

Immunologic and biochemical properties of the major mouse urinary allergen (Mus m I)

J. R. Lorusso, B.A., Scott Moffat, M.S., and John L. Ohman, Jr., M.D.
Boston, Mass.

Rabbit antiserum to the mouse major urinary protein identified a single antigen that was also found in mouse serum and pelt extract. The skin test reactivity of mouse-pelt extract and mouse urine in two mouse-allergic subjects was significantly reduced after immunoabsorption with the gamma globulin fraction of this antiserum. The antigen defined by this antiserum was designated mouse allergen 1 (MAI). An immunoelectrophoretic procedure was set up to measure its concentration. MAI had a molecular weight of approximately 19,000 on Sephadex gel filtration and 18,000 to 21,000 on sodium dodecyl sulfate polyacrylamide gel electrophoresis. Isoelectric focusing identified at least four bands with antigenic activity; the major band had an isoelectric point of 3.9. Significant antigenic and allergenic activity of MAI was retained on reduction and digestion with papain and pepsin. Heating at 90° C for periods up to 180 minutes resulted in a progressive loss, but not abolition, of activity. Serum and urine derived from male mice contained approximately fourfold more MAI than samples derived from female mice. Urine contained at least 100-fold more MAI than serum. Of the tissue extracts studied, liver extract had the highest amount of MAI. The immunochemical properties of MAI, its tissue distribution, and sex differences in its concentration provide strong evidence that MAI is identical to the previously described mouse major urinary protein. (J ALLERGY CLIN IMMUNOL 78:928-37, 1986.)

Respiratory and cutaneous symptoms of immediate hypersensitivity on exposure to laboratory animals are observed in 11% to 30% of workers who are regularly exposed to laboratory animals.¹⁻³ Asthma has been reported in nearly 50% of these workers.⁴ Symptoms on exposure to the laboratory mouse have been reported in a third of those individuals regularly exposed.² Mouse epithelium,^{1, 4-6} urine,⁵⁻⁸ and serum,^{5, 8} have been the sources of allergen that have been demonstrated to elicit rise to immediate hypersensitivity or IgE antibody binding in mouse-allergic individuals.

Mouse albumin has been implicated as one allergen involved in these reactions.^{6, 8} However, lower molecular weight allergenic activity (17,000 to 22,000 daltons) appears to be of more importance in mouse serum, urine, and epithelial extract.⁷⁻¹¹ These lower molecular weight allergens have a similar molecular

Abbreviations used

MAI:	Mouse allergen 1 (Mus m I)
SDS:	Sodium dodecyl sulfate
PAGE:	Polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
BSA:	Bovine serum albumin
HRP:	Horseradish peroxidase
DTT:	Dithiothreitol
Tris:	Tris(hydroxymethyl)aminomethane
pI:	Isoelectric point
Ova:	Ovalbumin
Chymo:	Chymotrypsinogen
Cyt:	Cytochrome
CIE:	Crossed immunoelectrophoresis
IEP:	Immunoelectrophoresis

weight to a family of mouse urinary proteins described by Finlayson in 1965 and labeled "mouse major urinary protein complex." These proteins have received much recent study.

Mouse major urinary protein is synthesized predominantly in the liver and excreted in large quantity in the urine.¹² Similar proteins are also secreted in the saliva.¹³ Structural genes of the mouse major urinary protein are located on chromosome 4.¹⁴ Synthesis of these proteins in the liver is stimulated by androgen.¹⁵

This article further describes the properties, distri-

From the Allergy Research Laboratory, Veterans Administration Outpatient Clinic, Boston, Tufts University School of Medicine, Boston, and Tufts University School of Veterinary Medicine, Boston, Mass.

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Reprint requests: John L. Ohman, Jr., M.D., VA Outpatient Clinic, 17 Court St., Boston, MA 02108.

bution, and standardization of the low-molecular-weight mouse urinary allergen and describes an immunochemical procedure to measure it. The immunochemical properties of this allergen, its tissue distribution, and sex differences in its concentration provide strong evidence that it is identical to the mouse major urinary protein originally described by Finlayson.

METHODS

Sources of mouse allergen

Serum, urine, and tissue samples were obtained from 12-week-old male and female outbred CD1 mice, and additional urine samples were obtained from male inbred BALB/c and C57 mice (Charles River Breeding Laboratories, Wilmington, Mass.). After collection of urine samples by bladder massage, three male and three female CD1 mice were anesthetized with ether and exsanguinated by cardiac puncture. Serum was separated from clotted blood by centrifugation. Serum and urine samples were collected from 10 male and 13 female mice. Samples of liver, kidney, heart, spleen, lungs, skeletal muscle, duodenum, bladder, proximal colon, testes, ovaries, uterus, brain, salivary gland, eye, perigonadal fat, pelt, and feces were collected by systematic dissection of three male and three female mice. Tissue proteins were eluted in distilled water (1:2 wet weight, gram, per volume, milliliter) for 48 hours after mincing of the tissues. Extract was separated from solid by centrifugation.

Preparation of antisera

Purified major urinary protein was prepared from CD1 male mouse urine in the following manner: Crude urine was dialyzed in 200 volumes of phosphate-buffered (0.01 mol/L, pH 7.5) saline (0.15 mol/L) with 3500 molecular weight cut off Spectrophor membrane tubing (Thomas Scientific, Philadelphia, Pa.) and subjected to gel filtration on a Sephadex G-75 column (Pharmacia Fine Chemicals, Uppsala, Sweden).

