

Repeated Subcutaneous Administration of Dermatophagoides Allergen Does Not Cause Immune or Non-immune Glomerular Injury in Wistar-Albino Rats

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ABSTRACT

We aimed to investigate the risk of immune or non-immune mediated glomerular injury associated with immunotherapy with dermatophagoides allergen. Three groups of 7 male rats aged 10 weeks were gathered. Group 1 received 1250 units of dermatophagoides allergen injection 3 times with an interval of 2-weeks via subcutaneous route, group 2 received 2500 units of dermatophagoides allergen injection, and the control group received isotonic saline solution equal in volume to the antigen. Kidneys were evaluated by both light and immunofluorescence microscopy. Urine was analyzed with urinalysis strips and light microscopy. Some cases demonstrated

mesangial proliferation as well as an increase in mesangial matrix and tubulointerstitial inflammation. However, these changes did not differ statistically significantly between the groups ($P>0.05$ for mesangial proliferation, increase in mesangial matrix and tubulointerstitial inflammation). Neither hematuria nor proteinuria was detected in control or treatment groups at the beginning or at the end of the study. This study demonstrated that repeated inoculation of dermatophagoides allergen, as required for subcutaneous allergen specific immunotherapy, did not result in any non-immune or immune complex induced glomerular injury. Thus, despite the fact that dermatophagoides allergen are exogenous macromolecules, subcutaneous allergen specific immunotherapy using these purified allergens may not have any risk for glomerular injury.

INTRODUCTION

Exogenous macromolecules trapped or planted within the glomerulus may become antigenic targets for antibodies. At the same time, these trapped or planted exogenous macromolecules may lead to non-immune mediated glomerular injury due to the electrostatic, physical or physicochemical interactions with the glomerular basement membrane (GBM). In case of immune mediated glomerular injury, interactions of antigens and antibodies lead to in situ formation of immune complexes. This mechanism may account for some forms of immune complex nephritis occurring in humans after exposure to certain therapeutic materials (eg, vaccines, parenteral immunotherapeutics, drugs, and toxins), as well as malignancy or microorganisms.¹⁻⁴ Several experimental procedures have been used to trap parenterally administered antigens in the glomerulus. Immune complex nephritis begins with the appearance of antibodies against the planted antigens to glomerulus. These antibodies may form naturally or may be contrived by parenteral injection of the antibody.⁵⁻⁸

Systemic conventional subcutaneous allergen specific immunotherapy (SIT) has been frequently used for the treatment of some forms of allergies (eg, bronchial asthma, allergic rhinitis, and insect allergies), especially when an allergen cannot be avoided. It is a desensitization strategy for managing allergies by habituating the immune system to the presence of an antigen that is a natural allergen and a macromolecule.⁹ Allergen immunotherapy prevents the body's allergic response by numerous mechanisms, such as activating regulatory-T helper lymphocyte, increasing IL-10 secretion from activated T- lymphocyte, T-h2 lymphocyte anergy, and many other possible mechanisms to the allergen, which triggers the allergic reaction.¹⁰ Tolerance to an allergen is achieved by

gradually administering increasingly higher doses of the allergen until exposure to allergen no longer provokes symptoms. Allergen immunotherapy is available for grass, weed (eg, ragweed), tree pollen, bee stings, fire ants, dust mites, dander, and mold.⁹ Allergens, content of immunotherapeutic material, are exogenous macromolecules having high molecular weight in the light of the above mentioned mechanisms, which may lead to glomerular injury when trapped within the glomerular capillary wall. Therefore, allergen immunotherapy may cause an immune or non-immune mediated glomerular injury by trapped or planted allergen within the glomerulus. However, to our knowledge, there has not been a study about allergen immunotherapy and its renal effects including immune or non-immune mediated glomerular injury.

In this study, because the allergens used in conventional subcutaneous allergen SIT are macromolecules, we aimed to investigate whether immunotherapy has the risk of immune or non-immune mediated glomerular injury, therefore dermatophagoides allergen, which is the house dust mite, is administered to Wistar-Albino rats.

