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Recombinant Major Urinary Proteins of the Mouse in Specific IgE and IgG Testing

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Key Words
Recombinant allergen · Animal allergen · Animal allergy · Specific IgE antibodies · IgG antibodies · IgG4

Abstract
Background: Recombinant allergens are preferred over natural allergen extracts in measuring antibodies. We tested the use of recombinant variants of the major mouse allergen Mus m 1 in detection of mouse-specific antibodies in sera of laboratory animal workers and children. ***Methods:*** Six recombinant major urinary proteins (MUPs) were produced and antibody-binding capacity was compared to natural Mus m 1 and to mouse urine extract. In a specific subset, cross-reactivity of MUP with Mus m 1 and between the different recombinant MUPs was determined. ***Results:*** For IgE antibodies, MUP8 showed high cross-reactivity with Mus m 1. MUP8-specific IgE was found in 55% of the mouse urine IgE-positive sera. Specific IgG and IgG4 antibodies against natural Mus m 1 correlated strongly with antibodies against recombinant MUP8 and were cross-reactive. IgG4 levels against MUP8 and mouse urine extract correlated, but detection of mouse urine-specific IgG4 in the absence of MUP-specific IgG4 was not uncommon. Cross-reactivity of IgG antibodies between MUP8 and Mus m 1 as well as between the different MUPs was high and inhibition varied between 54 and 99%. ***Conclusion:*** The mouse allergen Mus m 1 can be replaced in antibody testing by recombinant MUP8. Other MUPs, except MUP4, are interchangeable with MUP8. However, mouse urine extract showed better detection of both mouse-specific IgE and IgG4 levels. Other components in the mouse urine, like mouse albumin and other yet unidentified components, also induce IgE and IgG(4) antibodies.

Introduction

Exposure to mouse allergens is an established risk factor for development of mouse allergy among laboratory animal workers, affecting 10–26% of exposed employees [1–3]. However, sensitization to mouse allergens is more widespread, affecting for instance children in both inner cities and suburbs [4–6]. In Baltimore, 9–26% of children with asthma in both suburbs and inner city showed evidence of allergic sensitization to mice [4–6], identifying mouse allergens also as an environmental allergen. Symptoms of occupational and environmental allergy against mice are comparable to symptoms induced by environmental allergens and consist of allergic rhinoconjunctivi-

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tis as well as allergic asthma. Most mouse allergens are found in mouse urine, but they are also present in mouse serum and mouse dander [1]. The major mouse allergen Mus m 1 is predominantly found in mouse urine and is actually a complex of similarly sized mouse urinary proteins called major urinary proteins (MUPs) [7–9]. MUPs are small soluble proteins, belonging to the lipocalin family, that bind pheromones and play a role in the regulation of the release of pheromones [10, 11]. The family of lipocalin proteins also contains other allergens like the major rat allergen Rat n 1, dog allergens Can f 1 and Can f 2, cow allergen Bos d 5, horse allergen Equ c 1 and cockroach allergen Bla g 4 [12]. Lipocalins can also be produced in the lachrymal and salivary glands [13]. There they might assist in the capturing of pheromones entering the nasal cavity [14]. Although similarities in the structure of the allergenic lipocalins were found [15], no cross-reactivity or comparable ligand-binding properties are known that would explain their allergenicity.

MUPs are mostly synthesized in the liver under hormonal influence, then transported via the blood and secreted in urine [10]. The genes for MUPs are clustered on chromosome 4 and are highly homologous [16]. The 35 known MUP genes are subdivided into 4 groups based upon sequence homology and origin [13]. The majority of the functional MUP genes belongs to group 1 [13, 16]. There are 15 group 1 MUP genes and their transcripts make up about 5% of male mouse liver RNA [17]. Initially, the purified major urinary mouse allergen was named Mus m 1 and for reasons not obvious, the amino acid sequence of MUP6 is listed as major mouse allergen Mus m 1 in allergen databases.

IgE antibodies are related to clinical symptoms, while IgG antibodies are a biomarker for exposure [18]. They might play a role in reducing allergic symptoms or even prevent development of allergy [18, 19]. Production of IgG4 antibodies, referred to as the modified Th2 response, may induce tolerance indicating the importance of monitoring IgG and especially IgG4 levels [18, 19]. The use of single, often recombinant, allergens in diagnostics and research is preferred above the use of natural allergen extracts for measuring both IgE and IgG antibodies [20, 21]. Extracts are heterogeneous mixtures of both allergens and nonallergic components and may vary in composition. Sometimes they lack certain components and testing with extracts only identifies general allergen sources against which a patient is sensitized. Contamination with allergens from other sources can be a risk of natural allergens. Recombinant allergens are produced under defined conditions and more easily purified. They can often be expressed at high levels and are simply purified and standardized. In the last decade several recombinant allergens have been produced and characterized [for review, see 20].