Fractions representing the major protein peak (molecular weight approximately 19,000 daltons) were collected, dialyzed, and subjected to preparative isoelectric focusing in a sucrose gradient (see below). The major protein peak representing a pI of approximately 4.5 was collected, dialyzed against 200 volumes of 0.05 mol/L of ammonium bicarbonate, and freeze-dried. One milligram of purified urinary protein from CD1 mice was prepared in complete Freund's adjuvant and used to immunize 5-pound New Zealand white rabbits by multiple subcutaneous injections. Seventeen weeks after immunization, the rabbits were boosted with the same amount of mouse allergen in incomplete Freund's adjuvant. One week after the boost, the rabbits were bled, and the resultant antisera were tested by Ouchterlony gel diffusion. Antiserum was similarly prepared against dialyzed whole CD1 male mouse urine.

Gel filtration

Sephadex G-75 was prepared in PBS and poured into 2.5 by 61 cm columns that were then calibrated with BSA, ova, chymo A, and cyt C as previously described.¹⁶

SDS-PAGE

SDS-PAGE was performed in 9% acrylamide gels containing 0.1% SDS as described by Laemmli.¹⁷ Samples were either unreduced or reduced in 0.125 mol/L of Tris, pH 6.8, containing 0.125 mol/L of DTT. Immediately after electrophoresis, gel tracts containing reduced and unreduced allergen were excised and subjected to CIE.¹⁶ Molecular weight estimates were made by comparing the migration of proteins under study with standard proteins (β -galactosidase, phosphorylase- β , BSA, ova, chymo A, cyt C, and myoglobin) that were subjected to electrophoresis simultaneously.

Isoelectric focusing

Analytical isoelectric focusing was performed as previously described by use of an LKB Multiphor apparatus (LKB Producter, Bromma, Sweden).¹⁶

Preparative isoelectric focusing was performed in a 110 ml LKB isoelectric focusing column with a sucrose gradient and 5% ampholytes producing a pH gradient of 3.5 to 10.0. A constant voltage was applied across the column for 24 hours at 4° C. Fractions (1.2 ml) were then collected and tested for pH and protein content.

Quantitative IEP (Laurell rocket assay)

Quantitative IEP was performed after the method of Laurell as previously described^{16, 18} with an LKB Multiphor apparatus. Protein standards were included on each plate.

Immunoabsorbant techniques

Cyanogen bromide-activated Sepharose 4B was coupled to the gamma globulin fraction¹⁶ of rabbit antisera to MA1 and to normal rabbit gamma globulin according to manufacturer's instructions (Pharmacia Fine Chemicals) and packed in 2.5 by 4 cm columns equilibrated with PBS. Samples of dialyzed male CD1 urine containing 30 units of MA1 were applied to each column, and the effluent was collected and concentrated eightfold by Diaflow Filtration (Amicon Corp., Danvers, Mass.). Similarly, samples of CD1 pelt extract containing 12 units of MA1 were applied to each column, and the effluent was concentrated thirteenfold.

Stability testing

Solutions containing 70 units per milliliter of MA1 in distilled water were heated at 60° C or 90° C for 30, 60, or 180 minutes. An identical solution was kept at 37° C for 180 minutes. For reduction, similar solutions were suspended for 30 or 60 minutes in 1% DTT in Tris (0.007 mol/L, diethyl barbituric acid (0.003 mol/L) buffer (pH 7.5) at 20° C. All of the above allergen-containing solutions were tested for MA1 content by the Laurell rocket assay after appropriate volume adjustment.

Enzymatic digestions were conducted with soluble papain and pepsin (Sigma Chemical Co., St. Louis, Mo.). Papain, at a concentration of 6.75 mg/ml in 0.2 mol/L of phosphate buffer, was activated with ethylenediaminetetraacetic acid and incubated with 100 units of MA1 at 25° C for 24 hours. The reaction was stopped by the addition of ascorbic acid.

