THE STAGE-SPECIFICITY OF THE IgA RESPONSE TO NEWBORN LARVA AND TSL-1 ANTIGENS OF *TRICHINELLA SPIRALIS* IN HUMANS INFECTED WITH THE PARASITE

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Summary :

Characterization of human IgA responses to newborn larva (NBL) and TSL-1 antigens was carried out by ELISA assays. Relevant and differential IgA antibody responses to these antigens were detected in humans infected with *T. spiralis*. The inhibition ELISA results showed that the IgA response to NBL antigens was inhibited significantly by both NBL and TSL-1 antigens and to a lesser extent when phosphorylcholine (PC) was used as inhibitor. In contrast, the IgA response to TSL-1 antigens was inhibited by the homologous antigen and to a lesser extent by the NBL and PC. Thus, the early IgA antibodies developed in trichinellosis patients contained a portion of IgA antibodies directed to PC which is present in TSL-1, A and NBL components. Another portion of antibodies to NBL are directed to other common non-defined epitopes present in TSL-1 and NBL antigens. All together these results suggest that the IgA response to common epitopes in antigens of both stages of the parasite may be useful for early diagnosis and epidemiological studies of human trichinellosis.

KEY WORDS : human trichinellosis, IgA response, TSL-1 antigens, newborn larva.

The study of immune responses to common or stage-specific antigens from *T. spiralis* is a neccessary step in the understanding of the host-parasite interplay (Parkhouse & Clark, 1983). In particular, the identification of these antigens can be useful for diagnosis or vaccination. For diagnosis an accurate assay should also include the determination of the isotype produced against antigens of the different stages of the parasite. This may be relevant particularly for an early diagnosis of the infection. Our previous studies in human have shown differential antibody responses during *T. spiralis* infection to the three stages of the parasite (Salinas *et al.*, 1993a,b; Salinas *et al.*, 1996). In this, a relevant IgA antibody response to NBL and TSL-1 antigens was observed. Similarly, studies by Almond & Parkhouse, (1986) using rodents experimentally infected with the parasite showed an early detection of this antibody isotype. In this study a further characterization of the IgA response to NBL and TSL-1 antigens was carried out and its stage-specificity was defined in a follow-up study of the antibody responses in humans involved in a trichinellosis outbreak.

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MATERIALS AND METHODS

DESCRIPTION OF THE OUTBREAK

The trichinellosis outbreak took place in February 1989 in Toluca, State of Mexico where 22 adults were infected by eating undercooked sausages prepared from pork meat. The description of the outbreak was described by Salinas *et al.*, (1996).

SERUM SAMPLES

At least, three serum samples were collected from each of the 22 individuals involved in the trichinellosis outbreak. Serum samples were taken at different intervals after infection at weeks: 3, 4, 5, 7, 12, 15, 37 and 57. Control serum samples were collected from 26 healthy adults with no history of helminthic infection and 30 samples from adults infected with other parasites (Salinas *et al.*, 1996).

PARASITES

T. spiralis was maintained by several passages in outbred BALB/c mice. Recovery of muscle larvae (ML), adult worms (A) and NBL was performed as described by Dennis *et al.*, (1970).

ANTIGENS

(a) total soluble extracts (TSE) were prepared from 3-days-old A, 18-h-old NBL and ML following the method described by Philipp *et al.*, 1981. (b) Purified ML surface/stichosomal antigens (TSL-1) were obtained as described by OrtegaPierres *et al.* (1989).

