

Purification of *Ascaris suum* antigen: Its allergenic activity in vitro and in vivo

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Crude aqueous extracts of Ascaris suum (CE) have been used widely to study IgE-mediated reactions in various experimental preparations. Because some CE may contain a polypeptide, a mast cell degranulating peptide (MCDP), that degranulates mast cells by nonimmunologic mechanisms,^{1,2} various protocols have been used to ensure that the Ascaris preparation used did not contain MCPD. In general, these protocols have assumed MCDP had been removed without providing proof. Even protocols designed to isolate the major antigenic determinants from CE have usually been designed to evaluate immunogenic characteristics of the purified Ascaris; thus, few systematic comparisons of CE with purified Ascaris exist concerning mast cell degranulation, and few studies have demonstrated that MCDP has been removed during purification.³⁻⁶ Since Ascaris has proved to be useful in a variety of studies of IgE-mediated reactions, particularly in large animals (dog and sheep), we have developed a protocol to purify CE and MCDP and characterize their physicochemical and immunologic properties. We compared the allergenic activity of our purified Ascaris to that of CE and MCDP in skin and lung of natively sensitized dogs and in unsensitized rat peritoneal mast cells. Our results indicate that MCDP probably contaminates CE by <1.0%. However, the biologic activity of MCDP in dog lung appears insignificant and probably contributes little to CE-induced reactions in doses of CE commonly used (≤ 100 mg injected). If a purified Ascaris preparation is essential, our protocol will yield an Ascaris preparation that has potent IgE-mediated effects in dog preparations with insignificant contamination by MCDP. (J ALLERGY CLIN IMMUNOL 77:443-51, 1986.)

It has been well established that extracts of *A. suum* degranulate mast cells of several animal species.^{3, 6, 7} Kessler et al.⁸ demonstrated that when natively sensitized dogs were exposed to extracts of *Ascaris*, mast cells were degranulated by an IgE-dependent mechanism. In contrast, Uvnäs and Wold¹ and, subsequently, Thompson² demonstrated that some extracts of *A. suum* also contain a small peptide capable of degranulating rat peritoneal mast cells by a nonimmunologic mechanism. Some workers have raised

Abbreviations used

CE:	Crude aqueous extract of <i>Ascaris suum</i>
MCDP:	Mast cell degranulating peptide (Uvnäs and Wold ¹ product A)
Asc-1:	National Institutes of Health reference standard <i>Ascaris</i> allergen
SDS:	Sodium dodecyl sulfate
PBS:	Phosphate-buffered saline
DEAE:	Diethylaminoethyl
pI:	Isoelectric point

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Supported in part by United States Public Health Service Program Project Grant HL-24136, and by **Boehringer Ingelheim, Federal Republic of Germany.

Received for publication Aug. 8, 1983.

Accepted for publication Aug. 8, 1985.

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*L. W. Greenspon, M.D., was the recipient of United States Public Health Service National Pulmonary Faculty Training Center Award (HL-07159) and a Medical School Pulmonary Faculty Training Award (HL-00535).

concern about the use of *Ascaris* extracts for the study of immunologically induced bronchoconstriction.^{9, 10} Although previous workers have purified extracts of *A. suum*,³⁻⁵ none has compared the relative biologic activity of the purified material to the crude extract on a systematic basis, nor has it been proved by immunologic or biologic criteria that the nonspecific degranulating peptide has been removed. Therefore, we describe the preparation of a purified *Ascaris* allergen and its physicochemical and immunologic properties. In addition, we report a comparison of the allergenic activity of our purified *Ascaris* to the activity of *Ascaris* crude extract and the MCDP of Uvnäs and Wold¹

in the skin and pulmonary mast cells of natively sensitized dogs and in unsensitized rat peritoneal mast cells.

MATERIAL AND METHODS

Material and reagents

Asc-1 was kindly supplied by the National Institute of Allergy and Infectious Diseases Research Resources Branch, Bethesda, Md. Whole *Ascaris* worms were obtained from Goehring Meat Packing Plant, Stockton, Calif. DEAE 52 ion exchanger was obtained from Whatman Inc., Clifton, N. J.; Sephadex G-75 and gel filtration standards were from Pharmacia Fine Chemicals, Piscataway, N. J.; bovine serum albumin, Coomassie blue, agarose, urethane, chloralose, and compound 48/80 were from Sigma Chemical Co., St. Louis, Mo; acrylamide was from Bio-Rad Laboratories, Richmond, Calif.; Freund's adjuvant was from Difco Laboratories, Detroit, Mich.; Evans blue dye was from J. T. Baker Chemical Co., Phillipsburg, N. J.; and gallamine triethiodide (Flaxedil) was from Davis-Geck, American Cyanamid Co., Pearl River, N. Y. S-adenosyl-L-(methyl-[^{14} C])methionine (50 μ Ci/2 ml) and 2,5 [^{3} H]histamine dihydrochloride (250 μ Ci/250 μ l) used in the assay were obtained from Amersham, Arlington Heights, Ill. All other chemicals used were of the highest chemical grade available.

