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ANTIGEN RECOGNITION BY IgG4 ANTIBODIES IN HUMAN TRICHINELLOSIS

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Summary :

The antibody isotype response to *Trichinella spiralis* excretory/secretory (ES) products of muscle larva was examined using sera from patients with confirmed trichinellosis. Using Western blots we identify components of the ES antigen that are recognized by IgM and IgG antibodies. A 45 kDa component was strongly recognized by different antibody classes and subclasses. We observed a 45 kDa-specific IgG4 response that was detected exclusively using sera of patients with trichinellosis and not of patients with echinococcosis, filariasis, cysticercosis, ascariasis, strongyloidiasis or toxocariasis. These results are relevant for the diagnosis of human trichinellosis.

KEY WORDS : human trichinellosis, ES antigen, 45 kDa, IgG4.

Monitoring programs for detection of trichinellosis in slaughterhouse animals have dramatically decreased the incidence of human trichinellosis. However, in many parts of the world, including Europe, it continues to occur. Recent outbreaks of trichinellosis have been reported from Germany, France, Spain and Italy (Ancelle *et al.,* 1998; Rodriguez *et al.,* 1999); (Pozio 1998).

In our laboratory the immunodiagnosis of trichinellosis is performed by using an enzyme-linked immunosorbent assay (ELISA) in which the excretory/secretory (ES) products of *Trichinella spiralis* muscle larvae are used as antigen (van Knapen *et al.,* 1982). Analysis of this ES antigen by SDS-PAGE revealed that this antigen comprises a complex mixture. The use of this antigen in an ELISA may give rise to false-positive results due to the presence of antigenic components shared with other helminths. Therefore, to confirm results from the ELISA we introduced the Western blotting technique. Identification of parasite antigens recognized by different antibody classes and subclasses can be a valuable tool for the immunodiagnosis of helminth infections (Magnaval *et al.,* 1991; Gadea *et al.,* 1999). We observed that the IgG4 response directed to a 45 kDa component of ES products of muscle larva was *Trichinella*-specific. The measurement of this response can be used for the immunodiagnosis of trichinellosis.

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MATERIALS AND METHODS

ANTIGENS

The excretory/secretory (ES) antigen was prepared using viable muscle larva *of T. spiralis* as previously described by Gamble (1985). The 45 kDa antigenic component was purified from freshly obtained muscle larva using two-step affinity chromatography as described by Homan *et al.* (1992)

SERUM SAMPLES

Serum samples from trichinellosis patients recently received at our laboratory and from an outbreak of trichinellosis that took place in Slupsk, Poland in 1991 were used in this study. The patients here studied were all at an early stage of trichinellosis. All serum samples used tested positive for IgM and/or IgG by means of ELISA using *T. spiralis* ES antigen. Infection was confirmed for several patients from the outbreak in Poland and for the other patients used in this study by identification of *Trichinella* larva in muscle biopsy. The source for *T. spiralis* infection for the outbreak was undercooked pork. Sera from our laboratory from echinococcosis, filariasis, cysticercosis, ascariasis, strongyloidiasis or toxocariasis patients were also included.

WESTERN BLOTTING PROCEDURE

The ES antigen or the 45 kDa protein was solubilized under reducing conditions and electrephorized on

10 % polyacrylamide gels in SDS according to Laemmli (1970). For Western blot analysis, antigens were electroforetically transferred onto nitrocellulose membrane according to the procedure of Towbin *et al.* (1979). Blots were incubated at 4°C overnight in 0.05 % Tween 20/phosphate-buffered saline (PBS/Tween) containing 1 % low fat milk (Protifar, Nutricia). Immunodetection was performed by incubation of the blots with serum samples diluted in 0.05 % PBS/Tween containing 1 % low fat milk. After sequential washing with PBS/Tween the blot was incubated with peroxidase conjugated monoclonal antibodies directed to the various IgG subclasses (CLB, The Netherlands) or with peroxidase conjugated goat anti-human IgM and IgG (Sigma). Conjugated antibodies were diluted in PBS/Tween containing 1 % low fat milk. After washes in PBS/Tween, tetramethyl benzidine/di-octyl-natrium-sulpho-succinate (TMB/DONS, 0.06 % and 0.2 %, respectively in Dimethyl sulfoxide, DMSO) was added as substrate.

