

Analysis of allergenic components of Bermuda grass pollen by monoclonal antibodies

Z. N. CHANG, L. C. TSAI, C. W. CHI, M. C. WANG, H. D. SHEN, D. T. LEE* & S. H. HAN

Department of Medical Research, Veterans General Hospital Taipei, and *Section of Allergy, Immunology and Rheumatology, Far Eastern Memorial Hospital, Taipei, Taiwan, Republic of China

A panel of 16 monoclonal antibodies (MoAbs) directed against Bermuda grass (*Cynodon dactylon*) pollen (BGP) were generated for identification and purification of the major allergenic components of the eliciting antigen (Ag). Radioimmunoprecipitation (RIP) analysis revealed that there were at least eight antigenic components with molecular weights (MW) ranging from 12 kilodalton (12 kDa) to 200 kDa. Each of these components has distinct biochemical characteristics based on sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF). Among them, *Cyn d* Bd67K and *Cyn d* Bd58K were basic proteins, *Cyn d* Bd35K consisted of at least four isomeric components with isoelectric points ranging from 6.2 to 7.2. The other antigens (*Cyn d* Bd68K, 48K, 38K, *Cyn d* Bd200K, *Cyn d* Bd46K, *Cyn d* Bd25K and *Cyn d* Bd12K) were all acidic proteins. The IgE binding capacity of all these antigens was determined with sera from 11 BGP-allergies by using a modified radioallergen sorbent test. All but one of the antigens (*Cyn d* Bd200K) were found to react with human IgE from sera of BGP-allergic patients. Among those human IgE-binding molecules, *Cyn d* Bd35K reacted with allergic sera most frequently (10 of 11), followed by *Cyn d* Bd58K (8 of 11) and *Cyn d* Bd46K (7 of 11) respectively. Our results suggest that *Cyn d* Bd35K, *Cyn d* Bd58K, and *Cyn d* Bd46K are major allergens of BGP, and the MoAbs we obtained should be valuable tools for further purification of these allergens.

Keywords: allergenic components; Bermuda grass pollen; monoclonal antibody; radioimmunoprecipitation.

Accepted for publication 22 January 1991

CLINICAL ASPECTS

Three major allergens of BGP (*Cyn d* Bd35K, *Cyn d* Bd58K and *Cyn d* Bd46K) recognized by MoAbs in the present study are very similar to the major allergenic components identified by other studies using immunoblot technique or immunoelectrophoresis analysis. MoAbs generated in the present study should be useful tools for purification of these specific allergenic components from BGP extracts. With large quantities of these purified allergens, its biochemical-physical characteristics could be well established and used for clinical diagnosis as well as immunotherapy. In addition, the present study found that some MoAbs reacting with minor allergen(s) of BGP also cross-reacted with four other grass pollens tested but not the MoAbs against major allergens. This suggests that major allergens of BGP have unique epitopes which are distinct from those of other grass pollens.

Bermuda grass is one of the important allergy-related grasses (2, 22, 25, 28). In Taiwan, about 27% of the asthmatic patients showed

positive immediate skin reaction to the crude extract of BGP (2).

Although major allergens of various grass-pollen Ags, for example, *Lolium perenne*, have been purified and well characterized (1, 5, 7, 8, 10, 13), progress in the study of allergens from BGP has been limited. Some years ago, Orren & Dowdle (25) first reported that BGP-1, with a molecular weight of 30 kDa, is the major allergen of BGP. Later, Karlstam & Nilsson (16) found that allergens of BGP showed a high affinity for Con-A. Recently, the allergenic component of BGP has been examined in detail using immunoblotting technique by Ford & Baldo (11) as well as our group (27). A complex composition was found in BGP extract. There were at least 14 IgE binding components with MW ranging from 8 kDa to 94 kDa. Among these, a 32-34 kDa component showed the highest frequency of IgE binding. Similar results were also obtained by Matthiesen et al. (21) using the crossed radio-immunoelectrophoresis analysis.

