

Influence of MHC Background on the Antibody Response to Detergent Enzymes in the Mouse Intranasal Test

Katherine Sarlo,*¹ Jennifer S. Parris,* Edwin D. Clark,* Patricia A. Horn,* Michael K. Robinson,* J. Ann McCay,†
Vanessa L. Peachee,† Yvonne L. Veloso,† and Kimber L. White, Jr.†

*The Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 538707, Cincinnati, Ohio 45253; and †ImmunoTox, Inc., Richmond, Virginia 23219

Received July 17, 2000; accepted September 8, 2000

The mouse intranasal test (MINT) was developed to assess the immunogenic potential of detergent enzymes. The BDF1 mouse (H-2^{b/d}), a cross of C57Bl/6 (H-2^b) × DBA/2 (H-2^d) has been used for most of the development work. Preliminary data in the CB6F1(H-2^{db}), a cross of Balb/c (H-2^d) × C57Bl/6 showed that this strain was similar to the BDF1 in its response to enzymes. These data also showed that the parental strains responded differently to the enzymes. To understand better the influence the major histocompatibility complex (MHC) background has on immune responses to enzymes, 3 different enzymes were tested in 4 inbred strains (C57Bl/6, DBA/2, Balb/c, and C57Bl/10), 2 hybrid strains (BDF1 and CB6F1), and 2 congenic strains (Balb.B10 and B10.D2). BDF1 mice rank enzymes the same as the guinea pig, which in turn correlates with sensitization in occupationally exposed humans. The ranking is based upon the dose of enzyme needed to give one-half maximal IgG1 antibody response (ED₅₀) where Termamyl is more potent than Alcalase, which is equipotent to Savinase. The H-2^d strains ranked the enzymes the same as the BDF1 but generated ED₅₀s for the proteases that were one order of magnitude greater than the BDF1 ED₅₀s. The response to Termamyl was the same in the two F1 strains and the H-2^d strains. The H-2^b strains did not rank the enzymes the same as BDF1 but the ED₅₀s for the proteases were similar to the ED₅₀s in the F1 strains. The response to Termamyl in the H-2^b strains was lower than the response in the F1 and H-2^d strains. Initial data show that the inbred strains will make enzyme-specific IgE antibody to high doses of enzyme with DBA/2 > Balb/c > C57Bl/6 in terms of the robustness of the response. The IgG1 responses in the congenic strains were similar to the responses in the H-2 matched strains. In addition, the antibody response to enzymes was consistent regardless of the source of BDF1 mice. The responses to these enzymes are clearly MHC linked with a role for Class II I-E molecules indicated. The current data strongly support the use of the F1 hybrid as an appropriate strain for evaluating allergic responses to enzymes.

Key Words: allergy; hypersensitivity; animal models; enzymes; MHC.

Occupational IgE antibody-mediated allergic responses and asthma to enzymes used in the detergent industry were first described by Flindt (1969) and Pepys *et al.* (1969). These reactions were noted shortly after the start of extensive manufacture of enzyme-containing detergents (Newhouse *et al.*, 1970; Greenberg *et al.*, 1970; Shapiro and Eisenberg, 1971). As a result, enzyme manufacturers developed less dusty enzymes and the industry instituted extensive dust control measures and exposure guidelines. The incidence of allergic responses and occupational asthma caused by detergent enzymes is now a rare event (Juniper and Roberts, 1984; Flood *et al.*, 1985; Sarlo *et al.*, 1990; Schweigert *et al.*, 2000).

A guinea pig intratracheal (GPIT) test was developed to assess the relative allergenic potential of new enzymes when compared to a reference protease, Alcalase (Ritz *et al.*, 1993). This enzyme was chosen as the reference since the bulk of our human experience has been with this protease and the ACGIH established a threshold limit value of 60 ng pure protein/m³ (ACGIH, 1998). The model has been used to rank enzymes as more potent, less potent or equipotent to Alcalase and exposure guidelines established for these materials based upon the guideline for Alcalase. Prospective evaluation of skin prick test reactions among workers exposed to these different enzymes is consistent with the ranking obtained in the GPIT model (Sarlo *et al.*, 1997). The GPIT model is a very useful method for evaluation of enzyme allergenic potential. However, it is time consumptive and expensive. This limits its use as a screening tool, and development of alternative models that are faster and cheaper was initiated.