In our study we aimed to test the usefulness of different recombinant forms of the mouse allergen Mus m 1 in allergy testing and in the detection of IgE and IgG antibodies. Therefore, 6 recombinant Mus m 1 isoforms were produced in *Escherichia coli* [11]. We studied the IgE and IgG antibody profiles against recombinant MUP allergen, purified Mus m 1 and crude mouse allergen extracts. Furthermore, we examined cross-reactivity between the recombinant MUPs and Mus m 1 as well as between the different recombinant MUPs.

Material and Methods

Sera

Sera of laboratory animal workers were collected from 2 previously performed studies concerning laboratory animal allergy [1, 2] and from a cohort of laboratory animal workers in the Netherlands. In total, 317 sera of laboratory animal workers were tested. Sera of 130 preschool children were collected in an inner-city study in Baltimore [6]. In these sera of children, the amounts of MUP8- and Mus m 1-specific IgG and IgG4 were analyzed. The volume of the sera of the children was too little to also determine other specific antibodies.

Allergens

MUP2, MUP4, MUP7, MUP8 and MUP9 were expressed in *E. coli.* The amino acid sequences of MUP1, MUP2, MUP7, MUP8 and MUP9 differ approximately by only 0.6–1.9%, while MUP4 differs approximately 25% from the others [11]. Purification of the MUPs was done as previously described [11, 22]. In short, Ni-affinity chromatography was followed by removal of the His(6) tag by treatment with factor Xa protease. Treatment with Ni-nitrilotriacetic acid resin removed uncleaved fusion protein and purification was done by anion exchange chromatography. The purified proteins were homogeneous as assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and estimated to be >98% pure. MUP8 and natural Mus m 1 (purity >95%; Indoor Biotechnologies Inc., Charlottesville, Va., USA) were labeled with ^{125}I.

Mouse urine was collected during the handling of over 200 mice, both males and females and varying in age. Urine was dialyzed against phosphate-buffered saline (PBS) and freeze dried. In the present article the allergens derived from urine are referred to as mouse urine. Mouse serum was obtained from our animal facility and mouse dander extracts were from HAL Allergy (Haarlem, The Netherlands).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Analysis

One-dimensional electrophoresis of mouse urine in 10% SDS-PAGE gels was performed as previously described [23]. Protein

spots were excised manually and analyzed on a Biflex III matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer equipped with a 337-nm nitrogen laser (Bruker Daltonics, Bremen, Germany) as described [23].

Radioimmunoassay of IgG and IgG4 with Labeled Allergen

Specific IgG and IgG4 to Mus m 1 and MUP8 were measured using a solid-phase antigen-binding assay as previously described [24]. Briefly, for the detection of IgG, serum was incubated overnight with protein G (CNBr-activated Sepharose 4B; Pharmacia, Uppsala, Sweden). Per test, 1–20 μl serum was added to protein G Sepharose (2 μl packed gel/test) in a total volume of 800 μl (PBS/0.3% human serum albumin/0.1% Tween 20) and radiolabeled MUP8 or Mus m 1 was added for detection. After washing, the amount of bound radioactivity was measured and compared to a standard curve, a mouse-specific IgG-positive human serum of an environmentally exposed child.

For detection of specific IgG4, 1–20 μl serum was incubated overnight with anti-IgG4 solid phase (CNBr-activated Sepharose 4B; Pharmacia) in a total volume of 800 μl. After washing, samples were incubated overnight with radiolabeled MUP8 or Mus m 1. Samples were washed and radioactivity was measured. Results were read from the above-mentioned standard curve and expressed in arbitrary units (AU) per milliliter serum. The detection limit of the antigen binding assay was 0.5 AU/ml for IgG and 2 AU/ml for IgG4. We will show that 1 AU IgG4 equals 1 ng IgG4.

Radioallergosorbent Test with Labeled Antigen

Specific IgE levels were only determined in sera of laboratory animal workers (n = 277) with the IgE-radioallergosorbent test (RAST). Freeze-dried mouse allergen extracts were dissolved in PBS and coupled onto a solid phase (100 μg protein to 100 mg Sepharose; CNBr-activated Sepharose 4B; Pharmacia). Recombinant MUP8 and Mus m 1 were similarly coupled to a solid phase.