MATERIALS AND METHODS

Study Design

Three groups, each containing 7 male rats aged 10 weeks, were studied. dermatophagoides allergen 1250 units was administered to group 1, 2500 units was administered to group 2, and isotonic saline solution equal in volume to the antigen was administered to the control rats (group 3). In all 3 groups, injections were made 3 times at 2-week intervals via subcutaneous injection to the abdominal region. All the rats were killed by decapitation under ether anesthesia 2 weeks after the third injection of allergen or saline. Urine samples from all rats were analyzed for proteinuria,

Table 1. Renal Histopathology Findings in Group 1

Rat no.	Mesangial proliferation	Increase in mesangial matrix	Thickness of basal membrane	Tubulointerstitial inflammation	Vascular changes	Focal tubular atrophy
1	+	+	-	+	-	-
2	+	+	-	+	-	-
3	-	+	-	-	-	-
4	-	+	-	-	-	-
5	-	-	-	+	-	-
6	-	-	-	-	-	-
7	+	-	-	-	-	-

hematuria, and pyuria at the beginning and the end of the study.

Experimental Animals, Allergens, and Urine Collection

Wistar-Albino rats weighing 220 to 245 g were purchased from Experimental Animals Research Laboratory of Ege University. All rats were housed in a stable environment (maintained at $22 \pm 1^\circ\text{C}$ with a 12-hour light/day cycle) and they were fed a standard laboratory diet. *Dermatophagoides pteronyssinus* (*Der p*) and *dermatophagoides farinea* (*Der f*) mixture allergens (Code 708 and 725) (Allergopharma, Reinbek, Germany) were administrated to the rats in the study.

Special metabolism cages were used to collect urine from rat for urinalysis.

Urinalysis

Urinalysis was made with Combur10-TestR M urinalysis strips (Boehringer, Mannheim, Germany) and light microscopy was used for examination of urine sediment.

Histological and Direct Immunofluorescence Evaluation

The kidneys were examined both by light and immunofluorescence microscopy. After the rats were killed, the kidneys were removed and fixed for 1 hour in phosphate buffered solution for routine conventional light and

immunofluorescence microscopy.

Longitudinally cut halves of the mice kidney were made for evaluation. For immunofluorescence the tissues were snap frozen in OCT embedding matrix (125 mL, crioembedding matrix). Frozen sections were cut at a thickness of 5 micrometer. The sections were stained with commercially available fluorescent antisera against IgG, IgA, IgM in 1:20 dilution for 30 minutes and again rinsed twice with phosphate-buffer-saline. Sections were examined at 400-x magnification using a Nikon Eclipse E600 fluorescence microscope. For light microscopy, renal specimens were fixed with 10% neutral buffer formalin and embedded in paraffin using routine procedures. Sections 4 to 5 micrometer in thickness were stained with hematoxylin/eosin, periodic acid-Schiff reagent (PAS), and Masson trichrome. All biopsy specimens were interpreted in a blinded fashion and graded for glomerular proliferation, mesangial expansion, increased thickness of the glomerular basement membrane and the degree of interstitial cellular infiltration, tubular atrophy was assessed. In addition, interstitial fibrosis was assessed as mild, moderate or severe.

STATISTICS

Differences between the treatment groups were evaluated with Kruskal Wallis variance analysis. The significance

Table 2. Renal Histopathology Findings in Group 2

Rat no	Mesangial proliferation	Increase in mesangial matrix	Thickness of basal membrane	Tubulointerstitial inflammation	Vascular changes	Focal tubular atrophy
1	-	+	-	-	-	-
2	-	+	-	+	-	-
3	+	+	-	-	-	-
4	+	-	-	+	-	-
5	-	-	-	+	-	-
6	+	-	-	+	-	-
7						
(Exitus)						

of the differences between treated and control rats were determined with Fisher chi-square test as post-hoc test. *P* values less than 0.05 were accepted as significant.