A mouse intranasal test (MINT) method was developed to assess the immunogenic potential of enzymes (Robinson *et al.*, 1996). In this model, BDF1 mice receive 3 intranasal doses of enzyme in saline or detergent matrix on days 1, 3, and 10. Sera are collected on day 15 and the enzyme-specific IgG1 antibody response is measured by ELISA. This method of dosing in this murine strain resulted in a robust dose-response to the reference allergen Alcalase. The model was able to detect the adjuvant effect of detergent matrix on the immune response to Alcalase. Continued work in the model showed that the MINT was able to rank other bacterial and fungal enzymes used in the

¹ To whom correspondence should be addressed. Fax: (513) 627-0400 or -0583. E-mail: sarlo.k@pg.com.

detergent industry in the same manner as the GPIT test (Robinson *et al.*, 1998). This was key to the continued development and validation of the mouse model as a means to determine the allergenic potencies of enzymes. During the course of this work, the immune response to selected enzymes in other murine strains was assessed. This preliminary work showed very similar responses to Alcalase in BDF1, CB6F1, and C57Bl/6 strains but lowered dose-responses in the CD1, Balb/c, and Swiss strains (Robinson *et al.*, 1996). Other work showed that the C57Bl/6 strain did not recognize the bacterial amylase, Termamyl, in the same way as the BDF1 mouse (unpublished observation). These data suggested that the ability to distinguish enzyme potencies in a manner similar to the guinea pig and man was linked to the major histocompatibility complex (MHC). In order to better understand the influence of MHC on immune and allergic responses to enzymes, we evaluated 3 immunologically distinct enzymes in two F1 strains, 4 inbred strains, and 2 congenic strains. The enzymes were tested in the hybrid BDF1^(b/d) and CB6F1^(db) strains along with the parent strains, C57Bl/6^(b), DBA/2^(d) and Balb/c^(d). The congenic strains were the B10.D2 (C57Bl/10 background with DBA/2 MHC Class II) and Balb.B10 (Balb/c background with C57Bl/10 MHC Class II). We also tested the enzymes in the C57Bl/10 strain. Finally, we examined whether the source (e.g., different suppliers) of BDF1 mice had an influence on the response to enzyme allergens.

MATERIALS AND METHODS

Animals. Female BDF1, CB6F1, Balb/c, DBA/2, and C57Bl/6 mice weighing 18–20 g were obtained from the Charles River Breeding Laboratory (Portage, MI). Female BDF1 mice were also obtained from the Charles River facility in Raleigh, NC, Harlan Sprague-Dawley (Indianapolis, IN), and Harlan SeraLab, (Bicester, UK). The female C57Bl/10, B10.D2 (H-2^d), and Balb.B10 (H-2^b) mice were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were housed in cages with wood-chip bedding. Female Sprague-Dawley rats weighing 250–275 g were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and housed in cages with wood-chip bedding. All of the animals were kept in rooms controlled for humidity (30–70%), temperature (67–77°F), and 12-h light and dark cycles. Animals were allowed feed (Purina mouse or rat chow) and water *ad libitum*. All animal treatments were done in accordance with procedures approved by an Institutional Animal Care and Use Committee. The NIH guidelines for the care and use of laboratory animals were followed throughout the course of these studies. Five to 8 mice were included in each dose group across all studies.

Test chemicals. Alcalase (serine protease), Savinase (serine protease), and Termamyl (amylase) were obtained from Novo Industri A/S (Bagsvaerd, Denmark). Protein levels were determined by Kjeldahl nitrogen analysis and were as follows: Alcalase [35.4%], Savinase [21.2%], and Termamyl [52%]. All enzymes were diluted (on a protein weight basis) in 0.9% sodium chloride containing 3 mg/ml of a detergent matrix. The detergent matrix used consisted primarily of anionic and nonionic surfactants, silicate builders, and perborate bleach and was formulated by The Procter & Gamble Company.

Intranasal dosing method. Animals were anesthetized by an intraperitoneal (ip) injection of a mixture of Ketaset (11.1 mg/kg) and Rompun (8.33 mg/kg). The anesthetized animals were placed in a supine position, backs down at a slight angle and dosed intranasally with enzyme in saline plus detergent matrix. Dosing solutions were administered in volumes of 10 μ l/

mouse divided equally between the 2 nostrils. The dosing solutions were gently placed on the outside of each nostril and inhaled by the mouse. Following administration of the enzyme solutions, the animals were placed on their backs upon a mound of slightly angled bedding with their heads angled slightly downward. This orientation prolongs the retention of the dosing solution in the nasal cavity (Robinson *et al.*, 1996). Dosing was repeated on days 3 and 10 and blood was collected on day 15, unless otherwise indicated.

Enzyme linked immunosorbent assay (ELISA) for mouse IgG1 antibody. Enzyme-specific IgG1 in mouse serum was measured by a capture ELISA method. The assays were either conducted manually or using the Zymark robotics system (Zymark Corp., Boston, MA). Briefly, 96-well plates (Maxisorp, Nunc, Netherlands) were coated with rabbit anti-enzyme antibody in a carbonate coating buffer. Optimal concentrations of enzyme protein were added to the coated wells followed by diluted mouse sera. A mouse IgG1 reference serum was generated for each enzyme by injecting mice with enzyme in alum adjuvant. The reference was used to standardize the test conditions for all ELISA plates. Goat anti-mouse IgG1 alkaline phosphatase conjugated antibody was added at a 1:2000 dilution (Southern Biotechnology) followed by p-nitrophenyl phosphatase substrate (Kirkergaard and Perry, Gaithersburg, MD). Absorbance of the wells was measured by a microplate reader (Molecular Devices, Sunnyvale, CA) at 405/490. For each dilution of serum tested, the optical density (OD) of normal sera tested at a 1:8 dilution was subtracted from the OD of the test sera. A plot of the titration curve for the test sera was created using a log-log curve fit (Softmax, Molecular Devices). A linear portion of each curve was derived and the serum dilution at an absorbance of 0.5 OD was calculated by interpolation. Titers for serum samples were expressed as log 2 of the interpolated dilution.

