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A FOLLOW-UP STUDY OF THE HUMAN CLASS AND SUBCLASS ANTIBODY RESPONSE DEVELOPED AGAINST THE ADULT STAGE OF *TRICHINELLA SPIRALIS*

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Summary :

We report the analysis by ELISA of class and subclass antibody response against a total soluble extract from *T. spiralis* adult stage (TSE-A) during a year after the infection in 17 symptomatic trichinellosis patients (SI) and five asymptomatic individual (AI) involved in an outbreak of trichinellosis occurred in the State of Mexico. Serum samples from 20 healthy individuals (HI) and 24 patients with other parasitosis were included as control. All SI showed a polyisotypic antibody response against the TSE-A, during the infection. Higher response of IgA, IgE, IgM were detected in SI during the acute phase of the infection, but only IgE remained at high levels all along the infection. None or a lower reactivity against TSE-A was observed in sera from AI and from HI. Some patients with trichuriosis and ascariosis showed a higher cross-reactivity against TSE-A when IgG and their subclasses were analyzed.

KEY WORDS : *Trichinella*, adult stage, human, antibody, diagnosis.

In some parasitic diseases, the analysis of human class and subclass antibody responses has improved the serological methods for diagnosis (Brito *et al.*, 2000) and conferred prognosis value (Hu *et al.*, 1999). Based on this, we analyzed the human antibody response against *Trichinella spiralis* adult stage with the aim to determine the usefulness of isotypic and subtypic antibody detection in the diagnosis of human trichinellosis.

MATERIAL AND METHODS

Parasite and infection

T. spiralis (pig strain) was maintained by serial passage in outbred BALB/c mice. Infective muscle larvae (ML) and adult worms (A) were collected as described by Dennis *et al.* (1970).

Antigen

A total soluble extract of adults (TSE-A) was obtained in 10mM Tris-HCl, pH 8.13 added with protease inhibitors as described Parkhouse, 1984.

Serum samples

These sera were collected from 22 individuals involved in an outbreak of trichinellosis occurred in the State of Mexico. Seventeen out of twenty two individuals displayed clinical symptoms of the disease (SI); 13 of them had confirmed parasite infection by muscle biopsy (SB^+) and four were negative (SB^-) while the remaining five did not exhibit any symptom and were negative by ELISA (AI). Serum samples from these individuals were collected at weeks 3, 4, 5, 7, 12, 15, 37, and 57 postinfection (pi). Control serum samples were collected from 20 healthy individuals (HI), and from 39 patients infected with other parasites (PI), whose diagnosis were confirmed by parasitological or serological tests.

ELISA assays

These assays were performed to detect class and subclass antibody responses using 5 µg/mL of TSE-A and serum samples were diluted at 1:320 for IgM, IgG and IgG subclasses detection or 1:100 for IgA and IgE. Indirect ELISA (I-ELISA) assays were carried out to detect IgM and IgG antibodies as described by Chapa-Ruiz *et al.*, 1989, using a peroxidase-conjugated goat anti-human IgM and IgG (heavy-chain reactive, Cappel Lab.) at 1:20000 and 1:5000 dilutions, respectively. Indirect amplified ELISA (Ia-ELISA) assays were carried out to analyze IgE, IgA, and IgG subclasses according to Au *et al.* (1983) and Ljungström *et al.* (1988). Goat anti-human IgE and IgA (heavy-chain reactive Cappel, Lab.) were diluted 1:32000 and 1:2000 respectively, and peroxidase-conjugated rabbit anti-goat IgG (heavy-chain reactive Cappel, Lab.) diluted 1:8000 for IgE and IgA detection. Monoclonal antibodies (MoAbs) to human IgG_1, IgG_2, IgG_3 or IgG_4 (Boehringer, Mannheim) were used at 1:2000 dilution. Goat anti-mouse IgG (heavy-chain reactive Cappel, Lab.) was diluted 1:8000, while peroxidase-conjugated rabitt anti-goat