Crude extracts of allergens which are still being used clinically for diagnosis and treatment may contain very low concentrations of the true allergenic components. In addition, the composition may vary from lot to lot. Being a practical tool for standardisation and purification, MoAbs have been used to solve this problem and have been applied successfully for the study of pollen allergy (1, 7, 9, 15, 24). To identify and purify the major allergenic components of BGP, anti-BGP MoAbs were generated in the present study. The Ag molecules recognized by these anti-BGP MoAbs were analysed, and the major allergenic components of BGP identified.

MATERIAL AND METHODS

Pollen extracts. Dried defatted pollen of BGP, annual blue grass (*Poa annua*), corn (*Zea mays*), canary grass (*Phalaris minor*) and cane (*Sorghum vulgare* var. *saccharatum*) were obtained from International Biologicals, Inc. (Piedmont, OK, USA). Pollens were extracted with 0.15 M phosphate buffered saline (PBS, pH 8.0) as previously described (27). Lyophilised extracts were stored at 4°C until use.

Sera were collected at the Allergy Clinic, Veterans General Hospital-Taichung (Taichung, Taiwan, ROC) from allergic patients with positive skin

test to BGP. They were further selected on the basis of strong positivity in the radioallergosorbent test (RAST). Sera from individuals with negative skin reactions were used as negative controls.

RAST was performed as described by Ekramoddoullah et al. (8) with some modifications. Briefly, the BGP extract was diluted in 50 mM bicarbonate buffer (pH 9.6) and coated onto wells (1 µg/50 µl) of 96-well polyvinyl plate (Costar, Cambridge, MA, USA) at 4°C overnight. The remaining binding sites were blocked by incubation with PBS containing 5% bovine serum albumin (BSA) at room temperature for at least 30 min. Serum samples (50 µl) from various sources were then added and incubated at 37°C for 4 h. The plate was washed with PBS containing 1% BSA (PBS-BSA), followed by incubation with ¹²⁵I-labelled anti-IgE MoAb (5 × 10⁴ cpm/well) at room temperature for 1 h. The anti-IgE MoAb E17-58 was prepared as previously described (3). The reaction was stopped by washing with PBS-BSA at least five times and the radioactivity in each well was determined by a gamma counter (LKB, 1271 RIAGAM-MA, Turku, Finland).

Iodination. One hundred µg of proteins were labelled with 1 mCi of Na¹²⁵I (Amersham Corp., Arlington Heights, IL, USA) by the chloramine T method (12). Free ¹²⁵I was removed by gel filtration through a Sephadex G 25 column (Pharmacia, Uppsala, Sweden).

Production and characterization of MoAbs

Immunization. Eight- to 12-week-old BALB/c mice were immunized by intraperitoneal injection of 100 µg of the BGP extract emulsified with complete Freund's adjuvant (Difco, Detroit, MI, USA). They were boosted with 50 µg of the BGP extract in incomplete Freund's adjuvant (Difco) three times at 2-week intervals.

Hybridoma technique. Spleen cells from hyperimmunized BALB/c mice were fused with NS-1

myeloma cells as described by Rector et al. (26). The fused cells were suspended in 200 ml RPMI with hypoxanthine-aminopterin-thymidine and 15% fetal calf serum (Flow Laboratories, Svenska AB, Stockholm, Sweden). One hundred μ l aliquots of the cell-suspension were added to 96-well PVC plates and then incubated at 37°C in 5% CO₂ for 7–10 days. Supernatants from each well with growing colonies of hybridoma were then harvested and screened for anti-BGP antibody production by radioimmunoassay (RIA)(3). Hybridomas which secreted MoAb reactive to BGP but not to ragweed pollen were chosen. While a few cells from each positive well were cloned and subcloned, the rest of the cells were transferred to a 48-well plate for further growth. The reaction pattern of the MoAb in culture supernatants against BGP was preliminarily analysed by immunoblot as previously described (27), and further confirmed by RIP and gel electrophoresis as described below. Only the clones which showed different patterns were saved for further study. After two rounds of subcloning, one clone was selected from each mother well to ensure that each clone was from a different mother hybridoma. The MoAbs secreted by these hybridomas were designated by the original plate number-well number when they were first screened.