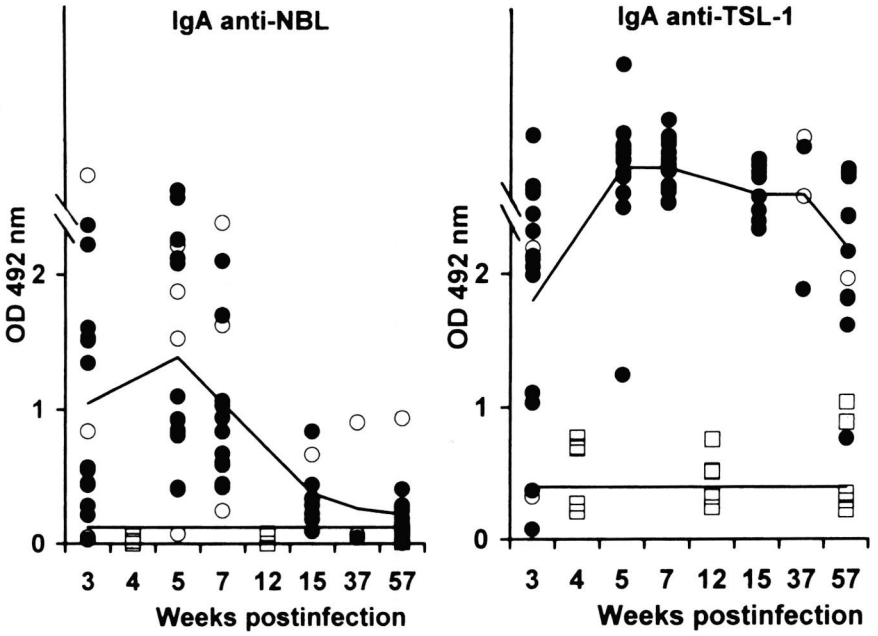

Fig. 1. – IgA response to NBL and affinity-purified TSL-1 antigens in individuals involved in a trichinellosis outbreak. ELISA assays were performed using a total soluble extract of newborn larvae (a) or affinity-purified TSL-1 antigens (b) of *Trichinella spiralis* and serum samples taken at different time intervals after infection from individuals with positive (●) or negative (○) biopsy and from asymtomatic individuals (□). Continued horizontal lines indicate X ± 1σ of OD values of serum samples from healthy individuals.

ELISA ASSAYS

Indirect amplified ELISA assays were done for IgA anti-NBL or anti TSL-1 antigens detection as previously described by Salinas *et al.*, (1996), with some modifications. These included the use of goat IgG anti-human IgA at a 1/4000 dilution and peroxidase-conjugated rabbit anti-goat IgG at a 1/8000 dilution for IgA anti-NBL antigens detection. Inhibition ELISA assays of the IgA response to NBL or TSL-1 antigens were done as above except that serum samples were diluted 1/20489 for IgA binding to TSL-1 antigens. Diluted serum samples were pre-incubated with the following inhibitors: 64 μg ml^{-1} of TSE of AD, NBL, ML, TSL-1 antigens or 10 mM PC in borate buffer, pH 8.3 as described by Peters *et al.*, (1999). Absorbances were determined by ELISA plate reader (Dynatech Industries). Binding of antibodies to the ELISA plate in the presence of inhibitor was expressed as a percentage by dividing the mean OD reading in the presence of the inhibitor by the mean OD reading in the presence of the diluting buffer, multiplied by 100.

STATISTICAL ANALYSIS

Statistical significance of the data was analyzed by t-Student or Mann-Whitney tests.

RESULTS

The analysis of antibody responses to *T. spiralis* antigens showed a much lower IgA response to NBL as compared with that observed to TSL-1 antigens throughout the infection. The IgA response to NBL was associated with the acute phase while IgA to TSL-1 was not distinctive from the acute or chronic phase of infection. Also, the detection of IgA to NBL (80 %) was as sensitive as that of IgA to TSL-1 antigens (75 %), by week 3 after infection (Fig. 1). To determine if IgA responses to NBL and TSL-1 antigens were stage-specific, inhibition ELISA assays were performed. In these, serum samples collected by week 3 or 5 pi and 64 μg ml^{-1} *T. spiralis* antigens were used, as well as 10 mM PC, since this epitope is widely distributed in nature, including *T. spiralis*. Inhibition ELISA results showed that NBL proteins inhibited ≥ 50 % of the serum IgA binding activity to NBL proteins in 11/12 serum samples taken from trichinellosis patients by week 3 and in 14/15 by week 5 while TSL-1 antigens in 10/12 serum samples by week 3 and in 13/15 by week 5 pi (Fig. 2). Moreover, IgA binding to NBL was inhibited in ≥ 50 % in 10/15 serum samples when ML TSE was used. In contrast, IgA binding to NBL was inhibited by TSE of A and PC in 5/15 and 7/15 serum samples by week 5 pi, respectively (Fig. 2). Based on these data further assays were performed using a dilution which gave approximately half-maximal absorbances and the same inhibitor concentration. The results obtained showed that IgA binding to NBL was inhibited from 10-15 % at 1/320 serum dilution to 85-95 % at 1/1280 serum dilution by TSL-1 or NBL proteins while the inhibition by A and PC was similar to the one previously observed (data not shown). On the other hand, TSL-1 antigens inhibited more than 50 % IgA antibodies in 11/12 serum samples to solid-phase TSL-1 components while NBL inhibited at this percentage only in 1/12 serum samples by week 3 after infection (Fig. 3). Similar results were obtained