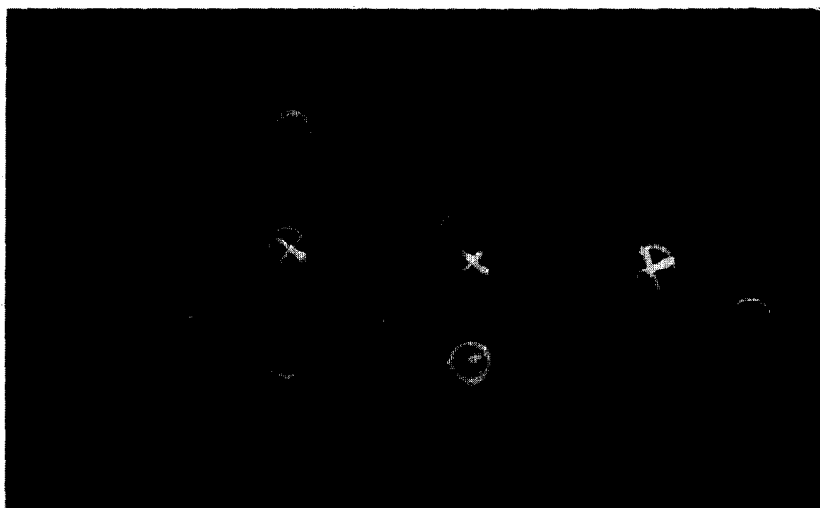


FIG. 1. Gel diffusion analysis of antiserum to mouse urinary allergen. Antiserum to purified mouse urinary protein was placed in the central wells (*marked with an x*). Wells 1 to 6 were filled with preparations from CD1 mice as follows: No. 1, 1/100 male urine; No. 2, 1/10 female urine; No. 3, 1:1 w/v male pelt extract; No. 4, 1:1 w/v female pelt extract; No. 5, undiluted male serum; No. 6, undiluted female serum. Wells 7 to 13 were filled with undiluted serum from the cow, cat, dog, rat, horse, rabbit, and guinea pig, respectively.

Pepsin, at a concentration of 0.07 mg/ml in 0.1 mol/L of acetate buffer, was incubated with 100 units of MA1 at 37° C for 26 hours. This reaction was terminated by the addition of solid Tris until the solution reached pH 8. Parallel digestions were carried out with human IgG and assayed by SDS-PAGE. The expected fragmentation of IgG took place under these conditions. The digested MA1 preparations were tested for biologic activity in the avidin-biotin ELISA inhibition described later in this article. Serial dilutions of the digested MA1 preparations, as well as control undigested MA1 subjected to identical conditions, were used as the inhibitor in the assay.

Skin testing

Prick tests were performed as previously described with extracts prepared in 50% glycerol.¹⁹ Informed consent was obtained from each volunteer who had a history of allergic symptoms on exposure to mice. The average diameter of the wheal in millimeters was noted after 15 minutes. All materials were tested in nonallergic control subjects to rule out irritative reactions.

Avidin-biotin micro-ELISA

The ELISA was performed with 96-well Immunolon II microtiter plates (Dynatech, Alexandria, Va.). Purified antibody to cat allergen 1 prepared as previously described¹⁶ was first added to the wells (5.52 mg/ml; 0.1 ml per well) and allowed to incubate for 30 minutes at 37° C. After four washes with PBS containing 0.05% Tween 20 (PBS-Tween), each well was similarly incubated with 0.1 ml of a preparation of purified urinary protein from male CD1 mice in a concentration of 40 µg/ml. The remaining steps were carried out as described by Rao et al.²⁰ with the fol-

lowing modifications: Biotin antihuman IgE and avidin-HRP were used in dilutions of 1/250 and 1/1000, respectively (Tago Inc., Burlingame, Calif.). For inhibition experiments, patient serum was diluted 1/4, and 0.1 ml was added to 0.1 ml of various dilutions of purified mouse urinary allergen, incubated for 30 minutes at 37° C, and added to the wells. Percent inhibition was calculated by use of the following equation:

$$\% \text{ Inhibition} = [1 - (V1 - V2/V3)] \times 100$$

where V1 is the value obtained with inhibited serum, V2 is the value with inhibitor alone, and V3 is the value for the uninhibited serum.

In order to test the specificity of the Tago antihuman IgE, purified human IgG²¹ was mixed with biotin antihuman IgE and incubated for 30 minutes before addition to wells that contained bound human IgE. Up to 13.6 µg of IgG per well resulted in no significant inhibition. This was accepted as evidence for the specificity of the anti-IgE reagent.

RESULTS

Gel diffusion analysis of antiserum to mouse major urinary protein

Fig. 1 illustrates the results of an Ouchterlony gel diffusion of the rabbit antiserum (*center wells*) against male and female urine and serum and pelt extract (*upper peripheral wells*). A single precipitin line identifies an antigenic component that is found in all of these sources. Because of the high concentration of this antigen in urine, it was necessary to dilute the urine samples extensively. Serum from seven other mammalian species were tested in the lower peripheral

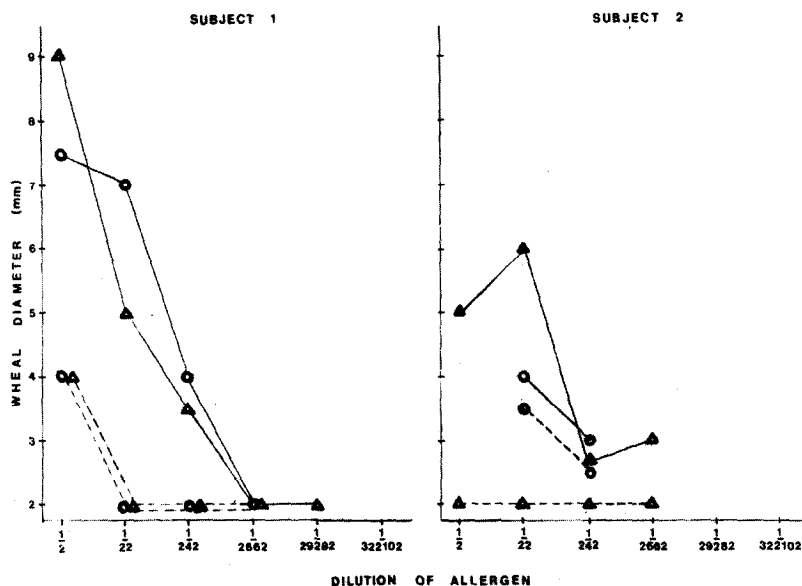


FIG. 2. Prick test responses in allergic subjects to absorbed and unabsorbed preparations of crude mouse urine and pelt extract. Samples of crude CD1 male mouse urine containing 30 U/ml and pelt extract containing 30 U/ml were absorbed with a gamma globulin fraction of anti-MA1 antiserum and normal rabbit gamma globulin. Fractions were tested in two mouse-allergic subjects (1 and 2). The average wheal diameter (ordinate) is plotted against the log dilution of the sample (abscissa). Absorbed preparations (dotted lines) of pelt (circles) and urine (triangles) were consistently less reactive than control preparations (solid lines).

