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Host Genetic and Adjuvant Factors Influence Epitope Specificity to a Major Recombinant Grass Allergen

Abstract

The role of host genetic and adjuvant factors in the induction of immune responses to a major recombinant Kentucky bluegrass allergen was examined utilizing five strains of mice and two different adjuvants. Analysis of the recombinant allergen-specific antibodies induced in these strains indicated that the antibodies of various isotypes were differentially regulated. In terms of IgE antibody response, BDF1 and DBA/2 were characterized as high responder, whereas BALB/C, CBA/J and C57BL/6 were intermediate and SJL was a low responder. In different strains, both dextran sulfate (DS) and complete Freund's adjuvant (CFA), as adjuvants, induced recombinant allergen-specific IgE antibodies of similar titer, however, CFA induced higher IgG2a and lower IgM antibodies compared to DS. Further, analysis of T cell proliferative responses of the splenocytes of different strains demonstrated that these strains varied also in their capacity to respond to synthetic peptides. Furthermore, utilizing a panel of synthetic peptides corresponding to the recombinant allergen, we demonstrated that the antibodies induced by the recombinant allergen with CFA in different strains vary with respect to their epitope specificity. In the BDF1 strain, compared to DS, CFA as adjuvant induced recombinant allergen-specific antibodies of additional peptide specificity. Taken together, these results suggest that both host genetic background and adjuvants govern the fine specificity of antibodies produced against this recombinant Kentucky bluegrass allergen.

Key Words

Adjuvants
IgE antibody response
Mouse strains
Host genetic factor

Introduction

Allergic immune response in humans results from the interplay between the host genetic factors and a number of environmental factors. The host factors include genes governing both (i) the specific responsiveness to allergens such as the genes of the HLA complex and of the TCR, which are involved in antigen recognition [1, 2] and (ii) the total serum IgE antibody responses [3]. The environmental factors include the exposure to allergens, smoking, nutritional fac-

tors, which may directly or indirectly increase the propensity of the individual to develop an allergic condition [4].

Similar to that of the humans, the IgE antibody response has been reported to vary with strains of mice [5] and rats [6]. Although the general immune responsiveness is controlled by the MHC genes, IgE-isotype-specific immune responsiveness appears to be controlled by non-MHC genes as well [7]. A large body of evidence has indicated that the nature of the priming antigen (e.g. native vs. denatured), and the genetic makeup of the animal host (H-2 haplotype)

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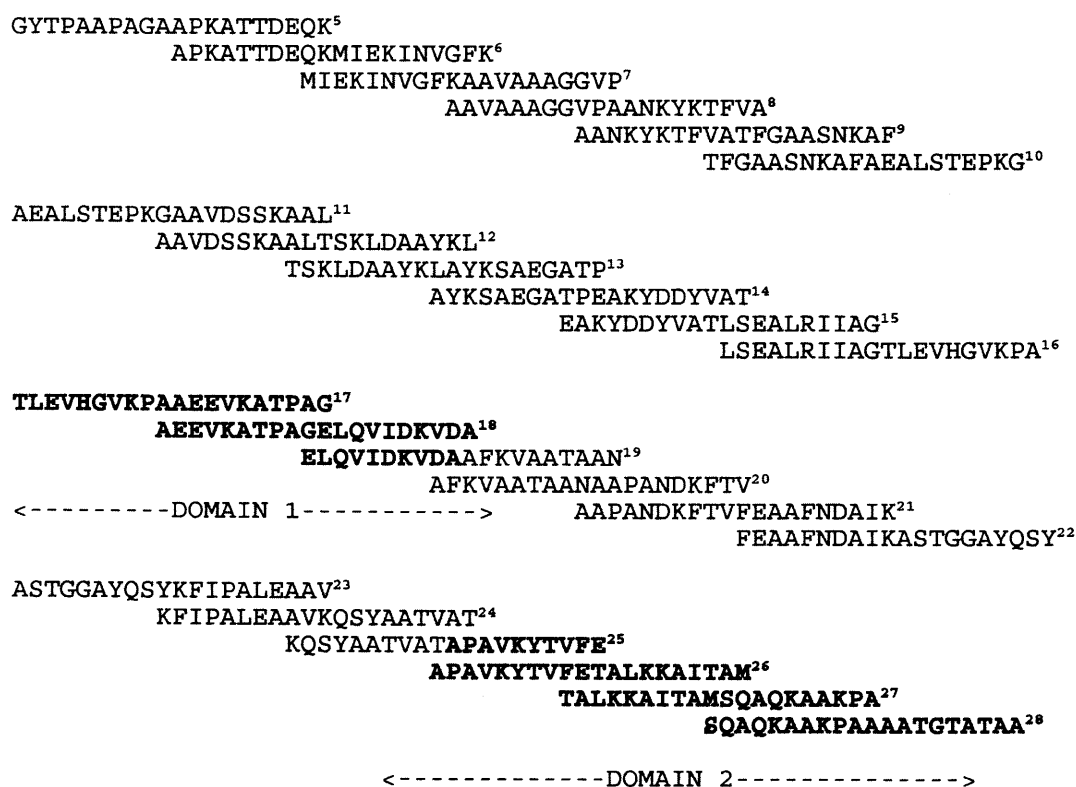


