Journal of Clinical Microbiology (2009) 47(10):3191-3196.

Serological Monitoring of Progression of Alveolar Echinococcosis with Multiorgan Involvement by Use of Recombinant Em18

Ishikawa, Yuji ; Sako, Yasuhito ; Itoh, Sonoyo ; Ohtake, Takaaki ; Kohgo, Yutaka ; Matsuno, Takeo ; Ohsaki, Yoshinobu ; Miyokawa, Naoyuki ; Nakao, Minoru ; Nakaya, Kazuhiro ; Ito, Akira

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2009, p. 3191–3196 Vol. 47, No. 10
0095-1137/09/$08.00+0 doi:10.1128/JCM.01111-09

Serological Monitoring of Progression of Alveolar Echinococcosis with Multiorgan Involvement by Use of Recombinant Em18[▽]

Yuji Ishikawa,[1,2] Yasuhito Sako,[1] Sonoyo Itoh,[1] Takaaki Ohtake,[2] Yutaka Kohgo,[2] Takeo Matsuno,[3] Yoshinobu Ohsaki,[4] Naoyuki Miyokawa,[5] Minoru Nakao,[1] Kazuhiro Nakaya,[1,6] and Akira Ito[1]*

Department of Parasitology,[1] Department of Internal Medicine,[2] Department of Orthopedic Surgery,[3] Respiratory Center,[4] Department of Surgical Pathology,[5] and Animal Laboratory for Medical Research,[6] Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa 078-8510, Japan

Received 8 June 2009/Returned for modification 8 July 2009/Accepted 13 July 2009

Two cases of alveolar echinococcosis (AE) with multiple-organ involvement (the liver, lungs, and bone) were monitored by imaging and serology for 20 years. Resection of the bone lesion was complete in one case but incomplete in the other case. Albendazole treatment was markedly to moderately effective against hepatic and pulmonary AE lesions in both cases, whereas it had almost no effect against the bone lesion in one case. The results of the serological tests with recombinant Em18 antigen coincided with the clinical findings in each case. An enzyme-linked immunosorbent assay for the detection of immunoglobulin G (IgG) responses, especially IgG4 responses, is expected to be a real-time indicator of the dynamics of active AE.

Alveolar echinococcosis (AE), caused by the fox tapeworm, *Echinococcus multilocularis*, is one of the neglected, emerging, or reemerging infectious diseases listed by WHO with cysticercosis, rabies, brucellosis, etc., and is often misdiagnosed as hepatocellular carcinoma (4, 17). It is one of the most lethal parasitic infections, and areas contaminated with this parasite are becoming wider and wider in the majority of the Northern Hemisphere, other than tropical and subtropical areas (10, 11, 19, 20, 24). When local people living in contaminated areas accidentally ingest eggs of this parasite expelled from foxes and dogs, the embryos develop into metacestodes, so-called alveolar echinococcus, by asexual proliferation, mainly in the liver (in more than 97% of cases of AE). Infected persons become symptomatic, usually 10 to 20 years later. Patients with active lesions are estimated to die within 15 years after the initial appearance of symptoms, whereas cases with only abortive or inactive AE lesions are rarely found (10, 12, 19, 25). Calcification occurs either at the early stage and requires no treatment (abortive or inactive cases) or at the late stage (the majority of advanced AE cases). Early diagnosis with early treatment, mainly by surgery, has strongly been recommended, since complete excision is so far the only curative treatment (19).

Therefore, the development of sensitive and specific diagnostic tools is crucial. Imaging tools that use ultrasonography, computed tomography (CT), and magnetic resonance imaging have been applied for the diagnosis and monitoring of the progression of AE (19). Most recently, positron emission tomography has also been introduced for the detection and characterization of the active lesions (3).

Serology for the detection of specific antibody responses was developed independently by several groups in Germany, Switzerland, Australia, and Japan by characterization with antigens EM10, Em2/III, EM4, and Em18, respectively (24). It later became evident that all of these were the same ezrin-like protein encoded by *elp* gene, which has a high degree of homology with human ezrin-radexin-moesin (ERM) (2, 10, 22). It is very interesting that specific diagnostic antigen candidates have a very high degree of homology with the host protein (ERM), and AE is a typical chronic disease. Among these four antigens, Em18 is the smallest component of the ezrin-like protein and is degenerated by a cysteine protease(s), but it has the lowest degree of homology with ERM (10, 22). Therefore, serology by the use of Em18, especially recombinant Em18 (recEm18), is expected to show the lowest level of cross-reactivity with human components (1, 5, 7, 8, 10, 13, 17, 22, 24, 25, 28). Furthermore, it has been shown that a positive result by serology with Em18 is a good indicator of active AE, and immunoglobulin G4 (IgG4) is the predominant subclass involved (9, 12).

