A Study of Intracutaneous Skin Tests and Radioallergosorbent Tests on 1,000 Asthmatic Children in Taiwan*

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SUMMARY **One thousand asthmatic and 281 normal children were skin tested with a panel of 31 allergens. The most commonly seen allergens were housedust (93.4%) and *Dermatophagoides farinae* (90.2%), followed by cotton (37.5%) *Candida albicans* (34.2%), rice straw (31.2%) and a variety of moulds (around 20%). The skin reactions to pollens and animal danders were relatively uncommon. For both housedust and *D. farinae*, there was a good correlation between the skin test and RAST, but the correlation was poor for moulds and cockroach dander. Moreover, not only did the wheal size elicited by housedust correlate very well with that elicited by *D. farinae* (r=0.74), the housedust RAST also correlated highly with mite RAST (r=0.80). In addition, a total of 12,143 allergen injections of housedust given to 533 patients elicited 176 positive reactions (1.45%) in 102 cases (19.13%). The skin tests of some normal children also were positive to housedust but with much less frequency (20.6%) and weaker reactivity. It is concluded that housedust and *D. farinae* were the two most important inhalant allergens in childhood asthma in Taiwan and *D. farinae* may be an important source of housedust antigen.**

ASIAN PACIFIC J ALLERG IMMUN 1984; 2 : 56-60.

Bronchial asthma is a disease characterized by hyper-responsiveness of the bronchial tree to various stimuli. Among the many factors capable of triggering asthmatic symptoms, the specific allergen-IgE antibody reaction still plays an important role in the pathogenesis of the disease. However, because the kinds of allergen differ from place to place, it is mandatory, therefore, for an allergist to know the relative preponderance of the various allergens in his geographic area in order to treat successfully patients with allergic disease.

This communication describes the results of an allergen survey in 1,000 asthmatic and 281 normal children in Taiwan by using skin tests and radioallergosorbent tests (RAST).

MATERIALS AND METHODS

Study populations. One thousand newly diagnosed asthmatic children, aged from 1 to 16 years with a male-to-female ratio of 2.5 to 1, were examined consecutively at the allergy clinic of the Department of Paediatrics, National Taiwan University Hospital, during the four-year period from January 1980 to December 1983. The diagnosis of bronchial asthma was made if there was a history of recurrent, paroxysmal attacks (at least three times) of reversible obstructive airway disease which resolved either spontaneously or after treatment with bronchodilators. Two hundred and eighty-one non-asthmatic normal children, who were undergoing routine health check-ups, were included as controls.

Intracutaneous skin testing. Aqueous allergen extract (0.04 ml) and buffered saline as control, were injected into the volar surface of the forearm using a 27-gauge needle and tuberculin syringe to raise a 5 mm bleb. The allergen concentration used was 1:1,000 (weight/

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volume, W/V), except for moulds (1:10,000) and for *D. farinae* (1: 50,000). All allergen extracts were purchased from Torii & Co. of Japan, except for the housedust extract which was prepared in our laboratory using locally collected materials. The same dust extract was used for both skin testing and preparing allergen discs for RAST. The skin tests were read 15 minutes after administration and the results were recorded according to the following criteria: (−), wheal smaller than 6 mm in diameter; (1+), wheal of 6-9 mm; (2+), wheal of 10-14 mm; (3+), wheal larger than 14 mm; and (4+), wheal with pseudopods. The intake of certain drugs before testing was prohibited: three days prior to testing in the case of antihistamine and six hours before testing in the case of β_2 agonists. In this study, a skin test of (1+) or greater was considered to be positive.

Radioallergosorbent test (RAST). Specific IgE antibodies to housedust, *D. farinae*, cockroach dander and moulds were determined by using the RAST method. Allergen discs were supplied through the generosity of Pharmacia Diagnostics of Sweden; other reagents such as ^{125}I-labelled antihuman IgE were purchased from the same company. For comparison, allergen discs coupled with locally collected housedust were prepared in our laboratory according to the method of Ceska *et al.*[1] A RAST score of (1+) or greater was considered to be positive.

RESULTS

The results of the skin test with 31 allergens on 1,000 asthmatic children are shown in Table 1. The most commonly encountered allergens were housedust (93.4%) and mite dander (90.2%), followed by cotton (37.5%), *Candida* (34.2%), rice straw (31.2%), other moulds (16.7-26.7%), ragweed (22.8%) and cockroach dander (17.9%). Foods elicited a positive skin test much less frequently (usually less than 10%) compared with the common inhalants.