Fig. 1. Schematic representation of the amino acid sequence of the 20-residue synthetic peptides encompassing rKBG60. Peptides with residues in bold letters indicate the peptides or segments thereof which comprise the two major antigenic domains of the recombinant allergens, rKBG60 and rKBG8.3.

can affect the epitope specificity of the immune response [8, 9]. Moreover, the adjuvants are known to influence isotype specificity of immune response [10, 11]. However, whether adjuvant can influence the antibody specificity has received little attention. The latter constitutes an important consideration in the design of synthetic and recombinant vaccines.

We have previously reported molecular cloning and sequencing of a group of major allergens from Kentucky bluegrass (KBG) (*Poa pratensis*) pollen [12–15], *Poa p* IX (referred to as *Poa p* 5 according to the new nomenclature), which constitutes one of the major environmental aeroallergens worldwide. Furthermore, the antibody binding sites of the *Poa p* 5 recombinant allergens, rKBG60, and the conserved C-terminal fragment encoded by cDNA, KBG8.3 [16], were defined using overlapping cDNA clones [15], peptides synthesized on polypropylin pins [17] and synthetic peptides with the aid of murine antisera to rKBG60 [18]. Moreover, our previous studies with the recombinant β -galactosidase-KBG8.3 chimeric protein indicated that adjuvants played a significant role in modulating the develop-

ment of specific immune response to the recombinant KBG allergen [19]. In an effort to gain further insight into the effect of host genetic and adjuvant factors in shaping the immune response to the recombinant *Poa p* 5 allergen, in this study we have examined fine specificity of antibodies induced by rKBG8.3 in six different strains and with two different adjuvants utilizing overlapping peptides encompassing this allergen. Our results demonstrate that host genetic background and adjuvant factors significantly influence not only the isotype specificity but also fine specificity of antibodies elicited to the recombinant KBG allergen.

Materials and Methods

Mice Strains. Female, 6- to 8-weekold BALB/C, CBA/J, C57BL/6, DBA/2 and SJL mice were purchased from Jackson Laboratories, Bar Harbor, Mich., USA. Female, BDF1 mice (6–8 weeks old) were purchased from the Central Animal Care Facility, University of Manitoba.

Synthetic Peptides. The sequences of overlapping pentadecapeptides encompassing rKBG60 (amino acid position 30–280) that are

used in this study are shown in figure 1. Peptides 12–28 correspond to rKBG8.3. The peptides were synthesized using an automated peptide synthesizer (model ABI 430) essentially based on the methods described by Merrifield [20]. After cleavage, the peptides were purified with reverse-phase HPLC and stored as lyophilized powder at -20°C .

Expression and Purification of rKBG8.3. The recombinant antigen rKBG8.3, which comprised the C-terminal part of KBG60, was used in this study and was cloned as described [21]. Briefly, overnight cultures were diluted 1 in 10 into 1 liter of L-broth containing 50 $\mu\text{g}/\text{ml}$ of ampicillin, incubated at 37°C for 1–4 h and then isopropyl thiogalactopyranoside was added at 0.5 mM to induce high-level expression of the fusion protein. After 4 h of incubation, cells were pelleted, resuspended in 20 ml of buffer containing 20 mM phosphate, pH 7.3, and 1% Triton X-100, and frozen at -70°C . The cells were quick-thawed at 37°C , sonicated and the lysate was cleared by centrifugation at 18,000 g for 20 min at 4°C . Supernatant was mixed with swollen and washed glutathione agarose beads (Sulphur Linkage, Sigma, St. Louis, Mo., USA) in the ratio 10:1 (v/v) to allow specific binding of the GST moiety of the fusion proteins to the glutathione on the beads. After incubation on a nutator for 30 min at 4°C , the beads were subsequently washed by 5×15 ml of phosphate buffer and 1×15 ml of 50 mM Tris-HCl, pH 8.0. The recombinant polypeptide was cleaved by thrombin from its GST fusion partner while the latter was still bound to the gel. The washed beads were suspended in 50 mM Tris-HCl, pH 8.0, containing 150 mM NaCl and 125 mM CaCl_2 , and subsequently thrombin (1:500; w/w) was added at room temperature. The cleaved protein was obtained in the supernatant after centrifugation of the beads. Protein concentration of the product was measured by the Lowry method and by dot blotting diluted samples onto nitrocellulose membrane (Schleicher and Schull, 0.45 μm), followed by staining with 0.1% amido black in isopropanol/acetic acid (25%/10%). Bovine serum albumin, diluted in the same solvent, served as the protein standard. Eluted materials were aliquoted and stored at -20°C .

