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The sequential appearance of IgG subclasses and IgE during the course of *Trichinella spiralis* infection

INGER LJUNGSTRÖM, L. HAMMARSTRÖM*, WANDA KOCIECKA† & C.I.E. SMITH* *Department of Parasitology, National Bacteriological Laboratory, Stockholm, *Department of Immunology, Stockholm University and Department of Clinical Immunology, Karolinska Institute at Huddinge Hospital, Sweden and †Department of Parasitic and Tropical Diseases, Medical Academy of Poznan, 60–355 Poznan, Poland*

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SUMMARY

Earlier studies have shown that IgG1 and IgG4 are the dominant IgG subclasses in the specific response during a chronic helminthic infection. It has also been suggested that IgG4 production results from chronic or repetitive antigenic stimulation and a correlation between IgG4 and IgE levels exists. An outbreak of *Trichinella spiralis* infection in Poland provided the opportunity to follow the sequential appearance of the IgG subclass and IgE responses in 15 patients during the early stage of *Trichinella* infection and to compare these observations in sera obtained one year later from the same patients. The results show that the sequential appearance of the IgG subclasses were IgG1 before IgG3 and IgG3 before IgG4. IgG1 antibodies dominated the immune response in all patients. A statistically significant increase in the number of IgG4 positive sera was observed in patients during the chronic stage compared to the findings during the early stage of infection (13% vs 73%; $p < 0.001$), supporting the view that IgG4 results from a chronic antigenic stimulation. A correlation between the appearance of IgG4 and IgE was not found. The highest levels of IgE were seen in the first serum samples obtained, with a decrease during the course of infection.

Keywords *Trichinella spiralis* human IgG subclasses IgE.

INTRODUCTION

Trichinosis is a disease caused by a parasitic nematode, *Trichinella spiralis*, with world-wide distribution. The worm is capable of infecting all mammals, and humans get infected when consuming raw or insufficiently cooked *Trichinella* infected meat. A specific IgG, IgA and IgM response accompanies trichinosis (Ljungström, 1974; Boczon *et al.*, 1981; Van Knapen *et al.*, 1982; Au *et al.*, 1983; Feldmeier 1987). Specific IgE antibodies and increased levels of total IgE, however, seem not to be obligatory findings in patients suffering from trichinosis (Ljungström, 1974; Patterson *et al.*, 1975; Van Knapen *et al.*, 1982; Au *et al.*, 1983; Feldmeier *et al.*, 1987).

Previous studies have shown that the dominant IgG subclasses in the specific response during parasitic infections are IgG1 and IgG4 (Iskander *et al.*, 1981; Ottesen *et al.*, 1985; Magnusson *et al.*, 1986) and it has been suggested that the IgG4 production results from chronic or repetitive antigenic stimulation (Iskander *et al.*, 1981; Aalberse *et al.*, 1983; Ottesen *et al.*, 1985; Magnusson *et al.*, 1986). Interleukin 4 (IL-4), a B-cell stimulatory factor, has been shown to increase the production of IgG1 (Vitetta *et al.*, 1984; Vitetta *et al.*, 1985; Sideras *et al.*, 1985) and IgE (Coffman *et al.*, 1986) in mouse B cells. IL-4 has also been demonstrated to be involved in IgE production in mice infected with a parasitic nematode, *Nippostrongylus brasiliensis* (Finkelman *et al.*, 1986). Recently, human IL-4 has been identified (Yokota, *et al.*, 1986). This B-cell stimulatory factor and the murine IL-4 mediate several similar activites such as the ability to induce IgE secretion by activated B cells (Coffman *et al.*, 1986; Pène *et al.*, 1988).

The sequential appearance of specific antibodies of the various IgG subclasses during the early stage of a parasite infection has so far to our knowledge not been reported. Nor has a comparison between such observations and the findings one year later in sera from the same patients been described. An outbreak of trichinosis in West Poland, caused by consuming raw or lightly smoked sausage prepared from fresh wildboar meat, provided this opportunity. Sera were collected at various times after early infection and one year after infection. In addition to the measurement of the specific IgG subclass responses, the *total* IgE level and the *specific* IgE response were also determined.