Rat passive cutaneous anaphylaxis (PCA) test. Shaved female rats were intradermally injected with dilutions of mouse sera in isotonic saline. Mouse sera from the high-dose groups were tested in the PCA. After 48 h, the animals were challenged via an intravenous injection of enzyme (1mg protein/kg) in 1% Evans blue dye (Sigma, St. Louis, MO). The titer of specific IgE antibody was defined as the log 2 of the reciprocal of the last dilution of sera that produced a blue spot at the injection site. Sera were randomly selected and heated at 56°C for 90 min, then re-tested in the rat PCA. Loss of reactivity at the injection site confirmed the presence of IgE antibody.

Data analysis. The ED₅₀ values (the dose of enzyme producing a half-maximal serum IgG1 titer) were determined from the IgG1 response versus enzyme-dose curve. These were determined using the SigmaPlot logistics curve fitting software program (SigmaPlot for Windows, Version 2.35, Jandel Scientific, San Rafael, CA). Relative enzyme potency determinations were made by dividing the ED₅₀ value obtained for Alcalase by the ED₅₀ value obtained for the test enzyme.

RESULTS

IgG1 Response to Alcalase, Savinase and Termamyl in CB6F1 and Inbred Strains

The calculated ED₅₀s for the 3 enzymes tested in all the F1 and inbred strains are shown on Table 1. In CB6F1 mice, Termamyl was approximately 3–5 fold more potent than Alcalase or Savinase and the 2 proteases were equivalent (less than 2-fold difference). The ED₅₀s to Alcalase, Savinase, and Termamyl were very similar to those obtained in BDF1 mice (Table 1; Robinson *et al.*, 1998). The H-2^d strains (Balb/c, DBA/2) recognized Termamyl as more potent than Alcalase or Savinase and generated ED₅₀s for this enzyme similar to the ED₅₀s calculated from the F1 mice. These inbred strains recognized the 2 proteases as equivalent in potency but the ED₅₀s were about 10-fold greater than the ED₅₀s for these proteases in

TABLE 1
The ED₅₀ to Alcalase, Savinase, and Termamyl in Six Strains of Inbred and Hybrid Mice:
Potency and Association with H-2 and I-E

	BDF1	CB6F1	DBA/2	Balb/c	C57Bl/6	C57Bl/10
H-2	b/d	d/b	d	d	b	b
Alcalase ED ₅₀	0.25	0.16	7.6	1.9	0.22	0.16
Savinase ED ₅₀	0.3	0.2	3.7	2.7	0.15	0.2
Potency: Savinase vs. Alcalase	0.83×	0.8×	2.1×	0.71×	1.5×	0.8×
Termamyl ED ₅₀	0.05	0.05	0.03	0.02	0.28	>1
Potency: Termamyl vs. Alcalase	5×	3.2×	253×	95×	0.78×	—
I-E	+	+	+	+	—	—

Note. ED₅₀s given in $\mu\text{g}/\text{mouse}$.

the F1 mice. Therefore, the potency difference between Alcalase and Termamyl was 253 $\times$ in DBA/2 mice and 95 $\times$ in Balb/c mice. The H-2^b strains recognized Alcalase and Savinase as equivalent in potency with ED₅₀s very similar to the ED₅₀s in the F1 strains. Termamyl was not recognized as more potent than the proteases in the 2 H-2^b strains. The C57Bl/6 strain recognized Termamyl as similar to Alcalase and Savinase. The C57Bl/10 strain recognized Termamyl as less allergenic than the 2 proteases.

IgG1 response to Alcalase, Savinase and Termamyl in Congenic and H-2 Matched Strains

The IgG1 antibody response to Alcalase, Savinase and Termamyl administered to the C57Bl/10 H-2^b and DBA/2 H-2^d mice along with the H-2 matched congenic strains are shown in figures 1 and 2. The responses to Alcalase, Savinase and Termamyl in the Balb.B10 mice were very similar to the

responses to these 3 enzymes in the H-2 matched C57Bl/10 mice (Fig. 1). The responses to these enzymes in B10.D2 mice were similar to the responses in the H-2 matched DBA/2 mice (Fig. 2).