RESULTS

WESTERN BLOT ANALYSIS OF IGM AND IGG REACTIVITY TO ES ANTIGEN

Analysis of Western blots using *T. spiralis*-ES antigen indicates a specific banding pattern represented by bands of 125 kDa, 90 kDa, 78 kDa, 38 kDa, 31 kDa and 22 kDa that are recognized by IgM and IgG antibodies present in sera from a *Trichinella*-infected patient (Fig. 1). Bands of 45 kDa, 55 kDa and 58 kDa recognized by IgM and total IgG antibodies of trichinellosis patients appeared occasionally using sera from patients with other helminth infections. We observed however, that IgM and total IgG antibodies present in sera from *T. spiralis*–infected patients reacted strongly to a 45 kDa antigenic component. The IgG response to this component was predominantly of the IgG1 and IgG2 subclass with a minor IgG3 response. Interestingly, a clear 45 kDa-specific IgG4 response was detected (Fig. 2). Therefore, we became interested to investigate further the parasite-specific IgG4 response of patients with trichinellosis.

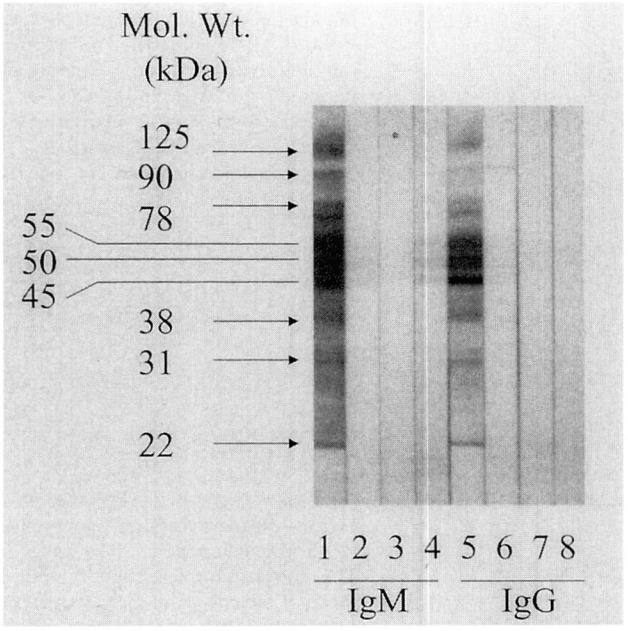

FIG 1. – Western blot revealing bands recognized by *Trichinella*-specific IgM and IgG antibodies. The ES antigen electrephorized on 10 % polyacrylamide gels in SDS was electroforetically transferred to nitrocellulose membrane. Indicated by arrows are the specific bands with molecular weight (Mol. Wt.) of 125 kDa, 90 kDa, 78 kDa, 38 kDa, 31 kDa and 22 kDa recognized by IgM (lane 1 to 4) and total IgG (lane 5 to 8) in serum from a trichinellosis patient from the outbreak that took place in Poland in 1991 (lane 1 and 5). Also indicated by lines are bands of Mol. Wt. 45 kDa, 50 kDa and 55 kDa which are recognized by IgM and IgG in sera of trichinellosis patients but are also ocassionally recognized by antibodies from other helminth infected patients (lane 4); echinococcosis (lanes 2 and 6), filariasis (lane 3 and 7), cysticercosis (lane 4 and 8).