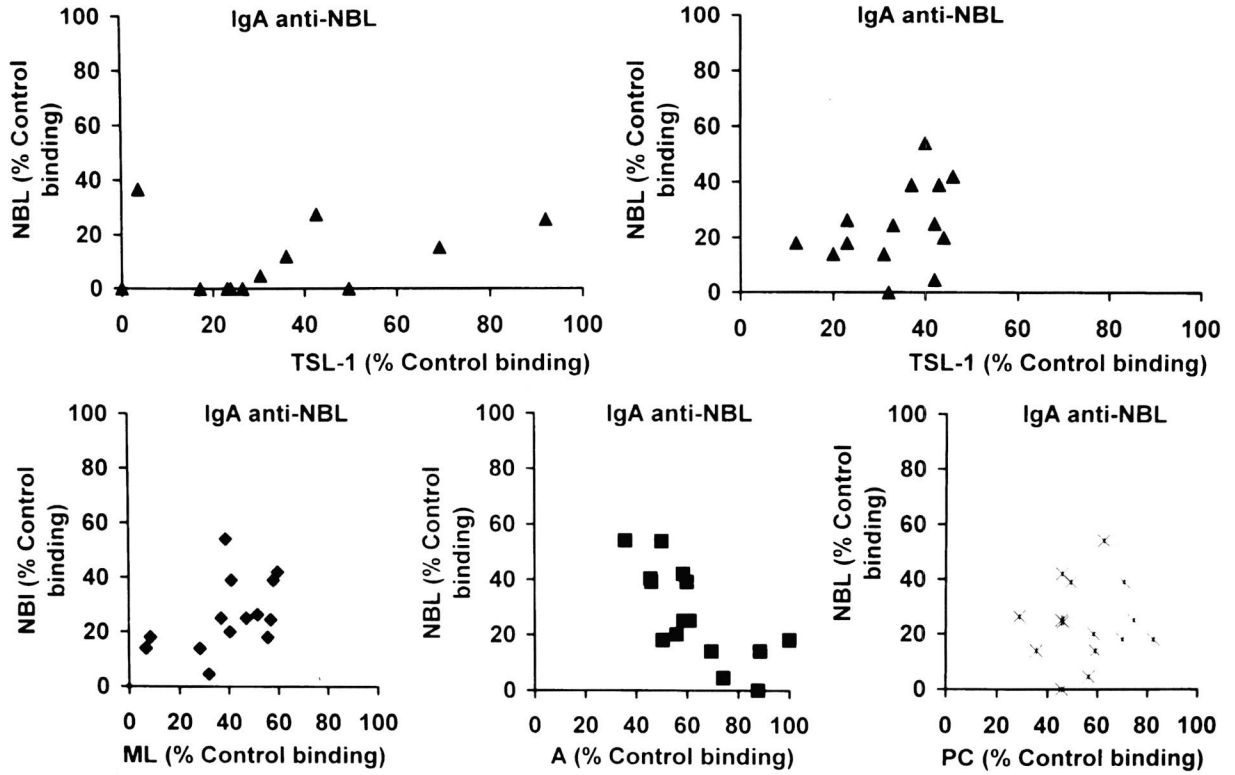

Fig. 2. – Inhibition of human IgA binding to NBL by *T. spiralis* antigens or PC.
Inhibition ELISA assays were performed using serum samples taken from trichinellosis patients by weeks 3 (a) or 5 (b,c,d,e) diluted 1/320 and preincubated with 64 μml^{-1} of affinity-purified TSL-1 antigens (▲), NBL, ML (◆), A (■) TSE of *T. spiralis* as well as 10 mM PC (*). Binding as % of control was calculated as indicated in *Materials and Methods.*