Table 1 Results of skin tests in 1,000 asthmatic children in Taiwan

Inhaled allergens	% +ve	Ingested allergens	% +ve
Housedust	93.4	Crab	9.1
D. farinae	90.2	Oyster	4.6
Cotton	37.5	Tunny	1.0
Rice straw	31.2	Sardine	0.8
Feather	10.1	Cuttlefish	0.3
Dog	8.2	Pork	0.2
Cat	7.4	Beef	0.1
C. albicans	34.2	Rice	0
A. fumigatus	26.7	Wheat	0
Cladosporium spp.	22.8	Buckwheat	0
Penicillium spp.	22.3	Eggwhite	10.3
Alternaria spp.	16.7	Milk	8.1
Ragweed	22.8	Tomato	0
Spinach	5.5	Radish	0
Red pine	8.8		
Black pine	4.5		
Cockroach*	17.9		

The allergen concentration used was 1:1,000 (W/V), except for moulds (1:10,000) and for mite (1:50,000).
*Only 78 cases were tested.

The influence of age on the frequency of positive skin tests to housedust and *Candida* in asthmatic and normal children is presented in Table 2. In both the patients and normal children, the positive rates to housedust and *Candida* rose with increasing age. In patients, the positive rate to housedust increased from 44.4 per cent at one year of age to higher than 95 per cent after six years of age. The skin tests of some normal children also were positive to housedust, but the skin reaction appeared much later (in one-year-old patients and in five-year-old normal subjects) and in much lower frequency (93.4 per cent for patients and 20.6 per cent for normal subjects). Furthermore, the magnitude of skin reactivity was much greater in patients than that in normal subjects (13.4 ± 2.8 mm vs 5.0 ± 1.4 mm, average wheal size, $p < 0.0001$; 23.2±7.6 mm vs 9.2±3.2 mm, average flare size, $p < 0.0001$). The same tendency was also noted for *Candida* (data not shown).

To determine the specificity of intracutaneous skin testing, RASTs were performed on sera drawn from skin test-positive individuals. For housedust, the RAST was positive in 72.1 per cent (673/934) of skin test-positive individuals when allergen discs coupled with locally collected housedust were used, but the figure decreased to only 53.3 per cent (16/30) when commercially available allergen discs from Pharmacia Diagnostics were used. There was a good correlation between mite skin tests and mite RAST, as 90 per cent of sera from individuals with skin test-positive to mite dander also gave positive mite RAST. The correlation between skin test and RAST was very poor for cockroach dander and moulds.

In order to study the relationship between mite antigen and

Table 2 Results of housedust and *Candida* skin tests in asthmatic and normal children according to age

Age (yr)	Asthmatics			Normal		
	No. tested	No. positive (%)		No. tested	No. positive (%)	
		Housedust	*Candida*		Housedust	*Candida*
1	9	4 (44.4)	0			
2	27	20 (74.1)	2 (7.4)			
3	89	82 (92.1)	16 (18.0)			
4	80	74 (92.5)	23 (28.8)	3	0	0
5	134	125 (93.3)	43 (32.1)	14	2 (14.2)	0
6	223	216 (96.9)	73 (32.7)	20	4 (20.0)	1 (5.0)
7	152	140 (92.1)	56 (36.8)	43	8 (18.6)	2 (4.7)
8	87	81 (93.1)	36 (41.3)	64	15 (23.4)	5 (7.8)
9	45	42 (93.3)	18 (40.0)	31	6 (19.4)	3 (9.7)
10	44	42 (95.5)	18 (40.9)	19	5 (26.3)	0
11	29	28 (96.6)	14 (48.2)	20	4 (20.0)	1 (5.0)
12	36	36 (100)	16 (44.4)	15	4 (26.7)	0
13	18	18 (100)	9 (50.0)	21	4 (19.0)	2 (9.5)
14	8	9 (100)	5 (62.5)	13	2 (15.4)	0
15	10	9 (90.0)	7 (70.0)	18	4 (22.2)	1 (5.6)
16	9	9 (100)	6 (66.6)			
Total	1,000	934 (93.4)	342 (34.2)	281	58 (20.6)	15 (5.3)