Methods

The experimental procedure followed adhered to the published "Guiding Principles in the Care and Use of Animals" approved by the Council of The American Physiological Society and specific protocols approved by the committee on Animal Care of the University of California, San Francisco.

CE preparation

Whole worms were washed clean with tap water and homogenized (Waring blender; Waring Products Div., New Hartford, Conn.) in an equal volume of PBS, pH 7.4. The homogenate was centrifuged at $1000 \times g$ for 20 minutes, and then the supernatant was centrifuged at $30,000 \times g$ for 30 minutes and designated CE.

Purified CE preparation

The CE was treated with ammonium sulfate (70% for 18 hours at 0°C), centrifuged at $10,000 \times g$ for 20 minutes, and then the supernatant was dialyzed against PBS (48 hours, four changes). This product was then treated with trichloroacetic acid (2.5%, 30 minutes), centrifuged at $10,000 \times g$ (20 minutes), and the supernatant was dialyzed against PBS (48 hours, four changes). The dialysate was boiled for 10 minutes and centrifuged, and the supernatant was dialyzed against double distilled water (48 hours, four changes). This product was lyophilized and designated partially purified *Ascaris*. Partially purified *Ascaris* was reconstituted in sodium phosphate, 0.01 mol/L, and absorbed on a DEAE cellulose (Whatman No. 52) ion exchange column (7.0 by 2.5 cm).

Preparation of MCDP

The MCDP described by Uvnäs and Wold was prepared according to their published methods.¹ The material (MCDP) we isolated from crude *Ascaris* extract represents their product A.

Analysis of chemical purity and molecular weight

Column chromatography. Molecular weight of purified *Ascaris* was determined by its elution profile from a Sephadex G-75 column (30 by 1.5 cm) with a flow rate of 12 ml/hour. The column was calibrated with the following standards: blue dextran, 2×10^6 daltons; ovalbumin, 4.3×10^5 daltons; chymotrypsinogen A, 2.5×10^5 daltons; and ribonuclease A, 1.37×10^4 daltons.

SDS gel electrophoresis. A 12% to 18% acrylamide gradient gel was used (0.1% SDS; 0.375 mol/L of Tris HCl, pH 5.8).¹¹ All proteins were treated with 1% SDS and 5% 2-mercaptoethanol (3 minutes at 95°C). Gels were stained with Coomassie blue (0.1% in 50% methanol to 10% acetic acid) and destained with a solution containing 14% acetic acid to 10% ethanol. The gels were calibrated with purified standards: blue dextran, 2×10^6 daltons; ovalbumin, 4.3×10^5 daltons; chymotrypsinogen A, 2.5×10^5 daltons; ribonuclease A, 1.37×10^4 daltons; aprotinin, 0.65×10^4 daltons; and, in some experiments, with insulin, 3.0×10^3 daltons.

Isoelectric focusing. Proteins were applied to a 6.5% acrylamide slab gel containing 2.4% ampholine solution (pH range 3 to 10) and 1.0% agar in 0.05 mol/L of barbital buffer. Current was applied horizontally across the gel during each run for 12 hours. At the end of each run, gels were stained and destained as described above.

Carbohydrate content. The carbohydrate content of purified *Ascaris* was determined colorimetrically by the phenolsulfuric acid reaction¹² with glucose as a standard.

Analysis of immunologic purity

Antibodies to CE and MCDP were raised in New Zealand white rabbits. Initial immunization consisted of subcutaneous injection of 0.25 mg of protein in complete Freund's adjuvant at four sites per animal. This was followed by biweekly injections of the same dose of antigen mixed with incomplete Freund's adjuvant. The antisera used for immunoelectrophoresis were obtained by bleeding the rabbits 10 weeks after initial immunization. Immunoelectrophoresis was run on 1% agar gels in 0.05 mol/L of barbital buffer (pH 8.0).¹¹

Analysis of allergenic activity

Skin testing of dogs. Twenty minutes after administration of 1% Evans blue (0.25 ml/kg, intravenously), serial tenfold dilutions of allergen were injected (0.1 ml, intradermally) into the skin of the anterior chest and abdomen. The end point was defined as the minimum amount of protein injected that caused a positive reaction (blue staining of the skin associated with a wheal larger than 5 mm in diameter).