IgE Response to Alcalase, Savinase and Termamyl in Inbred Strains

Figures 3, 4, and 5 show IgE titers in individual serum samples to selected doses of Alcalase, Savinase, and Termamyl in 3 inbred strains, respectively. DBA/2 (H-2^d) mice showed distinctly higher IgE titers to these enzymes vs. the other 2 strains. The Balb/c (H-2^d) mice showed equal or slightly higher titers vs. the H-2^b C57Bl/6 mice. Lower doses of Termamyl (1 to 10 μg) induced high IgE titers in the Balb/c and DBA/2 mice, whereas high doses of the proteases (30 μg and 60 μg) were needed to induce comparable IgE titers. Comparable IgE titers to equivalent protein doses (30 μg) of Termamyl, Alca-

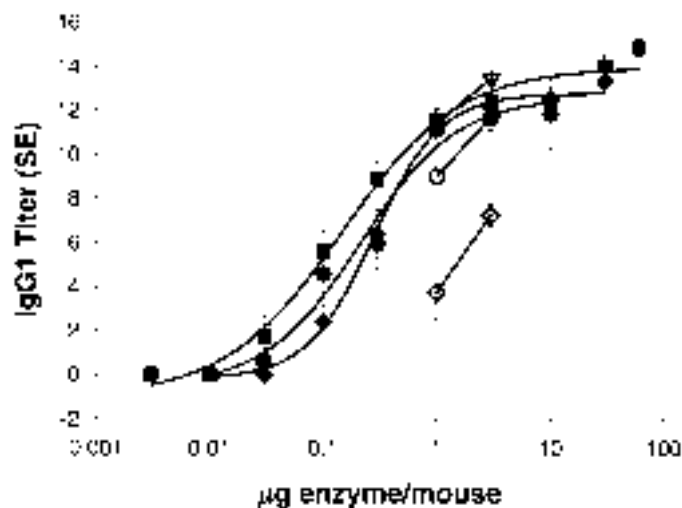


FIG. 1. IgG1 antibody response to Alcalase (circles), Savinase (squares), and Termamyl (diamonds) in C57Bl/10 (solid symbols) and Balb.B10 (open symbols) mice. The ED₅₀s for Alcalase and Savinase in the C57Bl/10 mice were 0.16 $\mu\text{g}/\text{mouse}$ and 0.21 $\mu\text{g}/\text{mouse}$, respectively.

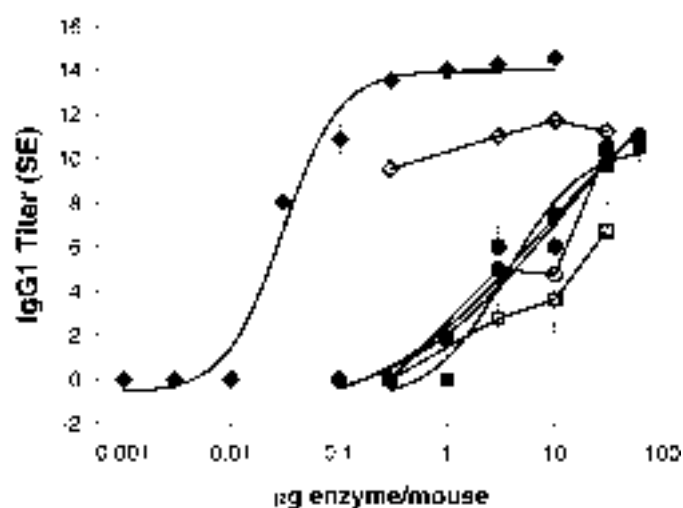


FIG. 2. IgG1 antibody response to Alcalase (circles), Savinase (squares), and Termamyl (diamonds) in DBA/2 (solid symbols) and B10.D2 (open symbols) mice. The ED₅₀s for Alcalase, Savinase, and Termamyl in the DBA/2 mice were 7.8 $\mu\text{g}/\text{mouse}$, 3.7 $\mu\text{g}/\text{mouse}$, and 0.03 $\mu\text{g}/\text{mouse}$, respectively.

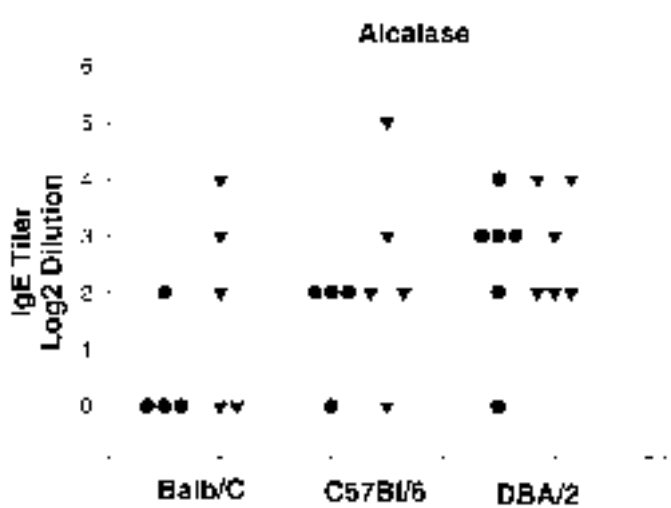


FIG. 3. IgE antibody titers to 30 µg (circles) and 60 µg (triangles) Alcalase in Balb/c, C57Bl/6, and DBA/2 mice.