SDS-PAGE and Silver Staining. SDS-PAGE was performed in a discontinuous system according to Laemmli [22] in a Bio-Rad mini slab gel apparatus. The rKBG 8.3 and thrombin were prepared in sample buffer containing 0.06 M Tris-HCl, pH 6.8; 2% SDS and 5% 2-mercaptoethanol and boiled for 5 min. The gel was stained with highly sensitive photochemical silver staining technique [23]. Low-molecular-weight prestained protein standards (Life Technologies) were used to calibrate the gels for the determination of the relative molecular weights (MWs) of the electrophoresed components.

Immunization of Mice. For all strains, mice were divided into two groups of 4 each. In group 1, mice were immunized with 5 μg of either cleaved rKBG8.3 or GST-rKBG8.3 fusion protein in 0.2 ml of 2.5 mg dextran sulfate (DS; Sigma) dissolved in PBS on day 1, and then boosted with the same dose of rKBG8.3 in 0.2 ml of 1 mg DS on day 22 subcutaneously. In group 2, mice were injected with 5 μg of rKBG8.3 protein emulsified in 0.1 ml of complete Freund's adjuvant (CFA) on day 1 and subsequently boosted subcutaneously with antigen and incomplete Freund's adjuvant on day 22. The mice were bled on days 8, 15, 29 and 35 individually and their sera were stored at -20°C . The immunization experiments were repeated at least twice. Antibodies to β -galactosidase-KBG8.3 was produced in BDF1 as described [14, 19, 24].

Detection of Specific Antibodies. The specificity of antibodies induced by the rKBG8.3 in mice were examined by ELISA. Microtiter plates were coated either with 0.1 μg per well of the rKBG8.3 or thrombin, or with 10 μg per well of the synthetic peptide, in 100 μl carbonate and bicarbonate buffer, pH 9.6, overnight at 4°C . The plates were then

washed three times with PBS containing 0.5% of Tween-20, blocked with 3% of BSA in PBS, washed and incubated with twofold serially diluted sera overnight at 4°C . Following three washes the plates were incubated with biotinylated goat anti-mouse total immunoglobulin (mixture of IgG + IgA + IgM) antibodies (Zymed, Mississauga, Ont., Canada).

To determine the isotype specificity of antibodies to rKBG8.3 the plates were incubated for 2 h with the conjugates of biotinylated goat anti-mouse IgG1 or IgG2a or IgM antibodies. The plates were then washed and incubated with alkaline phosphatase-streptavidin conjugate (Zymed). Finally, the plates were washed and the enzyme substrate [1 mg/ml of *p*-nitrophenyl phosphate in 1 mM diethanolamine (Sigma) and 0.5 mM MgCl_2 , pH 9.8] was added to the plates. The reaction was terminated 30 min later by the addition of 3 M NaOH solution and the optical density (OD) of each well was determined with an ELISA reader (Dynatech MR 600) at 405 nm. The antibody titer was defined as the reciprocal of the highest sample dilution giving an OD of 0.4.

Passive Cutaneous Anaphylaxis (PCA). Anti-rKBG8.3 IgE antibody titer was determined by the PCA assay in random bred hooded rats as described [24]. Volumes of 0.05 ml of two-fold serially diluted serum were injected intradermally onto the backs of the rats. Male rats were challenged 24 h later by an intravenous injection (penal vein) of 1 mg of rKBG8.3 in 1 ml of PBS containing 0.5% Evans blue. The IgE (PCA) titer of each serum was expressed as the reciprocal of the highest dilution of the serum, giving a wheal-and-flare reaction (blue spot) of at least 5 mm in diameter. Each serum was assayed in 2 rats.

Lymphocyte Proliferation Assay. The spleens were removed 7 days after secondary immunization from different strains of mice that had been primed with rKBG8.3 in CFA. Proliferation of spleen cells in the presence of peptide was carried out using the procedures as described [14]. Briefly, a single cell suspension of spleen was prepared in RPMI 1640 medium. The viability of the cells was checked by their ability to exclude trypan blue. The cells were finally suspended at a concentration of $5 \times 10^6/\text{ml}$ in freshly made culture medium (10% FCS, 5×10^{-4} mM of 2-mercaptoethanol, 2 mM of *L*-glutamine, 1 mM of sodium pyruvate, 100 U penicillin, and 50 μg of streptomycin), 100 μl of this cell suspension (1×10^6 cells) were then pipetted into each of 96 flat-bottomed wells of tissue culture plates (Flow Laboratories, Mississauga, Ont., Canada). The stock solution of various peptides, rKBG8.3 and KBG pollen proteins were diluted to 25–100 $\mu\text{g}/\text{ml}$ with the culture media. The cells were cultured in triplicates at 37°C with 5% CO_2 for 5 days. The extent of lymphocyte proliferation was measured by the degree of incorporation of [^3H]-thymidine [37 MBq/ml] (ICN Biomedical, Irvine, Calif., USA) added at 1 μCi per well during the last 12 h of culture and was expressed as counts per minute. Proliferation assays were repeated thrice.