Histamine release from dog lung fragments in vitro. Mongrel dogs (15 to 30 kg) were anesthetized (intravenously)

with chloralose (100 mg/kg) and urethane (500 mg/kg) and ventilated mechanically. The dogs were skin tested, paralyzed with gallamine triethiodide (2 mg/kg, intravenously, intermittently), placed in the supine position, and a right-sided thoracotomy was performed. Lung biopsy specimens were obtained from the periphery of the lung, placed immediately in oxygenated Krebs-Henseleit buffer (NaCl, 118 mmol/L; KCl, 4.6 mmol/L; NaHCO₃, 24.8 mmol/L; CaCl₂, 2.5 mmol/L; MgSO₄·7H₂O, 1.2 mmol/L; and glucose, 5.6 mmol/L, in distilled deionized water), and bubbled with 95% O₂ and 5% CO₂ to maintain pH 7.4. Lung tissue was cut with fine eye scissors, washed with Krebs-Henseleit buffer, and then divided into a series of test tubes (approximately 100 mg wet weight per tube). Increasing concentrations of CE, purified *Ascaris*, or MCDP were added to each test tube with Krebs-Henseleit buffer to a final volume of 2 ml. Tubes were incubated at 37° C for 10 minutes and then placed on ice (20 minutes) to stop the reaction. Percent histamine released by allergen was calculated by the following formula:

$$\text{Release (\%)} = \frac{\text{Histamine released by allergen}}{\text{Total histamine content}} \times 100$$

where total histamine content equals histamine released by allergen plus histamine released spontaneously plus residual tissue histamine. Histamine released by allergen was determined by assay of the supernatant of each tube and subtracting a value equal to the amount of histamine released spontaneously. Residual tissue histamine was determined by placing the remaining lung tissue in 2 ml of 50 mmol/L of sodium phosphate buffer, boiling for 10 minutes, centrifuging at 10,000 × g for 20 minutes, and assaying of the supernatant for histamine. All samples were assayed in duplicate by an enzymatic double isotope dilution method.¹³

Histamine release from rat peritoneal mast cells. To assay for nonspecific mast cell degranulating activity, we tested CE, purified *Ascaris*, pure MCDP, and compound 48/80 for their ability to release histamine from peritoneal mast cells isolated from parasite-free Wistar rats (130 to 160 gm). Bovine serum albumin was used as a nonspecific protein control. Peritoneal washings from two animals were collected for each experiment in a sterile ice-cold medium (Dulbecco solution A and B, Oxoid Ltd., London, England, supplemented with 100 mg of gelatin, 11 mg of glucose, and 0.1 ml of decomplexed rat serum per 100 ml). The cells were washed twice, counted, and then divided into aliquots containing 5 × 10⁴ mast cells per milliliter. Histamine release was calculated as described above. For these experiments histamine was determined by a spectrofluorometric method.¹⁴

Protein determinations. Protein content was determined by the method of Lowry et al.¹⁵ To compare allergenic activity, CE and purified *Ascaris* were normalized to contain equivalent amounts of protein.

Statistics

Allergenic activity between CE and purified *Ascaris* was analyzed by Wilcoxon's paired sample test.