The ascites was produced by intraperitoneal injection of hybridoma cells, and MoAbs were purified with a protein A column (Pharmacia) (14).

Isotype determination. The isotype of MoAbs was determined using a commercial solid-phase enzyme immunoassay kit (Zymed, San Francisco, CA, USA) as described by the manufacturer.

Cross-reactivity of the anti-BGP MoAbs to four other major grass pollens in Taiwan (canary, cane, corn and annual blue) was tested by RIA. The 96-well polyvinyl plate was coated at 4°C overnight with 1 μ g pollen extract in 50 mM bicarbonate buffer (pH 9.6). It was then saturated with 5% BSA and followed by the addition of 50 μ l supernatant of the hybridoma or NS-1 cells. After incubation at 37°C for 1 h

and washing 4 times with PBS-BSA, the bound antibody was measured by incubation with ¹²⁵I-labelled rabbit-anti-mouse Ig(G + M) (5×10^4 cpm/well) (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA).

Characterization of antigenic components recognized by MoAbs

RIP. Immunoprecipitation was performed by the method of Chorney et al. (4) with some modifications. Briefly, the rabbit-anti-mouse Ig (G + M) antibody was coupled to Sepharose 4B beads (Pharmacia) according to the description of the manufacturer. Ten μ l of ascites fluid was then added to 100 μ l of rabbit-anti-mouse Ig (G + M)-Sepharose 4B bead suspension, and the mixture shaken for 30 min at room temperature. The antibody-bead complex was then washed three times with 15 ml aliquots of ice-cold PBS containing 0.5% nonidet P-40 (Sigma). ¹²⁵I-labelled BGP (5×10^6 cpm) was then added to the bead pellet, and the mixture was again shaken for 30 min at room temperature. Reaction was stopped by washing the bound immune complexes four times with PBS containing 0.5% nonidet P-40, and once with a low-salt detergent buffer (2 mM Tris, 0.5% nonidet P-40, pH 7.4). After the final wash, the beads were centrifuged through a cushion of low-salt buffer containing 20% sucrose, followed by extraction with 100 μ l Laemmli (17) or O'Farrell (23) sample buffer at 95°C for 3 min.

Gel electrophoresis. SDS-PAGE was performed according to the method of Laemmli (17) and IEF by the method of O'Farrell (23) with some modifications. The IEF gel (4.75% acrylamide, 8 M urea and 2% pharmalyte, pH 3–10) was pre-electrophoresed for 350 volt-h, and the radioimmunoprecipitates were isoelectrically focused for 3700 volt-h. After focusing, a slice of the control IEF gel was cut into 0.5 cm pieces and immersed in degassed distilled water. After extraction at room temperature for 1 h on a shaker, the pH of each extract was determined.

For two-dimensional (2D) gel electrophoresis, the IEF gel was equilibrated in a buffer contain-

Table 1.
Characteristics of monoclonal antibodies against Bermuda grass pollen

Category	Monoclonal antibody	Immunoglobulin		Cross-reaction with other grass pollens*
		class	type	
I	9-13	IgG ₁	lambda	yes
	15-15	IgG ₁	lambda	yes
	26-11	IgG _{2b}	lambda	yes
	3-12	IgG ₁	lambda	yes
	16-3	IgG ₁	lambda	yes
II	14-8	IgM	kappa	no
III	5-10	IgG _{2a}	kappa	no
IV	1-52	IgG _{2a}	kappa	no
V	17-31	IgG _{2a}	kappa	no
	14-16	IgG ₁	kappa	no
VI	1-61	IgG ₁	kappa	no
	4-37	IgG ₁	kappa	no
	10-7	IgG ₁	kappa	no
	11-7	IgG ₁	kappa	no
VII	20-4	IgG ₁	kappa	no
VIII	4-45	IgG ₁	kappa	no