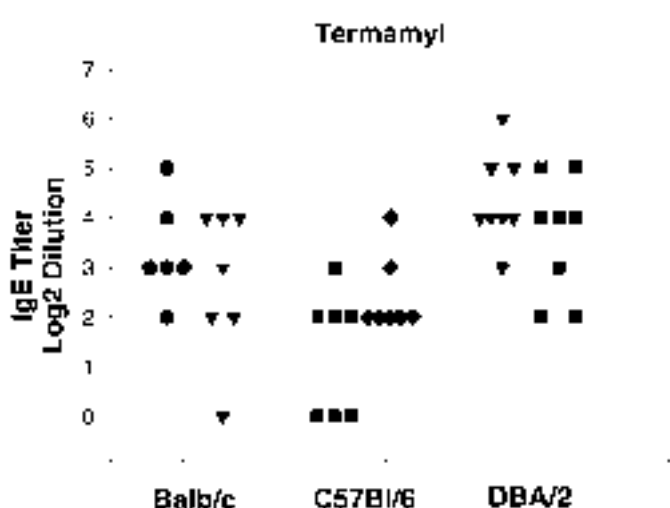


FIG. 5. IgE antibody titers to 1 µg (circles), 3 µg (triangles), 10 µg (squares) and 30 µg (diamonds) Termamyl in Balb/c, C57Bl/6, and DBA/2 mice.

lase, and Savinase were obtained in C57Bl/6 mice. Heat inactivation of randomly selected sera abrogated the rat PCA response confirming the presence of IgE antibody in the samples (data not shown).

IgG1 Response to Alcalase and Savinase in BDF1 Mice from Different Suppliers

The IgG1 antibody dose-response curves to Alcalase and Savinase administered to BDF1 mice obtained from different laboratory animal suppliers in the U.S. and the U.K. are shown in Figure 6. The responses to both enzymes were consistent among mice obtained from 2 different breeding sites from different suppliers in the U.S. and U.K. In addition, the response to these enzymes was consistent among BDF1 mice

obtained from different breeding sites within the same supplier in the U.S. (data not shown).

DISCUSSION

The guinea pig intratracheal test has been used to assess the relative allergenic potential of enzyme allergens used in the detergent industry. The data from this test are used to establish occupational exposure guidelines. These guidelines are used as part of a total program to control exposure and reduce occu-



FIG. 4. IgE antibody titers to 30 µg (circles) and 60 µg (triangles) Savinase in Balb/c, C57Bl/6, and DBA/2 mice.

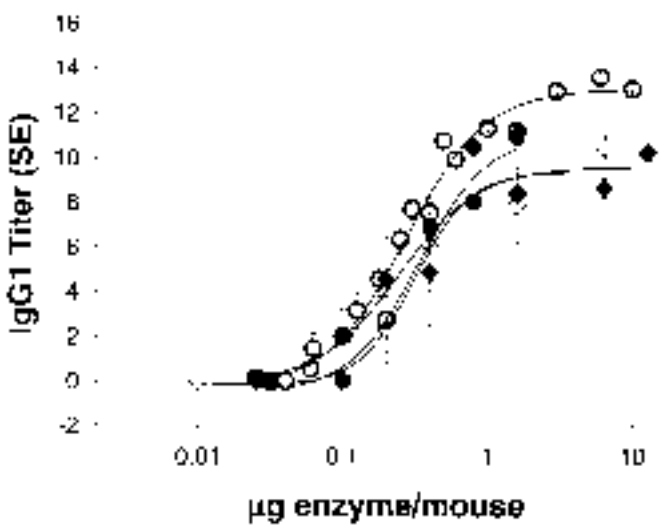


FIG. 6. IgG1 antibody response to Alcalase (circles) and Savinase (diamonds) in mice obtained from Harlan, UK (closed symbols) and Charles River, U.S. (open symbols). The ED₅₀s for Alcalase for U.K.- and U.S.- sourced mice were 0.34 µg/mouse and 0.27 µg/mouse, respectively. The ED₅₀s for Savinase for U.K.- and U.S.- sourced mice were 0.36 µg/mouse and 0.32 µg/mouse, respectively.

pational sensitization and allergy to enzymes (Peters and MacKenzie, 1997; SDA, 1995). The model has been used to evaluate non-enzyme protein allergens; therefore, it also has utility outside of the detergent industry. The mouse intranasal test is being developed as a simpler and more cost-effective alternative or supplement to the guinea pig model. Previous work in the mouse showed that different strains will respond differently to enzymes. However, the BDF1 strain generated responses to enzymes that were closely linked to the data generated in guinea pig tests, which in turn correlates with observations in man. The initial data from different strains prompted us to examine the role of MHC haplotype in the immune response to enzyme allergens. Also, since the BDF1 strain is generated via the cross of 2 inbred strains, it became important to demonstrate consistency of immune responses among animals obtained from different sources. The data presented clearly show the importance of strain selection for development and validation of murine models of allergy. The data also provide clues to understanding why some enzyme allergens are more potent than others.