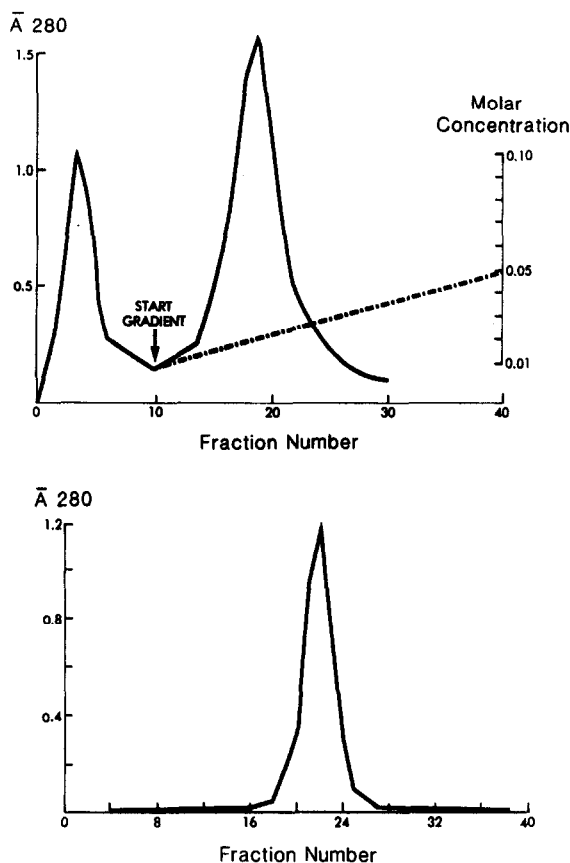


FIG. 1. (Top) DEAE cellulose ion exchange chromatography. Partially purified *Ascaris* (60 mg) was eluted from DEAE cellulose (Whatman No. 52) column (7.0 by 2.5 cm) with a linear ionic gradient (0.01 to 0.10 mmol/L of sodium phosphate, pH 7.4). Absorption ($\bar{A}$) at 280 nm (left ordinate) is indicated by the solid line versus fraction number collected (abscissa). The molar concentration of the elution gradient (right ordinate) is indicated by the broken line, starting at the arrow. The second peak (fractions 10 to 30) contained purified *Ascaris*. (Bottom) Sephadex gel filtration of purified *Ascaris*. A G-75 column (30 by 1.5 cm) was used to elute purified *Ascaris* (30 mg) at a flow rate of 12 ml/hr. The solid line represents absorption ($\bar{A}$) at 280 nm. The single peak collected between fractions 16 to 28 (fraction volume 1.0 ml) corresponds to a molecular weight of 18,100 daltons, based on calibration of the column with 1 μ g each of the following standards: blue dextran, 2×10^6 daltons; ovalbumin, 4.3×10^4 daltons; chymotrypsinogen A, 2.5×10^4 daltons; and ribonuclease A, 1.37×10^4 daltons.

RESULTS

Analysis of chemical purity and molecular weight

Since partially purified *Ascaris* might still contain one of the low-molecular-weight bands of MCDP because of the high I_p of the peptide, we purified the *Ascaris* further by ion exchange chromatography. Elution with a linear ionic gradient (0.01 to 0.10 mol/L of sodium phosphate, pH 7.4) produced the chro-



FIG. 2. (Left) SDS polyacrylamide gel electrophoresis comparing CE, purification steps for *Ascaris*, purified *Ascaris*, and Asc-1. From left to right, columns 1: CE, 60 μ g; 2: CE after ammonium sulfate precipitation, 60 μ g; 3: CE after ammonium sulfate and trichloroacetic acid precipitation, 50 μ g; 4: CE after ammonium sulfate, trichloroacetic acid, and boiling, 60 μ g; 5: calibration of the column with the following standards (1 μ g each): ovalbumin, 4.3×10^4 daltons; chymotrypsinogen A, 2.5×10^4 daltons; ribonuclease A, 1.37×10^4 daltons; and aprontinin, 6.5×10^3 daltons; 6: purified *Ascaris*, 2 μ g; 7: purified *Ascaris*, 5 μ g; and 8: Asc-1, 5 μ g. A 12% to 18% acrylamide gradient gel was used, pH 5.8. All proteins were treated with 1% SDS and 5% 2-mercaptoethanol, and gels were stained with Coomassie blue and destained with acetic acid (14%) ethanol (10%) solution. CE and purified *Ascaris* demonstrated similar bands corresponding to the major *Ascaris* allergen (9000 daltons) with some larger bands also evident, particularly when large amounts of protein were applied to the gel. There were also low-molecular-weight bands (3000 to 6000 daltons) in CE corresponding to those evident in MCDP, but none were present in our purified *Ascaris*. Absence of 2-mercaptoethanol during SDS gel electrophoresis did not affect the location of the major *Ascaris* band. (Right) SDS polyacrylamide gel electrophoresis comparing CE, purified *Ascaris*, and MCDP. Columns 1 and 2: CE, 100 μ g; 3 to 6: sequentially increasing amounts of purified *Ascaris*, 1, 2, 5, and 10 μ g; 7: calibration with standards, as used in Fig. 3, including insulin (MW 3000), 5 μ g; 8 and 9: purified *Ascaris* at 25 and 50 μ g, respectively; and 10 to 12: MCDP, 2, 25, and 42 μ g, respectively. CE and purified *Ascaris* demonstrated similar bands corresponding to the major *Ascaris* allergen (9000 daltons) with some larger molecular weight bands evident, particularly when large amounts of protein were applied to the gel. There were also low-molecular-weight bands (3000 to 6000 daltons) in CE corresponding to those evident in MCDP, but none were present in purified *Ascaris*. Absence of 2-mercaptoethanol during SDS electrophoresis did not affect the location of the major *Ascaris* band.