Correspondence: Inger Ljungström, Department of Parasitology, National Bacteriological Laboratory, S-105-21 Stockholm, Sweden

Table 1. Clinical course and treatment schedule. The day of infection, the clinical course of illness and the treatment schedule of the various patients

Patient No.	Day of Infection	Clinical course	Treatment (days after infection)		
			Prednisolone	Mebendazole	Pyrantel
1	85-03-27	Severe	19-31 days	13-16 days	28-29 days
2	85-03-28	Moderate	19-31 days	11-14 days	—
3	85-03-24	Very severe	17-56 days	12-15 days	30-31 days
4	85-03-24	Moderate	20-36 days	11-14 days	—
5	85-03-27	Very severe	22-42 days	16-19 days	—
6	85-03-30	Severe	16-28 days	7-10 days	—
7	85-03-25	Severe	25-50 days	—	29-30 days
8	85-03-28	Severe	14-41 days	9-12 days	—
9	85-03-28	Moderate	21-41 days	16-19 days	—
10	85-03-25	Moderate	32-44 days	—	—
11	85-03-24	Moderate	35-47 days	—	—
12	85-03-26	Severe	15-42 days	13-15 days	—
13	85-03-28	Mild	20-38 days	20-23 days	—
14	85-03-28	Severe	25-60 days	—	29-39 days
15	85-03-24	Moderate	25-37 days	—	34-37 days

MATERIALS AND METHODS

Patients

The outbreak of trichinosis took place in West Poland, 100 km south east of Poznan, in the end of March 1985. In all, 17 persons were infected by eating raw or lightly smoked sausage prepared from fresh wildboar meat. Examination of the meat revealed 30 *Trichinella spiralis* larvae/10 mg. The severity of the clinical course was evaluated as suggested by Kassur *et al.*, (1978) and all patients were treated by prednisolone and antihelminthic drug(s) (Table 1). Sera were not available from two patients, one did not survive the infection (Kociecka *et al.*, 1988) and one miscarriaged and refused co-operation. From each of the other 15 patients (nine females and six males), members of four families and two single individuals (cousins), at least three sera were obtained. A total of 83 sera were investigated. The medium age of the patients was 24 years. The eldest was 68 and the youngest 7 years old (Table 2). The sera were stored at −20°C until used.

Antigen

A soluble, saline extract of muscle larvae was used as antigen (Ottesen *et al.*, 1975). The muscle larvae were isolated from rats which had been infected 6 to 8 weeks earlier (Ljungström, 1974). The protein concentration of the soluble antigen was 3·7 mg/ml.

ELISA

The antigen was coated on polystyrene microtitre plates (Nunc, Roskilde, Denmark) at a concentration of 3 μg/ml. The subclass distribution of specific antibodies was determined as described previously (Persson *et al.*, 1985). Briefly, after incubation of the serum samples (diluted 1/100) overnight on antigen-coated plates, commercially available monoclonal antibodies against the various human IgG subclasses (Unipath, London) were added in optimal concentrations. Rabbit anti-mouse Ig (DAKO Immunoglobulins, Copenhagen, Denmark) was thereafter added. After incubation for 4 h, alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Company, St Louis, MO, USA) was added and the plates were again incubated overnight. After washing, substrate was added and the plates were incubated for 10–20 min. Absorbance (OD) was measured at 405 nm using a Titertek multiscan (Elflab OY, Helsinki, Finland).

Sera were considered to contain specific IgG antibodies, when showing an optical density (OD) of ≥0·3 in ELISA and considered to contain a trace of the antibody, when the OD was

Table 2. The patients. The age and sex of the members of the four families and two single individuals investigated with regard to the IgG subclass responses against *Trichinella spiralis* infection

Family	Patient No.	Age	Sex	Group
—	2	24	F	I
A	11	12	F	I
A	7	37	F	I
A	15	40	M	II
A	10	14	M	II
A	14	7	F	II
B	12	37	F	II
B	13	17	M	II
C	1	45	F	I
C	9	46	M	III
C	8	18	M	III
D	4	18	F	III
D	—	41	M	—
D	3	42	F	III
D	6	11	M	IV
—	5	68	F	IV

Group I; IgG4 OD-value <0·3 during both stages of infection; Group II; IgG4 OD-value <0·3 during the early stage and >0·3 during the chronic stage of infection; Group III; see group II besides, IgG3 OD-value >0·3 during both stages of infection; Group IV; IgG3 and IgG4 OD-values >0·3 during both stages of infection.

< 0.3, but still above background. As definition, the various IgG subclasses were regarded as positive for that particular subclass showing an OD ≥ 0·3. The background values for IgG1, IgG2, IgG3, IgG4 were 0·056, 0·020, 0·042, 0·064, respectively. The majority of the sera were analysed twice.

IgE Determination

The determination of total IgE and specific IgE in the sera was performed by Pharmacia Diagnostica, Uppsala, Sweden. The range of total IgE in a normal population has been found to be between 2·5–122 KU/l. Thus, values above 122 KU/l were regarded as increased. Total IgE was assayed by Phadezym IgE PRIST and specific IgE by Phadezym RAST methodology using the same antigen as above.