wells, and no precipitin lines were observed. The antigen identified by this antiserum is designated MA1. Antiserum similarly prepared against whole male mouse urine failed to react to any antigens other than MA1.

Development of an arbitrary unit designation for MA1

A pool of male CD1 mouse urine was collected. A 1/100 dilution of this pool was assigned a value of 10 U/ml. A precise weight estimate of this allergen is not yet available, but we estimate that 1 unit is approximately equal to 5 to 10 μ g. This is based on the optical density of the purified urinary protein at 280 nm.²² Serial dilutions of this urine pool were used as standards for all quantitative IEP procedures.

Demonstration of the antiallergenic properties of antiserum to MA1

In order to demonstrate the antiallergenic properties of antisera to MA1, identical samples of crude male CD1 urine and crude pelt extract were passed through immunoabsorbent columns containing either the gamma globulin fraction of the antiserum or normal rabbit gamma globulin as described in Methods. The concentrated effluent of the control columns con-

tained 3.5 U/ml for the urine sample and 4.1 U/ml for the pelt extract. Similarly, concentrated effluent from the anti-MA1 column contained an undetectable amount (<0.03 U/ml) for the urine sample and 0.6 U/ml for the pelt extract. The effluents were then diluted and prepared for prick testing.

Fig. 2 illustrates the prick test results obtained in two volunteers who were allergic to laboratory mice. In subject 1, for each of the fractions tested, there was an approximately linear relationship between average wheal diameter and the log reciprocal dilution of the fraction. It is apparent that extracts of pelt and crude urine were approximately 100-fold less allergenic when extracts were passed through the anti-MA1 column compared with the control column. In subject 2, there was again a very significant removal of allergenic activity after absorption of the crude urine. In this patient a smaller reduction in allergenic activity was observed when the crude pelt extract was absorbed with the antiserum.

Fig. 3 illustrates the relationship between the concentration of MA1 in the various preparations and the wheal size in subject 1. A highly significant correlation was observed, suggesting that in this individual the allergenic activity of both pelt and urine was due to the presence of MA1. Wheal sizes of 3 to 5 mm

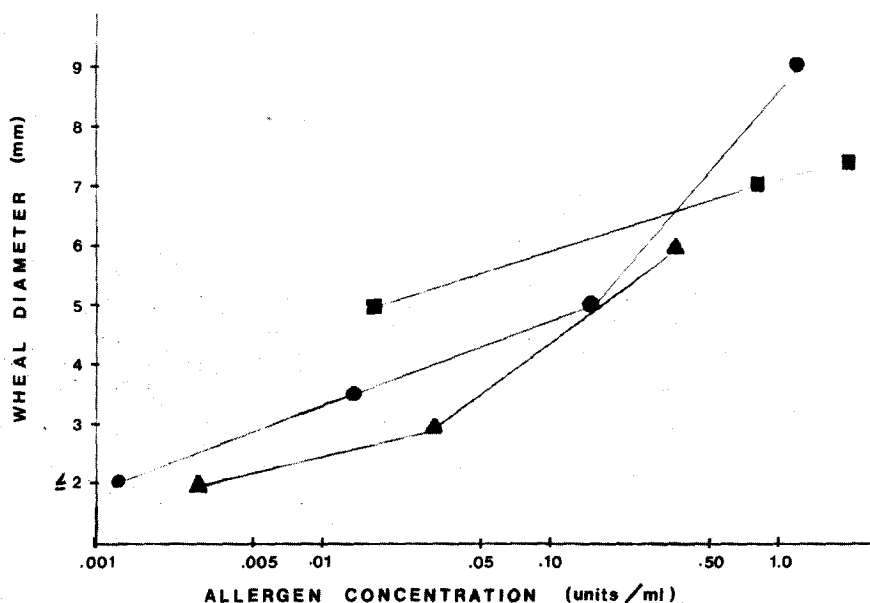


FIG. 3. Relationship between average prick test wheal size and MA1 content of various mouse products in subject 1. Dilutions of Sephadex G-75 fractions of male CD1 urine (see Methods) (triangles) and crude CD1 urine (circles) and pelt (squares) extracts with known concentrations of MA1 (abscissa) were plotted against the wheal diameter in subject 1. A strong correlation between MA1 content and wheal size was observed ($r = 0.94$; $p < 0.01$).