*Cross-reaction with four other grass pollens (canary, cane, corn and annual blue) was tested by radioimmunoassay.

ing 125 mM Tris, 10% (v/v) glycerol, 4% (w/v) SDS, 10 mM dithiothreitol for 1 h at room temperature, and then subjected to electrophoresis on 10% gel for 1000 volt-h. Low MW standards (Bio-Rad, Richmond, CA USA) included rabbit muscle phosphorylase b (97kDa), bovine serum albumin (66kDa), ovalbumin (43kDa), carbonic anhydrase (31kDa), soybean trypsin inhibitor (21kDa), and hen egg white lysozyme (14kDa). Following electrophoresis, the gels were stained with Coomassie blue R-250, fixed, dried and exposed to the X-ray film (Kodak XAR-5, Kodak, Rochester, NY, USA) at -70°C. In the meantime, the protein profile of BGP extract in 2D gel electrophoresis was demonstrated by a silver stain kit (Bio-Rad) according to the manufacturer's instruction.

Modified RAST. The allergenicity of the antigenic components reactive to the MoAbs was evaluated based on their IgE binding capacity by a modified RAST which was simplified from

the four-step sandwich RIA of Ley et al. (18). In brief, antigens were coated indirectly onto polyvinyl plates by interaction with specific MoAbs. Fifty µl aliquots of the pre-determined optimal concentration of MoAbs was coated to each well and 50 µl aliquots of BGP (100 µg/ml) was then added and incubated at 37°C for 1 h. After washing, sera (50 µl) from BGP-allergic patients or from normal persons was added and further incubated at 37°C for 4 h. At the end of incubation, the plate was washed again and reacted with ¹²⁵I-labelled anti-human IgE MoAbs (5 × 10⁴ cpm/well) for 1 h and radioactivity in each well was determined.

RESULTS

Sixteen MoAbs reactive to BGP were selected from two separate cell fusions. They are grouped into eight categories (Table 1) according to the reaction pattern with Ags they recognized. In the first fusion, more than 50 hy-

bridomas were generated and 10 were randomly analysed. It was found that seven hybridomas secreted MoAbs belonging to Category I MoAbs, and three were kept for further study. In the second fusion, even though the mice were boosted with Category I MoAb-absorbed BGP, two of the 10 MoAbs analysed still belonged to Category I.

The five MoAbs in Category I formed multiple bands with BGP in RIP and cross-reacted with the four other grass pollens (canary, cane, corn and annual blue) in RIA (Table 2). An unexpected finding was that their light chains were all of the rare lambda type. MoAbs of the seven other categories (Categories II-VIII), all of kappa type, were specific for BGP.

The MW and pI of these MoAb recognized components are summarized in Table 3. Designation of the antigens was according to the nomenclature system recommended by the International Union of Immunological Societies (19). By performing RIP assay, Fig. 1 b-h illustrates the representative 2D autoradiograph patterns of these antigenic components. Crude extract of BGP was also included for comparison (Fig. 1a). As shown, *Cyn d* Bd67K (Fig. 1 d) and *Cyn d* Bd58K (Fig. 1e) were basic proteins. *Cyn d* Bd35K (Fig. 1g) consisted of at least four isomeric proteins with pI ranging from 6.2 to 7.2. *Cyn d* Bd68K, 48K, 38K (Fig. 1 b) and *Cyn d* Bd200K (Fig. 1 c), *Cyn d* Bd46K (Fig. 1 f), *Cyn d* Bd25K (Fig. 1

h) and *Cyn d* Bd12K (Fig. 1 i) were all acidic proteins.