RESULTS

The amount of specific IgG subclass antibodies

The patients could be divided into four groups according to the amounts of specific IgG1, IgG3 and IgG4 antibodies observed during the early stage and one year later of *Trichinella spiralis* infection. In Group I and II only IgG1 appeared to be positive (OD > 0·3) during the early stage of infection. In Group III, in addition to IgG1, a positive response of IgG3 was observed. Finally in Group IV IgG1, IgG3 and IgG4 were all noted as positive during the same period of time (Fig. 1).

One year later, in the chronic stage, a shift of specific antibodies to IgG4 was observed in all sera from patients belonging to Group II and III (OD > 0·3). In group II also three of five patients had become positive for IgG3. No shift in subclass responses were noted in the sera from patients in Group I (OD < 0·3; Fig 1).

Thus, during the early stage of infection only 13% (2/15) of the patients appeared to be positive for IgG4, while 73% (11/15) of the patients were positive one year later ($P < 0{\cdot}001$). A positive specific IgG3 response appeared in 40% (6/15) of the patients early in infection and one year later 67% (10/15) had become positive ($P > 0{\cdot}05$). The figures for specific IgG2 were 27% (4/15) and 13% (2/15), respectively ($P > 0{\cdot}05$; Table 3).

Interestingly, it was recognized that the families A and B consisted of patients only belonging to Group I and II and that all members of family C and D belonged to Group III and IV, with one exception, patient no. 1 (Table 2).

The specific peak response during the early stage

The peak response was evaluated in 14 of the 15 patients, as only two serum samples were available from patients No. 13. In 10 patients, all belonging to Groups II–IV, a sequential appearance of IgG1 and IgG4 was observed and specific peak response of IgG1 appeared before IgG4. A simultaneous peak response of IgG1 and IgG3 was observed in six of these patients. The peak response of both IgG1 and IgG3 was generally obtained