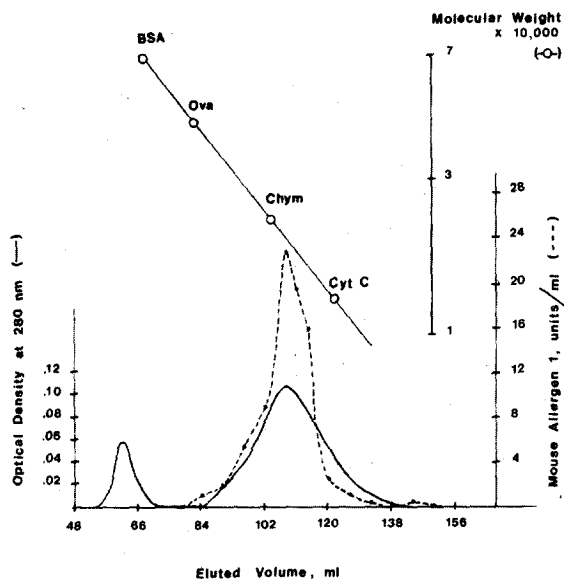


FIG. 4. Estimation of the molecular weight of MA1 by gel filtration. Dialyzed male CD1 urine was subjected to gel filtration on a Sephadex G-75 column. Protein standards that were used included BSA, Ova, Chym, and Cyt C. The molecular weight was estimated at 19,000.

were observed in fractions that contained very small amounts of MA1 (in the range of 0.01 to 0.1 U/ml). The differences in the slope of the lines with use of the two preparations may indicate the presence of allergens other than MA1.

Molecular weight estimates of MA1

One half milliliter of crude male mouse urine was subjected to gel filtration in PBS on a Sephadex G-75 column. After a small void peak, a major protein peak was observed that contained protein with a molecular weight of $19,000 \pm 2000$ (Fig. 4). The peak concentration of MA1 corresponded to this protein peak.

Purified MA1 (see Methods) was tested by SDS-PAGE with and without reduction with DTT (Fig. 5). Unreduced allergen appeared as two bands with molecular weights of 18,000 and 21,000. Reduced MA1 appeared as only a single band with a molecular weight of 21,000, corresponding to the lighter of the two bands observed with unreduced MA1. CIE was carried out with both reduced and unreduced allergen (results not presented). Antigenic activity was associated with the 18,000 molecular weight band of unreduced MA1 and the 21,000 molecular weight band of the reduced allergen. It was not possible to determine with certainty whether the 21,000 molecular weight band in the unreduced preparation reacted with the antisera, perhaps because of the small amount of protein associated with this band.

Isoelectric focusing

Purified MA1 (see Methods) was subjected to analytical isoelectric focusing in an acrylamide gel. Gel tracts containing MA1 were excised and subjected to CIE. Fig. 6 illustrates a stained gel tract containing 3

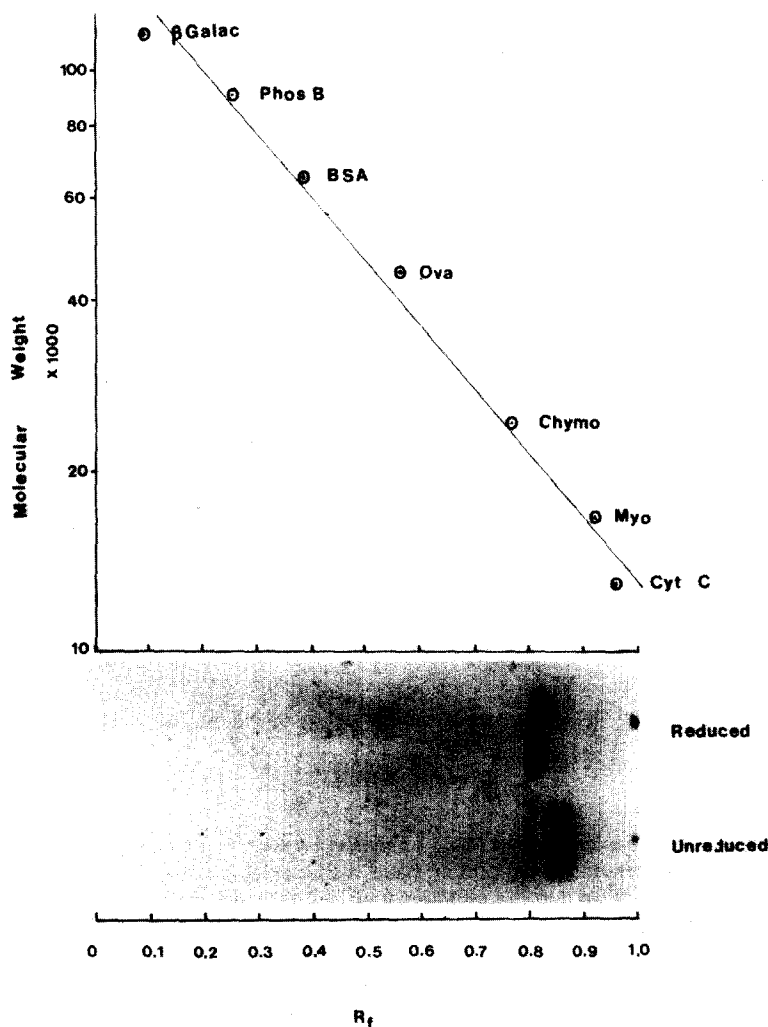


FIG. 5. Estimation of the molecular weight of MA1 by SDS-PAGE. Purified MA1 was subjected to electrophoresis in a 9% acrylamide gel containing SDS. Molecular weight markers included β -galactosidase (β Galac), phosphorylase-b (phos B), BSA, ova, chymotripsinogen A (Chymo A), myoglobin (Myo), and Cyt C. The sample was run with and without prior reduction with DTT with the cathode on the left. Unreduced allergen appeared as two bands: a heavier band with a molecular weight of 18,000 and lighter band with a molecular weight of 21,000. Reduction resulted in only a single band with a molecular weight of 21,000.