In this report, we describe two AE cases with lesions in the liver, lungs, and bone who were admitted for surgery of the bone lesion but who showed completely different clinical and serological outcomes after 20 years of monitoring, until the end of 2008. Data on the IgG responses of case patients 1 and 2 detected before 2000 were briefly reported by Fujimoto et al. (5), who designated these patients cases 6 and 7, respectively. In this study, we applied a recEm18-specific enzyme-linked immunosorbent assay (recEm18-ELISA) to the analysis of the serological dynamics of the responses to IgG4, IgG1, IgG (Fab), IgG (H+L), and IgG recognized by recombinant protein G to evaluate which one is the best for monitoring of the progression of AE.

MATERIALS AND METHODS

Serology for the detection of antibodies specific to Em18, the best diagnostic antigen for the detection of active AE, was applied for the monitoring of these

* Corresponding author. Mailing address: Department of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa, 078-8510 Hokkaido, Japan. Phone: 81-166-68-2420. Fax: 81-166-68-2429. E-mail: akiraito@asahikawa-med.ac.jp.

▽ Published ahead of print on 5 August 2009.

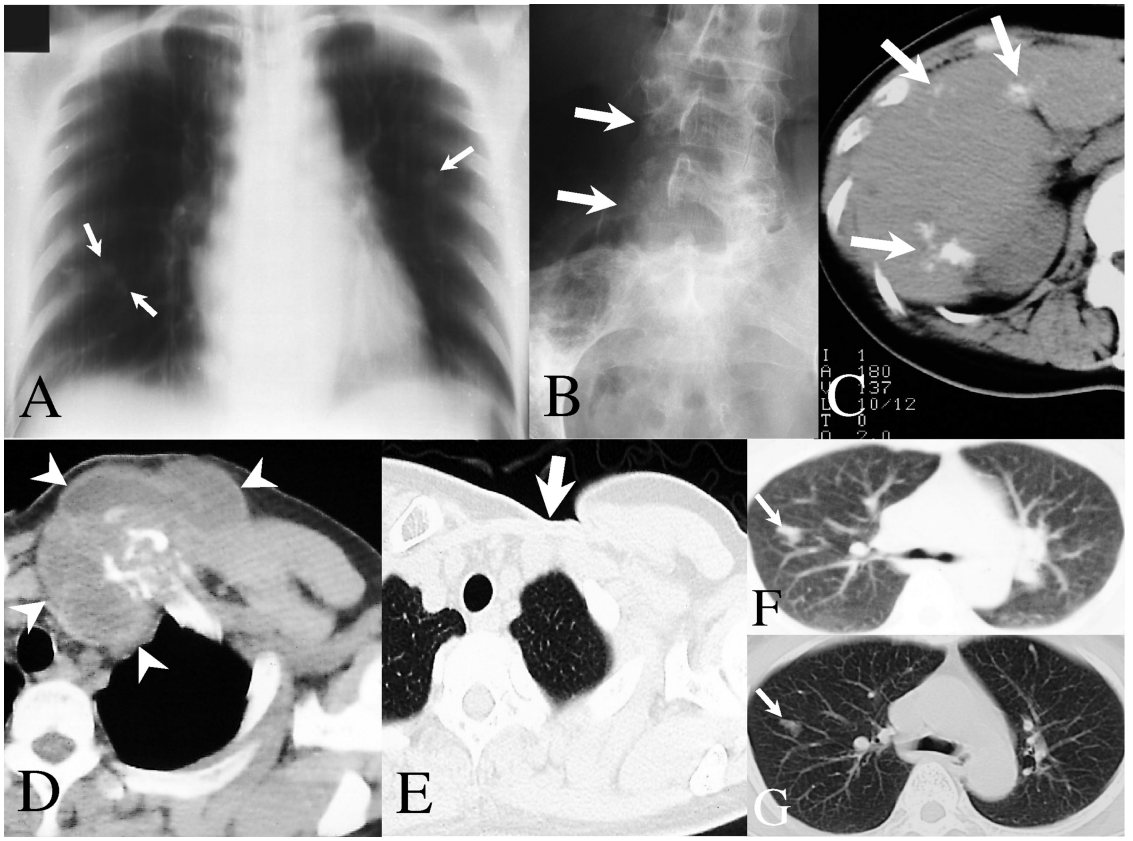

FIG. 1. Imaging figures of case 1 (A to C) and case 2 (D to G). (A) Several nodular densities demonstrated by a chest X-ray tomogram (arrows). (B) Osteolytic destruction of the right lower lumbar vertebrae resulting from AE invasion of the pelvis (arrows), demonstrated by X ray 143 months after ABZ chemotherapy (September 2007). (C) Reduction in size of hepatic AE foci with calcification (arrows), demonstrated 130 months after ABZ chemotherapy (July 2000). (D) Chest CT scan demonstrating a mass with a heterogeneous density (arrowheads) at the left medial head of the clavicle. (E) CT scan image obtained 81 months after radical resection of the bone AE and ABZ chemotherapy (December 1999). A defect after complete removal of the lesion without relapse is demonstrated (arrow). (F) Chest CT scan image demonstrating multiple nodular densities of pulmonary AE (the arrow points to one of the lesions). (G) Chest CT scan image demonstrating multiple nodular densities with few changes 81 months after the time of diagnosis (December 1999) (the arrow points to one of the lesions).

