Immunoglobulin E and G4 Antibodies in Cysticercosis

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The importance of immunoglobulin G (IgG) subclass responses in different infections has been elucidated for a number of organisms, but few parasitic organisms have been examined in this regard. In the current study, quantitative radioimmunoassays were used to examine the IgE and IgG4 subclass responses to larval Taenia solium. Patients were divided into clinically infected (CI) and probably uninfected (PU) groups. Unexposed normal subjects were used as controls. The CI group had elevated geometric mean levels of total IgE in serum (28.6 IU/ml) and specific IgG4 antibodies (438.8 arbitrary units [AU/ml] compared with controls [8.3 IU/ml and 50.1 AU/ml, respectively]. The CI group also had significantly elevated levels in cerebrospinal fluid of total IgG4 (18.6 µg/ml) and specific IgG4 antibodies (86.0 AU/ml) compared with the PU group (2.5 µg/ml and 1.6 AU/ml, respectively). There was no specific IgE antibody response detected in either the CI or PU patient group. The marked IgG4 response of CI patients to T. solium merits further investigation.

A predominance of certain immunoglobulin G (IgG) subclasses has been noted in the immune response to several antigens. IgG1 and IgG3 usually constitute the principal immunoglobulin response to viral antigens and to some unencapsulated bacterial antigens (13, 25). Polysaccharide antigens of encapsulated organisms stimulate principally IgG2 but also may elicit IgG1 and IgG4 antibodies (13). While much research has been done on bacterial and viral antigens, the only parasite antigens examined with regard to IgG subclass responses in human beings are those of malaria (24, 27), filariasis (23), and schistosomiasis (18). The IgE responses in parasitic infections have been well documented and serve an important role in host resistance to certain parasites (3). Interrelationships between IgE and IgG4 have been noted in several systems (17, 18). However, the exact physiological role of IgG4 is unclear. Some investigators report that IgG4 blocks IgE (30), while others suggest that IgG4 itself may be a reaginic antibody (29). The purpose of the current study was to determine total and specific IgE and IgG4 subclass responses to larval Taenia solium antigens in sera and cerebrospinal fluids (CSFs) of patients with cysticercosis and human controls.

MATERIALS AND METHODS

Specimen selection. Serum and CSF specimens were obtained from routine submissions to the Harbor-UCLA immunology research laboratory for serologic evaluation of suspected cases of neurocysticercosis caused by larval T. solium. The specimens were separated into two groups. The first group consisted of 21 patients and was designated clinically infected (CI) on the basis of symptoms, physical examination, and radiographic or imaging evidence of cysticercosis. The imaging data included skull X rays, computerized axial tomographic scans, and magnetic resonance images. The second group consisted of 17 patients and was designated probably uninfected (PU) on the basis of a lack of typical radiologic or imaging evidence. The control group consisted of 11 individuals selected from healthy, low-risk, presumably unexposed laboratory workers. No consider-

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reaction with 0.1 M glycine in 0.5 M sodium bicarbonate, tumbled for 6 h. The disks were washed three times each in acetate buffer (0.1 M, pH 4.0), sodium bicarbonate (0.5 M, pH 8.4), and phosphate buffer (0.1 M, pH 7.4). The coated disks were stored at 4°C in 1% fetal bovine serum–0.1 M phosphate buffer until used.

**Radioimmunoassay for total and specific immunoglobulin.**
The assays used for total IgG4 (2) and for IgE (5, 20) were described in previous publications. Briefly, isotype-specific capture antibody prepared in our laboratory (2, 20) was covalently coupled to cyanogen bromide-activated cellulose disks. Then 50 μl of serum diluted 1:10 or 50 μl of CSF diluted 1:2.75 in sodium phosphate buffer containing 20% fetal bovine serum was added to a test tube and incubated overnight with an appropriate disk. The following day the unbound proteins were washed off and 50,000 cpm of 125I-labeled mouse monoclonal anti-human IgG4 (Unipath Laboratory, London, United Kingdom) or affinity-purified rabbit anti-human IgE heavy chain was added (20). The specific activity of the labeled antibodies varied from 5 to 10 μCi/μg. The mixture was incubated overnight, and the unbound reagents were washed away. The percent radioactivity added which remained bound was determined with an automatic gamma counter. Each immunoglobulin was quantified by comparing the percent radioactivity bound for unknown specimens with a standard curve of percent radioactivity bound which was constructed from serial dilutions of a standard serum with a known high level of IgG4 or IgE. Each radioimmunoassay for specific antibody was similar to the assay described above, except that larval *T. solium* antigen was coupled to the cellulose disk. Quantitative results were obtained by comparing the percent radioactivity bound for unknown specimens with serial dilutions of a high-level antibody standard. Arbitrary units were assigned to the standards, permitting valid comparisons between each test specimen.