Per test, 5–40 μl serum was added to 500 μg Sepharose in a total volume of 300 μl (PBS/0.3% human serum albumin/0.1% Tween 20) and incubated overnight. After washing, ^{125}I-sheep anti-human IgE was added and again incubated overnight. Samples were washed and bound radioactivity was measured. Using a reference curve, results were expressed in international units per milliliter [25]. IgE levels above 0.35 IU/ml were considered positive.

Detection of specific IgG4 was performed with IgG4-RAST in the same way as described for IgE. One to twenty microliters of serum was used and radiolabeled monoclonal anti-IgG4 was used for detection. The radioactivity bound was measured and read from a standard curve and results were expressed in nanograms per milliliter [26]. The detection limit for this test was 10 ng/ml.

Inhibition Assays

Eight sera of laboratory animal workers with high levels of mouse urine-specific IgE were selected to study cross-reactivity for IgE between Mus m 1 and MUPs. Per serum, 50 μl was incubated with 5–2,500 ng of unlabeled MUP8 or with PBS/0.3% human serum albumin/0.1% Tween 20 (control) in a total volume of 100 μl. After 2 h incubation, Mus m 1 on solid phase was added to a total volume of 800 μl and all was incubated overnight. Samples were washed and labeled ^{125}I-sheep anti-human IgE was added for incubation overnight. After washing, bound radioactivity was measured. Autologous inhibition served as a control.

Ten sera with high levels (>500 AU/ml) of Mus m 1-specific IgG were selected. One to ten microliters of serum was incubated with 2 or 200 ng of MUP8 for 2 h or with PBS/0.3% human serum albumin/0.1% Tween 20 as uninhibited control. Per test, 500 μg protein G and radioactively labeled Mus m 1 were added in a total volume of 800 μl. This was incubated overnight. After washing, bound radioactivity was measured. Here also autologous inhibition served as control.

Cross-reactivity for IgG between the different recombinant MUPs was determined using the same method. This was done in 20 sera of laboratory animal workers using radiolabeled MUP8 and 20 ng (suboptimal inhibition) or 200 ng (optimal inhibition) of MUP1, MUP2, MUP4, MUP7 or MUP9. Unlabeled MUP8 served as control.

Results were blank corrected and inhibition was calculated as [1 – (inhibited results/uninhibited results)] × 100%.

Statistics

All analyses were performed with the SPSS software (version 11.5; Chicago, Ill., USA). Antibody levels were evaluated in terms of their log values. Values below the detection limit were allotted half the value of the detection limit. Parametric correlations between variables were expressed as Pearson's r, nonparametric correlations as Spearman's ρ. Regression analysis was used to estimate the amount of IgG4 per arbitrary unit. For comparison of levels of IgG4 between IgE-positive and IgE-negative sera, the Mann-Whitney test was used. $p < 0.05$ was considered significant.

Results

MUP in Mouse Urine

The protein composition of mouse urine was determined by electrophoretic separation onto a 10% SDS-PAGE gel and all distinct bands were subjected to MALDI-TOF analyses. The analyzed 8 specific bands represented either serum albumin or MUPs (data not shown).

IgE against Mouse Allergens

To study the cross-reactivity between Mus m 1 and MUPs, we inhibited Mus m 1-specific binding to IgE with MUP in 8 IgE-positive sera of laboratory animal workers. Cross-reactivity between Mus m 1 and MUP8 was high (fig. 1a). The highest concentration of MUP8 (2,500 ng/test) showed a mean inhibition of 91% (range: 85–94%), while autologous inhibition with 2,500 ng Mus m 1 gave a mean inhibition of 85% (range: 73–99%; online suppl. figure 1, www.karger.com/doi/10.1159/000106318). Unfortunately, there were inadequate amounts of specific MUP8 IgE-positive sera to test cross-reactivity between different MUPs.