matogram illustrated in Fig. 1, top. Peak No. 2 contained purified *Ascaris*. The Sephadex G-75 elution profile of purified *Ascaris* is illustrated in Fig. 1, bottom. This single peak corresponds to a molecular weight of 18,100 daltons. The protein bands obtained by the SDS gel electrophoresis of CE, the purification steps, Asc-1, and MCDP are illustrated in Fig. 2, left and right. Purified *Ascaris* has a single band at a molecular weight of 9000 daltons, similar to that found with Asc-1. Absence of 2-mercaptoethanol during SDS gel electrophoresis did not change the location of this band. MCDP had three bands ranging in molecular weight from 3000 to 6000 daltons. None of these bands was present in purified *Ascaris*. Isoelectric focusing demonstrated that both purified *Ascaris* and Asc-1 had two major bands at approximately $pI = 5.7$.

Analysis of immunologic purity

This purification protocol removed all but one major antigen, as evidenced by immunoelectrophoresis, by use of antibodies raised against CE (Fig. 3). Asc-1 and purified *Ascaris* appeared to have a major antigen in common that migrated anodically. Close examination of the photographs suggests that both preparations may have a minor antigen in common (Fig. 3), but this band is largely hidden by the major antigen. Antibodies raised against MCDP precipitated with CE and with MCDP, but not with purified *Ascaris* (Fig. 3).

Analysis of allergenic activity

Skin test results (Table I) demonstrated no loss in activity between CE and partially purified *Ascaris*, but a significant loss in biologic activity appeared to

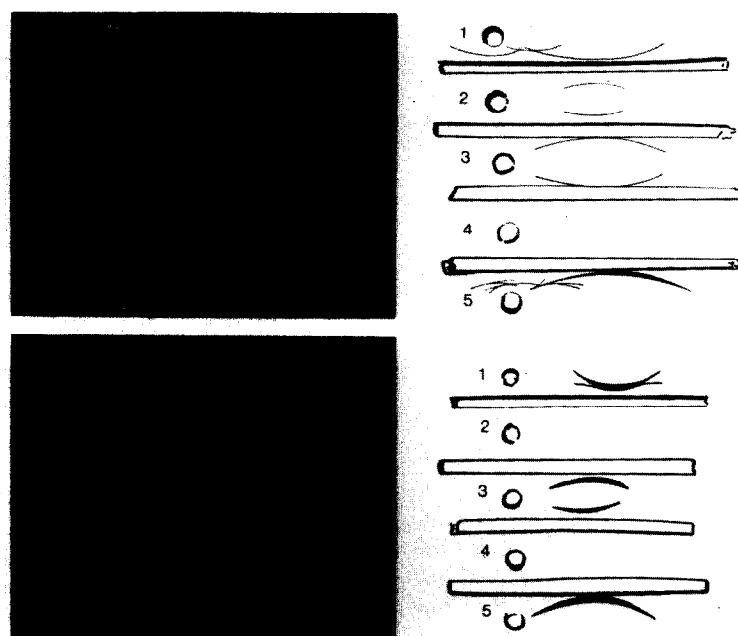


FIG. 3. (Top) Immunoelectrophoresis of CE, our purified *Ascaris*, Asc-1, and MCDP tested against anti-CE serum. In these experiments the anode was on the right; undiluted rabbit anti-CE serum was placed in the center rectangular wells. In the round wells, 1: CE, 35 $\mu\text{g/ml}$; 2: our purified *Ascaris*, 10 $\mu\text{g/ml}$; 3: Asc-1, 10 $\mu\text{g/ml}$; 4: MCDP, 30 $\mu\text{g/ml}$; and 5: CE, 70 $\mu\text{g/ml}$. Asc-1 and purified *Ascaris* appeared to have a major allergen in common that migrated toward the anode. Close examination suggests that both preparations may have a minor allergen, also in common, but largely hidden by the major precipitin band. (Bottom) Immunoelectrophoresis of CE, our purified *Ascaris*, and MCDP tested against rabbit anti-MCDP serum. In these experiments the anode was on the right, the undiluted rabbit anti-MCDP serum was placed in the center rectangular wells, and MCDP (10 $\mu\text{g/ml}$) was placed in the circular wells Nos. 1 and 5. CE (10 $\mu\text{g/ml}$) was placed in the circular well No. 3, and purified *Ascaris* (10 $\mu\text{g/ml}$) was placed in the circular wells Nos. 2 and 4. Antibodies raised against MCDP formed precipitin lines with MCDP and CE, but not with our purified *Ascaris*. Actual photograph (left); diagram (right).