RESULTS

Urinalysis

Neither hematuria nor proteinuria was detected in any control or treatment groups either at the beginning or at the end of the study (after 3 injections).

Light Microscopy

When the light microscopic findings of all 3 groups were compared, no statistically significant differences were found. All the light microscopic evaluations are presented in Tables 1, 2, and 3.

Mesangial proliferation, increases in mesangial matrix and tubulointerstitial inflammation, was observed in individual cases from different groups.

However, the differences in these observed changes among groups were statistically insignificant ($P > 0.05$ for mesangial proliferation, increase in mesangial matrix and tubulointerstitial inflammation). Thickness of basement membrane, vascular changes or focal tubular atrophy were not observed in this study.

Direct Immunofluorescence

While there was no immune deposition in the control rats, 1 rat in group 2 and 1

rat in group 1 exhibited granular IgG deposition in mesangium. These results were statistically insignificant ($P > 0.05$).

DISCUSSION

Exogenous therapeutic macromolecules are the most potent agents for immune or non-immune mediated glomerular injury since they are trapped or planted to glomerulus filtering the plasma.² Many studies have previously reported appearance of immune mediated glomerular injury (eg, IgA nephropathy) and non-immune mediated glomerular injury (eg, mesangial matrix deposition) as well as degeneration or mixed glomerular injury due to macromolecules including drugs, toxins, vaccine, microbial products, etc.^{1,2,11} Allergens that are used in conventional subcutaneous allergen (SIT) are macromolecules (eg, *Der p* and *Der f*) with a molecular weight approximately 170,000 Dalton, which may cause an important risk of glomerular injury via those mechanisms.^{12,13} Our study demonstrated that repeated inoculations of dermatophagoides allergen at 1250 units and 2500 units, as required for allergen SIT procedures, did not result in light microscopic or direct immunofluorescence microscopic findings of glomerular injury.

Non-immune mediated glomerular injury due to exogenous macromolecules may be constituted by several

Table 3. Renal Histopathology Findings in Group 3.

Rat no	Mesangial proliferation	Increase in mesangial matrix	Thickness of basal membrane	Tubulointerstitial inflammation	Vascular changes	Focal tubular atrophy
1	-	+	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	+	-	-	-	-
7	+	-	-	+	-	-

mechanisms such as electrostatic or physicochemical interaction of reabsorbed macromolecules with GBM, interaction of matrix with macromolecules that reached glomerular constructions or mesangial entrapment of these molecules.^{1-4,14} Moreover, all these mechanisms can act together with systemic or local immune response to cause immune mediated glomerular injury. Non-immune mediated glomerular injury can be frequently observed by light microscopic examination, mainly demonstrating mesangial proliferation, increases in mesangial matrix, tubulointerstitial inflammation, thickness of GBM, vascular changes or focal tubular atrophy. Some of these changes may also be observed in immune mediated glomerular injury due to macromolecules.^{1,2} Our results failed to demonstrate non-immune glomerular injury is associated with dermatophagoides allergen SIT, characterized by the light microscopic examination findings mentioned above. Electrostatic interaction of exogenous macromolecules with GBM may be the principal mechanism for non-immune mediated glomerular injury due to macromolecules because cationic antigens or molecules can bind GBM anionic sites.^{2,4,8} It generally leads to mesangial proliferation, matrix augmentation or tubulointerstitial degeneration. Dermatophagoides allergens are macromolecules with a molecular weight heav-

ier than 170,000 D and they have neutral electrostatic valance.^{12,13} Thus, they cannot bind to anionic sites of GBM and lead to non-immune mediated glomerular injury. Another important mechanism of non-immune mediated glomerular injury is physicochemical interactions between exogenous macromolecules and lectin-binding sites of glomerulus. Many pathogenic microorganisms or antigens may have lectin-like domains, such as Helix pomatia agglutinin or Concovalin-A, which may induce glomerular injury with immune complexes localized initially to the sub-endothelial aspects of the GMB.^{2,5} However, purified dermatophagoides allergens such as *Der p* and *Der f* do not have lectin binding sites or lectin-like domain naturally.^{12,13} Similar interaction may be considered between macromolecules and collagen-IV or heparan sulfate of GBM.² In addition, exogenous macromolecules may be phagocyted by mesangial cells and trapped in the mesangium. However, later mechanisms have not been proven definitively and their pathogenetic importance is unknown. Our results supported that dermatophagoides allergens, as exogenous macromolecules, do not interact with the matrix and are not trapped within mesangium. Mesangial and tubulointerstitial changes were observed in a few subjects. However, all these nonspecific and non-significant changes were attrib-