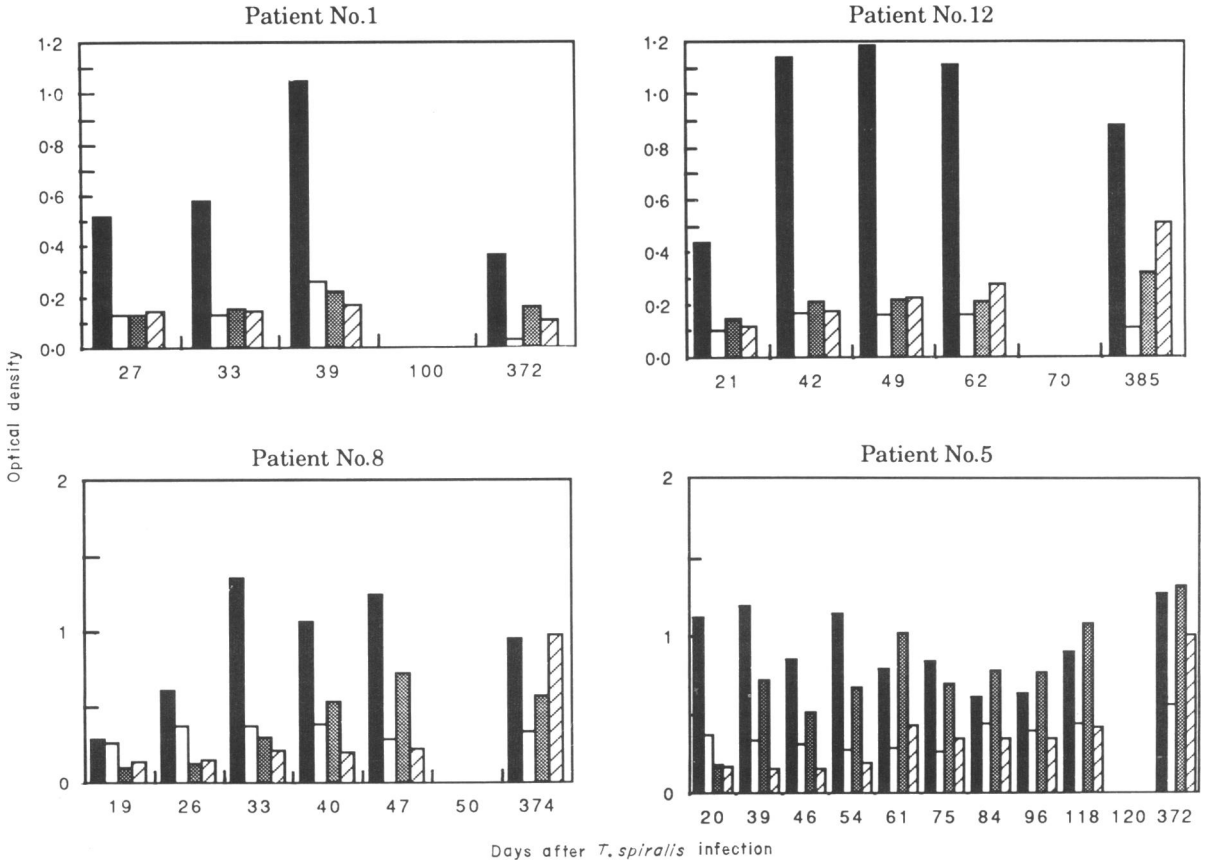

Fig. 1. The kinetics of the specific IgG subclass responses during the course of trichinosis. Groups I, II, III and IV are represented by patient No. 1, 12, 8 and 5 respectively. The values of optical density (OD) at serum dilution 1:100 are presented. Each bar represents the response of one subclass, ■ = IgG1, □ = IgG2, ▨ = IgG3 and ■ = IgG4, at the various times (days) after infection the sera were obtained from the patients. For definition of groups, see legend of Table 2.

Table 3. IgG subclass responses. The distribution of the specific IgG subclass responses in the various groups of patients during the early and chronic stages of *Trichinella spiralis* infection

Group of patients	Total number of patients	Number of patients with OD > 0·3							
		Early stage				Chronic stage			
		IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
I	4	4	1	0	0	4	0	1	0
II	5	5	0	0	0	5	0	3	5
III	4	4	1	4	0	4	1	4	4
IV	2	2	2	2	2	2	1	2	2
Total	15	15	4	6	2	15	2	10	11

Table 4. IgG subclass responses. The distribution of the specific IgG subclass responses during the early and chronic stages of *Trichinella spiralis* infection in relation to the clinical course of the patients

Clinical course	Total number of patients	Number of patients with OD > 0·3							
		Early stage				Chronic stage			
		IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
Mild	1	1	0	0	0	1	0	0	1
Moderate	6	6	0	2	0	6	0	3	4
Severe	6	6	2	2	1	6	1	5	4
Very Severe	2	2	2	2	1	2	1	2	2

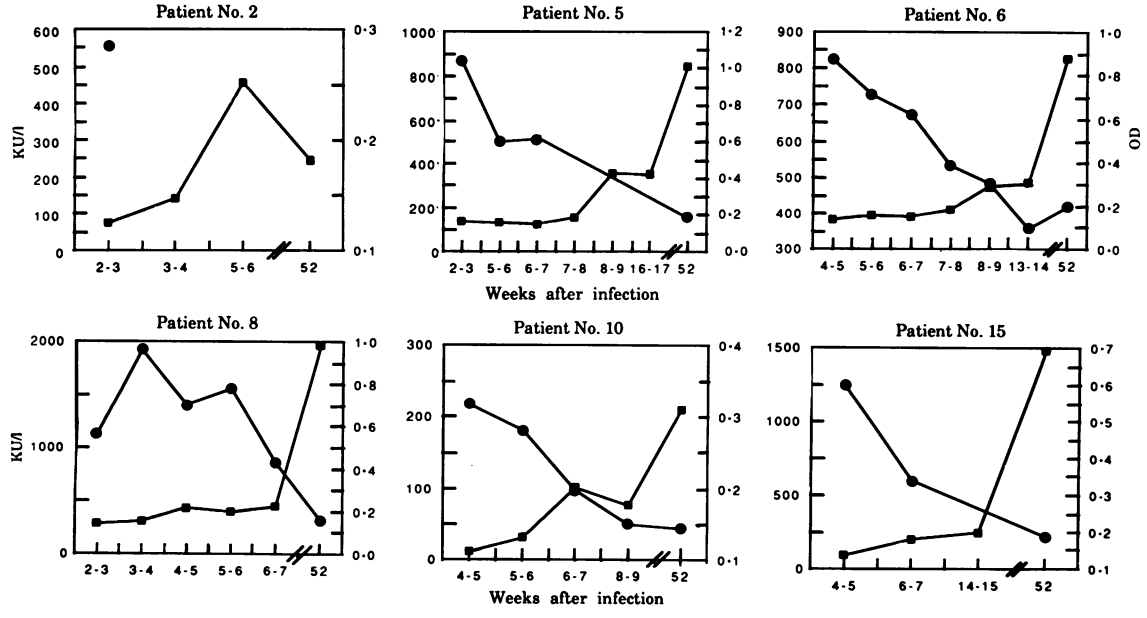

Fig. 2. The kinetics of total IgE levels ●——● (KU/l) and specific IgG4 value ■——■ (optical density; OD) during the course of *Trichinella spiralis* infection.

between 6 to 7 weeks after infection. In Group IV the specific peak response of IgG1 was already reached within 3 weeks after infection.