Results

The rKBG8.3 used in our study was purified from the GST fusion partner using thrombin. In order to eliminate the possibility of cross-contamination of recombinant protein with thrombin, we analyzed the cleaved rKBG8.3 by SDS-PAGE followed by silver staining. Results shown in figure 2 indicated that the fusion protein was resolved to near homogeneity. A major protein band of 20 kD corresponded to

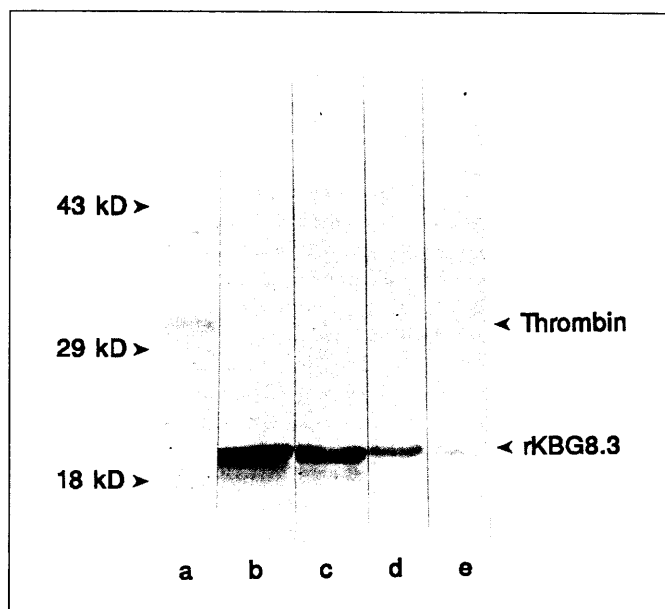


Fig. 2. SDS-PAGE and silver stain analysis of thrombin-cleaved products of rKBG8.3 (MW 20 kD). Lane a, 20 ng thrombin (MW 30 kD), lanes b–e represent serial dilutions of thrombin-cleaved rKBG8.3 (1, 0.25, 0.0625 and 0.0156 µg), respectively. The arrow heads in the left margin indicate MW standards.

rKBG8.3; no extra band corresponding to thrombin (30 kD) was seen. Further, in order to exclude the possibility that rKBG8.3-specific antibodies raised in mice contain antibodies against thrombin, we examined reactivities of rKBG8.3 and thrombin to rKBG-8.3-specific antibody by ELISA. The antibody raised against β -galactosidase-KBG8.3 fusion protein [14, 19, 24] and antibodies against cleaved rKBG8.3 bound specifically to purified rKBG-8.3, but not to thrombin (fig. 3). Moreover, the binding of cleaved rKBG-8.3 was specific and dependent on the dose of rKBG8.3 (data not shown). These results demonstrated that thrombin in the purified rKBG8.3 preparation was non-immunogenic and had little effect on rKBG-8.3-specific antibody production.

To examine the effect host background on antibody production to the recombinant protein we immunized different strains of mice with the rKBG8.3 in the presence of either CFA or DS as adjuvant. The titers of antibodies of different isotypes in the secondary bleeding are shown in table 1. The total rKBG8.3-specific antibody titers and the titers of rKBG8.3-specific IgG1 antibodies, which was the predominant isotype in the secondary response, did not differ among the strains examined. However, the titers of IgG2a antibodies varied among strains and was also influenced by the adjuvant used for immunization. All strains except

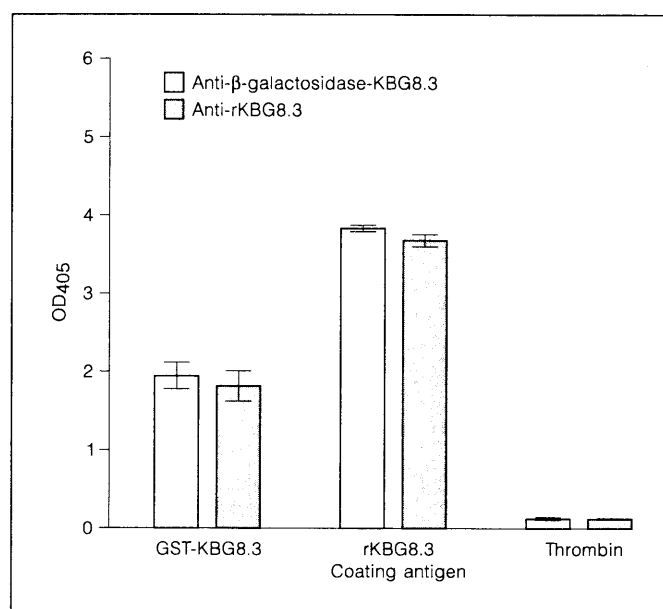


Fig. 3. Antibody reactivity against rKBG8.3 and thrombin was tested by ELISA. Uncleaved GST-rKBG8.3, thrombin-cleaved rKBG8.3 (rKBG8.3) and thrombin were used as coating antigen. The values represent means $\pm$ SEM of an ELISA performed in duplicates.