cases (12, 25). The recEm18-ELISA was applied to the analysis of the dynamics of the antibody titers throughout the treatment (22, 25). The serum samples used for the ELISA for either total IgG, IgG1, or IgG4 were used at a 1/200 dilution, whereas those used for immunoblotting (IB) were used at a 1/50 dilution (9, 12). Final ELISAs by the use three different tools were carried out in 2008 under the same conditions to show the dynamic changes throughout the follow-up studies. Five secondary antibodies were used for these ELISAs: horseradish peroxidase-recombinant protein G (Zymed Laboratories Inc.), horseradish peroxidase-monoclonal anti-human IgG1 and IgG4 (Zymed Laboratories Inc.), and peroxidase-conjugated goat IgG fraction to human IgG (Fab) and IgG (H+L) (Cappel).

RESULTS

Clinical follow-up. (i) Case 1. A 39-year-old woman was admitted to Asahikawa Medical College Hospital (AMCH) in August 1989. She lived in the eastern part of Hokkaido, known as the area of Japan where AE is the most endemic. On admission, she had a painful tumor in the right buttock with pus oozing through a fistula. Initially, beginning in September 1987, she felt only a dull pain in the right buttock, but she had a lump about the size of her fist afterwards. Even though the softened part of the lesion had been incised and drained during a previous hospitalization in April 1988, intractable pyorrhea followed. She underwent curettage of the sequestrum for chronic suppurative osteomyelitis of the ileum in July of the same year. She was then given antitubercular drugs owing to a misdiagnosis of tuberculous osteomyelitis, without effect. Another curettage of the sequestrum was performed in November 1988, but she was not cured of the persistent pyorrhea. For that reason, she was transferred to AMCH for a close examination of the refractory bone infection. Histological findings specific to AE were disclosed by microscopic examination of a biopsy specimen obtained at the time of the previous surgery. Multiple AE lesions were consequently detected in the liver (data not shown) and the lungs (Fig. 1A) by imaging diagnosis. Laparoscopy and a direct-vision hepatic biopsy revealed the endoscopic and histopathological picture of AE. She was finally diagnosed with AE involving the right ileum, the liver, and the lungs. As radical surgery of all the involved organs was considered impossible, administration of albendazole (ABZ; 400 mg orally twice daily) started on 18 October 1989 (6, 18, 23, 27). Continuous medication was opted for because of the severe condition of her illness, even though the intermittent administration of ABZ (a 28-day cycle followed by a 14-day ABZ-free interval) is the recommended regimen. The prolonged drainage stopped 6 weeks after the start of chemother-

