Relationship among presence of antibodies against ascaris suum, eosinophilia and autoantibodies (igm-rf)

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KEY WORDS: IgM-RF, habitat, gender, ascariosis seroprevalence, eosinophilia

ABSTRACT

The possible association among the presence of IgG antibodies against the parasitic helminth Ascaris suum, eosinophilia, and that of IgM-rheumatoid factors (IgM-RF) was analyzed in blood samples from 1264 persons. Three groups according to the values of eosinophils were considered, normal (G-C, <0.5 109 l-1), eosinophilic (G-I, 0.5-3 109 l-1) and hypereosinophilic (G-II, >3 109 l-1). The detection of IgG antibodies was done by using a modified enzyme-linked immunosorbent assay (ELISA) with A. suum excretory/secretory antigens, and IgM-RF autoantibodies by a latex agglutination test. Forty-one percent of the cases were positive to ascariosis and 15% had IgM-RF autoantibodies. The greatest values were reached in women from rural areas. The highest percentages of eosinophilic patients were found in men from rural areas. A positive and significant association between eosinophilia and IgM-RF autoantibodies was observed. We concluded that people living in rural regions have a higher risk for developing IgG antibodies against *A. suum*..

INTRODUCTION

Diagnosis of several helminthzoonosis like

Table I.- Distribution of the blood samples analyzed in the current investigation by considering the sex and the place where the patients are living (n= 1264).

	G-C			G-I			G-II		
	Country	Town	Total	Country	Town	Total	Country	Town	Total
Women	83	54	137	272	171	443	109	63	172
Men	23	31	54	169	129	298	86	74	160
Total	**106**	**85**	***191***	**441**	**300**	***741***	**195**	**137**	***332***

toxocariosis, trichinellosis or anisakiosis in human population toxocariosis is difficult because neither worms nor eggs are eliminated in faeces. Presumption of some human parasitic diseases is based on the presence of hypereosinophilia, and peripheral blood eosinophilia has been widely associated with helmintosis responsible for visceral larva migrans1, although this increase is non-specific and can be attributable to other diseases.

The finding that migrating larvae release excretory/secretory antigens in the host which stimulate the production of antibodies was employed to develop diagnostic probes focused on the detection of the humoral immune response against parasite antigens, such as the indirect enzyme-linked immunosorbent assay (ELISA) test[2].

The antibodies produced by the immune system do not attach to foreign substances only, and antibodies which binds other immunoglobulins are also released, the autoantibodies. Rheumatoid factors (RFs) are Fc-specific anti-IgG autoantibodies, usually of IgM isotype. Low titers of RFs are often detected in normal individuals, but RF high levels are detectable in up to 10% of normal individuals, 70-80% patients with several inflammatory diseases such as rheumatoid arthritis and in other systemic infections. RFs are used in clinical practice to diagnose some inflammatory disorders[3].

The main goal in this investigation was to elucidate the possible relationship among the presence of antibodies against *Ascaris suum*, eosinophilia and the production of IgM-RF autoantibodies.

MATERIALS AND METHODS

Sera collection

Sera from 1264 adult individuals were randomly collected from the Hospital Xeral (Lugo, Spain). No parasitological signs or rheumatologic diseases had been previously detected. Coprological tests were negative. Table I reflects the distribution of the samples according to their sex and habitat. Three groups were considered by taking account the values of eosinophils: normal, G-C (<0.5 109 l-1), eosinophilic, G-I (0.5-3 109 l-1) and hypereosinophilic G-II (>3 109 l-1).

IgM-RF detection

This test was carried out with the RF latex kit (Monlab, Barcelona, Spain). Macroscopic agglutination of IgG coated latex particles indicated a positive reaction. Quantification of positive results was not done. Positive and negative controls provided in the kit were included.

Excretory/secretory antigen preparation

Table 2 Risk analysis of the prevalence of antibodies against Ascaris suum, IgM-RF and eosinophilia.

		Women (*n*= 752)	Men (*n*= 512)	Country (*n*= 740)	Urban (*n*= 524)
Antibodies to *A. suum* Total cases: 621	*n*	415	206	218	403
	OR (95% CI)	1.8 (0.8-2.6)	0.5 (0.4-0.7)	1.7 (1.2-2.2)	0.6 (0.2-0.8)
	χ^2	18.947		14.303	
	p	0.001		0.001	
RF-IgM Total cases: 190	*n*	135	55	118	72
	OR (95% CI)	1.9	0.5	1.25	0.8
	χ^2	9.241		1.342	
	p	0.002		0.247	
Eosinophilia Total cases: 1073	*n*	616	457	633	440
	OR (95% CI)	0.5	1.8	1.2	0.85
	χ^2	14.045		0.787	
	p	0.001		0.675	

Table 3 Average values of IgG optical densities and eosinophil counts.