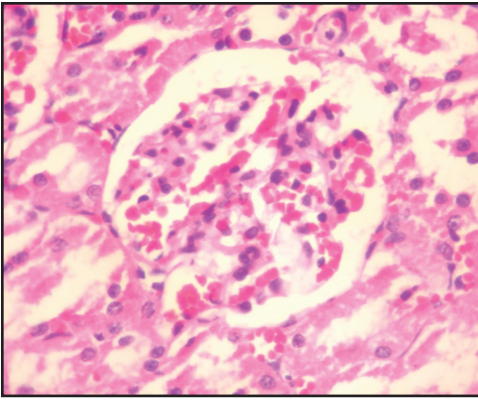


Figure. A normal glomerulus obtained rats from group 2 (400x).

deposits primarily due to antibody-mediated immune mechanisms.^{1,2} Thus, dermatophagoid allergens reabsorbed from SIT injection sites can be trapped by the glomerular wall and may result in immune complex formation. However, biochemical characteristics of circulating antigens have an important role in determining construction and biologic activity of immune complexes. These characteristics include antigen charge, size, valance, and clearance kinetics as well as the type of antibody response against exogenous antigen.^{1,2} GBM is negatively charged due to high content of heparan sulfate. Exogenous macromolecules with a positive charge are more likely to be trapped by the GBM. Thus cationic exogenous antigens that induce the most potent immune response against GBM, lead to an immune complex mediated disease in GBM such as IgA nephropathy, membranous glomerulonephritis, membranoproliferative or rapidly progressive glomerulonephritis. IgA, IgM, C3 and other immune elements are deposited in the mesangium.^{1,4} Many in vivo and in vitro studies have reported that immune complex diseases are caused by exogenous macromolecules (eg, drugs, toxins, vaccines or infectious agents).¹⁻⁸ For example, Kavukcu et al¹¹ reported that

conjugated *Haemophilus influenzae* type-b vaccine, given at 2-week intervals at a total of 6 doses, caused secondary IgA nephropathy in mice. Allergen extract of dermatophagoides for allergen SIT have neutral surface charge, therefore, they are not potent molecules to become trapped by the GBM and induce immune complex response.

Other important features of immune-complex mediated glomerular injury caused by antigenic exogenous macromolecules are antigenic characteristics, type of antibody response, application procedure and route of antigen entry.^{1-4,15,16} IgA antibodies that result in IgA nephropathy are produced in response to antigens which enter through the respiratory or gastrointestinal tracts. Similarly, antibody type and structure may influence the type and extent of injury. Repeated exposure to polysaccharide polymers can lead to the synthesis of IgM and IgG3 with too many antigen-binding sites.^{11,17-19} In dermatophagoid allergen SIT, dermatophagoid allergen extract is repeatedly introduced to subjects. All of those allergens are constituted by heavy proteins that block IgG1 antibody.⁹ Thus, weekly administration of allergen may lead to immune complex formation due to the mechanisms mentioned above. Additionally, our results supported that dermatophagoides allergen SIT does not result in immune complex deposition on GBM of mice experimentally. In conclusion, this study demonstrated that repeated inoculation of dermatophagoides allergen, like in allergen SIT procedure, did not result in any non-immune or immune complex mediated glomerular injury. Thus, despite the fact that dermatophagoides allergens are exogenous macromolecules, allergen SIT with these purified allergens may not have any risk for glomerular injury.

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