TABLE I. Skin test results in vivo

	CE	Partially purified <i>Ascaris</i>	CE	Purified <i>Ascaris</i>
Dose (μg)*	1.1 $\pm$ 0.24	0.64 $\pm$ 0.13†	15.7 $\pm$ 4.1	29.6 $\pm$ 11.4‡
Range (μg)	0.1 to 2.0	0.1 to 1.0	0.01 to 100	0.01 to 100
n	8	8	15	15

n = Number of dogs tested; CE was compared to partially purified *Ascaris* in one set of dogs and to purified *Ascaris* in another set of dogs.

*Minimum dose of antigen injected to cause positive skin test (5 mm of bluing, 20 minutes after 0.1 ml was injected intradermally) expressed as mean $\pm$ SEM.

†Not significantly different from CE ($p > 0.1$).

‡Significantly different from CE ($p < 0.02$).

result after the final purification step. Comparison of release from dog lung induced by CE and purified *Ascaris* in one dog is presented in Fig. 4. Table II summarizes the results in six dogs tested. In these experiments histamine released spontaneously equaled $1.8 \pm 0.5\%$ (mean $\pm$ 1 SEM, $n = 6$). Pu-

rified *Ascaris* had approximately 50% of the maximal histamine-releasing activity of equal doses (based on protein content) of CE. However, there was no significant shift in the dose-response curve, since the dose causing 50% maximal release was not significantly different for CE compared to purified *Ascaris*.

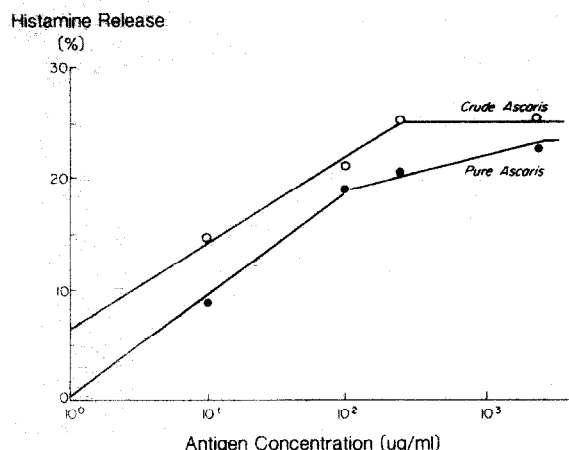


FIG. 4. Comparison of histamine release from dog lung fragments induced by CE and by purified *Ascaris* in one dog. Open circles, CE; closed circles, purified *Ascaris*. Histamine release expressed as percent of total content on the ordinate versus increasing concentrations of antigen (10^0 to 10^3 $\mu\text{g/ml}$) on the abscissa.

TABLE II. *Ascaris*-induced release of histamine from dog lung in vitro: Comparison of crude and purified *Ascaris*

Dog	Maximum histamine release (%)		Dose causing 50% release (μg)	
	CE	Pure	CE	Pure
32901	75	49	0.5	17
32366	25	24	5	16
31821	50	12	16	40
31707	25	16	10	10
32895	18	17	25	5
31596	39	13	250	250
Mean	38.7	21.8*	50.4	56.3†
SEM	9.5	6.2	43.9	42.8

Pure = our purified *Ascaris* extract (see Methods). In these experiments histamine released spontaneously equaled $1.8 \pm 0.5\%$ (mean $\pm$ SEM).

*Significantly different from CE ($p < 0.05$).

†Not significantly different from CE ($p > 0.5$).

MCDP had poor histamine-releasing activity compared to compound 48/80 in two dogs insensitive to *Ascaris* (Table III). In these experiments *Ascaris* insensitivity was defined on the basis of a negative skin test to 100 μg of CE injected intradermally and no significant release from lung fragments in vitro in response to incubation with 10^3 $\mu\text{g/ml}$ of CE. In contrast to these experiments in dog lung, MCDP caused significant release from rat peritoneal mast cells but at a concentration two logs greater than compound

TABLE III. Histamine release from dog lung in vitro: Comparison of compound 48/80, CE, and MCDP

Stimulus	Concentration ($\mu\text{g/ml}$)	Histamine release* (%)
Compound 48/80	1.0×10^2	3.5
	0.5×10^3	9.3
	1.0×10^3	17.5
CE	1.0×10^2	0.0
	0.5×10^3	0.0
	1.0×10^3	2.5
MCDP	1.0×10^2	0.0
	0.5×10^3	0.3
	1.0×10^3	0.6

*Mean histamine released (percent) from lung fragments obtained from two *Ascaris*-insensitive dogs (no cutaneous reactivity to CE, 100 μg , intradermally and no significant histamine release in vitro to CE, 1000 $\mu\text{g/ml}$).