The allergen concentration used was 1:1,000 (W/V) for house-dust and 1:10,000 for *Candida*

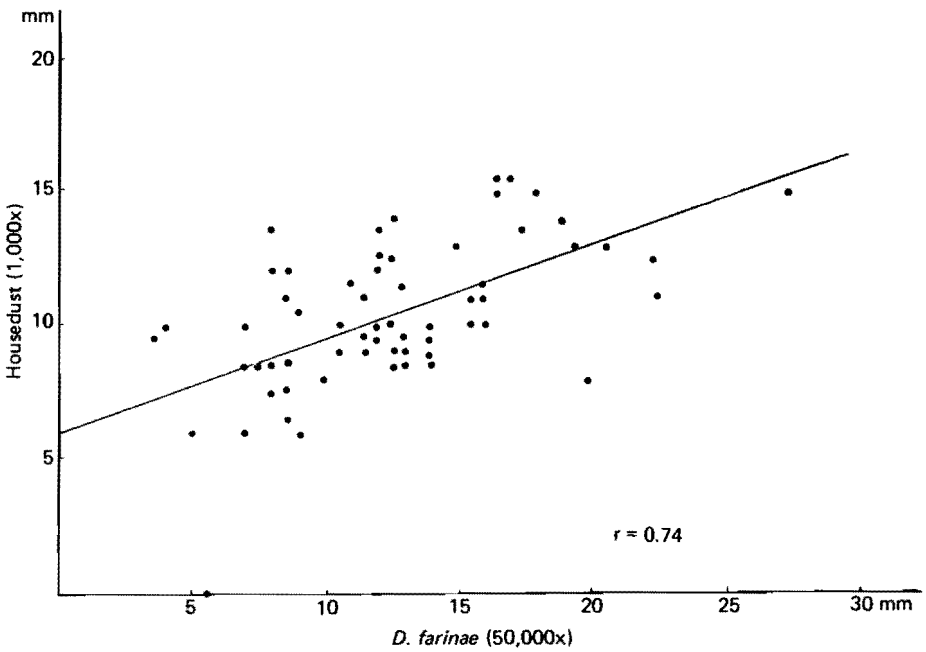

Fig. 1 Correlation between skin wheal size induced by house-dust extract (1:1,000, W/V) and that induced by *D. farinae* extract (1:50,000, W/V).

housedust antigen, the results of skin reactivity and RAST to housedust and those to *D. farinae* were compared. Figure 1 shows a linear relationship between the size of the wheal induced by housedust extract (1:1000, W/V) and that induced by *D. farinae* (1:50,000, W/V), with a correlation coefficient of 0.74. The correlation between housedust RAST and mite RAST was also high (r = 0.80; data not shown).

DISCUSSION

Since being introduced in 1873 by Blackley[2] as a method of clinical allergy diagnosis, immediate skin testing has been widely used as an *in vivo* procedure to detect specific allergens in patients with allergic diseases. Although the association between allergy skin tests and bronchial asthma has been recognised for some 60 years,[3] the specificity of the tests, their relationship to aetiology and clinical significance has been disputed. However, technology developed in recent years has repeatedly shown its important clinical implication in respiratory allergy. Stenius *et al*[4]

demonstrated a good correlation between the number of positive skin tests and total serum IgE level; also, the wheal size of the skin test correlated very well with the concentration of specific IgE antibody.[5] For a number of allergens, the skin test has also been shown to correlate closely with the bronchial provocation test.[6,7] Therefore, the skin test may be considered to be a reliable, rapid and specific method for demonstrating the presence of skin-sensitising antibody in the detection of allergens in atopic patients.

There are two general approaches to allergy skin testing. One approach is referred to as the intracutaneous test; the other, as the epicutaneous test. The latter includes the so-called scratch test, puncture test and prick test. Although the epicutaneous test has several advantages such as simplicity, safety, speed, being less injurious and, more importantly, offering better correlation with the occurrence of clinical disease, the intracutaneous technique exhibits the greatest degree of specificity.[8] In our clinic, intracutaneous testing is routinely used because experience shows it to be about 100 times more sensitive than scratch testing.