**Statistical analysis.** The results were analyzed by the Kruskal-Wallis statistic (19) for comparison of more than two samples and by the Mann-Whitney statistic (21) for comparison of two samples. Differences in the means were considered significant when *P* was less than 0.05.

**RESULTS**

Both the CI and the PU patients groups had total IgE levels in serum that were greater than those of the controls (Fig. 1, *P* < 0.05). There was no statistically significant difference in total IgE levels in serum between the CI and PU patient groups. Larval *T. solium*-specific IgE in serum was not detected in any of the three groups, all measurements being equivalent to those for buffer. Special assays using a 10-fold concentration of serum to increase assay sensitivity also failed to demonstrate specific IgE in any member of either patient or control groups.

The total IgE in CSF was not significantly different between the CI and the PU patients, with only two patients having detectable levels of IgE in the CI group and one patient with a detectable level in the PU group (Fig. 2). No specific IgE was detected in any of the CSFs examined. Specific IgE could not be detected following an additional fourfold increase in the amount of CSF assayed per antigen-coated disk.

There was not a statistically significant difference among the three groups with regard to total IgG4 in serum (Fig. 3). The level in serum of IgG4 specific for larval *T. solium* was elevated in CI patients versus PU patients (*P* < 0.05), as well as in CI patients versus controls (*P* < 0.05, Fig. 4). There was no statistically significant difference for specific IgG4 in serum in PU patients versus controls. Serum specimens from one PU patient and two controls had apparent elevations in the levels of specific IgG4 antibodies. Since none of these patients had evidence of active neurocysticercosis, it is presumed that they had prior unrecognized contact with *T. solium* or a related antigen.

The levels of both total and specific IgG4 in CSF were elevated in the CI patients compared with those of the PU patients (*P* < 0.05, Fig. 5 and 6).

**DISCUSSION**

The level of total IgE has been studied in relation to parasitic infections (30) and has been reported to be elevated in many developing countries, perhaps reflecting the level of parasitism in those regions. The uninfected control group in the current study consisted of individuals who work in the laboratory at Harbor-UCLA Medical Center and were presumably unexposed to larval *T. solium* infection, whereas the CI and PU patient groups consisted almost exclusively of patients who recently immigrated to the United States from endemic regions of Mexico or Central or South America. Therefore, the elevation in the levels of total IgE in both the CI and the PU patient groups actually may reflect environmental or ethnic differences in these two populations and may be unrelated to larval *T. solium* infection.

In contrast to the findings of Correa et al. (7), Grogl et al. (12), and Flisser et al. (10), no serum-specific IgE was
detected in the current study. These authors used the qualitative techniques of immunofluorescence, Western immunoblot, and immunoelectrophoresis, respectively, to demonstrate the presence of antigen-specific IgE. Espinoza et al. (8) were able to detect specific IgE in only 1 of 31 serum specimens and in only 2 of 60 CSF specimens by enzyme-linked immunosorbent assay, utilizing a crude antigen preparation. The three positive specimens were negative for IgE when tested with purified larval *T. solium* antigen B. Several possible explanations exist for the failure to detect a specific IgE response to the larvae of *T. solium*. The antigen used in this study may be qualitatively different from the antigen used in previously reported studies. Although other investigators used antigen prepared from fresh material and a lyophilized antigen from a commercial source was used in the present study, it seems unlikely that all antigens which stimulate an IgE response, as opposed to an IgG4 response, would be completely missing in the commercial preparation. It is especially noteworthy that nearly all CI patients had readily detected IgG and/or IgG4 antibodies to the commercial antigen used. The antigen concentrations used in most of the previous studies in which specific IgE was detected were considerably greater than that used in this investigation. Perhaps the use of a greater antigen concentration would have made specific-antibody detection more likely, since more antigen epitopes would be available and specific IgG antibody would be less likely to block the binding of a relatively low concentration of IgE antibody.

