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Research Note

Humoral and Cellular Immune Responses to *Acanthamoeba* in a Patient with Amoebic Meningoencephalitis and the Japanese Healthy Controls

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Acanthamoeba sp. is a free-living amoeba found in damp soil and fresh water. In some cases, those amoebae are pathogenic and cause keratitis or meningoencephalitis (Jones *et al.*, 1975; Stehr-Green *et al.*, 1989). It is well known that the cyst form of *Acanthamoeba* is detectable in our usual environmental conditions (Eldrige and Tobin, 1967, Yamaura *et al.*, 1993), and there is no doubt that almost all individuals are forced to contact with the amoeba. This finding raised a question why only a few persons developed clinical diseases, and whether any protective immunity against *Acanthamoeba* has been developed in healthy subjects. Recently we isolated *A. polyphaga* from a patient with meningoencephalitis, and this enabled us to test several parameters of cellular and humoral immunity to the pathogenic amoeba antigen. In this report, we describe results of survey for detecting cellular and humoral immunity to *Acanthamoeba* sp. in the patient as well as in healthy Japanese controls.

Acanthamoeba-specific IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA) (Min *et al.*, 1992), and cellular immunity was tested by antigen-driven proliferation of peripheral blood mononuclear cells (PBMN) in vitro (Ohta *et al.*, 1988). A patient tested was 28 yr old female, and was diagnosed as amoebic meningoencephalitis by detecting trophozoites of *A. polyphaga* in the spinal fluid and also by the characteristic computed tomography patterns. More than 6 months after the disease-onset, we obtained serum and PBMN from the patient. As controls, sera and PBMN from 19 healthy volunteers (27–47 yr) and serum from a 46 yr-male person with previous infection of *Schistosoma japonicum* were tested. Antigens used in this study were soluble extracts of *A. polyphaga* and *A. castellanii*, both of which were pathogenic because those materials were obtained from the spinal fluid of the tested patient and the cornea of amoebic keratitis patient, respectively. Amoebic antigens used were crude extracts of trophozoites which were kept in bacteria-free medium (Ishii and Ishibashi, 1989) with minor modification. Lyophilized materials were delipidized with ethylether, and were ground throughly in phosphate buffered saline (PBS). Soluble schistosome egg antigen (SEA) (Ishii and Owhashi, 1982) and purified protein derivative (PPD) of tuberculin (Nihon BCG Seizo Co., Tokyo) were used as a negative and a positive control antigen, respectively. Protein concentration was measured by the method of Lowry and coworkers (Lowry *et al.*, 1951).

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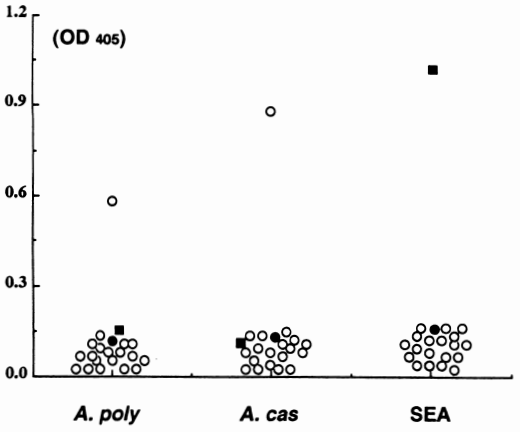

Fig. 1 ELISA for detecting human IgG antibodies specific to *A. polyphaga, A. castellanii* and schistosome egg antigen. Open circle; Healthy Japanese controls, closed circle; a patient with amoebic meningoencephalitis and closed square; an individual with previous infection of *Schistosoma japonicum*.