units of MA1. A major protein band is observed as well as several more acidic bands that are less intense. CIE of 1 unit of MA1 produced a major precipitin arc corresponding to the major band of stained protein at an approximate pI of 3.9 ± 0.1 . In addition, a smaller precipitin arc corresponded to the strongest of three more acidic bands. Repeated experiments with varying antigen concentrations strongly suggested that the other two bands were also associated with antigenic activity.

Antigenic stability of MA1

The effects of heating and reduction with DTT are summarized in Table I. It is clear that reduction and heating at 60° C for up to 180 minutes had minimal

effect on the antigenic activity of MA1. Heating at 90° C resulted in a progressive loss of activity with approximately a 70% loss at 180 minutes. Enzymatic digestion of 100 units of MA1 with pepsin resulted in no decrease in antigenic activity, whereas digestion with papain resulted in a 40% reduction in antigenic activity. We then tested the ability of these digests to inhibit in the ELISA system. A 1/1000 dilution of undigested MA1 inhibited 50%, whereas dilutions of 1/1700 and 1/2200 of pepsin digest and papain digest, respectively, elicited 50% inhibition in the ELISA. Pepsin alone inhibited 6%, and papain alone inhibited 16%. The allergenic activity of MA1 appears to be unaffected by digestion with either papain or with pepsin.

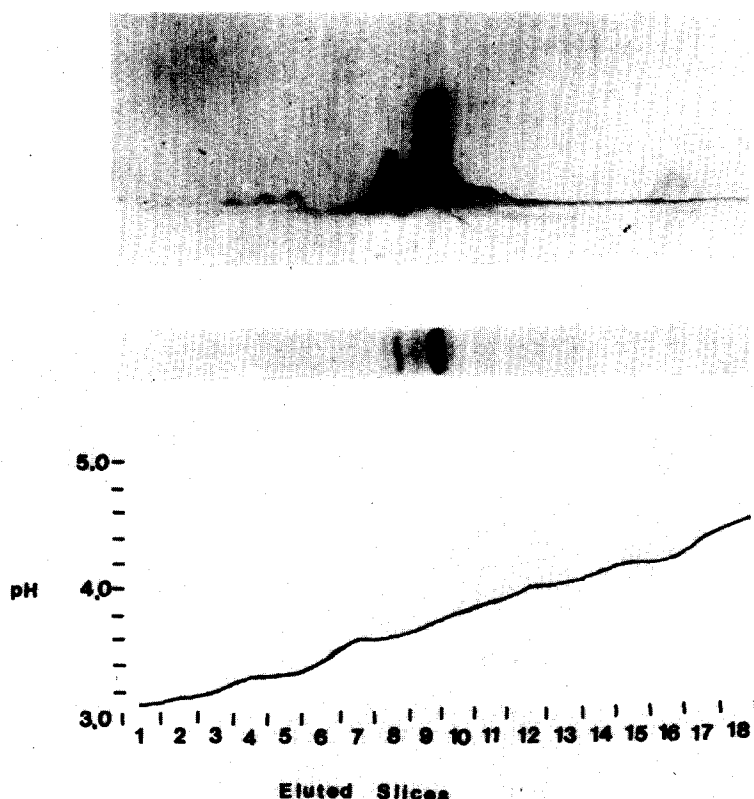


FIG. 6. Isoelectric focusing of purified MA1. Analytical isoelectric focusing of 3 units of MA1 in an acrylamide gel revealed a major protein band with a pI of 3.9 (a stained gel strip with the cathode to the left is indicated in the **middle panel**) and three minor bands with slightly more acidic pIs. Crossed electrophoresis of the protein in the acrylamide strip into an antiserum containing gel (**upper panel**) revealed a major precipitin arc corresponding to the major protein band as well as a smaller arc corresponding to the strongest of the three more acidic bands.

Distribution of MA1 in mouse tissues and fluids

Table II compares the concentration of MA1 in mouse serum and urine. Male mouse fluids contained approximately four times more MA1 than female mouse fluids. These differences were highly significant (Student's *t* test, two-tailed). Urine from both the male and female mouse contained 10 times more MA1 than serum.

Table III summarizes the measurement of MA1 in tissue extracts. Liver extract contained the highest concentration of MA1. It is of interest that the pelt extract contained very small amounts of MA1. Intermediate quantities of MA1 were observed in kidney and bladder extracts, probably indicating contamination with urine.