BDF1 exhibited a higher titer of IgG2a antibodies when immunized with CFA compared to DS. In contrast, mice immunized with CFA as adjuvant had reduced titers of IgM antibodies as opposed to those immunized with DS as adjuvant. Moreover, as shown in table 1, all strains except SJL were able to induce IgE antibody production. However, the titers varied widely among strains; BDF1 and DBA/2 induced the highest IgE titer, whereas CBA/J, C57BL/6 and BALB/C exhibited an intermediate titer and SJL induced no IgE titer. Little difference was seen between the adjuvants in terms of IgE antibody production. These results suggest that BDF1 and DBA/2 are high IgE responders, whereas CBA/J, C57BL/6 and BALB/C are intermediate and SJL is low responder for this allergen.

To elucidate the epitope specificity of the antibodies produced in different strains, the sera of mice following secondary immunization were examined for their binding with various overlapping peptides. The results are illustrated in figure 4. The peptide binding patterns varied with the antibodies induced in different strains with CFA as adjuvant. Antibodies produced by CBA/J and C57BL/6 showed binding with 5–8 peptides, whereas those produced by DBA/2 and BALB/C recognized only 2–3 peptides. Peptides 18, 21 and 28 bound to antibodies of three of four strains examined. Peptides 5–11, which do not encode rKBG8.3 did not

Table 1. Antibody titers of different strains of mice immunized with rKBG8.3

Strains (H-2 haplotype)	Adjuvant	ELISA titers ^a				PCA titers
		Ig(s)	IgG1	IgG2a	IgM	
BALB/C (H-2 ^d)	CFA	>20,480	>20,480	80	40	160
	DS	20,480	>20,480	0	320	80
BDF1 (H-2 ^{b/d})	CFA	>20,480	>20,480	320	160	1,280
	DS	>20,480	>20,480	2,560	640	1,280
CBA/J (H-2 ^k)	CFA	>20,480	>20,480	2,560	0	320
	DS	10,240	>20,480	1,280	640	320
C57BL/6 (H-2 ^b)	CFA	>20,480	>20,480	0	0	320
	DS	5,210	>20,480	0	320	40
DBA/2 (H-2 ^d)	CFA	>20,480	>20,480	2,560	40	1,280
	DS	10,240	>20,480	20	320	1,280
SJL (H-2 ^s)	CFA	>20,480	>20,480	320	40	0
	DS	20,480	>20,480	320	320	0

^a The titers of the pooled sera of day 35 were determined. Individual variation in the titers was in the range of $\pm 20\%$. A representative experiment is shown.

bind to any of these antibodies. Moreover, antibodies to rKBG8.3 induced with DS did not show significant binding to any of these synthetic peptides (data not shown).

We also examined the T cell proliferation in the splenocytes of BALB/C, CBA/J, C57BL/6 and DBA/2 mice following immunization with the rKBG8.3 with CFA as adjuvant. All strains induced proliferation of splenocytes in vitro in response to KBG extract or rKBG8.3 at doses of antigen ranging from 30 to 50 μ g (data not shown). The method of splenocyte culture was selected as opposed to T cell line, as the former provides an unbiased approach to identify the dominant T cell epitope. For each strain, the peptide with mean proliferative response twofold above the background was considered positive. To elucidate if strain difference existed at the T cell epitope level, the proliferative response of splenocytes of these mice was examined utilizing 10 selected synthetic peptides, which were previously shown to stimulate PBMC from allergic individuals [18] (fig. 5). The highest proliferative response was seen in cells of DBA/2 mice which recognized 4 (No. 19, 23, 25 and 27) of the 10 peptides examined. In contrast, all other strains exhibited a high background response and recognized only 1 or 2 peptides. Thus, cells of CBA/J recognized peptide No. 26, BALB/C recognized peptides No. 16 and 18, and C57BL/6 recognized peptide No. 16. It is inferred from these results that the cellular responses to the rKBG8.3 varied among different strains of mice.

To further examine the role of adjuvants in shaping the responses to different epitopes, the antibody binding of synthetic peptides was investigated in BDF1 mice and the results are shown in figure 5. Comparison of antibody specificities induced with different adjuvants revealed varying profiles. Thus, with CFA as adjuvant, rKBG8.3 induced antibodies which bound predominantly to peptides from two regions of the protein, i.e. domain 1 comprising peptides No. 16–19 and domain 2 comprising peptides No. 26–28 (fig. 6a). With DS as adjuvant rKBG8.3 induced antibodies that recognized only peptides of domain 1 and not those of domain 2 (fig. 6b). Similar binding patterns were observed when BDF1 mice were immunized with GST-rKBG8.3 fusion protein in CFA or DS (fig. 6c, d). The antibody binding of peptides was also examined using isotype-specific ELISA, thus the IgG2a antibodies induced by GST-rKBG8.3 and CFA bound to a number of peptides that bound IgG1 as well (data not shown). Notably, the binding pattern of these peptides to IgG2a antibodies was different from that of the IgG1 binding pattern, i.e. domain 2 peptides showed higher binding to these IgG2a antibodies than that of the domain 1 peptides. Analysis of the binding pattern of IgM antibodies indicated that the majority of the peptides bound to the antibodies to GST-rKBG8.3 irrespective of the adjuvant used (data not shown). It is inferred from these results that adjuvants play a significant role in shaping the type of humoral response produced to this recombinant allergen.