Table 4 shows the IgE binding capacity of each antigenic component of BGP using a modified RAST. Sera from 11 BGP-allergic patients were tested, but none reacted with *Cyn d* Bd200K. Among the other antigenic components, three (*Cyn d* Bd35K, *Cyn d* Bd58K and *Cyn d* Bd46K) were found to react with IgE antibodies in more than 50% of the sera from BGP-allergic patients. Among these three, *Cyn d* Bd35K had the highest binding capacity as well as reaction frequency (10 of 11), followed by *Cyn d* Bd58K (8 of 11) and *Cyn d* Bd46K (7 of 11). Others had less IgE binding capacity and their reaction frequency was < 50%. In comparison, five normal sera tested showed only negligible bindings.

DISCUSSION

Previous investigations using polyclonal antibodies to examine the antigenic specificity of BGP have found that BGP is unique in that it does not share common antigens with other grass pollens (20, 21). However, in the present study, in addition to specific anti-BGP MoAbs, we have also found that MoAbs in Category I not only recognized BGP but also cross-reacted with pollens from four different grasses. This cross-reactive epitope is only on a minor allergen of BGP (Table 4). Moreover,

Table 2.

Analysis of cross-reaction between Category I MoAbs and other grass pollens by radioimmunoassay

Monoclonal antibody	Binding of ¹²⁵ I-goat-anti-mouse IgG (cpm) to MoAbs reacting with extracts of pollen of:					
	Canary	Cane	Corn	Annual blue	BGP	Ragweed
Category I						
9-13	4898	3746	4422	4074	4280	78
15-15	4471	2988	3129	3010	4647	63
26-11	5818	4586	4234	4507	3771	94
3-12	2626	2391	2348	2252	3764	77
16-3	1006	1157	758	683	1243	70
Category VI						
4-37	63	55	54	25	3764	47

The cross-reactions between Category I anti-BGP MoAbs and other grass pollens were tested by RIA. Extracts of different pollens were coated to wells of 96-well polyvinyl plate at a concentration of 1 µg/well. MoAbs were added to wells and incubated at 37°C for 1 h. The banded MoAbs were then incubated with ¹²⁵I-goat-anti-mouse IgG (5 × 10⁴ cpm/well). Ragweed was used as a negative pollen control and MoAb 4-37 as a MoAb control. Data are the average of 3 replicates from a representative experiment.

Table 3.

Antigenic components of Bermuda grass pollen recognized by anti-BGP monoclonal antibodies

Monoclonal antibody	Antigen recognized	Molecular weight (kDa)	Isoelectric point
I	<i>Cyn d</i> Bd68K, 48K, 38K	68	5.4
		48	5.4
		38	5.5
		200	5.5
II	<i>Cyn d</i> Bd200K	67	≥ 8.0
III	<i>Cyn d</i> Bd67K	58	≥ 8.0
IV	<i>Cyn d</i> Bd58K	46	5.4
V	<i>Cyn d</i> Bd46K	35	6.2 - 7.2
VI	<i>Cyn d</i> Bd35K	25	5.5
VII	<i>Cyn d</i> Bd25K	12	5.5
VIII	<i>Cyn d</i> Bd12K		

Molecular weight and isoelectric point were determined by two-dimensional gel electrophoresis of radioimmunoprecipitates.

Kahn & Marsh (16) recently reported that anti-*Lol p* I MoAb cross-reacted with BGP to a certain degree. Our results confirm this finding and, further, indicate that an epitope or epitopes of BGP are shared by different grass pollens.

Another interesting point is that light chains of the five MoAbs in Category I were of lambda type, which is rare. In BALB/c mice, the ratio of kappa type immunoglobulins to lambda type immunoglobulins is about 20:1. Only four antigens have been reported to induce antibodies

