>Taenia</em> spp.</td>
</tr>
<tr>
<td>Anti-ES Ad.</td>
<td>25</td>
<td>155</td>
<td>37</td>
<td>CpaAg-ELISA 10–87</td>
<td>98</td>
<td><em>T. hyd.</em></td>
</tr>
<tr>
<td>Anti-ES Ad.</td>
<td>41</td>
<td>177</td>
<td>24</td>
<td>CpaAg-ELISA 29–92</td>
<td>97</td>
<td><em>Taenia</em> spp.</td>
</tr>
<tr>
<td>Anti-S Ad.</td>
<td>26</td>
<td>–</td>
<td>–</td>
<td>CpaAg-ELISA 77</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MoAb EmA9</td>
<td>4</td>
<td>83</td>
<td>11</td>
<td>CpaAg-ELISA 100</td>
<td>96</td>
<td><em>T. hyd.</em>, nematodes</td>
</tr>
<tr>
<td>Anti-S Ad.</td>
<td>34</td>
<td>60</td>
<td>21</td>
<td>CpaAg-ELISA 61–100</td>
<td>91</td>
<td><em>D. c., Taenia</em> spp.</td>
</tr>
<tr>
<td>Anti-ES Pn.</td>
<td>37</td>
<td>97</td>
<td>66</td>
<td>CpaAg-ELISA 78</td>
<td>93</td>
<td><em>D. c.</em></td>
</tr>
<tr>
<td>Anti-S Ad.</td>
<td>20</td>
<td>24</td>
<td>31</td>
<td>CpaAg-ELISA 100</td>
<td>98</td>
<td><em>T. hyd.</em>, <em>D. c.</em></td>
</tr>
</tbody>
</table>

ES: excretory-secretory products; S: somatic extract; Ad.: adults; Pn.: protoscoleces; MoAb: monoclonal antibody; CpaAg: ELISA for detection of coproantigens; *T. hyd.*: *T. hydatigena*; *D. c.*: *Dipylidium caninum*.

* Depending on the parasite burden.
purified oncosphere proteins in sera from experimentally infected sheep (Heath and Lawrence, 1996). Following this approach, a recombinant antigen vaccine was developed for use in ruminant intermediate hosts of *E. granulosus* (Lightowlers et al., 1996). The recombinant antigen, termed EG95, was cloned from mRNA obtained from the parasite oncospheres. The vaccine has been shown to be highly effective in animal trials (96–98% protection in immunized sheep), with almost complete immunity persisting for more than a year after vaccination (Lightowlers and Heath, 2004). In an attempt to identify the location of host-protective epitopes, truncated forms of EG95 have been prepared and tested for their ability to elicit host-protective responses (Woollard et al., 2001). Although each of the truncated forms of EG95 induced specific antibodies, none of them were able to elicit protective immune responses, suggesting that the host-protective epitopes of EG95 are conformational. This fact demonstrates that the major proportion of the protective efficacy of EG95 requires the intact molecule.

The gene encoding EG95, designated *eg95-1*, belongs to a small gene family which includes five other members that are also transcribed in the oncosphere (Chow et al., 2001). It has been suggested that the identification of the expressed protein in life-cycle stages other than the oncosphere may allow the development of potential vaccines against different stages of the parasite’s life cycle (Chow et al., 2004).

EG95 has also been used as a diagnostic antigen in serological tests for the detection of *E. granulosus* in sheep (Kittelberger et al., 2002), but a very poor diagnostic sensitivity was obtained (5.2%). This suggests that there are few specific serum antibodies present in the naturally infected sheep, demonstrating that EG95 is an unsuitable diagnostic marker of the disease in sheep.

6. Antigenicity associated to carbohydrates

The parasite cuticle surface is known to be largely composed of a carbohydrate-rich coat also known as the glycocalix. Glycoconjugates are highly immunogenic and are important mediators of host-parasite interactions (Dell et al., 1999). Carbohydrates have been characterized in *E. granulosus* metacestode (Dennis et al., 1993), protoscoleces (Khou et al., 1997; Baz et al., 1999), and adult (Casaravilla et al., 2003). These molecules are highly immunogenic, being able to elicit specific antibody responses in mice (Miguez et al., 1996) and infected humans (Sterla et al., 1997). Furthermore, *E. granulosus* carbohydrates are responsible for immunological cross-reactions with sera from patients infected with other helminths (Sterla et al., 1997).