The enzymes Termamyl, Alcalase, and Savinase were chosen for testing because these enzymes are immunologically distinct (Arlian *et al.*, 1990; unpublished observation), have been tested in the guinea pig model, and are widely used in the detergent industry. In addition, our use of these 3 enzymes has allowed us to collect prospective skin prick test data on exposed workers. Termamyl was ranked as approximately 3- to 10-fold more potent than Alcalase in the guinea pig test (Sarlo *et al.*, 1997) and the MINT (Robinson *et al.*, 1998). Prospective skin prick test data from workers exposed to both high and low levels of these enzymes confirm the greater potency of Termamyl (Sarlo *et al.*, 1997; Schweigert *et al.*, 2000). The 2 proteases are considered equivalent in potency in both the guinea pig and mouse models. Prospective skin prick test data from workers exposed to these enzymes under comparable conditions of exposure show similar rates and prevalence of sensitization (Sarlo *et al.*, 1997).

We evaluated these enzymes in two F1 strains that were H-2^{b/d} or H-2^{db} haplotype, the 3 parental strains that were either H-2^b or H-2^d, and 2 congenic strains. Since it was difficult to obtain an ample supply of congenic mice from C57Bl/6 stock, we used congenic mice developed from C57Bl/10 stock. For this reason, we also tested the 3 enzymes in the C57Bl/10 parental strain. Also, the supply of congenic mice within the required age and weight range was limited, so we were limited to testing a minimum number of doses of each enzyme.

The IgG1 antibody responses to Termamyl, Alcalase, and Savinase were very similar between the BDF1 and CB6F1 mice. The similarity was noted in both the shape of the dose-response curves and the calculated ED₅₀ values. Both F1 strains ranked Alcalase and Savinase as equipotent and ranked Termamyl as 3- to 5-fold more potent than the proteases. Both strains also recognize and rank a fourth enzyme, a cellulase, in a similar manner (data not shown). This was consistent with

the guinea pig and human databases (Robinson *et al.*, 1998). The F1 strains share the C57Bl/6 H-2^b parent strain but differ in the H-2^d parent strain.

The IgG1 antibody response to the enzymes was dependent upon the MHC haplotype of the mice. The IgG1 antibody response to Alcalase and Savinase was similar among the two H-2^b inbred strains (C57Bl/6 and C57Bl/10), the two F1 strains and the H-2^b congenic strain (Balb.B10). The ED₅₀ values for Alcalase and Savinase ranged from 0.15 µg to 0.30 µg for all the strains but could not be calculated for the congenic strain. Limited numbers of animals allowed us to test only 2 doses of enzyme. The antibody titers from the congenic animals were of similar magnitude to the antibody titers from the F1 and inbred strains. The IgG1 antibody response to Alcalase and Savinase was also similar among the two H-2^d inbred strains (Balb/c and DBA/2) and the H-2^d congenic strain (B10.D2). However, the ED₅₀ values for both proteases were at least one order of magnitude higher than the ED₅₀ values calculated from the responses generated in the H-2^b and F1 strains. The H-2^d strains recognized the 2 proteases as equipotent but required higher doses of protein to generate the dose response. Again, we could not construct a complete dose-response curve with the congenic mice, but the antibody titers overlapped with the titers generated in the H-2 matched DBA/2 strain. Although H-2^b and H-2^d mice were able to "rank" Alcalase and Savinase as equipotent, the H-2^d haplotype was associated with a shift in the threshold for responsiveness to these protease antigens.

The antibody responses and potency estimates to the amylase Termamyl were different among the strains. The two F1 strains correctly ranked and estimated the potency of this enzyme. Comparison of ED₅₀ values for Alcalase and Termamyl estimated Termamyl as 3- to 5-fold more potent than Alcalase. The H-2^d strains also ranked Termamyl as more potent than Alcalase. The Termamyl ED₅₀ values from Balb/c and DBA/2 mice were very similar to the ED₅₀s calculated from the two F1 strains. However, the potency estimate within the H-2^d inbred strains was well out of the range of potency estimates calculated from guinea pig and F1 strain data (Robinson *et al.*, 1998; Sarlo *et al.*, 1997). This was due to the higher ED₅₀ values calculated for Alcalase. The H-2^d congenic strain also recognized Termamyl as more potent than Alcalase or Savinase but the limited dose-response curve did not allow calculation of an ED₅₀ value.

The H-2^b inbred strains (C57Bl/6 and C57Bl/10) did not recognize Termamyl as more potent than either of the proteases. The Balb.B10 congenic mice, possessing the H-2^b MHC complex from C57Bl/10 mice, did not recognize Termamyl as more potent than Alcalase or Savinase. The response to Termamyl in this congenic strain overlapped with the H-2-matched C57Bl/10 strain. The C57Bl/6 mice recognized Termamyl as equipotent to the proteases with an ED₅₀ value comparable to the ED₅₀s calculated for the proteases. Interestingly, the C57Bl/10 and Balb.B10 strains recognized Termamyl as less potent than the proteases with ED₅₀s estimated

to be greater than the ED_{50} s for the proteases. While the C57Bl/6 and C57Bl/10 strains share H-2^b MHC haplotype and lack Class II I-E molecules, there are minor differences between the strains. The most notable difference is found in the IgH2 region which is linked to immunoglobulin diversity in the B-cell compartment (personal communications, Jackson Laboratories; Melchers and Rolink, 1999). It is possible that the differential response to Termamyl between the B6 and B10 mice is due to differential recognition of B cell epitopes. It is also possible that antigen uptake and processing may vary between these 2 strains. It is clear from these data that the H-2^b haplotype is associated with a shift in the threshold for responsiveness to Termamyl.