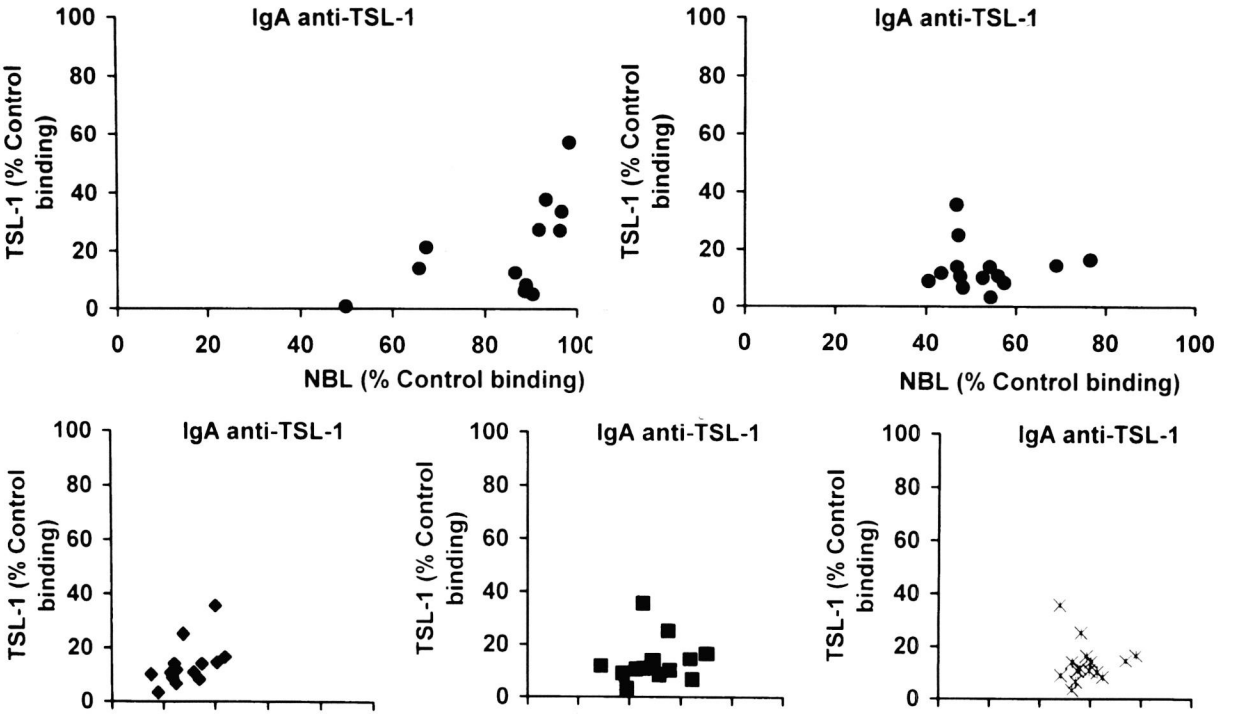

Fig. 3. – Inhibition of human IgA binding to TSL-1 antigens by *T. spiralis* antigens or PC.
Inhibition ELISA assays were performed using serum samples taken from trichinellosis patiens by weeks 3 (a) or 5 (b,c,d,e) diluted 1/320 (a) or 1/20,480 (bc,d,e) and preincubated with 64 μml^{-1} of affinity-purified TSL-1 antigens, NBL (●), ML (◆), A (■) TSE and of *T. spiralis* as well as 10 mM PC (*). Binding as % of control was calculated as indicated in *Materials and Methods.*

when serum samples taken by week 5 were analyzed except that IgA binding activity was not inhibited by NBL proteins and ≥ 50% inhibition by TSL-1 components in only 6/15 serum samples was observed (data not shown).