The finding in this study that the most common allergens were housedust and *D. farinae* is consistent with those reported for children by Pearson[9] and Smith[10] and with that reported by Hendrick *et al*[11] regarding a group of children and adults. However, the results obtained in this study showing that the next most common allergens were moulds rather than pollens and animal dander is in contrast to those reported by the aforementioned investigators.[9-11] The discrepancy may be due to such factors as high humidity, high temperature, mild seasonal differences and the crowded conditions (there is only limited space for plants and grasses to grow) on this island as well as cultural differences between Westerners and Chinese. The importance of housedust and *D. farinae* in the pathogenesis of childhood asthma in Taiwan is further supported by our previous work which showed that housedust was able to provoke bronchospasm accompanied by the occurrence of eosinophilia and the activation of complement.[7,12,13] Moreover, during this study, 12,143 allergen injections of housedust were given to 533 patients sensitive to housedust in terms of positive skin test and RAST; 176 systemic reactions occurred in 102 of these patients. The incidence of reaction per injection was 1.45 per cent (176/12,143); per person, 19.1 per cent (102/533). The reactions included 160 asthmatic attacks, five attacks of asthma and urticaria, three urticaria, three instances of anaphylactic shock and one occurrence each of asthma and rhinitis, rhinitis only, rhinitis and conjunctivitis, rhinitis and urticaria and conjunctivitis only (Table 4). Sixty per cent of the reactions occurred when the dose was increased (15 to 100%),

Table 3 Results of RAST in skin test-positive asthmatics

Allergens	No. tested	No. positive	%
Housedust	934	673	72.1*
	30	16	53.3**
D. farinae	60	54	90.0
Cockroach	14	1	7.1
Candida spp.	30	3	10.0
Aspergillus spp.	30	2	6.7
Cladosporium spp.	30	1	3.3
Penicillium spp.	30	4	13.3
Alternaria spp.	30	1	3.3

*Allergen discs were prepared using local housedust extract.
**Allergen discs were obtained from Pharmacia Diagnostics, Sweden.

Table 4 Reactions to allergen injections during hyposensitisation

Type of reaction	No. of cases	%
Asthma	160	1.3176
Asthma and urticaria	5	0.0412
Anaphylactic shock	3	0.0247
Urticaria	3	0.0247
Rhinitis	1	0.0082
Rhinitis and asthma	1	0.0082
Rhinitis and conjunctivitis	1	0.0082
Rhinitis and urticaria	1	0.0082
Conjunctivitis	1	0.0082
Subtotal	176	1.4494
No reaction	11,967	98.5506
Total	12,143	100.0000

*Because 533 cases were treated and a reaction occurred in 102 cases, the incidence of reaction per person was 19.13 per cent

24 per cent occurred when the dose was the same as at the previous visit and 16 per cent occurred even when the size of the dose was decreased due to a previous reaction.

It is interesting to note that 72.1 per cent of housedust-sensitive individuals were also RAST-positive to housedust when the allergen discs coupled with locally collected housedust were used, but the positive rate decreased to 53.3 per cent when commercially available allergen discs from Pharmacia Diagnostics were used. This result emphasises the absolute need for using the same allergen in performing skin testing and preparing allergen discs for RAST. This may explain the poor correlation between skin tests and RAST for moulds and cockroach dander, although non-specific irritants present in these allergen extracts might cause an unusually high skin reaction.

Because many clinical and laboratory data suggest that, in many areas of the world,[14-16] *Dermatophagoides* mites are a major source of housedust allergen, the importance of *D. farinae* in causing childhood bronchial asthma in Taiwan was studied. In this series, 90.2 per cent of the asthmatic children had a positive skin reaction to *D. farinae* and 90 per cent of the skin test-positive individuals showed a positive RAST. Furthermore, not only was there a good correlation between wheal size induced by housedust and that induced by mite dander (r=0.74, Fig. 1), there was also good correlation between housedust RAST and mite RAST (r=0.8) which was comparable to the figure of 0.87 obtained in a previous series.[7] Taken together, these data strongly indicate that *D. farinae* is an important allergen in Taiwan and that it may also be a major component of housedust antigen.

ACKNOWLEDGEMENT

The author would like to express his sincere gratitude to Miss Chuen-Horng Chen and Miss Li-Hwa Lee for their excellent technical assistance.