		IgG OD (492 nm) Cut-off:		Eosinophil counts (x 10^9 l^{-1})	
		x ± SD	F *p*	x ± SD	F *p*
Patients' sex	Women	0.8678 ± 0.3826	27.324 0.001	0.8977 ± 0.5886	6.591 0.010
	Men	0.7340 ± 0.3599		1.003 ± 0.6176	
Habitat	Urban	0.7205 ± 0.3074	39.393 0.003	0.9363 ± 0.6169	0.061 0.805
	Country	0.8795 ± 0.4102		0.9464 ± 0.5821	
Antibodies to *A. suum*	Negative	0.5347 ± 0.1474	1132.7 0.001	0.9592 ± 0.6222	3.160 0.076
	Positive	1.1029 ± 0.3252		0.8713 ± 0.5180	
FR-IgM detection	Negative	0.8295 ± 0.3919	8.894 0.003	0.93 ± 0.5243	1.528 0.217
	Positive	0.7242 ± 0.2817		1 ± 0.9281	

The use of excretory/secretory antigens from A. suum (AsES) was based on prior reports4. Adult worms were collected from swine guts at the slaughterhouse and washed several times in phosphate-buffered saline (PBS, pH 7.5). Females were identified and dissected under stereomicroscope, and A. suum-eggs collected from their uteri. After mechanical disruption of the eggs for obtaining the L2, the larvae were finally incubated in RPMI medium at 37ºC and 5% CO2 atmosphere for 6 hours. Eggs were removed by sieving and centrifugation, and the supernatant containing AsES products was collected and lyophilised. The protein concentration was estimated by the BCA technique described by Pierce®.

Detection of IgG against AsES

The antibody levels against the parasitic antigens were established by means of an indirect-ELISA. Wells of micro-plates were coated with 100 µl antigen (1 µg ml-1) and incubated overnight at 4ºC. Blocking of excess-binding sites was performed by incubation with 250 *u*l of PBS containing 0.05% Tween and 1% skimmed milk (PTM) for 30 min at 37ºC. Human sera diluted 1/50 in PTM were added in duplicate to the wells and incubated for 1 h at 37 ºC. After 6 washings 100 *u*l of horseradish-peroxidase-conjugated (HRP-conjugated) sheep anti-human IgG (H&L chains, Nordic Immunology Laboratories, Tilburg, Netherlands Nordic Immunology) were added diluted 1/1000 in PTM and incubated for 1 h at 37ºC. 100 µl of substrate consisting of 10 mg of ortho-phenylenediamine in 12 ml citrate buffer (pH 5.0) and 10 µl of 30% H2O2 were added to each well. The plates were incubated in the dark for 15 min at room temperature. The colour reaction was stopped by addition of 100 µl of 3N H2SO4, and absorbances were read using a spectrophotometer (Titertek Multiskan) at 492 nm.

In order to establish the cut-off point, positive values were the mean optical density (OD) of all negative sera plus three standard deviations5. Mean OD negative sera values were 0.3810 with a standard deviation of 0.0371. Thus, positive absorbance values were 0.4923 or higher.

Statistical methods

Statistical analysis was conducted using ANOVA (significance limit at p= 0.05). The non-parametric Spearman's correlation test was applied to evaluate the existence of correlation among the different variables considered. All tests were performed by the statistical package SPSS, version 14 (SPSS Inc., 2006).

RESULTS

ELISA

Forty-nine percent (95% CI, 46-52) individuals had positive IgG antibodies against AsES (Table II). A significant greater seroprevalence was observed in women (55%, 50-60) than in men (40%, 36-44) (x^2= 18.947, p= 0.001). The odds ratio (OR) value for the women was 1.8.

The seroprevalence was higher in the people living in rural areas (54%, 50-58) than those in the city (42%, 38-46). These differences were significant (x^2= 14.303, p= 0.001) and the OR was 1.7 for the country people (Table II).

As drawn in Table III, the values of absorbance were significantly greater in women than in men (F= 27.324, p= 0.001). ANOVA showed statistical differences in the absorbances between countryside patients and those from the city (F= 39.393, p= 0.003).

IgM-RF

The presence of IgM-RF was found in 15% (13-17) of the sera analyzed (Table II). The percentage of positive cases was higher in women (18%, 15-21) than in men (11%, 8-14) and these differences were significant (x^2= 9.241, p= 0.002). A significant OR value of 1.9 was estimated for the women.

The percentage of IgM-RF positive cases was similar in country (16%, 13-19) and urban individuals (14%, 11-17) and statistical differences were not obtained (Table II).

We observed 38% (73/190) of the patients with IgM-RF had also IgG against AsES. The value for OR of in these patients was 0.6 (0.4-08) (x^2= 7.238, p= 0.007).