A number of *E. granulosus* antigens have well characterized glycosylate moieties. The HCF antigen 5 has complex oligosaccharides covalently linked to its polypeptide backbone (March et al., 1991), and studies using chemical deglycosylation have demonstrated that saccharidic epitopes are the major immunodominant determinants of the antigen (Lorenzo et al., 2005b). The blood group P1 epitope (Galβ1-3GalNAc) has been identified in the protoscoleces tegument and the laminated layer of the cyst membrane (Makni et al., 1992). More recently, the carcinoma-associated Tn antigen (GalNAc-O-Ser/Thr) has been found to be expressed in both larval and adult worm preparations, with the highest levels observed in the adult excretory-secretory extract (Alvarez-Eitzico et al., 2001). High levels of circulating Tn antigen were also detected in serum samples from patients with hydatidosis.

Recent studies carried out by Elayoubi et al. (2003) and Elayoubi and Craig (2004) demonstrated that *E. granulosus* excretory-secretory products in faeces from dogs naturally or experimentally infected with the parasite comprised carbohydrates with α-L-fucose and/or β-galactose, β-galactose and N-acetyl-β-glucosamine residues. In coproantigen ELISA these carbohydrate moieties were associated with a significant proportion of the coproantigen activity. Two highly glycosylated fractions were identified by liquid chromatography, with different large molecular masses of >670 and 146–440 kDa, respectively. A glucidic molecule of 155 kDa was revealed by the hyperimmune anti-whole worm IgG in immunoblotting, suggesting that may be derived from the glycocalix of adult worms. These findings demonstrate the involvement of carbohydrate epitopes in coproantigen antigenicity, highlighting the necessity of a further characterization of the corresponding glycoproteins.

It is well recognised that parasite glycosylated moieties from excretory-secretory extracts (Lightowlers and Rickard, 1988) or associated to the tegument (Blaxter et al., 1992) are key modulators involved in processes like the suppression of the host immune response, the mimicry of host components, or the induction of type-1/type-2 cytokine responses. In *E. granulosus*, Dematteis et al. (2001) observed that the carbohydrate-rich fraction obtained from protoscoleces (named E4+) was involved in the modulation of the cellular immune response in experimental mice infection by stimulating IL-10 secretion and later induction of the type-2 cytokine response. A closely related molecule to E4+ in terms of antigenic
properties and functions has been identified in *E. multilocularis* by Walker et al. (2004).

7. Conclusions and perspectives

The main problems in the immunodiagnosis of echinococcal disease are the often unsatisfactory performances of the available tests and the difficulties associated with the standardisation of antigen preparations and techniques. The search of highly sensitive and specific antigens represents the greatest challenge to overcome these inconveniences. Therefore, during the last three decades an extensive effort has been made in order to characterize highly antigenic components derived from different developmental stages of *E. granulosus* to be used in serodiagnostic tests. As evident from the recent literature, recombinant proteins and synthetic peptides are proved to be more reliable for immunodiagnostic purposes than native antigens and their purified sub-units/fractions. Two are the reasons: firstly, recombinant antigens typically evidence higher diagnostic performances than their homologous native proteins. Secondly, recombinant technology can produce well defined polypeptides in large quantity, allowing the standardisation of the antigen source. Currently, a number of recombinant proteins are available as candidate antigens for the immunodetection of *E. granulosus*, mainly in man. Despite the unquestionable progress accomplished in the last years, much work must be carried out hereafter in order to improve the serodiagnostics of echinococcal disease. It will be interesting to evaluate novel native antigens, recombinant proteins, and synthetic peptides (individually or in combination) for their potential in the detection of different developmental stages of the parasite. Special attention deserves the identification and characterization of highly protective molecules, in order to develop vaccines against the infection. An effective vaccine against *E. granulosus* infection in the definitive host would imply a crucial step in the implementation of hydatid control programmes. In addition, research has to be directed to elucidate and/or clarify the biological role of those antigens, and the molecular basis by which these molecules are expressed and regulated. Finally, the identification of new antigen sources and molecules with high diagnostic features always remains an important task that have to be undertaken in order to maintain the progress in the immunodiagnosis of echinococcal disease.

Acknowledgements

The authors thank Prof. Peter Deplazes (Institute of Parasitology, University of Zurich, Switzerland), and Drs. Sonja Kock, Danielle Smyth, and David Guiliano (Department of Biological Sciences, Imperial College London, UK) for their critical revision of the manuscript.

References

Carmena, D., Martínez, J., Benito, A., Guisantes, J.A., 2005b. Shared and non-shared antigens from three different extracts of the...


D. Carmena et al. / Acta Tropica 98 (2006) 74–86