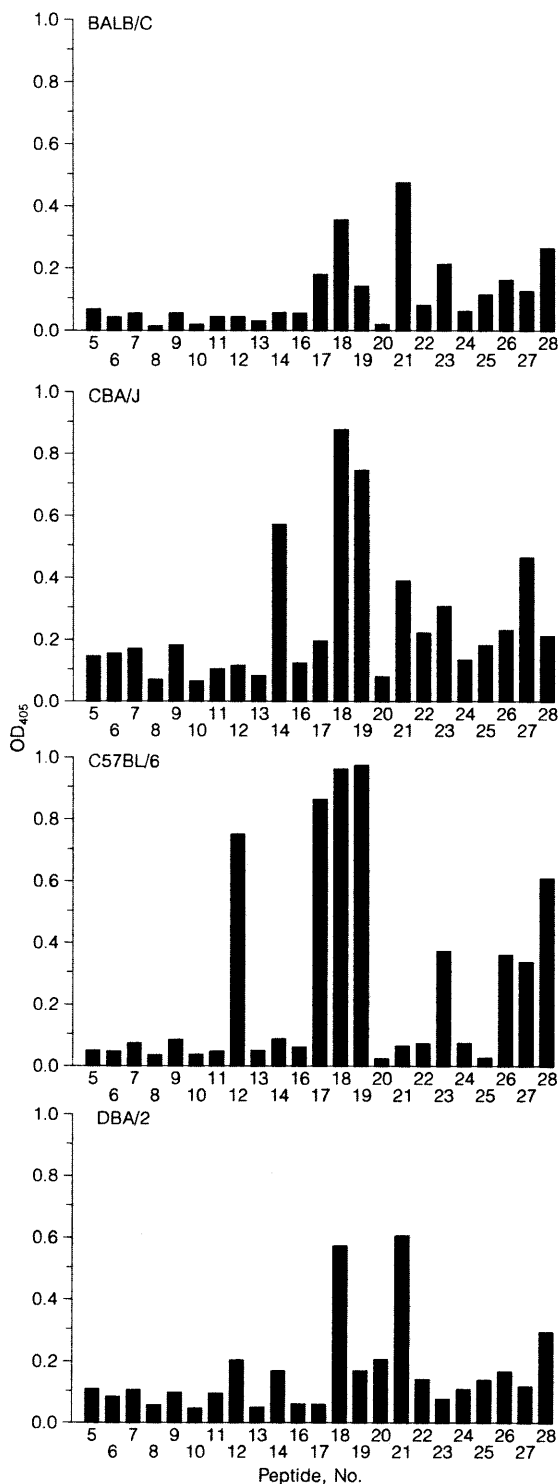


Fig. 4. Determination of fine specificity of total IgG antibodies produced by rKBG8.3 and CFA in four different strains using overlapping synthetic peptides encompassing rKBG60. The antibody binding assays were done by ELISA as described in Materials and Methods. The assays were repeated three times. A representative experiment is shown.