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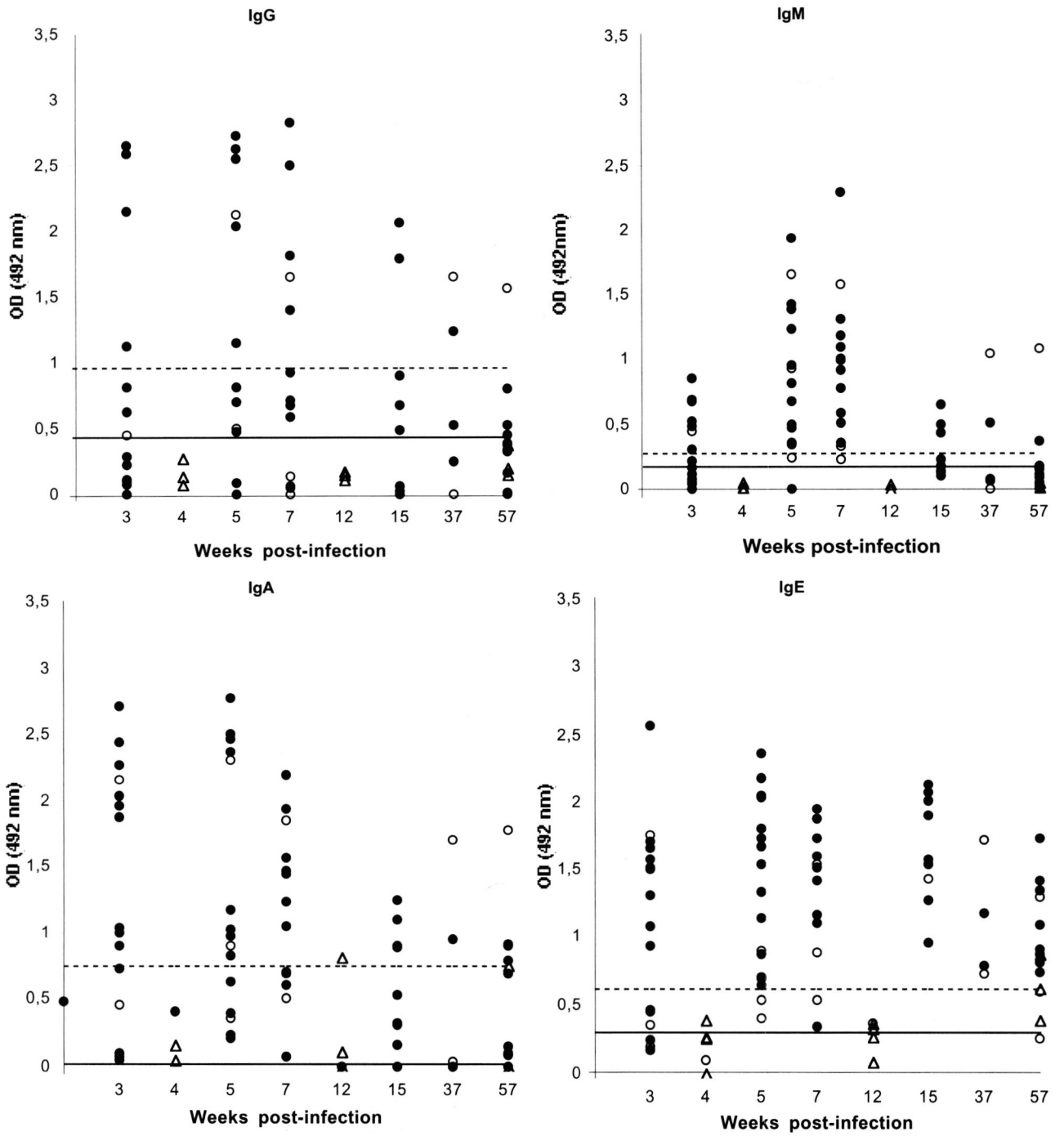

Fig. 1. – Analysis by ELISA of class antibody response against the adult stage of *Trichinella spiralis* in trichinellosis patients during the infection.

I-ELISA assays were carried out to detect IgM and IgG antibodies as described in material and methods. Serum samples from symptomatic trichinellosis patients with: ● positive or ○ negative biopsy and from ▽ asymptomatic individuals, were analyzed. Doted line: cut-off value (media + 3σ from patients with other parasitosis). Line: cut-off value from healthy individuals.

