ISOLATION AND CHARACTERIZATION OF A REAGINIC ANTIBODY FROM PIGS INFECTED WITH *Ascaris suum*[1]

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Um anticorpo reaginico análogo ao IgE da espécie humana, foi isolado de suínos infectados com *Ascaris suum.* Este anticorpo mostrou ser lábil ao calor, ao 2-mercaptoetanol, e teve um peso molecular de 220.000 daltons. Seu isolamento foi obtido pelo emprego das técnicas de cromatografia de troca ionica, de gel filtração, de cromatografia de afinidades e em eletroforese com gel de poliacrilamida. Sua caracterização foi obtida pelas provas de imunodifusão, imunoeletroforese e teste cutâneo de anafilaxia passiva.

TERMOS DE INDEXAÇÃO: Anticorpo reaginico, *Ascaris suum,* suínos.

ABSTRACT.- A reaginic antibody related to human IgE was isolated from pigs infected with *Ascaris suum.* This antibody was found to be labile to heat and 2-Mercaptoethanol, and had a molecular weight of 220,000 daltons. Its isolation was achieved by the use of ion exchange chromatography, gel filtration, affinity chromatography and polyacrilamide gel electrophoresis. Its characterization was obtained by inmunoelectrophoresis, immunodiffusion and passive cutaneous anaphylaxis tests.

INDEX TERMS: Reaginic antibody, *Ascaris suum,* pigs.

INTRODUCTION

The immediate type of hypersensitivity caused by an helminthic infestation and the antibody involved in this reaction (reaginic antibody), has been widely studied in experimental animals (Tada 1975). Kojima and Ovary (1975) provided evidence that the helminthic infestation led to a circumstance in which the reaginic antibody (RA) production was facilitated. However, a clear reason for the development of these high RA levels resulting from helminthic infestation and its function remains uncertain. It is probable that through the induction of the vasoactive compounds released by the mast cells, these antibodies contribute to protection against a parasitic infestation (Dobson 1972).

Pig RA was first identified by Barratt (1972) in an animal with a heavy *Metastrongylus spp.* infestation. This antibody was shown to have the same characteristics of the RA from other species. However, attempts to separate RA from IgA were only partially successful. Although the pig RA has been demonstrated by Barratt (1972), the measurement of such antibody was based on the ability of serum from helminth infested animals to produce a positive passive cutaneous anaphylaxis test (PCA) in normal pigs. This method, in spite of being sensitive, is difficult and expensive to perform. The experiments described in this work were undertaken to isolate the pig RA in a pure state, study its physico-chemical characteristics and produce a specific antiserum for further identification and measurement of this antibody.

MATERIALS AND METHODS

Pig reaginic serum

One adult Large White pig was orally administered with 10,000 *Ascaris suum* infective eggs followed by an equal dose 4 weeks later. Two weeks after the second challenge, the animal was slaughtered and blood collected. The gamma globulins were precipitated from the serum by addition of an equal volume of saturated ammonium sulphate solution. The precipitate was then centrifuged at 15,000 g for 30 minutes at 4°C and the sediment redissolved by extensive dialysis against phosphate buffered saline (PBS) pH 7.4.

Chromatography methods

The gamma globulins previously salt precipitated, were dialysed extensively against 0.002 M of phosphate buffer pH 7.4 and then loaded into a DEAE cellulose[3] column for ion exchange chromatography. The protein was eluted by several buffer changes varying from – 0.002 M to 0.1 M of phosphate buffer pH 7.4. Each eluted fraction was 10 times concentrated by Carbowax 20,000[4] and its anaphylatic activity tested by the PCA test, according Barratt (1972).

A concentrated pool of elutions from the DEAE-52 cellulose column which gave a positive PCA test, was loaded into Sephadex

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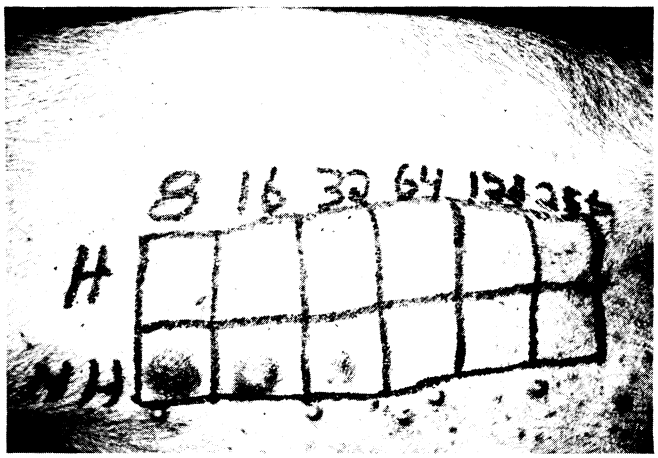