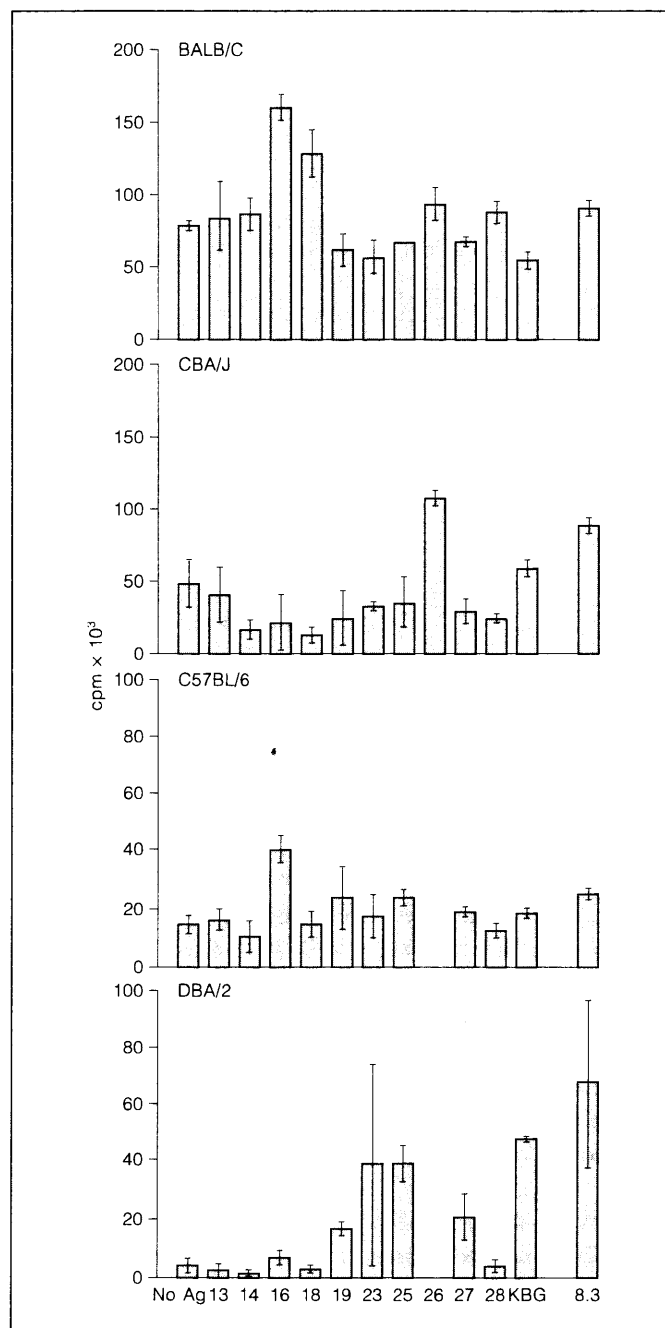


Fig. 5. T cell recognition of overlapping peptides of rKBG8.3 as determined by proliferation of splenocytes in four different inbred strains of mice. The assay was performed as described using [³H]incorporation in triplicate cultures and the cpm to peptides with background (~1,000 cpm) subtracted are shown. Proliferation assays were repeated thrice. A representative experiment is shown.

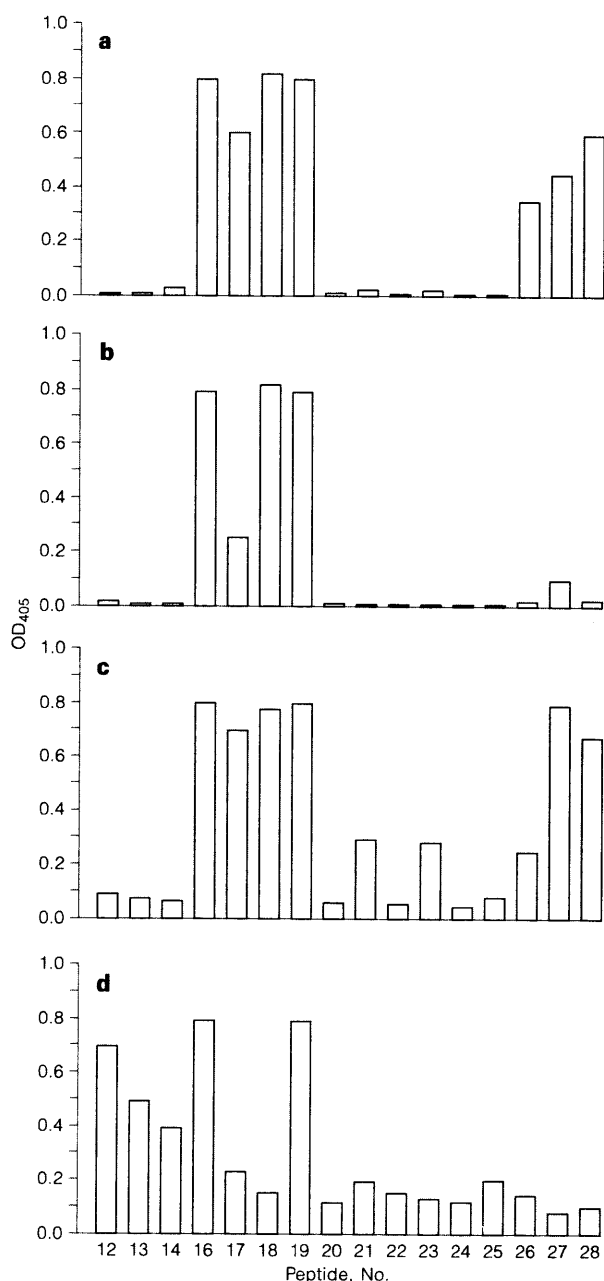


Fig. 6. Reactivities of synthetic peptides to the antibodies induced by cleaved rKBG8.3 (**a, b**) or GST-rKBG8.3 fusion protein (**c, d**) peptides in conjunction with CFA (**a, c**) or DS (**b, d**) as adjuvant on day 35. The immunization and binding experiments were repeated twice and a representative experiment is shown.

Discussion

Recently progress in the molecular characterization of allergens has led to the determination of the primary structures of the allergenic proteins, the synthesis of the corresponding recombinant allergens in virtually unlimited quantities in *Escherichia coli* and other hosts, identification of the structures of the T and B cell epitopes of the allergens, delineation of cross-reactivities among different allergens and their epitopes [25]. Clearly, however the development of new immunotherapeutic modalities with the aid of recombinant allergens and their epitopes is dependent on the comprehensive analysis of the intrinsic and extrinsic factors, such as, the structural properties of the B cell epitopes, the availability and specificity of T cell help, its dominance in relation to other epitopes and host immune responsiveness. In an effort to shed light on these aspects we have extended in this study our analysis of epitope-specific immune responses to the major recombinant grass allergen rKBG8.3.