Eosinophilia

Eighty-five percent (82-89) of the samples analyzed presented eosinophilia. From these cases, 69% (66-72) belonged to G-I and 31% (28-34) to G-II (Table I).

The highest percentages of eosinophilia were found in men (89%, 86-92), and statistical differences with the sex of the patients were observed (x^2= 14.045, p= 0.001) (Table II). An OR value of 1.9 was assessed for the masculine cases.

People living in the countryside achieved a greater prevalence (86%, 84-88) than those in the town (84%) (Table II), and by means of the Chi-square test significant differences were not proved (p> 0.05).

Eosinophilia and IgG antibodies to AsES was obtained in 45% patients (41-49), but 70% (64-76) cases with normal values of eosinophils were also positive to ascariosis (x^2= 28.014, p= 0.001). By using the Spearman's test, a negative and significant correlation between the levels of eosinophils and IgG was established (r= -0.186, p= 0.001).

The percentage of cases with eosinophilia was greater in the IgM-RF positive patients (91%), and by estimating the OR value a significant relationship between these parameters was achieved (OR= 2, 1-3.6; x^2= 4.583, p= 0.032).

Table III reflects that the eosinophil counts were significantly greater in men than in women. No differences were recorded in the eosinophil values regarding the habitat of the individuals or that of IgM-RF.

The relation among the eosinophilia, seroprevalence of antibodies against A. suum

Table 4 Analysis of the relation among the presence of IgG against Ascaris suum excretory/secretory antigens, eosinophilia and IgM-RF.

	Antibodies to *A. suum*			IgM-RF		
	OR (95% CI)	χ^2	p	OR (95% CI)	χ^2	p
Eosinophilia	0.4 (0.2-0.5)	28.266	0.001	2 (1.5-2.7)	6.022	0.049
IgM-RF	0.6 (0.4-0.8)	6.738	0.009			

and IgM-RF is represented in Table IV. A positive significant relation between IgM-RF and eosinophilia was found (OR= 3.1; x^2= 34.952, p= 0.001).

DISCUSSION

Eosinophilia may be attributable to several pathologies, including allergy, parasitic infections, neoplasms, vasculitis and some autoimmune diseases. For this reason, a case-study to gain more information on the influence of some factors in the eosinophilia, like the presence of IgG antibodies to the nematode A. suum or IgM-RF was conducted.

In the current work, we found 45% of patients with eosinophilia had positive IgG values against A. suum, but this percentage was 70% in the normal patients, which indicates the absence of relation between these two parameters. It is also remarkable that a negative and a significant correlation between the eosinophilia and the antibodies was obtained.

The percentage of patients positive both to eosinophilia and to autoantibodies was 91%, and a significant risk for eosinophilia in positive IgM-RF cases was established. In previous works eosinophil counts were significantly higher in the positive RF level group than those in a negative one6.

The presence of eosinophilia was significantly enhanced in men and the estimation of OR showed this population had a greater risk for eosinophilia, opposite to that reported in preceding investigations7.

The risk for ascariosis was higher in patients from rural areas which could be attributable to the pig farming conditions in the area of study. In most of rural farms from NW Spain, 1-4 pigs are maintained per year for family consumption. Treatment against parasites is seldom administered to swine livestock, and manure is manually eliminated. By following ancient traditions, pig guts are washed in water-courses for the elaboration of sausages. This implies that the possibility for sensitization with A. suum antigens is elevated in the population of this area. In people from Denmark all the examined patients acquired Ascaris-infections from domestic pigs, and ascariosis might therefore be considered a zoonotic disease8.

A significant risk for RF was found in women living in rural areas. There is a lack of information concerning the presence of autoantibodies. Although a relationship has been suggested between rheumatoid arthritis and contact with animals and soil, to date this has not been evident9.

Different mechanisms have been proposed to explain the production of autoantibodies, such as polyclonal B cell activation and molecular mimicry. Infection with helminth parasites induces a humoral immune response due to the activation of B cells10. It is tempting to speculate that the persistence of immune complexes, caused by inefficient clearance or by local production, may result in sustained T cell help to RF B cells. It is also noticeable the finding that the RFs may be present more than 10 years before the onset of clinical disease11, which suggests that important environmental factors are acting years before disease onset.

We concluded there is an association between eosinophilia and IgM-RF autoantibodies. People living in rural regions have a higher risk for developing IgG antibodies against A. suum. Further investigation is in progress to gain more knowledge about the influence of the residence place and the patients' gender on the presence of antibodies and autoantibodies.

ACKNOWLEDGEMENT

This work was partly supported by the Project XUGA PGIDIT06BTF26101PR (Consellería de Innovación e Industria, Xunta de Galicia, Spain). We are in debt with Mrs. B. Valcárcel for critically reading the manuscript.

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