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Contact Sensitivity in Fully Allogeneic Bone Marrow Chimera in Mice, [B6→C3H]*

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SUMMARY **Lethally irradiated C3H mice were successfully reconstituted with bone marrow cells from allogeneic C57BL/6 mice. The lymphoid cells of such chimeric mice showed an H-2 phenotype of the bone marrow donor and responded to PHA, Con A and LPS. On the other hand, no reactivity to stimulator cells from the recipient strain was observed. The results showed that the chimeric mice had lymphoid cells of donor origin and that the lymphocytes developed the proliferative reactivity to mitogens but not to alloantigens of the recipients.**

After being sensitised by painting their skin with DNFB, these chimeric mice showed typical delayed-type hypersensitivity which was manifested by swelling of the ear. However, a previous injection of DNBS failed to induce tolerance to the relevant antigen in allogeneic chimeric mice [B6→C3H]. These findings suggest that, in this chimera, the immune system or immunological competent cells may carry some obstacles to the regulatory mechanism involved in cellular immune response to contact sensitisation with DNFB as well as the humoral response.

ASIAN PACIFIC J ALLERG IMMUN 1984; 2 : 61-66.

Despite the widespread acceptance of the fundamental effectiveness of bone marrow transplants as a therapeutic maneuver, many problems remain to be overcome.[1] Irradiated bone-marrow-chimeric mice have enabled immunologists to recognise a new facet of the complicated immune system.[2-4] In this laboratory, several aspects concerning the immunological functions of such chimeric mice have been extensively studied.[5-9] A chimeric mouse can carry bone-marrow-derived cells of donor origin and survive without difficulty even when it has been reconstituted with allogeneic bone marrow cells. Not only lymphocytes but also macrophages were observed to bear the H-2 phenotype of the bone marrow donor, indicating that macrophages also differentiate from precursor cells in bone marrow (unpublished observations).

In earlier reports,[5-8] chimeric mice reconstituted with fully allogeneic bone marrow cells were shown with few exceptions to maintain immunological response from several points of view. One exception was the failure of 2,4-dinitrofluorobenzene (DNFB), by injection with 2,4-dinitrobenzene sulphonic acid sodium salt (DNBS), to induce a tolerant state to contact sensitisation; this was observed in irradiated AKR mice reconstituted with C57BL/6 (B6) bone marrow cells ([B6→AKR]).[5] Another exception was seen in the primary humoral response to sheep erythrocytes in [BALB/c→A/J][8] as well as in [B6→AKR].[5] These chimeric mice were shown not to have produced any antibodies upon primary immunisation with sheep erythrocytes; subsequent studies showed that antibody response was detected at a significant level only when recipient mice shared the K-end of the H-2 region with the bone marrow donor strain.[6,8]

In this report, C3H mice were demonstrated to be reconstituted with bone marrow cells from B6 mice; thereafter, it was shown that such [B6→C3H] chimeric mice

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Supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan (Nos. 57770279, 58480184 and 58770365).

were successfully sensitised by painting with DNFB, and that they did not develop tolerance to this sensitising agent by a prior intravenous administration of DNBS as was the case with [B6→AKR] chimeric mice.[5]

MATERIALS AND METHODS

Mice

Female B6 and C3H mice were obtained from Shizuoka Laboratory Animal Corporation (Hamamatsu, Japan). All except the chimeric mice were 6-12 weeks old. The mice were housed in laminar flow racks equipped with a HEPA filter and plastic walls to prevent microbial infection between the cages. Sterilised water and a conventional diet were given *ad libitum*.

Bone marrow chimera

Chimeric mice were prepared as described earlier.[5,6,10] Female mice 8-12 weeks old were used as recipients; mice 7-10 weeks old, as bone marrow donors. Recipient mice were placed in a plastic box and irradiated at a dose of 40 R/min with a total of 880-900 R of X-ray from a Toshiba KXC-2-18-2 source that was equipped with a 0.5 mm Cu and 0.5 mm Al filter. On the following day, bone marrow cells were obtained from the femur, tibia and humerus of donors. The cells were treated with monoclonal anti-Thy 1.2 antibody (F7D5, Olac) without complement. The retro-orbital sinus of each irradiated recipient mouse was injected with 25 x 10^6 bone marrow cells from the donors. All chimeric mice were allowed to rest more than eight weeks before immunisation; during first three weeks after irradiation, they were given 1 mg/ml of Terramycin (Taito-Pfizer Inc., Tokyo) in their drinking water. The chimera of the C3H mice reconstituted with bone marrow cells from the B6 mice is expressed as [B6→C3H], B6 with B6 cells as [B6→B6], and C3H with C3H cells as [C3H→C3H].