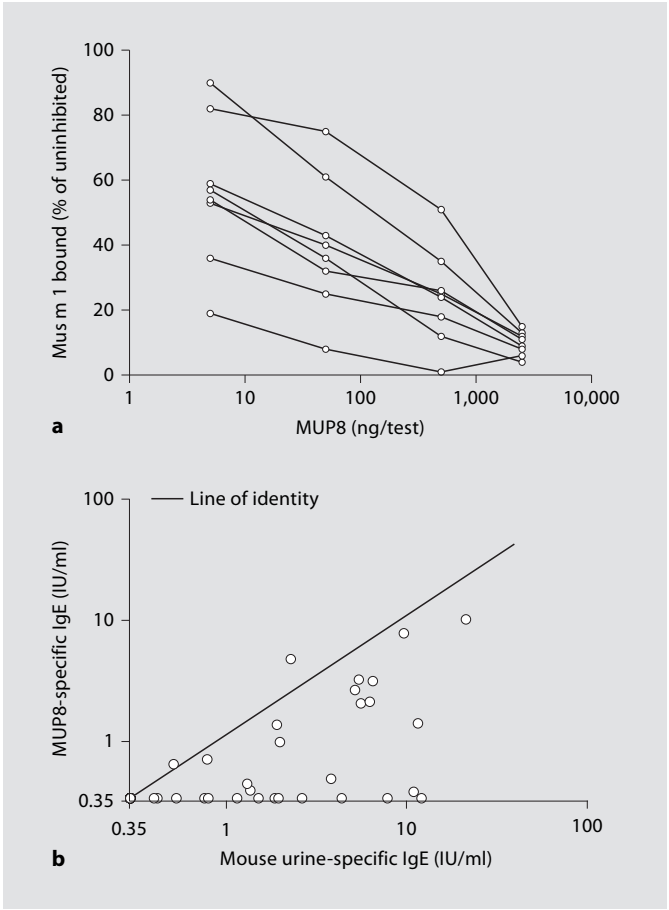

Fig. 1. a Inhibition of specific IgE binding to Mus m 1 with MUP8 in 8 IgE-positive sera. After inhibition with 2,500 ng MUP8, a mean of 91% of Mus m 1 binding was inhibited. **b** Mouse urine- and MUP8-specific IgE show a significant correlation ($r = 0.770$, $p < 0.001$).

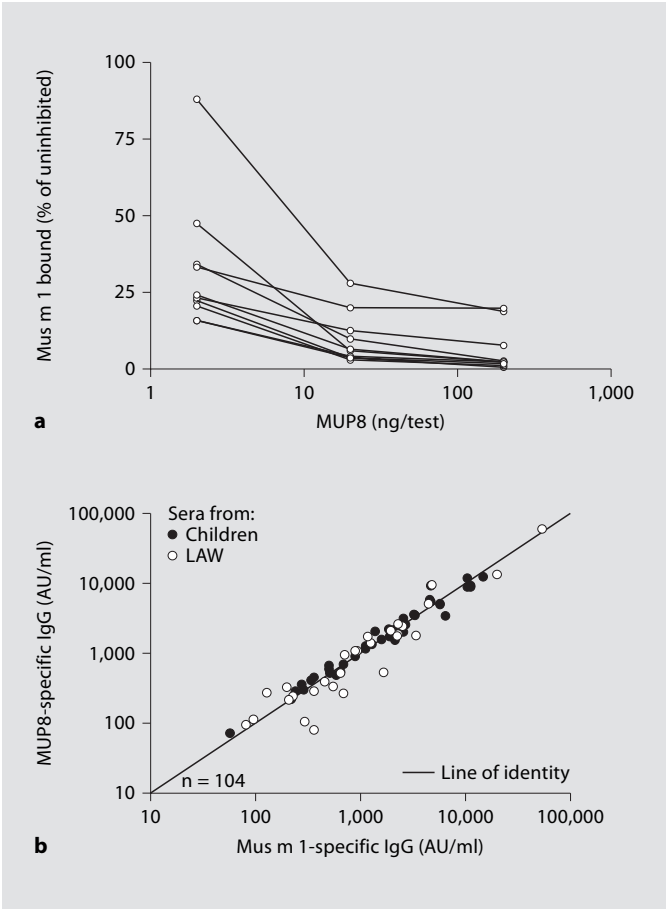

Fig. 2. a Binding of specific IgG to Mus m 1 after inhibition with MUP8 in 10 IgG high sera. The mean inhibition after blocking with 200 ng MUP8 is 94%. **b** MUP8- and Mus m 1-specific IgG, expressed as percentage binding in RIA, were compared in 177 sera of laboratory animal workers (n = 52) and children (n = 130). The majority of the sera (n = 104, boxed) were negative for both Mus m 1- and MUP8-specific IgG. We found a high correlation between the Mus m 1- and MUP8-specific IgG4 for the sera with measurable amounts of specific IgG4 ($\rho = 0.865$, $p < 0.001$). The line represents the relation 1 ng MUP8 = 1 ng Mus m 1 for the reference serum.