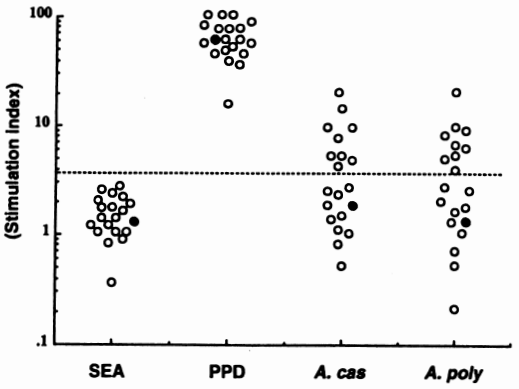

Fig. 2 Proliferative responses of PBMN obtained from an amoebiasis patient (closed circle) and 19 Japanese healthy subjects (open circle). Data are shown as stimulation index for respective antigen. All subjects showed vigorous response to PPD, however, nine out of 19 persons tested showed strong proliferative responses to Acanthamoeba antigens.

All sera except one from healthy controls showed no detectable IgG response to *Acanthamoeba* antigens as well as to SEA (Fig. 1). Serum of a schistosome-infected subject had a high OD value for SEA. It was interesting to note that serum of the amoebiasis patient also had no detectable IgG not only to SEA, but also to *A. polyphaga* which was thought to be a causative pathogen for the present meningoencephalitis.

All subjects tested showed strong cellular responses to PPD, while no detectable response was observed for SEA (Fig. 2). Nine out of 19 Japanese healthy controls (47.4%) showed strong proliferative responses to amoeba antigens. When the antigen concentration was changed between 1–80 μg/ml, any detectable response was still not observed in non-responders (data not shown). PBMN from a patient with amoebic meningitis failed to respond both to two amoebic antigens. The patient showed vigorous proliferation to PPD, indicating that the patient was not in non-specific immunodeficiency condition.

There was an apparent discrepancy between cellular and humoral immunity to *Acanthamoeba* antigens in the Japanese population. We should consider a possibility that the antigen used was not good for testing in ELISA, or that the protocol used was not optimal. We would exclude the first possibility because we obtained vigorous cellular responses to the same antigen in vitro for a half of healthy subjects tested, and one subject did show a high OD value to the amoeba antigen. It is not clear what factors provided such a high OD value in this case, and we are collecting detailed information of past history as well as environmental conditions of the subject. It seemed unlikely that the positive cellular responses were driven by amoebic super antigen because only $CD4^+$ cells were reactive, and because responding cells had critical MHC-restriction (unpublished data). There is still a possibility that some critical immunogenic components might have been lost during antigen extraction, and it is required to test antigens prepared by other protocols. For the second possibility, we tested ELISA at two different antigen concentrations. All sera were tested at 150 times-dilution; the serum dilution was enuogh for detecting specific IgG to usual antigens in our ELISA system. Solid phase antigens were prepared at the concentrations of 10 and 25 μg/ml in PBS. In those two different conditions, we obtained same results in case of the patient's serum (data not shown). This suggests that our ELISA protocol was, if not optimal, sufficient for detecting IgG antibodies of enough titers.

Almost 50% of healthy subjects showed T cell-mediated immunity against *Acanthamoeba* anti-

gens. The patient was also negative for *Acanthamoeba*-driven cellular immune response. Together with the results of ELISA, the patient might have been in impaired immunity to the causative pathogen. It is interesting to test whether such amoeba-specific non-responsiveness was involved in susceptibility to the amoebic disease. All T cell clones reactive to *Acanthamoeba* were of Th1 function (unpublished data) and those T cells were not active to produce enough quantity of cytokines for promoting antibody production (Mosmann and Coffman, 1989). Considering that Th1 cells seemed to be protective against several protozoan infections (Salata *et al.*, 1987), it could be possible that T cell-mediated immunity, but not specific antibodies, has important roles in protection against *Acanthamoeba* infection.

The present study is the first report on cellular as well as serological survey of immunity to *Acanthamoeba* sp. in healthy human population. The patient with amoebic meningoencephalitis showed impaired immunity to the causative *Acanthamoeba*, and this might be one of the important factors for determining susceptibility to the amoebic disease.

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