The results of this study demonstrated that differences existed among various strains of mice in terms of isotype-specific antibody responses to rKBG8.3. However, no difference was seen in terms of the IgG1 titers suggesting that the mechanism underlying the control of this isotype is complex. In contrast, however the IgG2a levels varied among different strains of mice and the levels of antibodies were dependent upon the adjuvant used. Among the strains examined, BDF1 and DBA/2 were considered as high responder for IgE antibody responsiveness, whereas BALB/C, CBA/J and C57BL/6 were considered intermediate in IgE antibody production. The strain SJL/J (H-2^s haplotype) was considered low responder. This is consistent with a number of previous reports [5, 10, 11] which utilized other antigens and our findings with a cocktail of allergens [7]. The differences seen among strains were attributed mainly to the differences in MHC genes. It is noteworthy, however, that BALB/C mice which were of the same haplotype as those of the DBA/2 did not show high IgE response which suggests that genes other than those of the MHC locus also play a role in IgE immune responsiveness. These results are consistent with our previous finding that the generalized IgE production differed among strains and the IgE responsiveness was inherited by a major and some modifier genes, not linked to the H-2 complex [7]. Moreover, variations in strain-dependent isotype-specific responses have been reported previously [10, 11]. Although the genetic mechanism underlying this isotype-specific variation of immune response has not been reasonably addressed, it is likely due to the differential expression of certain cytokine genes [26, 27], which are known to be under genetic control [28].

Previous studies showed that immune responsiveness to B cell epitopes can be regulated by the genes of the MHC complex [29]. However, MHC-dependent regulation of antibody response to this grass allergen has not been reported. Our results showed that the fine specificity of antibodies induced in different strains varied which suggests that the variations in humoral immunity observed among strains of mice were in part due to the differences at the epitope-specific antibody production. The responses to the majority of the epitopes, which localized on rKBG8.3, were similar among strains. However, there were exceptions, for instance, antibodies binding to peptide No. 12 but not to No. 21 were only found in C57BL/6 (fig. 4). Moreover, a sum total of the peptides recognized by these four strains represented also the peptides recognized by allergic humans [18]. It thus appears that in terms of immune recognition, the studies with different strains of mice together may reflect the human situation.

In addition to the differences in the humoral responses, the *in vitro* proliferation of splenocytes using peptides also showed variations in the cellular responses to the rKBG8.3 in these strains. The high IgE responder DBA/2 exhibited at least four epitopes, whereas the intermediate strains exhibited 1–2 epitopes. Furthermore, as with humans, T cell epitopes recognized by these strains differed [18]. It is noteworthy in this context that our previous study using PBMC of allergic humans suggested presence of a number of epitopes on rKBG8.3. In the light of the above findings, the results of this study indicate that individual inbred strains of mouse have limited potential for T cell epitope studies. Clearly, for determination of the complete profile of the T cell epitopes and for studies involving modulation of T cell responses, it is necessary to examine a number of responder strains or their F1 hybrids.

Priming of animal hosts *in vivo* usually requires the delivery of the antigen together with adjuvants. The results of this study indicate that the adjuvants not only influenced the isotype specificity of humoral immune response, but also

the fine specificities of antibodies produced to rKBG8.3. Comparison of fine specificities of antibodies produced to CFA in BDF1 mice suggested that GST-KBG8.3 fusion protein or cleaved rKBG8.3 induced antibodies against both domain 1 and domain 2 peptides (fig. 6). This is in marked contrast to DS which induced antibodies predominantly against domain 1 peptides. These results are consistent with a previous report on adjuvant-induced alterations in antibody specificity [30]. The mechanism by which adjuvants differentially affect the antibody specificities remains to be elucidated. It is likely that adjuvant affects antigen processing and presentation or alternatively, adjuvants may influence specific antigen-presenting cells possessing distinct endocytic and processing pathways [31, 32]. Either mechanism could result in presentation of alternative sets of T and B cell epitopes. Of note, however, previously we demonstrated that immunization of BDF1 mice with the β -galactosidase-KBG60 in DS as adjuvant elicited antibodies against both domain 1 and domain 2 peptides [17]. Therefore the possibility exists that adjuvant influences the physical and chemical environment which in turn alters the conformation of the immunogen [33].

Overall, our results lead to inference that the fine specificity of the antibody produced is dependent upon the interaction between factors, such as genetic makeup, the antigen and the adjuvant. The study of fine specificity of antibodies elicited by a specific allergen and adjuvant combination is important and such studies should precede formulation of any immunotherapeutic strategies against allergic disorders using recombinant and synthetic allergens.

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