IgE against MUP8 was compared with IgE against mouse urine in sera of laboratory animal workers with recorded positive skin prick test for mouse urine (n = 57). Of the 57 sera, 26 (46%) were negative for specific IgE against mouse urine and MUP8. Of the IgE-positive sera, 55% were found to be positive for both mouse urine and MUP8, while 45% were positive for only mouse urine (fig. 1b). None of the laboratory animal workers was positive for only MUP8. IgE against mouse urine and against MUP8 were significantly correlated ($r = 0.430$, $p = 0.018$).

IgG and IgG4 against Mouse Allergens

MUP8 inhibited binding of specific IgG to Mus m 1. The mean inhibition by MUP8 at the highest concentration (200 ng) was 94% (fig. 2a), while autologous inhibition was 99% (online suppl. figure 2, www.karger.com/doi/10.1159/000106318).

Cross-reactivity between MUP8 and other MUPs was studied with MUP8-specific IgG high (>500 AU/ml) sera of 20 laboratory animal workers. Autologous blocking with 20 ng of MUP8 showed 96.4% inhibition (SD: 0.027) and using 200 ng there was 99.9% inhibition (SD: 0.003). Most MUPs showed an inhibition similar to MUP8 (table 1). As expected on the basis of difference in amino acid sequence, the cross-reactivity with MUP4 was the

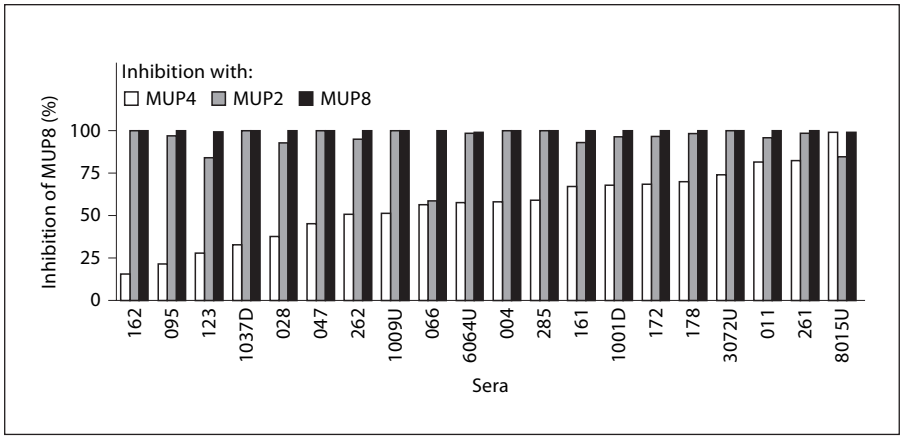

Fig. 3. Inhibition of MUP8-specific IgG by MUP4, MUP2 and MUP8 (control) in sera of 20 IgG-positive laboratory animal workers. For inhibition, 200 ng MUP was used. On the x-axis, sera codes are used.

Table 1. Inhibition of MUP8 with other recombinant MUPs in sera of 20 laboratory animal workers with levels of MUP-specific IgG >500 AU/ml

MUP	Inhibition with:			
	20 ng	SD	200 ng	SD
MUP1, %	87.6	5.7	97.0	4.5
MUP2, %	87.1	10.4	95.0	9.3
MUP4, %	42.1	25.5	56.2	21.5
MUP7, %	97.0	4.2	99.3	1.9
MUP8, %	96.4	2.7	99.9	0.3
MUP9, %	88.6	10.6	96.5	8.0

lowest (table 1). The second lowest inhibition was found using MUP2. Inhibition patterns of MUP2 and MUP4 are shown in figure 3. The inhibition patterns vary in the different sera, especially for MUP4, where they differs from 15.7 to 99.1% inhibition.

Levels of Mus m 1- and MUP8-specific IgG and IgG4 were assessed in sera of children (n = 130) and laboratory animal workers (n = 47) with radioimmunoassay (RIA). There was a significant correlation between IgG against MUP8 and natural Mus m 1 (fig. 2b) and between IgG4 against MUP8 and Mus m 1 (n = 153). If only sera with detectable IgG or IgG4 were considered, highly significant correlations were established for IgG ($r = 0.972$, $p < 0.001$) and IgG4 ($r = 0.959$, $p < 0.001$).