Due to limited volumes of serum, we were only able to measure IgE titers to the 3 enzymes at a few dose levels. Qualitatively, we found that the H-2^d mice (Balb/c and DBA/2) produced a range of IgE titers to lower doses of Termamyl that were comparable to the range of titers produced to higher doses of Alcalase or Savinase. This was consistent with the IgG1 antibody data. Similarly, the H-2^b C57Bl/6 mice produced a comparable range of IgE titers to equivalent protein doses of Termamyl, Alcalase, and Savinase. Again, the IgE response was consistent with the IgG1 antibody data. Taken together, the IgG1 and IgE antibody responses appear to be linked to the MHC haplotype. Additional testing for IgE antibody at other doses will allow us to make better quantitative comparisons of responses to the enzymes in various strains of mice.

We know that the H-2^b C57Bl/6, C57Bl/10, and Balb.B10 strains lack the MHC Class II I-E alpha chain (Marguiles, 1999). Therefore, these mice lack the I-E heterodimer that is involved in antigen presentation and thymic selection of T-cell repertoire. Unlike I-E positive strains, I-E negative mice do not have deletions of T cells that carry specific T-cell receptor V-beta phenotypes (Vacchio and Hodes, 1989). We theorize that the inability of the H-2^b I-E negative strains to recognize Termamyl as a more potent allergen is due to the T-cell repertoire engaged during the immune response to the amylase. Since these animals do not form I-E heterodimers, presentation of peptides along with selection of reactive T cells may be compromised. Hence the inherent potency of the protein is not detected in these strains. Conversely, the I-E positive H-2^d strains do recognize Termamyl as a more potent allergen. The ability to form both I-A and I-E heterodimers probably leads to selection of T cells that lead to a robust response to the amylase. In contrast, the H-2^d strains required much higher doses of protease to generate IgG1 antibody titers; the threshold for response to the proteases was higher in the H-2^d strains vs. the H-2^b or F1 strains. The presence of H-2^d Class II I-A and I-E molecules appears to be linked to the higher threshold response to the protease allergens. The data we have generated suggests that class II I-E is linked to the quality of the antibody responses to Termamyl and the proteases. Phenotyping enzyme-responsive T cells in the H-2^b strain and comparing that

to the phenotype of enzyme-responsive T cells in an H-2^d strain will allow us to examine this problem.

Why do the BDF1 and CB6F1 mice respond to Termamyl and the 2 proteases in a manner similar to guinea pigs and humans? Again, we need to look at the presence of I-E in these strains. While the BDF1 and CB6F1 mice carry H-2^d Class II I-A and I-E $\alpha\beta$ chains, they also carry H-2^b class II I-A $\alpha\beta$ chains and the I-E β chain. While the I-A can be of homologous $\alpha\beta$ chains, I-A heterodimers can form by mixing $\alpha\beta$ chain isotypes so that antigen-presenting cells can also present peptides in the context of I-A $\alpha^d\beta^b$ and I-A $\alpha^b\beta^d$. I-E heterodimers can also form by mixing I-E α^d with I-E β^b . Finally, there can be mixing of trans-isotypes so that an I-A α chain can pair with an I-E β chain. The mixing and matching of I-A and I-E $\alpha\beta$ chains from different haplotypes leads to a greater variety of antigen-presenting molecules (Malissen *et al.*, 1986; Matsunaga *et al.*, 1990; Natarajan *et al.*, 1992). Since T cells must recognize peptide epitopes in the context of the MHC I-A and I-E molecules, heterogeneity in the I-A and I-E can lead to recognition of all epitopes important to the inherent potency of enzyme allergens and the selection and expansion of a varied T-cell repertoire. Examination of the phenotype of responding T cells in the F1 strains will allow us to confirm our hypothesis.

The data we have generated to date confirm the F1 hybrid strains as the best choice to use for model development. This is based on the correlation of ranking and potency estimates between the F1 strains and the guinea pig data, which in turn correlate with skin prick test prevalence data in exposed humans. While we have a limited set of data in the CB6F1 strain, we have been able to correlate the BDF1 response to over 10 different enzymes with the response in the guinea pig model (Robinson *et al.*, 1998; unpublished data). The F1 strains possess both H-2^d and H-2^b Class II I-A and I-E heterodimers. The clues from the data generated in the inbred and congenic strains indicate how important these Class II molecules are for the recognition of the inherent potency of enzyme allergens. Continued evaluation of immune responses to different proteins in the F1 strain, with correlations to the response in exposed humans, will either support or refute the conclusions we have made and their predictiveness for humans.