Fig. 1. *Positive reaction of unheated (NH) pig RA to PCA up to a diluation of 1/32. Heated (H) RA did not react.*

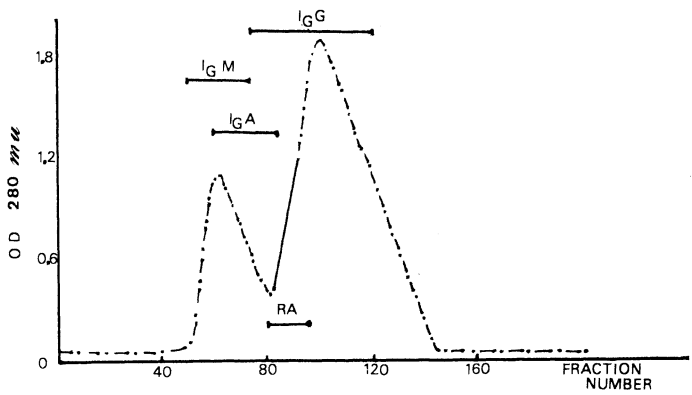

Fig. 2. *Gel-filtration in Sephadex G-200 of a sample containing pig reaginic antibody (RA). The continuous line on the trace represents the position where the RA was eluted.*

G-200[5] column for gel filtration chromatography. The fractions collected were 10 times concentrated in Carbowax 20,000 and its anaphylatic activity tested by the PCA test.

The samples fractioned by Sephadex G-200 and identified as containing RA were loaded in a series of three different immunosorbent columns containing anti-pig, IgA and IgM, according to the affinity chromatography method described by March et al. (1974). The final eluted protein was pooled, 10 times concentrated as before, dialysed in PBS pH 7.4 and its anaphylatic activity tested by the PCA test.

Polyacrylamide Gel Electrophoresis (PAGE)

A preparative acrylamide gel electrophoresis was used as described by Raymond (1968), to isolate pure sample of RA. At this stage SDS was not used. The samples, previously purified by chromatographyc methods and identified as containing RA, were applied in the gel plate and electrophoresis carried out. Part of the gel was stained with Comassie Blue to demonstrate the protein bands localization and by comparison the remaining gel was cut and the protein extracted in saline. The RA was then concentrated by microfilter Amicon B 15[6], and its anaphylatic activity was tested by the PCA test.

A sample of pig RA obtained from the preparative PAGE was diluted 1/1 with tris-acetate buffer pH 7.4 containing 0.1% SDS and assayed by analytical PAGE. The control samples with different known molecular weights (MW) (pig IgA; IgG; BSA; Ovalbumin; Myoglobin) were used on the same way. The analytical PAGE was used to check the purity of the sample isolated and to determine its molecular weight.

Specific antiserum to pig reaginic antibody

Purified pig RA obtained from the preparative PAGE was emulsified in incomplete Freund's adjuvant and injected subcutaneously into an adult New Zealand rabbit. Two and four weeks later the injection was repeated and the blood sample taken one week after the last injection constituted the specific antiserum. Immunodiffusion and immunoelectrophoresis tests were carried out with this antiserum according to the method described by Ouchterlony (1967).

Treatment of pig RA with 2-Mercaptoethanol (2-ME)

The method used was described by Bloch and Wilson (1968). Antisera dialysed against saline alone and against saline containing iodoacetamide, were used as controls. Finally, the anaphylatic activity of the pig RA was tested by the PCA test.

Immunoabsorption of the pig RA

Pig RA was absorved in solid phase with Sepharose 4B[7] gel, coupled to specific anti-pig RA and anti-human IgE as described earlier. After the anaphylatic activity of the pig RA was tested by the PCA test.

Heterologous RA tested by PCA test

Rabbits and guinea-pigs were injected intradermally with 0.05ml of pig RA. Forty eight hours later the animals were tested by the PCA test. Human IgE were also injected intradermally into a normal pig and 48 hours later a PCA test was carried out.

RESULTS

Chromatography purifications of pig RA

Positive reactions in the PCA test were observed in the 0.03 M, 0.04 M and 0.05 M phosphate buffer eluates from the ion exchange chromagraphy colum (Fig. 1). Positive reactions were abolished by prior heat treatment of the sample tested.

The eluted samples obtained from the gel filtration chromatography and tested by the PCA test to detect RA showed that positive samples were obtained between 19 S and 7 S fractions, in the ascending limb of the second peak (Fig. 2). The preparation obtained from the affinity chromatography process, also gave a positive result when tested by the PCA test.

Isolation and identification of pig RA by PAGE

The samples tested by analytical PAGE showed two bands of protein, one with a MW of 360.000 and another with MW of 220.000 (Fig. 3). The use of preparative PAGE on those two types of protein resulted a pure preparation of protein with a MW of 220,00 that gives positive reaction on the PCA test (Fig. 4).