IgG4 against mouse urine and against MUP8 were compared in 277 sera of laboratory animal workers. MUP8-specific IgG4 was determined with 2 techniques: IgG4-RAST (n = 277) and RIA (n = 86). There was a significant correlation between these techniques ($r = 0.675$, $p < 0.001$;

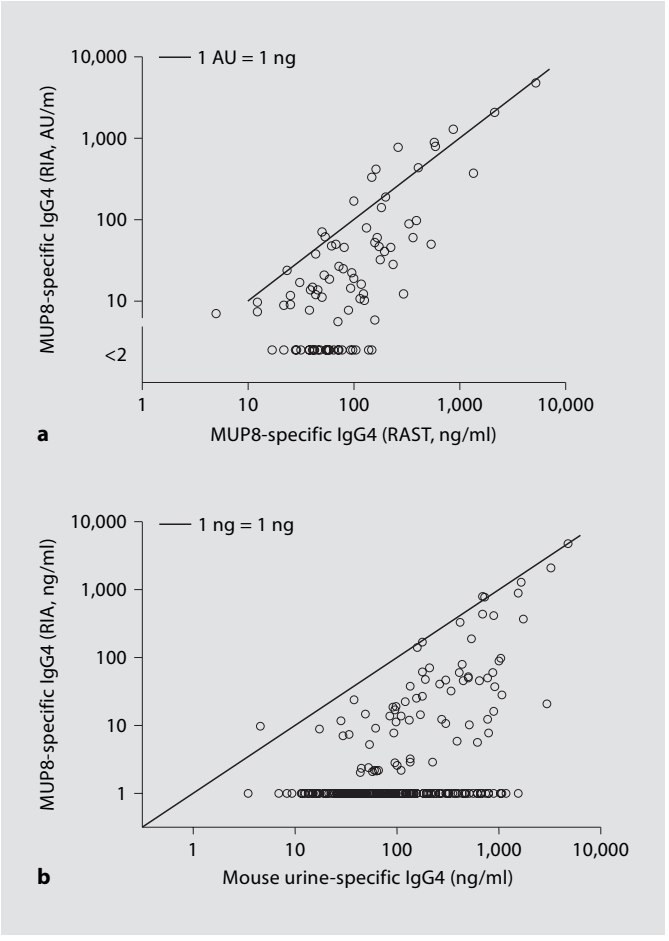

Fig. 4. a Two techniques to measure MUP8-specific IgG4 correlated ($r = 0.675$, $p < 0.001$). The line corresponds to the relation 1 AU MUP8 = 1 ng MUP8. **b** Relation between MUP8-specific IgG4, measured with RIA, and mouse urine-specific IgG4, measured with RAST, in sera of 277 laboratory animal workers. The majority of the sera contained more IgG4 against mouse urine than against MUP8.

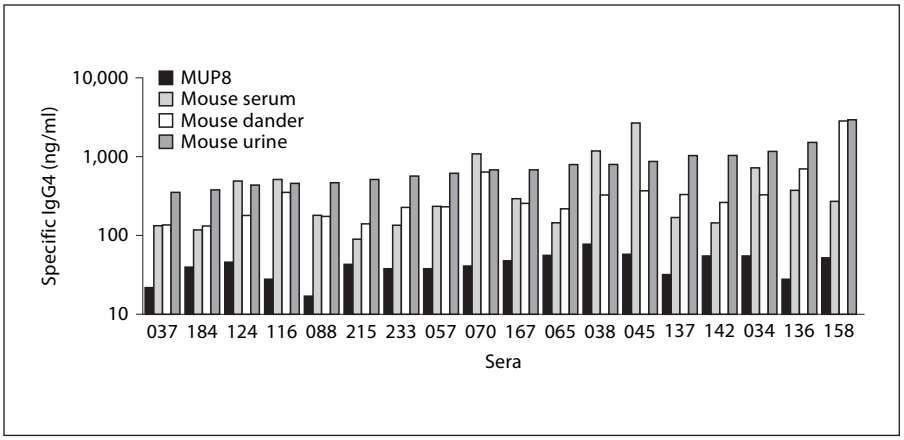

Fig. 5. Levels of IgG4 against MUP8, mouse urine, mouse serum and mouse dander extracts were determined in sera of laboratory animal workers (n = 18) with high responses to mouse urine extract but low responses to MUP8. IgG4 against MUP8 explained less than 11% of the mouse urine response. IgG4 against mouse urine (y-axis) could be explained by the response to MUP8, mouse serum proteins or mouse dander proteins. Sera are ordered based on their mouse urine-specific IgG4 level. The serum codes are given on the x-axis.

fig. 4a). With the data shown in figure 4a, we estimated in a regression analysis that 1 AU IgG4 in RIA corresponded to approximately 1 ng IgG4 in the IgG4-RAST ($p < 0.001$). Twenty-four samples were below the detection limit when tested with RIA, but had detectable levels of MUP8-specific IgG4 when tested with IgG4-RAST. Only 1 sample was below detection limit in IgG4-RAST, but had detectable levels of IgG4 when tested in RIA.