Due to the highest titers of IgA antibodies to TSL-1 antigens observed in trichinellosis patients by week 3 or 5 pi, the stage-specificity of these antibodies was determined using a dilution which yielded approximately 50 % maximal absorbance. When serum samples were tested, IgA binding to TSL-1 proteins was inhibited from ≥ 70% in 9/12 serum samples at 1/320 serum dilution to ≥ 83% in 13/15 serum samples diluted 1:20400 by the homologous antigen (Fig. 3a,b) while the NBL did inhibited it to a lesser extent (inhibition ≥ 50% in 7/15 serum samples at week 5). Similarly, the IgA binding to TSL-1 was also inhibited by A and PC in ≥ 50% in 8/15 and 2/15 serum samples, respectively (Fig. 3d,e). In contrast, ML TSE inhibited IgA binding to TSL-1 in the same way as TSL-1 antigens (Fig. 3c).

DISCUSSION

In this study, we have analyzed the IgA response in trichinellosis patients to NBL and TSL-1 components of the parasite. Interestingly, the IgA and IgG_3 antibody responses against TSL-1 glycoproteins (Salinas *et al.*, 1996) as well as the IgA to NBL components were detected in a high percentage of patients. However, a stronger IgA antibody response to TSL-1 antigens was observed as compared to that obtained to NBL proteins. As expected, polyclonal IgA antibody responses observed in trichinellosis patients included IgA antibodies to non-stage specific epitopes on the parasite components as shown by ELISA results. The inhibition patterns of IgA binding to NBL proteins by TSL-1 components and ML TSE were similar in most trichinellosis patients, likewise, inhibition of IgA binding to TSL-1 glycoproteins by TSE of NBL, indicated the presence of common epitopes on TSL-1 and NBL components. Similar inhibition patterns of IgA binding to NBL by TSE of A and PC but different to the other ones, suggested that a minor proportion of IgA antibodies to NBL were also directed against common epitopes on A components and PC which had developed in most trichinellosis patients. As far as the IgA binding to TSL-1 antigens is concerned similar inhibition patterns were observed using as inhibitor the homologous antigen or ML TSE. An inhibition of ≥ 50 % in most serum samples was evident. However, a more restricted and similar IgA binding to TSL-1 antigens was shown using NBL, A or PC.

The data obtained in this study may be related to the quantity or the quality of the epitopes expressed on TSL-1, A and NBL antigens (Ortega *et al.*, 1996). Furthermore, cross-reactive IgA antibodies to TSE of A, ML and NBL might be directed to non-defined epitopes and PC. Indeed, PC is increasingly becoming recognized as a structural component of a wide variety of prokaryotic and eukaryotic pathogens (Harnett & Harnett, 1999). In the eukaryotic parasite *T. spiralis*, PC is located in several subcellular structures of ML containing antigens that induce early antibody responses (Takahashi, 1997). Also PC is present in subcellular structures of NBL and adult worm as well, i.e., the embryonic sheath, or the adult exocrine glands which are possible sources of adult ES antigens (Appleton *et al.*, 1991; Takahashi, 1997). Furthermore, antigens located on the cuticular surface of ML, also contain PC (TSL-4 and 8 antigens). Thus, it is possible that affinity-purified TSL-1 antigens, which induce a strong antibody response late in infection (Denkers *et al.*, 1990), also contain PC.

Early IgA antibodies to PC observed in trichinellosis patients may be due to the presence of this epitope in such subcellular structures of the parasite that may reach the host immune system by PC-ES of A worms or when parasites dye. This is supported by studies in mice and rats in which a proportion of both early mucosal and systemic antibodies specific for PC bearing ML antigens were detected following infection with *T. spiralis* (De Vos & Dick, 1993; Peters *et al.*, 1999).

Although some studies indicate that PC does not play a major role in immune defense mechanisms (De Vos & Dick, 1993; Peters *et al.*, 1999), PC exhibits a surprising range of immunomodulatory properties that might be to the detriment of the host . This indeed has been demonstrated in PC containing-component filarial or *Ascaris suum* antigens (Harnett & Harnett, 1999). On the other hand, some studies performed in rodents experimentally infected with *T. spiralis* suggest a role for IgA in protective immunity (Almond & Parkhouse, 1986) but the immunological relevance of this isotype has not been elucidated in human. Therefore, further characterization of antigenic carrier proteins recognized by IgA anti-*T. spiralis* is essential to identify epitopes, different from PC, which are useful in early diagnosis of human trichinellosis. Also of great impact on the study of the host parasite interplay will be the elucidation of their functional role in this interaction.

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