apy. She underwent radical curettage of the sequestrum and bone grafting for the right ileum AE on 19 December 1989. As her clinical course was good for 3 years after the operation, she was not treated with ABZ from March 1993 to March 1994. However, the ileum AE recurred after the stop of chemotherapy, and ABZ chemotherapy was resumed, followed by a second operation. She underwent a third operation for AE invasion of the fifth lumbar vertebra in October 1999, and the dose of ABZ was increased to 600 mg. The dose of ABZ was returned to 400 mg due to mild liver dysfunction in September 2000, and intermittent medication was chosen 1 year later. Although pain in the right leg developed with the gradual progression of the bone AE, it subsided for several months with the use of continuous medication. She has so far needed surgical treatment four times and gradual increases in the dose of ABZ. Despite the combined medical and surgical treatment, the bone AE has hardly been controlled (Fig. 1B). Furthermore, she complained of bloody sputum in January 2005, and diagnostic imaging revealed the recurrence of pulmonary AE. However, this respiratory symptom was controlled by use of an increased dose of ABZ (1,000 mg daily), which had no marked adverse effects. In contrast, the hepatic AE remained in complete remission (Fig. 1C) and has not recurred.

(ii) Case 2. A 41-year-old woman living in the central part of Hokkaido felt a pain in her upper left chest at the end of July 1991. She was first diagnosed with sternoclavicular joint arthritis and shoulder periarthritis at AMCH on 14 August 1991. A tumor of 50 by 60 mm in the sternoclavicular joint was found by physical examination on 19 September 1991 (Fig. 1D). The pain disappeared when the tumor became large, and this was accompanied by central necrosis. A cytological examination and bacterial culture of an aspirate were performed on 13 February 1992. However, there were neither malignant cells nor bacterial growth. An open biopsy was performed on 12 February 1993, and a histopathological diagnosis of AE was made by microscopic examination. Multiple AE lesions in both the liver and the lungs (Fig. 1F) were subsequently detected by imaging diagnosis. She was diagnosed with AE with multiorgan involvement (the liver, the lung, and the clavicle) after 20 months of upper chest pain. She underwent radical resection of the left clavicular AE lesion (Fig. 1E). In addition, AE involvement of the liver was confirmed by laparoscopy and ultrasound-guided liver biopsy (data not shown). Chemotherapy with ABZ against AE of the liver and lungs started on 1 April 1993. After a remarkable reduction in the size of the hepatic AE lesion was detected by CT after 1 year with this chemotherapy, we recommended that the ABZ treatment be stopped and that the AE lesions be monitored. The hepatic AE remained in complete remission. However, the lung lesions may still have been viable and growing, as determined by CT on 4 January 1996 (data not shown). As summarized in serological follow-up studies (Fig. 2B), serology still indicated weakly positive results. We therefore resumed ABZ treatment from March 1996, and no exacerbation of AE lesions either in the lungs (Fig. 1G) or in the liver (data not shown) was detected by diagnostic imaging after that.

Serological follow-up. The clinical background information and serological data obtained by recEm18-ELISA are shown in Fig. 2. The antibody response to recEm18 showed highly dynamic and variable changes over 20 years, until October 2008, for case 1, who was referred to as case 7 before 2000 in the work of Fujimoto and others (5) (Fig. 2A). There was a certain correlation between the ELISA results and her clinical course mentioned above. The ELISA values remained positive after palliative surgeries of the iliac lesion and rose during the advanced stages of the disease, especially after 2000. We noticed a rise in the ELISA values after the third operation, and in October 2000, she complained of pain in the right hip and a relapse of the right iliac AE was found. It was supposed that the rise in the ELISA values mainly originated at the focus of the infection, because the increased ELISA values remained after the imperfect operation and because a relapse or the development of new AE lesions in other organs was not observed. In this study, we carried out ELISA for the detection of IgG4 and IgG1 (data not shown) as well as total IgG using several secondary antibodies, including recombinant protein G, anti-IgG (Fab), and anti-IgG (H+L). As shown in Fig. 2, it was clear that the ELISA values for the detection of antibodies specific to recEm18 by the use of recombinant protein G, anti-human IgG (Fab), and anti-human IgG4 were highly reliable for monitoring of the clinical courses. There were no critical differences in the antibody responses when anti-human IgG (Fab) and anti-human IgG4 were used. As the IgG1 responses were rather weak in these two cases and the IgG response that was detectable with anti-human IgG (H+L) showed highly nonspecific background responses, these results were not included in Fig. 2 and are not useful for the monitoring of progression of the disease (data not shown). Among these five tools, the detection of IgG4 was expected to be the most sensitive for follow-up studies.