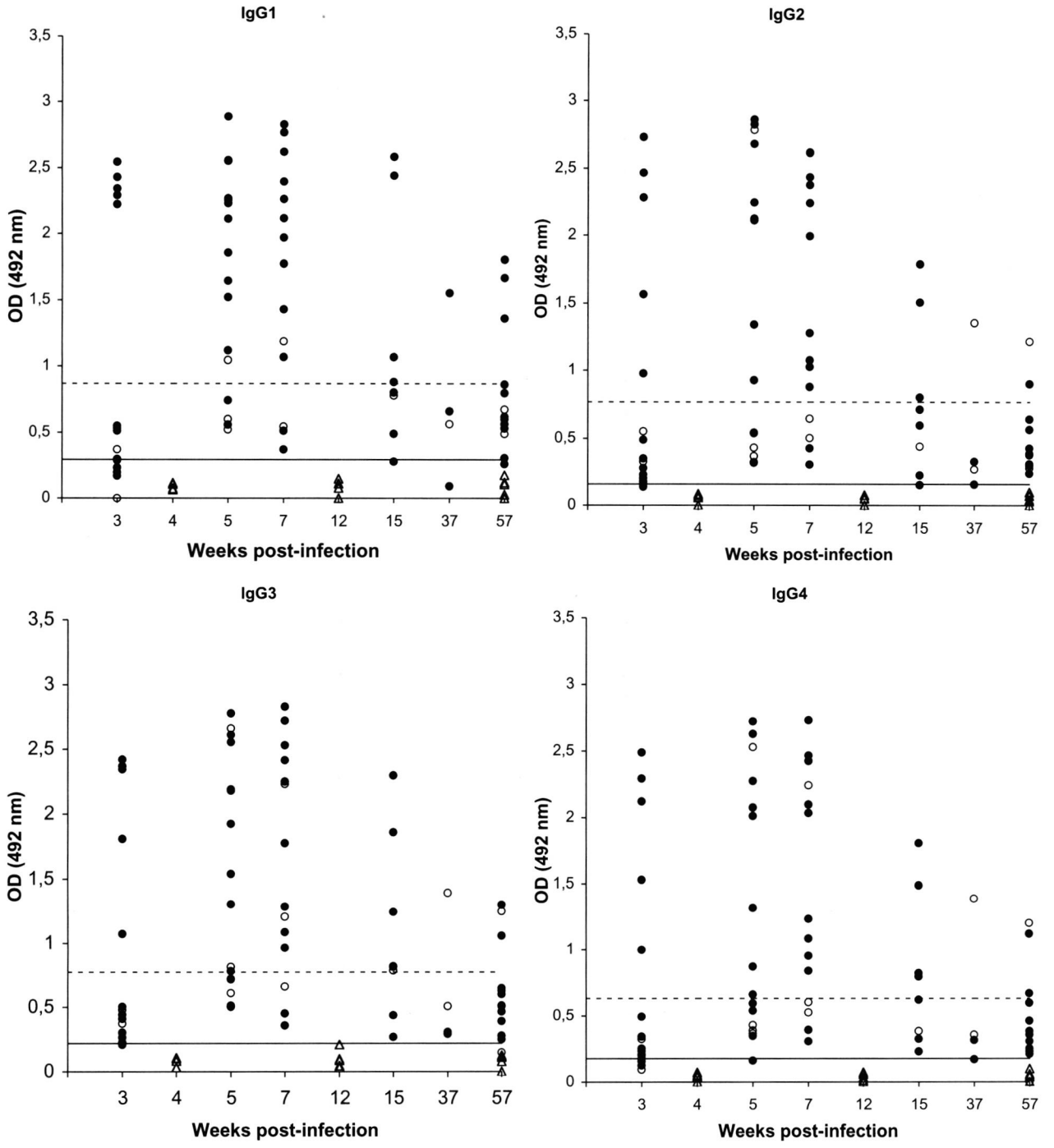

Fig. 2. Analysis by ELISA of subclass antibody response against the adult stage of *Trichinella spiralis* in trichinellosis patients during the infection.