Cytotoxic test

All chimeric mice supplied for the experiment were analysed on chimerism of lymphoid cells by using the cytotoxic test as described earlier.[5] Lymphoid cells were analysed for the H-2 phenotype. Spleen or lymph node cells were incubated with anti-H-2^b (E^k anti-EL-4), anti-H-2^k (hybridoma 5R3) or medium 199, followed by incubation with rabbit complement. (These antibodies were provided by Doctors F.W. Shen, E. Nakayama, N. Tada, and U. Hammerling of the Sloan-Kettering Memorial Cancer Center, U.S.A.). The percentage of dead cells was evaluated by the dye-exclusion method with 0.2% trypan blue; the cytotoxic index (CI) was calculated as follows:

$$CI = \frac{(\%\text{ of dead cells in experiment}) - (\%\text{ of dead cells in controls})}{100 - (\%\text{ of dead cells in controls})} \times 100$$

Mitogen response

Spleen cells (5 x 10^5) were cultured with 1 μl/ml of phytohaemagglutinin (PHA; Difco Laboratories, Detroit), 5 μg/ml of concanavalin A (Con A; Sigma Chemical Co., St. Louis) or 25 μg/ml of *E. coli* lipopolysaccharide (LPS; Difco Laboratories, Detroit) in 200 μl of RPMI 1640 supplemented with 10% foetal calf serum, 100 Units/ml of penicillin and 100 μg/ml of streptomycin in a round bottom 96-well plate (A/S NUNC, Denmark). The plate was cultured in a humidified 5% CO_2 atmosphere at 37°C for 72 hours. Twenty hours before harvest, 0.5 μCi of [methyl-^{3}H] thymidine (NET-027, New England Nuclear, Boston) was added to each well. Cells were harvested on a glass filter; the level of radioactivity incorporated by the cells was counted by a liquid scintillation counter. Arithmetic means of triplicate cultures were presented in counts per minute (cpm).

Mixed lymphocyte reaction

The 5 x 10^5 spleen cells were cultured with the same number of stimulator cells in a round bottom 96-well plate. Stimulator spleen cells were irradiated with 2,000 R of X-ray before culture. The plate was cultured in a humidified 5% CO_2 atmosphere at 37°C for 96 hours, with 0.5 μCi [methyl-^{3}H] thymidine added during the final 20 hours. Cultures were harvested onto a glass filter and processed for liquid scintillation counting. Arithmetic means of triplicate cultures were presented in cpm.

Contact sensitivity to DNFB

DNFB and DNBS were obtained from Wako-Junyaku Co., Tokyo. Mice were sensitised with DNFB by painting them twice daily with 25 μl of 0.5% DNFB on the shaved abdomen. Five days after the first painting, the mice were challenged with 20 μl of 0.2% DNFB painted on the right ear and with 20 μl of vehicle (4:1 acetone: olive oil) applied to the left ear as control. Twenty-four hours later, the thickness of each ear was measured with a dial thickness-gauge (Model G, Peacock Co., Japan). The response of mice to contact sensitisation was evaluated in terms of ear swelling, which was calculated by subtracting the thickness of the left ear from thickness of the right ear and expressing the results in units of 10^{-2} mm.

In some experiments, contact sensitivity was assayed *in vitro* in addition to *in vivo* with ear swelling. Regional lymph node cells (5 x 10^5) were incubated with 20 μg or 40 μg of DNBS in a round bottom 96-well plate for 72 hours, which procedure was followed by the same as that previously described for determining mitogen response.

Tolerance to DNFB was induced by intravenous injection of DNBS (750 mg/kg) according to Phanuphak *et al.*[11] Seven days later, the mice were sensitised with DNFB,

followed by a challenge as previously described.