When we applied the recEm18-ELISA to case 2 (Fig. 2B), a drastic drop in antibody titers was confirmed within 4 years before 2000 (5). In this study, we monitored the patients until October 2008. The IgG response still appeared to be positive until 2000. However, when we checked the IgG4 response, it was negative at that time and remained negative until October 2008. The result of the IgG-ELISA became negative by 2003 and remained negative until October 2008. All clinical findings, including those obtained by ultrasonography and magnetic resonance imaging, also supported a diagnosis of a complete cure, which corresponded to the serological data in this case.

DISCUSSION

The inoperable parasitic lesion of the bone in case 1 progressed even after continuous chemotherapy for 20 years, starting in 1989. This is because of the inoperable residual lesion in the bone. In contrast, case 2 is expected to have been cured after complete surgical removal of the lesion in the bone. Chemotherapy with ABZ appeared to be markedly to moderately effective against hepatic and pulmonary AE in these two cases. In contrast, more prolonged treatment may be required for AE at sites such as bone and the brain, as clearly described in the summary of product characteristics by SmithKline Beecham. The proportion of surgically resectable cases of AE was 40% or less, and the mortality rate within 10 years for patients with nonresectable cases was 90% in about 1980 (23). Therefore, the prognoses for these two AE patients with multiorgan involvement were presumed to be very poor, but they have survived over a long period of time. These outcomes are