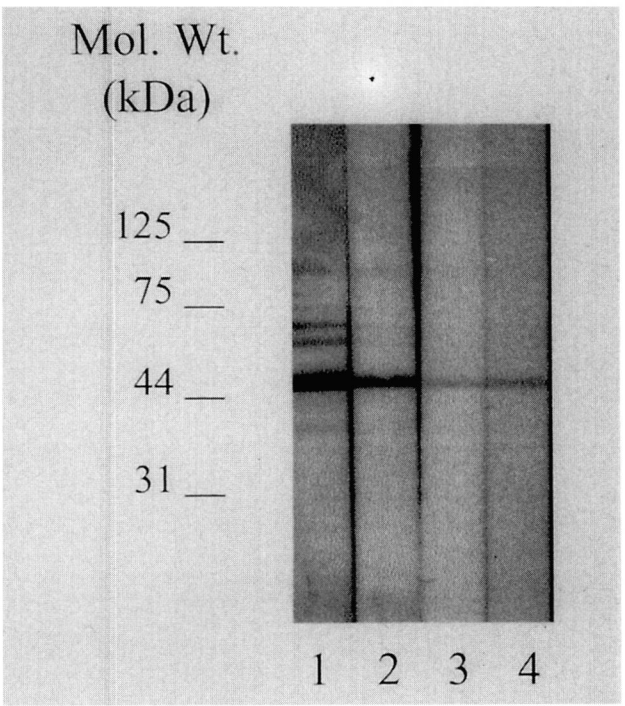

FIG 2. – Reactivity of IgG subclass antibodies to 45 kDa component of ES antigen. A partially purified fraction of the component of ES antigen with a molecular weight (Mol. Wt.) of 45 kDa was electrophorized on 10 % polyacrylamide gels in SDS was electroforetically transferred to nitrocellulose membrane. Indicated are results using serum from a trichinellosis patient from the outbreak that took place in Poland in 1991; IgG1 (lane 1), IgG2 (lane 2), IgG3 (lane 3) and IgG4 (lane 4).

Recognition of ES antigen by IgG4 antibodies

Fig. 3 shows the recognition of the ES antigen by IgG4 antibodies present in serum samples of trichinellosis patients. The intensity of this response varied from patient to patient and it was detected only when using sera from trichinellosis patients and not from patients with echinococcosis, filariasis, cysticercosis, ascariasis, strongyloidiasis or toxocariasis.

Recognition of 45 kDa component of ES by IgG4 antibodies

In Fig. 4, the recognition of the 45 kDa component of the ES antigen by IgG4 antibodies is shown. The intensity of this response varied from patient to patient and it was observed only when using sera from trichinellosis patients. In contrast, no reactivity was found using sera from echinococcosis, filariasis, cysticercosis, ascariasis, strongyloidiasis or toxocariasis patients. The serum samples used here were the same and in the same order as that described for Fig. 3. Noteworthy is the weak reactivity of IgG4 from patients shown in lane 15 and 16. However, no reactivity at all was observed using the ES antigen and this same serum samples (Fig. 3).

DISCUSSION

Infection with *Trichinella spiralis* evokes a humoral immune response, predominantly of the IgM, IgG and IgA classes (van Knapen *et al.,* 1982; Au *et al.,* 1983; Feldmeier *et al.,* 1987). In this study we report

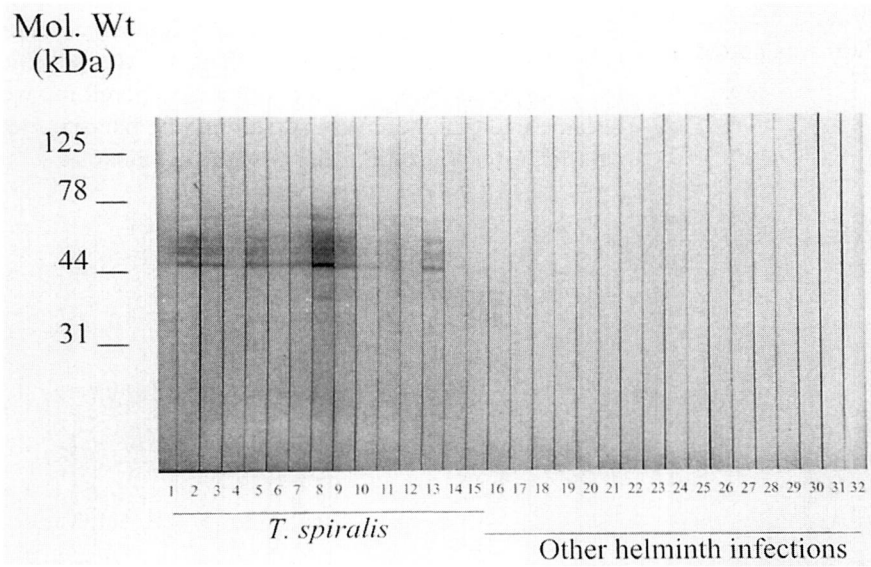

Fig 3. – Reactivity of IgG4 antibodies to ES antigen. The serum samples used included those of patients from the outbreak of trichinellosis taken place in Poland, 1991 (1-10), and serum samples received at our laboratory of patients with trichinellosis (11-15), echinococcosis (16-17), filariasis (18-19), cysticercosis (20-22), ascariasis (23-25), strongyloidiasis (26-28) or toxocariasis (29-32).