RESULTS

Bone marrow chimera

After 8-12 weeks of rest, chimeric mice were supplied for the experiment; all chimeras were evaluated for chimerism, proliferative response to mitogens and reactivity to the donor and recipient strains of bone marrow transplants. Spleen cells were estimated for H-2 phenotype by means of a cytotoxic test. As seen in Table 1, [B6→C3H] mice showed cytotoxic index (CI) values of 72 per cent on H-2^b and 3 per cent on H-2^k compared with normal B6 mice (74% on H-2^b and 6% on H-2^k). Syngeneic chimeric mice, irradiated B6 (H-2^b) or C3H (H-2^k) mice reconstituted with bone marrow cells from B6 ([B6→B6]) or C3H ([C3H→C3H]) strain also showed CI comparable to normal B6 or C3H mice.

Mitogens were employed for the sake of evaluating the maturity and nonspecific proliferative response of lymphoid cells in chimeric mice. Incorporation of ^{3}H-thymidine in response to T-cell mitogens (PHA, Con A) and B-cell mitogens (LPS) are shown in Table 2. As can be easily seen, there was a significant level of response of [B6→C3H] chimeric mice as well as [B6→B6] and[C3H→C3H] syngeneic chimeric mice to PHA, Con A and LPS. Although both net cpm and the stimulation indices in the [B6→C3H] mice were relatively low in this experiment, satisfactory responses were observed in repeated experiments (data not shown).

Whether lymphoid cells in chimeric mice could respond with proliferation to alloantigens (expressed on spleen cells from donor and recipient strains) was examined by mixed lymphocyte reaction (MLR). Table 3 shows that [B6→C3H] chimeric mice responded to the recipient strain, C3H, with 3,554 cpm, in contrast with the response of normal B6 to C3H mice (14,687 cpm). Similar responses were observed in [C3H→B6] and C3H mice.

Contact sensitivity and tolerance induction to DNFB

As shown in Table 4, [B6→C3H] chimeric mice were successfully sensitised by painting with DNFB. The thickness of the ear increased following a challenge with the same antigen (5.4 x 10^{-2} mm) was comparable to the reaction of the B6 mice (4.1 x 10^{-2} mm); regional lymph node cells from sensitised [B6→C3H] mice synthesised DNA in culture with antigen (17,718 cpm) was also comparable to that of B6 (12,837 cpm). Although lymph node cells of sensitised C3H mice showed significant proliferation without antigen (31,118 cpm), additional incorporation of ^{3}H-thymidine was observed in the presence of DNBS (73,639 cpm). Succeeding studies demonstrated that sensitised lymph node cells of certain strains significantly proliferate in the absence of antigen (data not shown), indicating that *in vitro* activity to proliferation may vary with the strain, although the meaning of this phenomenon remains unknown.

Similar *in vivo* manifestation of contact sensitivity was observed in [B6→C3H] chimeric mice (3.0 x 10^{-2} mm) as well as B6, [B6→B6], [C3H→C3H], and C3H mice (Table 5). Injection with DNBS is known to render a mouse tolerant to subsequent immunisation with DNFB.[12,13] As shown in Table 5, this was the case with B6, [B6→B6], [C3H→C3H] and C3H mice. In contrast, allogeneic [B6→C3H] chimeric mice showed significant ear swelling (4.2 x 10^{-2} mm) in spite of pretreatment with intravenous DNBS.

Table 1 H-2 phenotype of spleen cells.

Mouse	Cytotoxic Index (%)		Complement
	H-2^b	H-2^k	Control*
B6	74	6	87
[B6→B6]	71 (65-80)	0 (0-2)	87 (83-91)
[B6→C3H]	72 (66-85)	3 (0-7)	76 (69-84)
[C3H→C3H]	4 (0-8)	65 (53-78)	79 (75-83)
C3H	0	73	86

*Viability (%) of complement control.
Range of data is presented in parenthesis.

Table 2 Proliferative responses to mitogens

Mouse	Incorporation of ^{3}H-Thymidine (cpm)			
	Medium	PHA	Con A	LPS
B6	939	25,391 (27)	66,424 (71)	45,828 (49)
[B6→B6]	402	11,897 (26)	23,016 (57)	43,941 (109)
[B6→C3H]	3,958	18,300 (5)	52,370 (13)	22,534 (6)
[C3H→C3H]	2,494	29,083 (12)	94,092 (38)	66,390 (27)
C3H	1,647	51,223 (31)	158,614 (96)	58,382 (35)

Stimulation index is presented in parenthesis.

Table 3 Negative response to the donor and recipient strain in bone marrow chimera.