48/80 (Table IV). CE released only at high concentrations (10^3 $\mu\text{g/ml}$), whereas purified *Ascaris* released none at 10^3 $\mu\text{g/ml}$ and a small amount at 10^4 $\mu\text{g/ml}$.

DISCUSSION

Studies of anaphylaxis by use of extracts of *A. suum* have been difficult to interpret because two mechanisms of mast cell degranulation have been described, an immunologic, IgE-dependent mechanism and a nonimmunologic direct chemical mechanism. This article describes a purification protocol that allows large quantities of CE to be processed rapidly into a purified allergen that has physiochemical properties similar to purified *Ascaris* preparations described previously (Table V). We have demonstrated that our purified allergen has significant biologic activity in dog lung, although it appears less active than CE. More importantly, we have used biologic and immunologic tests to demonstrate that almost all nonspecific mast cell degranulating activity has been removed from our purified allergen (less than 1:1000 remains).

We purified *Ascaris* by physiochemical techniques designed to remove MCDP. Because partially purified *Ascaris* appeared to retain the major antigen by immunoelectrophoresis and had no loss in allergenic activity by skin testing, we believed that purification steps to this point did not destroy the major allergen. However, we cannot rule out the possibility that one of the major allergens was denatured. This is in agreement with Ambler et al.¹⁶ who demonstrated that *Ascaris* allergen A activity in the rat was resistant to

TABLE IV. Histamine release from rat peritoneal mast cells in vitro

Stimulus	Concentration ($\mu\text{g/ml}$)	n	Histamine release (%)
48/80	0.03	4	$4.3 \pm 1.6^*$
	0.10	5	39.0 ± 3.8
	0.3	5	69.0 ± 2.7
	1.0	5	83.0 ± 0.8
CE	10	4	NR
	100	4	NR
	1,000	4	17.7 ± 2.3
	10,000	4	75.4 ± 2.3
MCDP	1.0	4	3.4 ± 1.6
	10	4	30.0 ± 2.5
	100	4	75.0 ± 3.3
Bovine serum albumin	10	4	NR
	100	4	NR
	1,000	4	NR
	10,000	4	2.4 ± 2.1
Purified <i>Ascaris</i> [†]	1.0	2	NR
	10	2	NR
	100	2	NR
	1,000	2	NR
	10,000	2	23.4 ± 7.9

NR = no release.

*Mean $\pm$ SEM.[†]Our purified *Ascaris* preparation (see Methods).

boiling, 5% trichloroacetic acid and enzymatic digestion with trypsin. Since partially purified *Ascaris* may still contain one of the low-molecular-weight bands of MCDP demonstrated by SDS gel electrophoresis, we decided to purify the protein further by ion exchange chromatography. The loss in biologic activity that resulted from this step may be explained by two factors. Since the material eluted as peak No. 1 also had histamine-releasing activity, we may have removed other allergens^{17, 18} and immunogens⁴ from CE that are responsible for its total allergenic activity. This may account for the fact that, like some other purified allergens,¹⁹ our purified *Ascaris* had less biologic activity than the CE from which it was obtained. Alternatively, we may have removed MCDP. It appears unlikely that the conditions of ion exchange denatured the allergen. In preliminary studies to purify *Ascaris*, we found it difficult to remove MCDP by column chromatography alone. This result may be explained by the high pI of the peptide (pI = 12.3) and therefore its marked ionic affinity for other proteins. Only by ion exchange chromatography or isoelectric focusing could the peptide be separated successfully from the major *Ascaris* allergen.

By isolating MCDP we were able to approximate

the content of this material in CE or purified *Ascaris* and estimate its significance to the overall histamine-releasing activity of *A. suum*. Analysis of SDS gel electrophoresis suggested that MCDP made up less than 1% of CE.

We used the rat peritoneal mast cell as a bioassay for histamine-releasing activity. We found that equivalent amounts of histamine were released between MCDP and 100 times more concentrated than CE (1% contamination) and 1000 times more concentrated than purified *Ascaris* (0.1% contamination). Unfortunately, we could not obtain enough Asc-1 to test the nonspecific histamine-releasing capacity of this material. These results indicate that it is difficult to remove the MCDP from the major *Ascaris* allergen and that criteria for its removal on the basis of physiochemical methods are inadequate. If this same relationship holds with respect to canine anaphylaxis, we estimate that CE in excess of 50 mg/ml would be required to contain enough MCDP to cause significant release by nonimmunologic mechanisms (Table III).