Antiserum against pig RA

The antiserum against pig RA was found to contain antibodies to long chains which cross reacted with other immunoglobulin classes. This antibody activity was abolished

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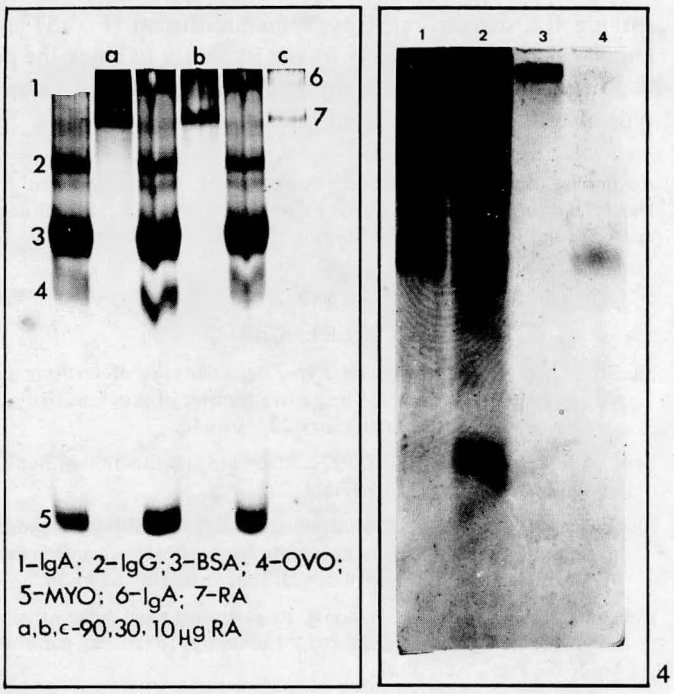

Fig. 3. *Polyacrilamide gel electrophoresis of a serum fraction containing pig RA at different concentrations (a, b, c) and its relationship to molecular weight markers (1-IgA with MW of 360,000; 2-IgG with MW of 150,000; 3-BSA with MW of 67,000; 4-ovalbumin with MW of 43,000; 5-myoglobin with MW of 16,000). The serum fraction tested contained two different classes of protein with molecular weight of 360,000 (6) and 220,000 (7) respectively.*

Fig. 4. *Polyacrilamide gel electrophoresis of a serum fraction containing pig RA. (1) and (2): 20 and 40 nl hyperimmune serum against* Ascaris suum *after preliminary chromatography purification. (3) and (4): protein isolation corresponding to the molecular weight of 360,000 and 200,000, respectively.*

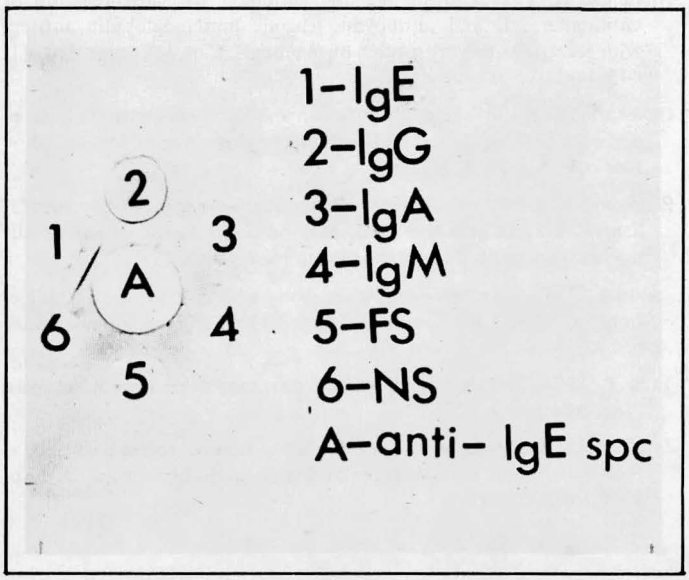

Fig. 5. *Specific antiserum (A) against pig RA (anti-pig IgE) tested by immunodiffusion against, 1-pig IgE (RA); 2-pig IgG; 3-pig IgA; 4-pig IgM, 5-fetal pig serum and 6-normal pig serum.*

by absorption of the antiserum with pig IgG and pig foetal serum. When retested against other classes of antibodies (Fig. 5), it was shown to be specific for IgE (pig RA). The specific antiserum to pig RA also showed a single precipitation line when tested by immunoelectrophoresis (Fig. 6) and completely inhibited the anaphylatic activity of the pig RA when tested by the PCA test.

Anaphylatic activity of the pig RA

The anaphylatic activity of the serum containing the RA was partially removed by immunoabsorption with anti-human IgE. No alteration in the anaphylatic reaction was noticed in the samples absorbed with antiserum to other immunoglobulin classes (Table 1). It was also observed that heat treatment and reduction with 2-ME followed by alkylation with iodecetamide, completely destroyed its ability to induce a positive PCA test. Furthermore, diluted samples (1/32) of hyperimmune serum containing pig RA were able to produce a positive PCA test up to 5 days after the animal had been sensitized. The reaction was very weak at 24 hours, maximal at 48 hours and minimal after 5 days (Fig. 1).