Comparison of ability of MA1 from three breeds of mice to inhibit human IgE binding to insolubilized urinary allergen

With the avidin-biotin micro-ELISA, varying concentrations of purified MA1 from three mouse strains

were used to inhibit the binding of IgE antibody from a mouse-allergic individual to insolubilized MA1 derived from CD1 mouse urine. MA1 from C57, BALB/c, and CD1 mice exhibited 50% inhibition in concentrations of 0.07, 0.045, and 0.055 U/ml, respectively. The slopes of the inhibition curves are very similar (Fig. 7). This suggests that the urinary antigens from these three strains have similar antigenic properties when antigens are compared with reactivity of human IgE antibody. When rabbit antisera was added as an inhibitor in an identical manner, it was found that 1/25, 1/125, 1/625, and 1/3125 dilutions inhibited 98%, 80%, 74%, and <0%, respectively, again verifying the antiallergenic specificity of the rabbit antisera.

DISCUSSION

This article describes the properties of the major antigen present in mouse urine. This antigen, which was found in mouse serum, urine, and extracts of pelt, was initially identified by the use of an antiserum prepared by the immunization of rabbits with the ma-

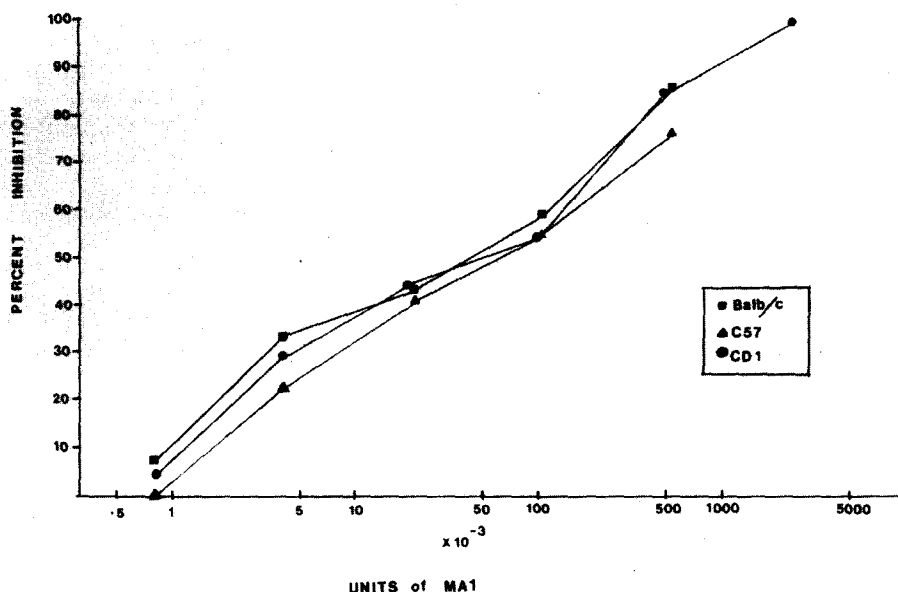


FIG. 7. Inhibition of IgE antibody binding with MA1 from three mouse strains. With the avidin-biotin micro-ELISA, varying concentrations of purified MA1 from three mouse strains were used to inhibit the binding of IgE antibody from a mouse-allergic individual to insolubilized MA1 derived from CD1 mouse urine. MA1 from C57 mice (triangles) and BALB/c mice (squares) inhibited as well as MA1 from CD1 mice (circles).

TABLE I. Stability of MA1 on heating and reduction

Procedure	Antigenic activity (U/ml)
Control	
Suspended in PBS at 37° C 1 hr	69
Reduction with DTT at 20° C	
30 min	68
60 min	68
Heating at 60° C	
30 min	68
60 min	68
180 min	68
Heating at 90° C	
30 min	43
60 min	29
180 min	23

TABLE II. Concentration of MA1 in serum and urine, units per milliliter (SD)

	Male (n = 10)	Female (n = 13)	Significance
Serum	40.3 (10.9)	6.8 (1.6)	$p < 0.001$
Urine	3865.0 (2846)	935.0 (553)	$p < 0.005$

major urinary protein of the mouse (Fig. 1). That this was the major antigen in mouse urine was demonstrated by the observation that antiserum obtained from rabbits immunized with dialyzed crude mouse urine failed to identify additional antigens.

In order to demonstrate that this antiserum was also directed against allergen, a series of immunoabsorption experiments were carried out (Fig. 2). There was a marked reduction in the skin test reactivity of crude

mouse urine and crude pelt extract in the two subjects after absorption of these allergen sources with this antiserum. The antigen identified by this antiserum was therefore designated MA1, and a quantitative IEP procedure was set up for its measurement with an arbitrary unit designation. The exact weight of protein that corresponds to a unit is not known, but it appears to be approximately 5 to 10 μ g. Because, strictly speaking, an antiserum measures antigenic sites on protein and not protein concentration, it appeared wise to retain the unit designation.

The measurement of MA1 will likely prove useful in the standardization of reagents for diagnosis and perhaps treatment of mouse allergy. Quantitative IEP or radial immunodiffusion have a number of obvious advantages when they are used in allergen standardization. These procedures are simple to carry out, relatively quantitative, and are readily performed by extract manufacturers. The reagents involved (mono-specific rabbit antiserum and an antigen standard) are easily prepared, and different preparations can be checked against each other by simple gel diffusion

TABLE III. Concentration of MA1 in various tissues in units per milliliter (SD) in a 1:1 w/v extract

Tissue	Male (n = 3)	Female (n = 3)	Significance
Pelt	1.9 (0.3)	2.4 (0.4)	ns
Liver	248.0 (86)	85.0 (46)	$p < 0.05$
Kidney	14.6 (8.1)	7.4 (3.0)	ns
Bladder	68.0 (40)	7.1 (3.6)	ns

ns = Not significant.