IgG4 against total mouse urine, measured with IgG4-RAST, significantly correlated with MUP8-specific IgG4 measured with both techniques (IgG4-RAST: $r = 0.570$, $p < 0.001$; RIA: $\rho = 0.419$, $p < 0.001$; fig. 4b). MUP8-specific IgG4, measured with RIA, was compared to MUP8-specific IgG in sera of 302 laboratory animal workers. Levels of MUP8-specific IgG4 and IgG correlated ($\rho = 0.636$, $p < 0.001$).

IgG4 against mouse urine and IgG4 against MUP8, both measured by IgG4-RAST, were compared. IgG4 against MUP8 was on average 37.5% of IgG4 against mouse urine (interquartile range: 11–55%). We selected 18 sera of which most IgG4 against urine proteins could not be explained by IgG4 against MUP8 (maximal 11% was explained by IgG4 against MUP8) and tested these sera for specific IgG4 against 2 other mouse allergen sources, mouse serum and mouse dander. Mouse urine contains detectable levels of mouse albumin, which could explain the difference in urine-specific and MUP8-specific IgG4. Antibodies against mouse serum explained the difference for 5 sera completely and partly for the other sera. Dander-specific IgG4 also explained a part of the difference between MUP8-specific and urine-specific IgG4 (fig. 5). Eight sera had higher levels of mouse dander-specific IgG4, while the rest showed higher levels of mouse serum-specific IgG4.

Discussion

We investigated the use of recombinant MUPs in serology against mouse allergens. Cross-reactivity between MUPs and Mus m 1 was high for both IgE and IgG, and inhibition assay for specific IgG confirmed a high degree of cross-reactivity between the different MUPs. Levels of specific IgG and IgG4 against recombinant MUP8 were highly related to levels of specific IgG and IgG4 against natural Mus m 1 ($r > 0.950$). However, testing with mouse urine revealed more IgE- and IgG4-positive sera than when tested with recombinant MUP8, suggesting other urine-derived allergens like albumin to contribute to specific IgE and IgG4 response. Additionally, 2 different tests for detection of specific IgG4, RIA and IgG4-RAST, were compared. Overall, we found similar results using these tests.

Major Urinary Proteins

The use of recombinant mouse allergens in antibody measurements has not been published before. In the present paper we show that recombinant lipocalins of the mouse can be used in immunoglobulin testing. Recombinant MUP8 is equally useful for IgE and IgG antibody determination as the natural major allergen Mus m 1, as MUP8 can inhibit Mus m 1 binding and antibody levels against both allergens correlate strongly ($r > 0.950$). In some sera, inhibition of Mus m 1 binding to IgE and IgG by MUP8 was over 95%, indicating that our recombinant MUPs are correctly folded. MUP8 inhibited IgE binding to Mus m 1 overall even better than autologous inhibition, while for IgG inhibition by the autologous allergen was better. As mentioned, natural Mus m 1 is a complex of different MUPs [7–9], while our recombinant MUP8

represents only 1 lipocalin. Our results may indicate that IgE in the sera we tested is directed more to MUPs related to MUP8 than to the other MUPs present in Mus m 1, while the IgG is directed to probably several different MUPs. This indicates differences in specificity for epitopes between IgE and IgG. It also proves that IgE- and IgG-producing B cells are from different origin as previously described [27].

The cross-reactivity of urinary MUPs (MUP1, MUP2, MUP7, MUP8 and MUP9) in IgG testing was high. MUP2 showed the lowest cross-reactivity with MUP8, but this was still 95%. MUP7 showed the highest cross-reactivity (99%). Both MUP2 and MUP7 differ by 2 amino acids from the sequence of MUP8 [11]. MUP1 and MUP9 both differ by only 1 amino acid from the sequence of MUP8 and both showed 97% cross-reactivity with MUP8. The nasal MUP4 belongs to another group of MUPs and amino acid sequence is about 75% identical [13]. The cross-reactivity between MUP8 and MUP4 was 56%. MUP4 is possibly involved in capturing pheromones in the nasal cavity [14]. It is probably not a relevant allergen in either occupational or environmental settings, but it can inhibit MUP8 binding to specific IgG antibodies over 90% in some sera of laboratory animal workers. It would have been interesting to test cross-reactivity between MUPs in IgE-positive sera. Unfortunately, amounts of IgE-positive sera were not adequate.