Table 1. *PCA titration of the pig RA before and after immunoabsorption with several specific antisera*

Antiserum to:	RA – dilution						
	1:2	1:4	1:8	1:16	1:32	1:64	1:128
None	XXXXX[a]	XXXX	XXXX	XXX	XX	–[b]	–
Pig IgC	XXXXX	XXXX	XXXX	XXX	XX	–	–
Pig IgA	XXXXX	XXXX	XXXX	XXX	XX	–	–
Pig IgM	XXXXX	XXXX	XXXX	XXX	XX	–	–
Human IgE	XXXX	XXX	XX	–	–	–	–

(a) X = 5mm diameter of positive PCA reaction.
(b) – = No PCA reaction.

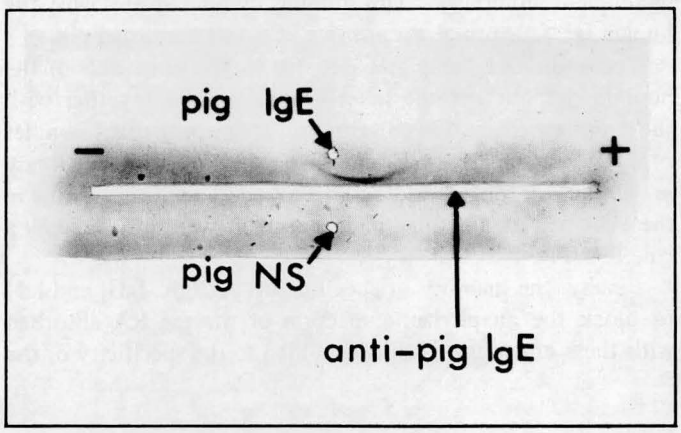

Fig. 6. *Immunoelectrophoresis of pig RA (IgE) and normal pig serum (NS), against a rabbit specific antisemm to pig IgE (anti-pig IgE).*

RA tested in different animal species

Human IgE injected into pig skin gave a negative PCA test reaction. The pig RA when tested by PCA test in rabbits and guineapigs also gave negative reactions. Those results indicate a lack of reactivity in the anaphylatic activity of the RA produced in different animal species.

DISCUSSION

Some of the physico-chemical characteristics of the pig RA originally described by Barratt (1972), such as heat lability, sedimentation coefficient, electrophoretic mobility and sensitivity to 2-ME, were also confirmed in this work. In addition, the elution characteristics of the pig RA on ion exchange and gel filtration chromatography observed in this experiment (Fig. 2), also agree with previous findings in man (Bennich & Johansson 1972) and several animal species (Sadun 1972). However, in the present study aditional features were observed, namely, the isolation of the RA in pure state, the determination of its molecular weight and the production of a specific antiserum.

The presence of detectable levels of IgG and IgA in all the RA samples obtained by chromatography methods (ion exchange and gel filtration), made the isolation of a pure RA difficult. The use of immunoabsorbents, although useful as a step in the purification of the RA, did not free the preparation from all impurities (Fig. 3). In order to purify the pig RA, PAGE was used (Fig. 4) resulting in a pure RA sample which had the same molecular weight of the RA observed in rabbits, as described by Zvaifler and Robinson (1969).

Different degrees of IgE cross reactivity between human antiserum and various animal species have been described in the literature (Ishizaka et al. 1969, Zvaifler & Robinson 1969, Halliwell et al. 1972, Neoh et al. 1973, Nielsen 1977). The results of the PCA test obtained in this experiment have confirmed these findings and demonstrated a partial homology between the anti-human IgE and the pig RA (Table 1). This observation showed the applicability of cross reactivity for a preliminary identification of the pig RA. However, the cross reactivity was at times not detectable, problably due to incomplete homology. This finding could explain why the human IgE failed to give a positive PCA test in normal pigs.

The ability of the pig RA to fix to the mast cells of the homologous but not the heterologous species, together with the complete absence of this reaction with heat-treated samples or those treated with 2-ME, and the persistence of this antibody in the skin for long periods, confirm that the positive results in the PCA test obtained in this experiment were produced by a true RA (Fig. 1).

Finally, the inability of specific anti-pig IgA, IgG, and IgM to block the anaphylactic reaction of the pig RA absorbed with these antiserum (Table 1), added to the specificity of the anti-pig RA demonstrated by immunodiffusion (Fig. 5) and immunoelectrophoresis (Fig. 6) and its ability to block the pig RA anaphylactic activity, suggests that the pig RA is a unique type of antibody related to the human IgE.

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