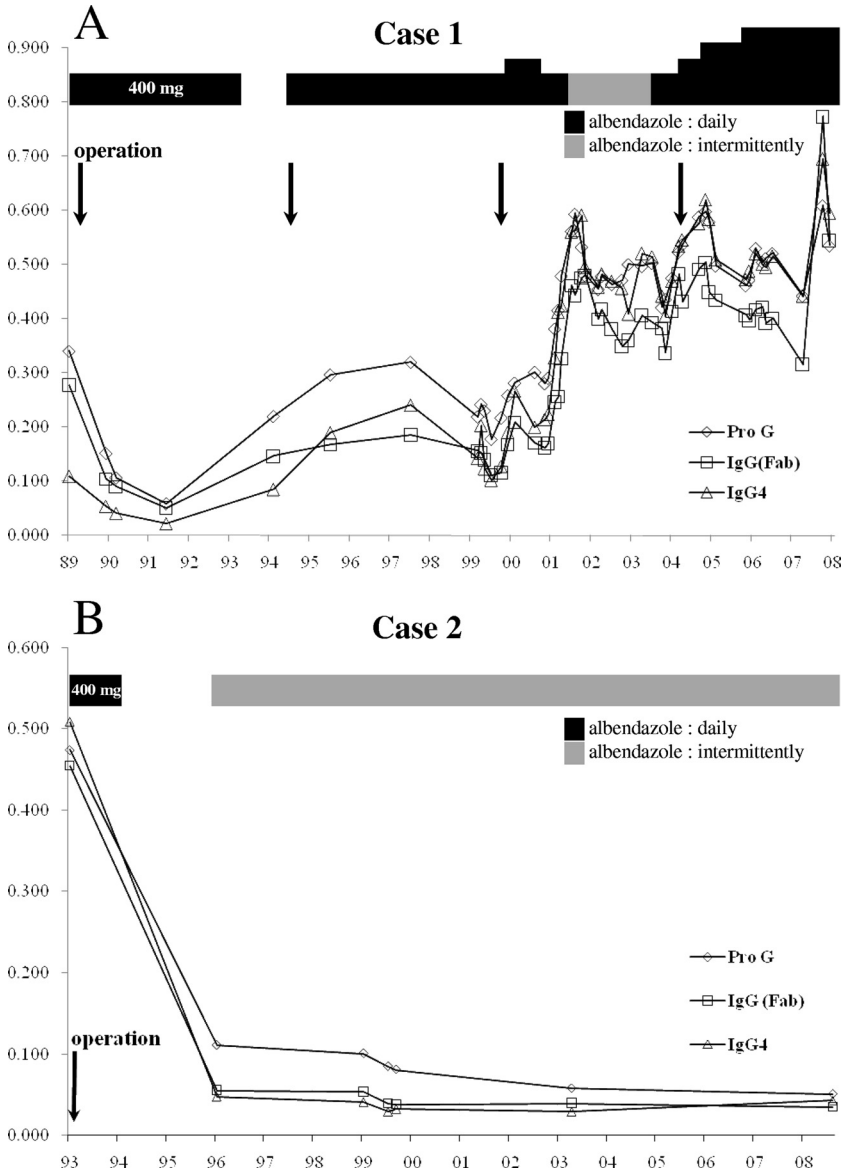

FIG. 2. Antibody responses to recombinant Em18 over the course of follow-up of the two AE cases. Four different secondary antibodies (anti-human IgG [Fab], anti-human IgG [H+L], anti-human IgG1, and anti-human IgG4) and recombinant protein G (Pro G) were applied for these follow-up studies. The data for the IgG (H+L) and IgG1 responses are not included.

very encouraging for patients needing long-term ABZ chemotherapy for the treatment of severe AE.

WHO has recommended combined imaging and serology for the diagnosis of AE and cystic echinococcosis (19). Imaging diagnosis of AE is comparatively easy in cases with distinctive findings, such as calcification and cavitations of lesions in patients with advanced AE, especially in areas of endemicity, although it is not always possible to make a conclusive specific diagnosis from imaging figures only. The addition of highly specific serology is strongly recommended. The use of Em2^{plus}-ELISA, recEm18-IB, and/or recEm18-ELISA, as well as several other ELISAs, has been recommended by WHO (19). However, a recent comparative analysis of the recEm10-ELISA, Em2^{plus}-ELISA, and recEm18-ELISA for monitoring of the progression of different pathological types of AE by the WHO criteria (14, 16, 25) revealed that the recEm18-ELISA exclusively showed strongly dynamic changes, including negative results for cured cases (25). The sensitivity of either recEm18-ELISA or RecEm1-IB was superior to that of other serological tools, including the Em2^{plus}-ELISA (1, 10, 25). Similar data were observed before and after liver transplantation for AE cases in France (S. Bresson-Hadni and A. Ito, unpublished data) and follow-up studies of hepatic AE cases with radical resections in Japan (5; H. Akabane and A. Ito, unpublished data). The most remarkable picture provided by the recEm18-ELISA is that the real-time increases in ELISA values correlated with relapses. As serology by either Em18-IB or Em18-ELISA was highly useful for the detection of approximately 95% of active AE cases (1, 10, 12, 21, 25), it is

further notable that the results becomes negative after a complete cure (5, 12, 13, 25; J. F. Wilson, P. M. Schantz, and A. Ito, unpublished data). After curative surgery, the results may become negative within a half year (Akabane and Ito, unpublished). Therefore, it is strongly recommended that the recEm18-ELISA by applied, especially for the detection of IgG4, for monitoring of the progression of AE. Several reports have stressed the usefulness of the detection of IgG4 (12, 26), as serum samples that gave optical density values at 405 nm that were greater than the mean ± 3 standard deviations were considered to be seropositive, Although IgG4 responses became negative a little bit faster than the IgG (Fab) responses (Fig. 2B), we consider that there is no crucial difference between the detection of IgG4 and IgG (Fab).

In order to detect the antibody response for outpatients or screening of patients in areas where AE is endemic, as well as inpatients, a rapid immunochromatographic test (ICT) which does not need any special facilities or experienced personnel has already been developed by the use of recEm18 (21). The disease in the two AE cases described here was also confirmed by ICT (data not shown). A commercially available ICT kit is ready from Adtec Inc. (Usashi, Oita, Japan). A quantitative ICT is under development for monitoring of the progression of AE (Y. Sako et al., unpublished data).

Although ABZ is still the preferred chemotherapeutic agent for the treatment of AE, continuous dosing may be essential (15), since some resistance to ABZ appeared to develop after the cessation of ABZ treatment (19). As demonstrated in this study, Em18 serology is a good marker for the presence of active AE lesions and inactive or abortive AE cases, as well as for the detection of seronegativity in cured AE cases after radical surgery. However, ABZ does not usually have a clear metacestocidal effect in vivo or clinically (15), and an indication of inactive or abortive AE does not always mean a complete cure (Bresson-Hadni and Ito, unpublished). Without direct evidence that an AE lesion is dead or completely calcified and has no germinal layer through histopathological examination of biopsy specimens or radical resection of whole lesions, it is still difficult to recommend when chemotherapy for AE cases should be stopped. Standards for the time of cessation of ABZ treatment remain unresolved, and the development of metacestocidal drugs is still needed.