Ia-ELISA assays were carried out to detect IgA and IgE antibodies as described in material and methods. Serum samples from symptomatic trichinellosis patients with: ● positive or ○ negative biopsy and from ▽ asymptomatic individuals, were analyzed. Doted line: cut-off value (media + 3σ from patients with other parasitosis). Line: cut-off value from healthy individuals.

IgG was used at 1:16000 dilution. Absorbances were determined by ELISA plate reader (Dynatech, Industries) at 492 nm.

RESULTS

This study revealed that all immunoglobulin isotypes and subclasses of IgG were detected in SI at week 3 pi, reached a peak by weeks 5 or 7 pi and diminished from week 15 up to the end of the study except for IgE, which remained at high levels during infection (Figures 1 and 2, respectively). These antibody responses were variable among SI with regard to time of analysis. In a previous study (Salinas *et al.*, 1996) negative serum samples from AI for TSL-1 antigens were tested in this work to demonstrate early production of antibodies to adults. Lower reactivity against TSE-A was detected in serum samples from AI and comparable to that from HI (OD < 0.3) (Figures 1 and 2). Higher cross-reactivity against TSE-A were obtained in PI (OD < 0.86) with ascariosis and trichuriosis when IgG and their subclasses, IgA and IgE were determined. ELISA assays revealed that a higher number of trichinellosis SI were detected with: IgA and IgE (69 and 62 %, respectively) at week 3 pi, IgE and IgM (88 % and 86 %, respectively) at week 5 pi, IgM (93 %) at week 7 pi and IgE (values around 100 %) from weeks 15 up to 57 when mean absorbance + 3 σ from PI were taken as cut-off values.

DISCUSSION

The analysis of the human class and subclass antibody response against adults of *T. spiralis* by ELISA revealed that adults antigens induce a high polyisotypic and polysubtypic antibody response in humans during the acute phase of the infection. This response showed to be variable among analyzed individuals and in each individual along the study. These could be due to genetic differences in histocompatibility antigens as detected in mice infected with the parasite (Almond *et al.*, 1986).

The analysis of the class and subclass antibody response against TSE-A and TSL-1 antigens by ELISA in the same group of patients revealed that adults antigens induce a higher polyisotypic and polysubtypic response during the acute phase of the infection, as the one observed against TSL-1 antigens from ML (Salinas *et al.*, 1996). Nevertheless, the response of SI against the TSE-A diminished from 15 up to 57 weeks pi, except for IgE, meanwhile the response against TSL-1 antigens remained elevated in the same period. This suggests that adult worm and TSL-1 antigens share epitopes as demonstrated by Takahashi & Homan (1993). These epitopes were recognized by IgE but not by the other isotypes and subtypes during the chronic phase of the disease. The high levels of isotypes and subtypes detected during acute phase of the infection in SI may be associated with the adults development in the intestine. IgE produced in rats gut after *T. spiralis* infection is preferentially transported to the gut lumen rather than to circulation (Negrao-Correa *et al.*, 1996) and consistently higher in rat strains that eliminate *T. spiralis* worms earlier in the infection (Negrao-Correa *et al.*, 1999). According to this, a similar IgE production and transport in human gut may occur, as low levels of IgE and IgA were detected in serum samples from trichinellosis patients by the methodology used in this study.

In SI, IgM response developed against adults was the most specific. It suggests that glycosilated antigens of adults are specific ones.

On the other hand, AI showed no reactivity to adults antigens; this suggests that AI were not infected with *T. spiralis* or the level of the infection was so low to elicit a detectable antibody by the techniques employed in this work.

In conclusion, the detection of IgA or IgE against antigens from *T. spiralis* adults is useful for the early diagnosis of human trichinellosis. Therefore, specific antigens from TSE-A recognized by these classes of antibodies could be identified and isolated in order to improve the early diagnosis of human trichinellosis. On the other hand, the detection of IgA and IgM against TSE-A could be used as markers of acute phase of the *T. spiralis* infection.

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