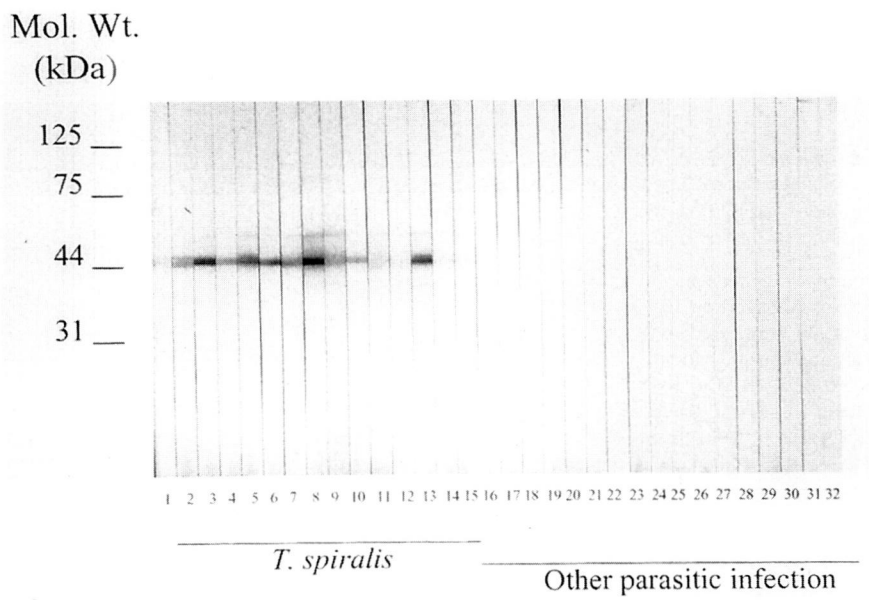

Fig 4. – Reactivity of IgG4 antibodies to 45 kDa component of ES antigen. The serum samples used included those of patients from the outbreak of trichinellosis taken place in Poland, 1991 (1-10), and serum samples received at our laboratory of patients with trichinellosis (11-15), echinococcosis (16-17), filariasis (18-19), cysticercosis (20-22), ascariasis (23-25), strongyloidiasis (26-28) or toxocariasis (29-32). The serum samples used here were the same and in the same order as that described in Fig. 3.

on the use of Western blots for the identification of component of the ES antigen recognized by IgM and IgG subclasses present in sera of patients at an early stage of trichinellosis.

An interesting result from our study is the detectable IgG4 response directed to a 45 kDa component of the ES antigen. This response was found to be *T. spiralis*-specific since it was not detected using sera from patients with other helminth infections.

An increase in the production IgG4 during infection with helminths has been previously described (Ottesen *et al.*, 1985; Magnusson *et al.*, 1986). However, increased IgG4 antibody levels has been generally associated with chronic antigenic stimulation (Magnusson *et al.*, 1986; Aalberse *et al.*, 1983).

Detection of IgG4 levels in human trichinellosis has not been extensively studied. However, from an earlier follow-up study it was shown that a significant higher number of IgG4 *Trichinella*-positive sera were found by means of ELISA, during the chronic stage of infection. The authors suggest that the IgG4 response can discriminate between an early and a late infection with *T. spiralis* (Ljungstrom *et al.*, 1988). By identifying the target antigens of IgG4 and using a purified a fraction of one of these antigens we observed that using Western blots, this response could also be detected during an early stage of trichinellosis. Taking together, monitoring the 45 kDa-specific IgG4 response can be a valuable tool for the immunodiagnosis of human trichinellosis.

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