ACKNOWLEDGMENTS

The study was supported in part by the Infection Matrix Fund; the Hokkaido Translational Research Fund, sponsored by the Ministry of Education of Japan; and the International Joint Research Project by the Japan Society for the Promotion of Science (grants 17256002 and 21256003) to A.I.

We sincerely thank P. M. Schantz for his crucial comments and suggestions and for amendment of the manuscript.

T.M. did surgery at the affiliated hospital of AMCH; and Y.I., T.O., Y.K., Y.O., and T.M. followed the clinical treatment after surgery at the affiliated hospital of AMCH and Ishikawa Clinic. Y.S., S.I., Y.I., and A.I. did the serology. Pathological examination was carried out by N.M., K.N., and M.N., Y.I., T.O., Y.K., and A.I. prepared the manuscript. All authors read through the manuscript.

We received written agreement from the two patients.

We declare that we have no conflicts of interest.

REFERENCES

1. **Bart, J. M., M. Piarroux, Y. Sako, F. Grenouillet, S. Bresson-Hadni, R. Piarroux, and A. Ito.** 2007. Comparison of several commercial kits and Em18 serology for detection of human alveolar echinococcosis. Diagn. Microbiol. Infect. Dis. **59:**93–95.
2. **Brehm, K., K. Jensen, P. Frosch, and M. Frosch.** 1999. Characterization of the genomic locus expressing the ERM-like protein of *Echinococcus multilocularis*. Mol. Biochem. Parasitol. **100:**147–152.
3. **Bresson-Hadni, S., E. Delabrousse, O. Blagosklonov, B. Bartholomot, S. Koch, J. P. Miguet, G. A. Mantion, and D. A. Vuitton.** 2006. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. Parasitol. Int. **55:**S267–S272.
4. **Craig, P. S., C. M. Budke, P. M. Schantz, T. Li, J. Qiu, W. Yang, E. Zeyhle, M. T. Rogan, and A. Ito.** 2007. Human echinococcosis: a neglected disease? Trop. Med. Health **35:**283–292.
5. **Fujimoto, N., A. Ito, Y. Ishikawa, M. Inoue, Y. Suzuki, M. Ohhira, T. Ohtake, and Y. Kohgo.** 2005. Usefulness of recombinant Em18-ELISA to evaluate efficacy of treatment in patients with alveolar echinococcosis. J. Gastroenterol. **40:**426–431.
6. **Horton, J.** 1989. Chemotherapy of *Echinococcus* infection in man with albendazole. Trans. R. Soc. Trop. Med. Hyg. **83:**97–102.
7. **Ito, A.** 2002. Serological and molecular diagnosis of zoonotic larval cestode infections. Parasitol. Int. **51:**221–235.
8. **Ito, A., and P. S. Craig.** 2003. Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. Trends Parasitol. **19:**377–381.
9. **Ito, A., L. Ma, M. Paul, J. Stefaniak, and Z. S. Pawlowski.** 1998. Evaluation of Em18-, Em16, antigen B-Western blots, Em2plus-ELISA and four other tests for differential serodiagnosis of alveolar and cystic echinococcosis patients in Poland. Parasitol. Int. **47:**95–99.
10. **Ito, A., M. Nakao, and Y. Sako.** 2007. Echinococcosis: serological detection of patients and molecular identification of parasites. Future Microbiol. **2:**439–449.
11. **Ito, A., T. Romig, and K. Takahashi.** 2003. Perspective on control options for *Echinococcus multilocularis* with particular reference to Japan. Parasitology **127:**S159–S172.
12. **Ito, A., P. M. Schantz, and J. F. Wilson.** 1995. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. Am. J. Trop. Med. Hyg. **52:**41–44.
13. **Ito, A., N. Xiao, M. Liance, M. O. Sato, Y. Sako, W. Mamuti, Y. Ishikawa, M. Nakao, H. Yamasaki, K. Nakaya, K. Bardonnet, S. Bresson-Hadni, and D. A. Vuitton.** 2002. Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for differential diagnosis of alveolar echinococcosis: results of a blind test. J. Clin. Microbiol. **40:**4161–4165.
14. **Kern, P.** 2006. Medical treatment of echinococcosis under the guidance of good clinical practice (GCP/ICH). Parasitol. Int. **55:**S273–S282.
15. **Liu, Y. H., X. G. Wang, J. S. Gao, Y. Q. Yao, and J. Horton.** 2009. Continuous albendazole therapy in alveolar echinococcosis: long-term follow-up observation of 20 cases. Trans. R. Soc. Trop. Med. Hyg. **103:**768–778.
16. **Ma, L., A. Ito, Y. Liu, X. Wang, Y. Yao, D. Yu, and Y. Chen.** 1997. Alveolar echinococcosis: Em2^{plus}-ELISA^{TM} and Em18-Western blots for follow-up after treatment with albendazole. Trans. R. Soc. Trop. Med. Hyg. **91:**476–478.
17. **McManus, D. P., W. Zhang, J. Li, and P. B. Bartley.** 2003. Echinococcosis. Lancet **362:**1295–1304.
18. **Moshimann, F.** 1980. Is alveolar hydatid disease of the liver incurable? Ann. Surg. **192:**118–123.
19. **Pawlowski, Z. S., J. Eckert, D. A. Vuitton, R. W. Ammann, P. Kern, P. S. Craig, K. F. Dar, F. De Rosa, C. Filice, B. Gottstein, F. Grimm, C. N. L. MacPherson, N. Sato, T. Todorov, J. Uchino, W. von Sinner, and H. Wen.** 2001. Echinococcosis in humans: clinical aspects, diagnosis and treatment, p. 20–71. *In* J. Eckert, M. A. Gemmell, F. X. Meslin, and Z. S. Pawlowski (ed.), WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. World Organization for Animal Health, Paris, France.
20. **Romig, T., A. Dinkel, and U. Mackenstedt.** 2006. The present situation of echinococcosis in Europe. Parasitol. Int. **55:**S187–S191.
21. **Sako, Y., K. Fukuda, Y. Kobayashi, and A. Ito.** 2009. Development of a rapid immunochromatographic test for the diagnosis of alveolar echinococcosis. J. Clin. Microbiol. **47:**252–254.
22. **Sako, Y., M. Nakao, K. Nakaya, H. Yamasaki, B. Gottstein, M. W. Lightowlers, P. M. Schantz, and A. Ito.** 2002. Alveolar echinococcosis: characterization of diagnostic antigen Em18 and serological evaluation of recombinant Em18. J. Clin. Microbiol. **40:**2760–2765.
23. **Schantz, P. M.** 1985. Effective medical treatment for hydatid disease? JAMA **253:**2095–2097.
24. **Schantz, P. M.** 2006. Progress in diagnosis, treatment and elimination of echinococcosis and cysticercosis. Parasitol. Int. **55:**S7–S13.
25. **Tappe, D., M. Frosch, Y. Sako, S. Itoh, B. Grüner, S. Reuter, M. Nakao, A. Ito, and P. Kern.** 2009. Close relationship of clinical regression with specific