Prior tests carried out in this laboratory with the Western blot technique showed specific IgE antibody in serum to be present in 1 of 10 patients infected with larval *T. solium* (patient not in the present study groups). The small number of patients in whom specific IgE antibody was detected by Espinoza et al. (8) and the results of the present investigation suggest that while specific IgE is sometimes formed in response to larval *T. solium* infection, it may be the exception rather than the rule.

Goldberg et al. (11) found that the level of total IgE in CSF was elevated in Mexican patients with cysticercosis compared with either Mexican patients without cysticercosis or non-Mexican controls. This is in contrast to our finding in a different cohort of patients that there was no difference in levels of total IgE in CSF between CI and PU patient groups. Only two patients in the CI group and one patient in the PU group had IgE levels above the lower limit of sensitivity for the current assay. The lower limit of sensitivity in the earlier study was 0.01 IU/ml, whereas the lower limits for the current study were 3.0 IU/ml for serum and 0.75 IU/ml for CSF. This difference in sensitivity may explain the lack of significantly elevated levels of IgE in CSF in the present work.

IgG subclasses have not previously been studied in larval *T. solium* infections. Although the mean levels for total IgG4 in serum followed a general pattern of CI > PU > controls, the differences were not found to be statistically significant. Larger numbers of patients and controls could lend statistical support to this pattern. The elevation of levels specific IgG4 antibody in the sera of CI patients compared with either PU patients or controls agrees with the findings for *Brugia malayi* (17) and *Schistosoma* spp. (18). Elevated IgG4 levels have been reported to result from chronic
antigen exposure (1). Certainly infection with larval *T. solium* represents a chronic exposure and may account for the higher levels of specific IgG4 in CI patients compared with PU patients and controls.

Five patients were observed to have total IgG4 levels in CSF equal to or greater than the levels in serum. This is in agreement with the findings of Miller et al. (22) that some neurocysticercosis patients exhibit considerable intra-blood-brain barrier synthesis of IgG. The elevation of levels of both total and specific IgG4 in CSF in *T. solium* infections suggests that this immunoglobulin isotype may play an important role in the immune response to this parasite. While the results suggest a specific IgG4 response to *T. solium*, data on specific-antibody levels in the other IgG subclasses are needed before the full significance of the IgG4 response can be assessed.

There has been little success in correlating laboratory immune responses with clinical manifestations of *T. solium* infection. Espinoza et al. (8) found no correlation between humoral immune responses and clinical or radiologic findings in infected patients. Flisser et al. (9) found no correlation between clinical manifestations of cysticercosis and antiglycercicus antibody as determined by immunoelectrophoresis. Corona et al. (6), on the other hand, reported a correlation between an enzyme-linked immunosorbent assay and the clinical classification of neurocysticercosis as benign or malignant (6). Further investigation of subclass responses in cysticercosis combined with an appropriate clinical stratification of patients may elucidate meaningful correlations for this disease.

Parallel IgE and IgG antibody responses have been described in both filariasis (16) and schistosomiasis (14). Evidence gathered from investigation of these diseases (17, 18) seems to support the idea that IgG4 antibodies somehow modulate the IgE immune response. This interpretation could also apply to the current study. A strong IgG4 antibody response may effectively turn off or prevent an IgE antibody response. Also consistent with the data are the notions that IgG4 may be similar in function to IgE and that in individuals with neurocysticercosis IgG4 may be the predominant antibody response. Perhaps IgE is protective and only those subjects with little or no specific IgE response to larval *T. solium* are likely to develop cerebral cysticercosis. The precise role of IgG4 antibodies to most antigens, however, is not well understood. Both a protective role (29) and an anaphylactic role (28) have been proposed. Each hypothesis is supported by several studies, and a dual function incorporating both roles also has been proposed (24, 27).

In conclusion, specific IgE antibodies, although occasionally detectable, are exceedingly low in titer and probably are not an important feature of the humoral immune response in patients with neurocysticercosis. The elevation in levels of total IgE in CI and PU patients compared with controls probably represents a naturally higher level of IgE in individuals from developing countries, where the level of parasitism is high. Larval *T. solium* stimulates a marked IgG4-specific response in both serum and CSF which is not reflected as a significant increase in the level of total IgG4 in serum but is reflected in the level of total IgG4 in CSF. The data from this study do not prove a particular role for IgG4 but suggest that it may be an important component of humoral immunity in patients with neurocysticercosis. Clearly, more research into the role of IgG4 and other subclass responses in this infection is warranted.

**LITERATURE CITED**