Extracts of heart, lungs, spleen, skeletal muscle, duodenum, colon, testes, ovaries, brain, salivary gland, eye, uterus, and feces all contained less than 6 U/ml.

techniques. Such advantages do not all clearly pertain to such procedures as RAST inhibition, crossed radioimmuno-electrophoresis, or quantitative skin testing procedures. The validity of quantitative IEP as a measure of allergen depends on the ability of rabbit antisera to identify allergenic sites on protein molecules. The rabbit antisera used in our present studies could readily be demonstrated to remove significant allergenic activity from crude preparations of urine and pelt extract.

The molecular weight of MA1, its polymorphic charge properties, and its relative distribution in mouse fluids and tissues strongly argue for an identity between MA1 and the previously described mouse major urinary protein complex.^{23, 24} This identity has been previously suggested by others.¹⁰ Mouse urinary proteins are synthesized in the liver.²⁵ Transcriptional control mechanisms responsive to androgen are believed to be the cause of an increased synthesis of the proteins in the male liver tissue.¹⁵ Sequence analysis of DNA may allow a detailed knowledge of the structure of this family of molecules.¹⁴ The stability of the antigenic sites on heating, reduction, and enzymatic digestion (see Results and Table I) suggests that it may be possible to determine the structure of these sites. This could be useful in future studies of antigen processing and perhaps immunoregulation in immediate hypersensitivity.

In the future, other allergens derived from the mouse may be demonstrated to be important in patients who are allergic to mice. There is some evidence that a higher molecular weight allergen, probably serum albumin, is allergenic.⁸ In our present studies, protein bands consistent with the charge and mobility of albumin were in only trace concentrations or undetectable in the urine. Further studies are needed to document more fully the predominant importance of MA1.

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Cutaneous mast cell heterogeneity: Response to antigen in atopic dogs

Allan B. Becker, M.D.,* K. Fan Chung, M.D.,** Donald M. McDonald, M.D., Ph.D., Stephen C. Lazarus, M.D., Oscar L. Frick, M.D., and Warren M. Gold, M.D.
San Francisco, Calif.

Because mast cells (MCs) in skin of atopic dogs are heterogeneous with respect to tissue fixation and staining properties, we determined the effect of antigen on each type of MC in vivo. Skin biopsies were done in anesthetized, ragweed-sensitized dogs before and at 1, 3, 6, and 24 hours after intradermal injections of ragweed antigen (n = 5) or glycerin diluent (n = 4). In each case, one biopsy specimen was fixed with formalin, and a second specimen from an adjacent abdominal site was fixed with basic lead acetate. In sections stained with Alcian blue, 49.7% more MCs ($p < 0.05$) were detected in tissue fixed with basic lead acetate ("typical" plus "atypical" MCs: $2916 \pm 581/\text{mm}^2$; mean $\pm$ SEM) than in tissue fixed with formalin ("typical" MCs: $1955 \pm 537/\text{mm}^2$). After antigen, the number of "typical" MCs detectable in tissue sections progressively decreased during the 24-hour period, whereas the number of "atypical" MCs was lowest at 1 hour and had increased at 24 hours. After diluent, MC numbers did not change significantly over time. A late-phase response (LPR), detected clinically as induration and edema, was present 6 hours after antigen in four of five dogs, but LPR was not detected after diluent. The size of LPR was correlated ($r = 0.85$; $p < 0.05$) with the decrease in the number of "typical" MCs at 6 hours. We conclude that the response of the "typical" and "atypical" MC to antigen in vivo differs markedly. The "atypical" MCs participate in the early, acute response to antigen, and the "typical" MCs may be associated with the development of the LPR. (J ALLERGY CLIN IMMUNOL 78:937-42, 1986.)

From the Cardiovascular Research Institute, Departments of Medicine, Pediatrics, and Anatomy, University of California, San Francisco, San Francisco, Calif.

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Reprint requests: Warren M. Gold, M.D., Cardiovascular Research Institute, 1327-M, University of California, San Francisco, San Francisco, CA 94143.

*Dr. Becker was a Traveling Fellow of the Medical Research Council of Canada Fellowship. Current address: Children's Hospital, Department of Pediatrics, The University of Manitoba, 678 William Ave., Winnipeg, Manitoba, Canada R3E 0W1.

MCs play a critical role in allergic disease. Research directed toward the understanding of MCs has clearly demonstrated morphologic heterogeneity,^{1, 2} biochemical heterogeneity,^{2, 4} and functional heterogeneity^{2, 3, 5} of these cells in vitro. These studies have been done primarily in rats, comparing MCs harvested from the gastrointestinal tract after parasitic infestation (mucosal or atypical MCs) with those cells from the peritoneal cavity (connective tissue or typical MCs). The typical MCs exhibit metachromatic stain-

**Dr. Chung was a Traveling Fellow of the Medical Research Council of Great Britain. Current address: The Cardiothoracic Institute, British Postgraduate Medical Federation, University of London, Fulham Road, Brompton, London SW3 6HP.