serology in the follow-up patients with alveolar echinococcosis in different clinical stages. Am. J. Trop. Med. Hyg. **80:**792–797.

26. **Wen, H., P. S. Craig, A. Ito, D. A. Vuitton, S. Bresson-Hadni, J. C. Allan, M. T. Rogan, E. Paollilo, and M. Shambesh.** 1995. Immunoblot evaluation and IgG-subclass antibody responses for immunodiagnosis of human alveolar echinococcosis. Ann. Trop. Med. Parasitol. **89:**485–495.
27. **Wilson, J. F., R. L. Rausch, B. J. McMahon, P. M. Schantz, D. E. Trujillo, and M. A. O'Gorman.** 1987. Albendazole therapy in alveolar hydatid disease: a report of favorable results in two patients after short-term therapy. Am. J. Trop. Med. Hyg. **37:**162–168.
28. **Xiao, N., W. Mamuti, H. Yamasaki, Y. Sako, M. Nakao, K. Nakaya, B. Gottstein, P. M. Schantz, M. W. Lightowlers, P. S. Craig, and A. Ito.** 2003. Evaluation of use of recombinant Em18 and affinity-purified Em18 for serological differentiation of alveolar echinococcosis from cystic echinococcosis and other parasitic infections. J. Clin. Microbiol. **41:**3351–3353.