An alternative analysis of this problem consistent with this conclusion is on the basis of the fact that the antigen dose of 100 mg of protein used by previous

TABLE V. Comparison of purified *Ascaris* preparations

<i>Ascaris</i> preparation	A	B	C	D	E	F
Molecular weight (daltons)	17,000 to 19,000	14,000	18,400	10,000 to 50,000	15,000	18,100
Sedimentation coefficient	1.86	1.85	---	---	---	---
pI	4.8 to 5.0	5.0 to 5.2	5.1	---	---	5.7
Reducing sugars (%)	8.6	1.0	5.4	1.0	<1.0	1.0

A, Hussain et al.⁴; B, Ambler et al.¹⁶; C, Herzig⁵; D, Hogarth-Scott²¹; E, Dandeu and Lux¹⁷; and F, present study.

investigators in vivo²⁰ is distributed not only to the lungs but also to the systemic circulation, releasing histamine from extrapulmonary sites. If the volume of distribution of the antigen in blood and tissue were only 400 ml (or 400 gm of tissue), the resulting concentration of antigen would be approximately 0.25 mg/ml or /gm of tissue. Since the wet weight of the lungs alone in large dogs is >400 gm,* CE exceeding 0.25 mg of protein per milliliter in the lungs of such experimental dogs appears highly unlikely. A dose of 0.25 mg of protein per milliliter of CE would be on the linear ascending limb of the antigen-histamine release dose-response curve as determined by studies in vitro from similar dogs, but the concentration of MCDP would only be 10⁻³ mg of protein per milliliter, a concentration that would barely cause release of in the rat peritoneal mast cell system (Table IV), let alone release from dog lung (Table III). Since most investigations of canine anaphylaxis with CE have used concentrations of CE far below this level, non-specific mast cell degranulation is probably not a significant factor in these studies.

Table V summarizes the physiochemical relationships of our purified *Ascaris* with those isolated by others. Herzig⁵ has isolated a purified allergen that has similar properties to Asc-1. He reported increased allergic activity by skin testing (intradermally) in one dog. Kuo and Yoo¹⁸ reported three allergenic fractions of CE isolated by isoelectric focusing. The most active fraction by passive cutaneous anaphylaxis in the rat had a pI = 6.0; however, these authors did not compare its activity relative to CE. Our attempts to isolate other allergens from the CE with greater activity than purified *Ascaris* have not been successful.

In conclusion, we have purified an allergen from CE that has significant mast cell degranulating activity. We have also confirmed the presence of a non-specific mast cell degranulating peptide in CE, as described by Uvnäs and Wold,¹ but have demonstrated

that its biologic activity in canine anaphylaxis induced by CE is probably insignificant. Studies with our purified *Ascaris* allergen in dogs are even less likely to be contaminated by this peptide to a degree that is physiologically significant.

We wish to thank Ryckje Wagner/Hall for her assistance in preparing this manuscript.

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Delayed anaphylactoid reaction in a worker exposed to chromium

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A 29-year-old male welder reported systemic reactions after exposure to chromium. Inhalation challenge testing to 29 µg/m³ of sodium chromate aerosol resulted in late appearing systemic urticaria, angioedema, and severe bronchospasm that occurred at the same time as a threefold rise in plasma histamine. A direct leukocyte inhibitory factor test to 5.5×10^{-6} mol/L Na₂CrO₄ was positive. Although the mechanism of this reaction is unknown, the positive leukocyte inhibitory factor and the general acceptance of hexavalent chromium as a contact skin sensitizer suggest that cell-mediated mechanisms could be involved. (J ALLERGY CLIN IMMUNOL 77:451-6, 1986.)

Occupational inhalants have rarely been demonstrated to cause anaphylactoid reactions, although systemic urticaria and wheezing have been reported for airborne exposure to animal allergens, penicillin, and platinum salts in the workplace.¹ Chromium is a transition metal that is widely used for electroplating processes, metal alloys, pigments, tanning of leather,

Abbreviations used

LHR: Leukocyte histamine release
PBMC: Peripheral blood mononuclear cells
LIF: Leukocyte inhibitory factor

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Supported in part by a Clinical Research Center Grant RR-0068-18, a *National Research Service Award Training Grant, and an *Asthma and Allergy Foundation of America Fellowship Award.

Received for publication Feb. 13, 1985.

Accepted for publication Aug. 1, 1985.

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cement, and production of chromate salts. Chromium metal is nonallergenic, but chromate salts have been investigated extensively as causative agents in occupational contact dermatitis.² Chromium salts exist naturally in three valence forms: two, three, and six. Bivalent compounds are unstable and have little commercial value. Hexavalent chromium compounds (e.g., chromium trioxide and mono- and bichromates), as compared to trivalent chromium compounds such as chromium chloride, have an increased potential for allergic contact dermatitis because they