Because of the high cross-reactivity found between the different MUPs and between MUP and Mus m 1, MUPs can be considered reliable recombinant allergens for measuring Mus m 1-specific antibody responses. In spite of the fact that most tested sera demonstrated a strong correlation between antibodies against MUP8 and against urine, responses against other (urinary) proteins of the mouse were found. Albumin is also a major component of mouse urine, as found by MALDI-TOF analysis, and it is a known allergen [7, 28]. For identifying sera positive for mouse-specific IgE in a clinical setting, the use of recombinant MUPs would underestimate the number of IgE-positive sera and is therefore not suitable. However, in combination with other (recombinant) mouse allergens, recombinant MUPs could be used in clinical settings for assessing allergic sensitization to mice.

When comparing IgG4 responses to mouse urine and MUP8, we found that the mean IgG4 response against MUP8 is 37.5% of the response against mouse urine. However, some sera showed a mouse urine-specific IgG4 response while almost no IgG4 against MUP8 was found. This was further investigated in 18 sera in which MUP8 explained less than 11% of the mouse urine response. The responses against mouse urine were, at least partly, explained by IgG4 responses against mouse serum proteins and mouse dander proteins. Five of the tested sera showed a higher IgG4 response to mouse serum than to mouse urine (fig. 5). These sera probably have high levels of IgG4 against mouse albumin. The amount of albumin on the solid phase that is loaded with mouse serum is higher than the amount on the solid phase coated with mouse urine, explaining the difference. In 12 sera, the response to mouse urine was higher than the response to MUP8, mouse serum and mouse dander together. This specific mouse urine reaction might be directed against MUPs with low cross-reactivity to MUP8, for example MUP4, or unidentified mouse urine components like the low allergenic mouse dander fractions described by Schumacher [7]. We did not identify other components than MUPs or albumin in mouse urine, but other components could not be excluded.

IgG4-RAST versus RIA

The 2 tests for MUP8-specific IgG4, IgG4-RAST and RIA, showed a significant correlation, but some sera better reacted to MUP8 in one test than in the other. After adjusting for the standards (fig. 4a), comparison of the tests was possible. The 27 sera that were below detection limit for MUP8-specific IgG4 in RIA had measurable levels when tested with IgG4-RAST (fig. 4a). A possible cause for this phenomenon is that radioactive labeling of MUP8 influences the structure of 1 or more IgG4 binding sites and thereby reduces binding in RIA. Another possibility is that because the IgG4-RAST is more sensitive [29], minor components or contamination of the allergen contribute to the results. However, we used a purified recombinant allergen, so the contribution of such minor components is unlikely.

Both tests have their advantages and disadvantages. IgG4-RAST can be used in antibody detection against crude allergen extracts, as the total extract can be bound to solid phase. The disadvantage is that high amounts of allergen are required. Heterologous calibration in IgG4-RAST also allows results to be expressed in nanograms per milliliter [26]. RIA uses a single purified allergen for detection but can be performed when the allergen is limited. Overall, the IgG4-RAST is more sensitive while the RIA has higher specificity [29]. For measuring total IgG, the RAST approach proved to be unreliable due to nonspecific antibody binding [29]. For determining ratios of IgG and IgG4, IgG4 should be measured with the same technique. Study-specific considerations determine which technique to use.

Children versus Laboratory Animal Workers

Some of the children from the inner city had levels of mouse urine- and MUP8-specific IgG and IgG4 comparable to laboratory animal workers. However, the allergen sources might be different. Laboratory animal workers are exposed to mouse allergens through direct contact with mice so that they may have greater exposure to urine and serum allergens than individuals who are exposed to mouse allergens as a result of infestation in their homes. It is possible that household infestation leads to greater exposure to dander allergens. It would therefore be interesting to look into the possible differences in specificity of both IgE and IgG(4) of people with occupational exposure and with environmental exposure.

Cohort studies of laboratory animal workers, like those we are conducting, can improve insight into the development of allergy against high molecular weight allergens on both clinical and cellular levels.

Conclusion

In summary, our recombinant MUPs have a high degree of cross-reactivity with Mus m 1 and with each other. They show similar antigenicity as their natural counterparts and can be used to measure the antibody response against the major mouse allergen Mus m 1. However, there are also antibody responses against mouse-derived proteins other than Mus m 1. Serum proteins, dander components and other urinary proteins might act as allergens. More (recombinant) purified allergens are necessary to determine the specificity of the antibody response to mouse allergens.

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