REFERENCES

- ACGIH (1998). *Threshold Limit Values and Biological Exposure Indices*, p. 38. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.
- Arlan, L. G., Vyszynski-Moher, D. L., Merski, J. A., Ritz, H. L., Nusair, T. L., Wilson, E. R. (1990). Antigenic and allergenic characterization of the enzymes Alcalase and Savinase by crossed immunoelectrophoresis and crossed radioimmunoelectrophoresis. *Int. Arch. Allergy Appl. Immunol.* **91**, 278-284.
- Flindt, M. L. H. (1969). Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* **1**, 1177-1181.
- Flood, D. F. S., Blofield, R. E., Bruce, C., Hewitt, J. I., Juniper, C. P., and Roberts, D. M. (1985). Lung function, atopy, specific hypersensitivity, and

- smoking of workers in the enzyme detergent industry over 11 years. *Brit. J. Ind. Med.* **42**, 43–50.
- Greenberg M., Milne, J. F., and Watt, A. (1970). Survey of workers exposed to dust containing *Bacillus subtilis*. *Brit. Med. J.* **2**, 629–633.
- Juniper, C. P., and Roberts, D. M. (1984). Enzyme asthma: Fourteen years clinical experience of a recently prescribed disease. *J. Occup. Med.* **34**, 127–132.
- Malissen, B., Shastri, N., Pierres, M., and Hood, L. (1986). Cotransfer of the $E^d\alpha$ and $A^d\beta$ genes into L cells results in the surface expression of a functional mixed isotype Ia molecule. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 395–398.
- Marguiles, D. H. (1999). The major histocompatibility complex. In *Fundamental Immunology* (W. E. Paul, Ed.), pp. 267. Lippencott-Raven, Philadelphia.
- Matsunaga, M., Seki, K., Mineta, T., and Kimoto, M. (1990). Antigen reactive T-cell clones restricted by mixed isotype $A^d\beta/E^d\alpha$ Class II Molecules *J. Exp. Med.* **171**, 577–582.
- Melchers, F., and Rolink, A. (1999). B-lymphocyte development and biology. In *Fundamental Immunology* (W.E. Paul, Ed.), pp183–224. Lippencott-Raven, Philadelphia.
- Natarajan, K., Burstyn, D., and Zauderer, M. (1992). Major histocompatibility complex determinants select T-cell receptor α chain variable region dominance in a peptide-specific response. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8874–8878.
- Newhouse, M. L., Tagg, B., Pocock, S. J., and McEwan, A. C. (1970). An epidemiologic study of workers producing enzyme washing products. *Lancet* **1**, 689–693.
- Pepys, J., Longbottom, J. L., Hargreave, F. E., and Faux, J. (1969). Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet* **1**, 1181–1184.
- Peters, G., and MacKenzie, D. P. (1997). Worker safety: How to establish site enzyme capability. In *Enzymes in Detergency* (J. H. van Ee, O. Misset, and E. J. Baas, Eds.), pp. 327–340. Marcel Dekker, New York.
- Ritz, H. L., Evans, B. L. B., Bruce, R. D., Fletcher, E. R., Fisher, G. L., and Sarlo, K. (1993). Respiratory and immunological responses of guinea pigs to enzyme containing detergents: A comparison of intratracheal and inhalation modes of exposure. *Fundam. Appl. Toxicol.* **21**, 31–37.
- Robinson, M. K., Babcock, L. S., Horn, P. A., and Kawabata, T. T. (1996). Specific antibody responses to subtilisin Carlsberg (Alcalase) in mice: Development of an intranasal exposure model. *Fundam. Appl. Toxicol.* **34**, 15–24.
- Robinson, M. K., Horn, P. A., Kawabata, T. T., Babcock, L. S., Fletcher, E. R., and Sarlo, K. (1998). Use of the mouse intranasal test to determine the allergenic potency of detergent enzymes: Comparison to the guinea pig intratracheal test. *Toxicol. Sci.* **43**, 39–46.
- Sarlo, K., Clark, E., and Ryan, C. (1990). ELISA for human IgE antibody to subtilisin A (Alcalase): Correlation with RAST and skin tests results with occupationally exposed individuals. *J. Allergy Clin. Immunol.* **86**, 393–399.
- Sarlo, K., Fletcher, E. R., Gaines, W. G., and Ritz, H. L. (1997). Respiratory allergenicity of detergent enzymes in the guinea pig intratracheal test: Association with sensitization of occupationally exposed individuals. *Fundam. Appl. Toxicol.* **38**, 44–52.
- Schweigert, M., MacKenzie, D. P., and Sarlo, K. (2000). Occupational asthma and allergy with the use of enzymes in the detergent industry: A review of epidemiology, toxicology, and methods of prevention. *Clin. Exp. Allergy* (in press).
- Shapiro, R. S., and Eisenberg, B. E. (1971). Sensitivity to proteolytic enzymes in laundry detergents. *J. Allergy* **47**, 76–79.
- SDA (1995). *Work Practices for Handling Enzymes in the Detergent Industry*. Soap and Detergent Association, New York.
- Vacchio, M. S., and Hodes, R. J. (1989). Selective decreases in T-cell receptor VB expression: Decreased expression of specific VB families is associated with expression of multiple MHC and non-MHC gene products. *J. Exp. Med.* **170**, 1335–1346.