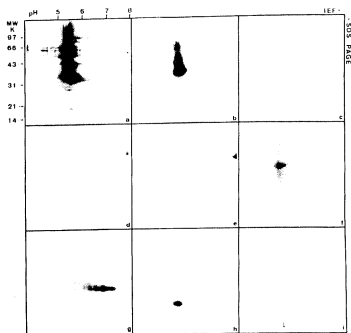


Fig. 1. Two-dimensional gel analysis of antigenic components of Bermuda grass pollen. a) crude extract; b) *Cyn d* Bd68K, 48K, 38K recognized by Category I MoAb 9-13; c) *Cyn d* Bd200K recognized by Category II MoAb 14-8; d) *Cyn d* Bd67K recognized by Category III MoAb 5-10; e) *Cyn d* Bd58K recognized by Category IV MoAb 1-52; f) *Cyn d* Bd46K recognized by Category V MoAb 14-16; g) *Cyn d* Bd35K recognized by Category VI MoAb 4-37; h) *Cyn d* Bd25K recognized by Category VII MoAb 20-4; i) *Cyn d* Bd12K recognized by Category VIII MoAb 4-45.

Table 4.
The IgE binding capacity of antigenic components of Bermuda grass pollen
recognized by monoclonal antibodies

Tested sera	<i>Cyn d</i> Bd68K, 48K, 38K	<i>Cyn d</i> Bd200K	<i>Cyn d</i> Bd67K	<i>Cyn d</i> Bd58K	<i>Cyn d</i> Bd46K	<i>Cyn d</i> Bd35K	<i>Cyn d</i> Bd25K	<i>Cyn d</i> Bd12K
Allergic								
a	602	181	682	1693	860	2306	330	325
b	104	78	37	730	179	164	87	56
c	810	124	407	654	765	822	168	161
d	163	22	158	744	333	728	132	102
e	111	87	126	105	158	399	60	94
f	332	94	629	632	610	768	677	215
g	148	67	166	302	358	412	105	116
h	134	83	396	161	126	486	380	81
i	382	72	465	194	1165	1680	301	117
j	160	56	184	337	166	372	134	86
k	136	46	171	375	693	581	119	74
Normal								
a	54	108	112	114	138	120	135	117
b	18	32	19	60	32	84	68	11
c	106	50	52	94	50	86	72	72
d	42	72	64	65	72	60	98	79
e	52	14	30	40	4	90	104	69

*IgE binding capacity (cpm) determined by modified radioallergosorbent test. Data are the mean of duplicate determinations from a representative experiment.

that contain a high proportion of lambda chains, namely, the 4-hydroxy-3-nitrophenyl-acetyl (NP) group, dextrans having an alternating (1-3), (1-6) backbone, the 2,4-dinitrophenyl (DNP) group and the 5-dimethylaminoaphthalene-1-sulfonyl (dansyl) group (6). The reason why all Category I MoAbs were the rare lambda type is not clear at present.

The composition of BGP is complex. A large number of antigenic components with different MW have been identified by immunoblotting (11, 27). In the modified RAST, antigenic components recognized by seven of the eight categories of MoAbs described reacted with IgE antibodies in some allergic sera. These antigens are presumably allergenic. In this study, a total of nine allergens with different MW (12 - 68 kDa) were identified with anti-BGP MoAbs. The MW of these allergenic components being recognized were all < 70 kDa, this is in agreement with the results of other reports (11, 27).

Among the three allergenic components which reacted with IgE antibodies in more than 50% of the sera tested, *Cyn d* Bd35K showed the strongest reaction and the highest binding

frequency in the modified RAST. In an earlier report (27), we found that a 32 kDa component of BGP was the major allergen which reacted with IgE antibodies in sera from 75% BGP-allergic patients in an immunoblotting study. Similar findings have also been observed by other investigators. Orren & Dowdle (25) isolated a 30 kDa allergen from BGP that elicited positive skin test in 100% of BGP-allergic patients. Ford & Baldo (11) reported a 34 kDa allergen reactive with IgE in all BGP-allergic patients in immunoblotting analysis. Matthiesen et al. (21) also found that IgE in sera from 97% BGP-allergic patients reacted with a 32 kDa allergen in BGP. The size of the major allergen of BGP reported by different laboratories is similar to *Cyn d* Bd35K described in this report. In spite of this, controversy in the pI of the major allergen remains to be solved. The pI of BGP-1 from Orren & Dowdle (25) was 5.4. In contrast, Matthiesen et al. (21) reported that pI of the major allergenic component of BGP was distributed from 6.0 to 6.8. Using RIP, the present study demonstrated clearly that *Cyn d* Bd35K had at least four isomeric components,