Specific subclass response vs clinical course

In all patients the specific IgG1 was the dominant subclass. The specific IgG2 response was only positive (OD > 0·3) in patients with severe or very severe illness. Presence of IgG3, but not IgG4, seemed to correlate with severity of disease (Table 4).

Total and specific IgE responses

Sera from all patients were investigated for total IgE and six of the patients (40%) had reacted with an increase of total IgE (> 122 KU/l). The clinical course of these patients was from moderate to very severe illness. In contrast to IgG4, the highest amount of IgE was observed in the beginning of the disease with a decrease during the course of infection (Fig 2).

The specific IgE was analysed during the early stage of infection. None of the patients had measurable specific IgE antibodies. The range (OD) of normal Swedes ($n = 12$) was 0·014–0·041 with a mean value of 0·032. The values for the sera ($n = 70$) from the *Trichinella spiralis* infected patients was 0·009–0·058 with a mean value 0·021.

DISCUSSION

The sequential appearance of the specific IgG subclasses was followed up to four months after *T. spiralis* infection. It was found that the peak response of IgG1 appeared before IgG4 and in about half of the patients simultaneously with IgG3. Specific IgG1 antibodies dominated the immune response and the other IgG subclasses mostly appeared in trace amounts. During the year following the infection, a statistically significant shift of specific antibodies to IgG4 was noted. In addition to specific IgG4 antibodies, specific IgG3 antibodies also appeared in high amounts. These observations suggest that the expression of the IgG subclasses parallels the sequence, 5′-μ-γ3-γ1-α1-γ2-γ4-ε-α2-3′ (Flanagan & Rabbitts, 1982), of the immunoglobulin heavy-chain genes on chromosome 14. Thus, a succesive downstream utilization of the constant region genes may be an important feature in immune response to a helminth infection. The observation is consistent with findings in malaria infected persons (Wahlgren *et al.*, 1986) and supports the hypothesis that IgG4 antibodies become prominent upon chronic antigenic stimulation (Iskander *et al.*, 1981; Aalberse *et al.*, 1983; Ottesen *et al.*, 1985; Magnusson *et al.*, 1986).

The classification of the clinical course of trichinosis should only be used with reservation, as the intensity of symptoms at the onset of illness might depend more on the host's reactivity (e.g. eosinophilia, fever, oedema) than on the intensity of invasion (Kassur *et al.*, 1978). However, although specific IgG1 dominates, our findings indicate that the severity of illness is reflected in the IgG2 and IgG3 responses of the patients, while the IgG4 response seems to discriminate between an early and late infection with *T. spiralis*.

Genetic differences in the specific IgG subclass responses to *T. spiralis* infection between the various families may result in that two of the families belonged to group I and II and the other two families to group III and IV. Earlier studies in mice infected with *T. spiralis* have clearly shown the importance of the genetic factors in the antibody response (Wakelin & Denham, 1983). However, we cannot exclude the possibility that the families exhibit different food habits or that environmental factors may have contributed to the different patterns.

Some studies have shown a correlation between the IgE and IgG4 levels (Iskander *et al.*, 1981; Magnusson *et al.*, 1986), while others have not (Merrett *et al.*, 1983). In this study, level of total IgE showed an inverse correlation to levels of specific IgG4 antibodies, the latter appearing late during infection. Specific IgE antibodies were not detected in any of the sera obtained during the early stage. These results might indicate a polyclonal activation of IgE secreting cells during the first months of infection.

Due to the ability of anti-IL-4 antibody to inhibit IgE secretion, but not the IgG1 secretion, in their study using *Nippostrongylus* infected mice, Finkelman *et al.*, (1986) speculated that either a higher IL-4 concentration is required for the stimulation of IgE secretion than for IgG1 secretion or that IL-4 is required for stimulation of IgE, while IgG1 secretion may be induced by other stimuli. It has been shown *in vitro* that high concentrations of IL-4 are needed for IgE stimulation (Paul & Ohara, 1987) and it is tempting to speculate that IL-4 may be involved in the induction of IgG1 and IgE responses seen after *Trichinella* infection. The different kinetics of the IgG4 could argue against an IL-4 effect. However, the down-regulation of IgE may be caused by other interleukins and the possible effect of IL-4 on IgG4 still remains elusive.

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