and the pIs ranged from 6.2 to 7.2. This corresponds well with the result of Matthiessen et al. Moreover, it seems that the major allergen identified in this study is the same as that found by other methods, such as immunoblot, crossed radioimmuno-electrophoresis and modified RAST.

Using crossed radioimmuno-electrophoresis, Matthiessen found that the 32 kDa major BGP allergen has precipitates and IgE-binding characteristics similar to many major grass allergens (21). In this study, all four Category VI MoAbs which reacted with *Cyn d* Bd35K neither cross-reacted with the four grass pollens tested nor with ryegrass pollen (data not shown). This suggests that the epitope on *Cyn d* Bd35K is unique and absent in the grass pollens tested.

Cyn d Bd58K and *Cyn d* Bd46K, reacting strongly and frequently with IgE antibodies, should also be important allergens. The former may be the same as a 60 kDa allergen found in immunoblot analysis by Ford & Baldo (11) and our group (27) which reacted with 43–66% of the sera tested. The latter reacted with IgE in 33% of BGP-allergic sera in our previous report (27). Ford & Baldo (11) also found a 46 kDa allergenic component in BGP which reacted with IgE antibodies in the sera derived from 50% BGP-allergic patients.

Due to technical difficulties, most of the allergens of BGP have not been purified nor well-characterized. With anti-BGP MoAbs available, we hope that this problem will soon be solved. In addition, the four anti-BGP MoAbs in Category VI, which are against the major allergen *Cyn d* Bd35K, will be useful tools for studies of epitope specificity and IgE binding capacity of the epitope(s) on the major allergen of BGP.

ACKNOWLEDGEMENTS

The authors thank Dr. C. K. Ho of Department of Medical Research, Veterans General Hospital-Taipei for his comments on the manuscript. This work was supported by grant NSC 79-0418-B075-06 from the National Science Council, Republic of China.

REFERENCES

1. Bose R, Rector ES, Fischer J, Taronno R, Delespesse G. Production and characterization of mouse monoclonal antibodies to allergenic epitopes on *Lol p I* (Rye I). *Immunology* 59, 309–315, 1986.
2. Chang HN, Lin FM, Chang YF. Correlation of skin test to RAST, and Prist in asthma patients. *Clin Med J* 34, 376–383, 1984.
3. Chang ZN, Chang LD, Wang MC, et al. Monoclonal antibodies specific for human IgE and their clinical applications. *Proc Natl Sci Comm B.R.O.C.* 12, 140–145, 1988.
4. Chorney MJ, Tung JS, Bushkin Y, Shen FW. Structural characteristics of Tla products. *J Exp Med* 162, 781–789, 1985.
5. Cottam GP, Moran DM, Standring R. Physicochemical and immunochemical characterization of allergenic proteins from ryegrass (*Lolium perenne*) pollen prepared by a rapid and efficient purification method. *Biochem J* 223, 305–310, 1986.
6. Eisen HM, Reilly EB. Lambda chains and genes in inbred mice. *Ann Rev Immunol* 3, 337–365, 1985.
7. Ekmamoddoullah AKM, Kisil FT, Bundesen PG, Fisher JM, Rector ES, Schon AH. Determination of ryegrass pollen cytochrome c recognized by human IgE and murine monoclonal antibodies. *Mol Immunol* 21, 375–382, 1984.
8. Ekmamoddoullah AKM, Kisil FT, Schon AH. Partial characterization of an antigenic site of high molecular weight basic antigen, a ryegrass pollen allergen, using a monoclonal antibody. *Mol Immunol* 23, 111–117, 1986.
9. Ekmamoddoullah AKM, Kisil FT, Schon AH. Isolation of a Kentucky bluegrass pollen allergen using a murine monoclonal antibody immunosorbent. *Int Arch Allergy Appl Immunol* 80, 100–106, 1986.
10. Ford SA, Baldo BA. A reexamination of ryegrass (*Lolium perenne*) - pollen allergens. *Int Arch Allergy Appl Immunol* 81, 193–203, 1986.
11. Ford SA, Baldo BA. Identification of Bermuda grass (*Cynodon dactylon*) - pollen allergens by electroblotting. *J Allergy Clin Immunol* 79, 714–720, 1987.
12. Greenwood FC, Hunter MW, Glover JS. The preparation of ^{125} I labelled human growth hormone. *Biochem J* 89, 114–122, 1963.
13. Howlett BJ, Charke AE. Isolation and partial characterization of two antigenic glycoproteins from ryegrass (*Lolium perenne*) pollen. *Biochem J* 197, 695–706, 1981.
14. Hudson H, Hay FC. Isolation of IgG subclasses using protein A - sepharose; In Hudson H, Hay FC (eds): *Practical immunology*, pp. 223–224. Blackwell, Oxford, 1980.
15. Kadan CR, Marsh DG. Monoclonal antibodies to the major *Lolium perenne* (Rye grass) pollen allergen *Lol p I* (Rye I). *Mol Immunol* 23, 1314–1318, 1986.
16. Karlstam B, Nilsson B. Characterization of grass pollen allergens by affinity chromatography on Concanavalin A - Sepharose. *J Immunol Methods* 54, 119–128, 1982.

17. Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 22, 680-685, 1970.
18. Ley V, Corbi AL, Sanchez-Madrid F, Garreira JC. A four-step sandwich radioimmunoassay for direct selection of monoclonal antibodies to allergen molecules. *J Immunol Methods* 84, 265-270, 1985.
19. Marsh DG, Gooderlend L, King TP, Lowenstein H, Platts-Mills TAE. Allergen nomenclature. *Allergy* 43, 161-168, 1988.
20. Marsh DG, Haddad ZH, Campbell DH. A new method for determining the distribution of allergenic fractions in biological materials: its application to grass pollen extracts. *J Allergy* 46, 107-121, 1970.
21. Matthieson F, Schumacher MJ, Lowenstein H. An immunoelectrophoretic analysis of the allergens of *Cynodon dactylon* (Bermuda grass) pollen. *J Allergy Clin Immunol* 83, 1124-1134, 1989.
22. Meyers RL, Berns-mason AW, Thayer KH, Feldman BH, Rosengren HN. Isolation and characterization of the dialysate from Bermuda grass pollen. *J Allergy* 47, 100-101, 1971.
23. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250, 4007-4021, 1975.
24. Olson JR, Klapper DG. Two major human allergenic sites of ragweed pollen allergen antigen E identified by using monoclonal antibodies. *J Immunol* 136, 2109-2115, 1986.
25. Orren A, Dowdle EB. Studies on Bermuda grass pollen allergens. *S Afr Med J* 51, 586-591, 1977.
26. Rector E, Nakajima T, Rocha C, et al. Detection and characterization of monoclonal antibodies specific to IgE receptors on human lymphocytes by flow cytometry. *Immunology* 55, 481-488, 1985.
27. Shen HD, Wang SR, Tang RB, Chang ZN, Su SN, Han SH. Identification of allergens and antigens of Bermuda grass (*Cynodon dactylon*) pollen by immunoblot analysis. *Clin Allergy* 18, 401-409, 1988.
28. Trinca JC. Pollens used in the treatment of hay fever and asthma in eastern Australia. *Med J Aust* 49, 969-972, 1962.

Address:

Dr. Shou-hua Han

Department of Medical Research

Veterans General Hospital - Taipei

Taipei, Taiwan 11217 (Republic of China)

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.