Mouse	Incorporation of ^{3}H-Thymidine (cpm)		
	Medium	Stimulator	
		B6	C3H
B6	4,137	1,863	14,687
[B6→C3H]	2,931	2,089	3,554
[C3H→B6]	5,220	5,514	3,826
C3H	6,163	13,651	5,828

Table 4 Contact sensitivity to DNFB in [B6-C3H] chimera *in vivo* and *in vitro*.

Mouse	Sensitisation with DNFB	Ear swelling ($x10^2$ mm) mean ± S.D.	Incorporation of ^{3}H-Thymidine (cpm)	
			medium	DNBS (20 μg)
B6	+	4.1 ± 1.4	3,500	12,837
B6	–	0.1 ± 0.3	1,387	2,577
[B6→C3H]	+	5.4 ± 1.2	1,534	17,718
C3H	+	7.6 ± 0.5	31,118	73,639
C3H	–	0.5 ± 0.2	4,629	3,936

Table 5 Contact sensitivity and tolerance induction to DNFB.

Mouse	Prior injection of DNBS	Ear swelling ($x\ 10^2$ mm) mean ± SD
B6	–	4.8 ± 0.8
B6	+	1.1 ± 1.2
[B6→B6]	–	8.1 ± 2.9
[B6→B6]	+	1.2 ± 0.2
[B6→C3H]	–	3.0 ± 1.4
[B6→C3H]	+	4.2 ± 2.6
[C3H→C3H]	–	10.0 ± 1.4
[C3H→C3H]	+	1.5 ± 0.7
C3H	–	18.7 ± 2.9
C3H	+	1.6 ± 1.4

DISCUSSION

In the present study, irradiated C3H mice reconstituted with bone marrow cells from allogeneic B6 strain mice, [B6→C3H], were employed to examine contact sensitivity to DNFB, using a typical model of cell-mediated immunity in mice. The method for producing irradiated bone marrow chimera had already been established in this laboratory.[5,6,10] Difficulties due to graft-vs-host reaction by T-lymphocytes were overcome by means of pre-treating bone marrow cells with anti-Thy 1 antibody in the absence of complement. Although T cells were not killed by this treatment, T cells and T-cell function to promote antibody production were not detected in irradiated recipients following adoptive transfer of lymphocytes treated in such a way.[10]

As a routine, chimeric mice supplied for experimentation are evaluated on some of the following points on or after use: Firstly, whether lymphoid cells are derived from the donor strain; secondly, whether spleen cells respond with proliferation to T- and B-cell mitogens; and thirdly, whether spleen cells remain tolerant to the donor and recipient strains. Derivation of lymphoid cells in chimeric mice is estimated according to H-2 or Thy 1 phenotypes expressed on the cells, assuring that H-2 or Thy 1 genotypes direct the relevant phenotypes. As clearly presented in Table 1, spleen cells of [B6→C3H] mice manifested H-2^b antigen on their surface, indicating that lymphoid cells in [B6→C3H] had been replaced by the cells derived from bone marrow cells of B6 mice. Macrophages in peripheral blood, spleen, lymph node, liver, lung and peritoneal cavity of [B6→C3H] mice also showed the H-2^b phenotype (to be published). Taken together, the major components of the immune system (lymphocytes and macrophages) were indicated to be reconstituted by transplanted bone marrow cells in allogeneic [B6→C3H] chimeric mice. Furthermore, spleen cells of [B6→C3H] mice showed comparable proliferative responses with normal B6 and C3H mice after stimulation of both T-cell (PHA, Con A) and B-cell mitogens (LPS) (Table 2). Such results suggest that lymphoid cells in [B6→C3H] mice retain the capability of proliferating in response to non-specific stimulators in the same fashion as B6 and C3H mice. On the other hand, no proliferation was observed in [B6→C3H] spleen cells when they were cultured with irradiated stimulator cells of B6 or C3H strain mice (Table 3). This suggests that the spleen cells of the chimera were tolerant to the alloantigens of both the donor and recipient strains. Other experiments demonstrated that the spleen cells of allogeneic chimeric mice, after incorporation of ^{3}H-thymidine, responded to the alloantigens of mice other than those of the donor and recipient strains (data not shown).

The results show that the lethally irradiated C3H mice were successfully reconstituted by tran-

splantation of B6 bone marrow cells with lymphoid cells of B6 origin, preserving reactivity to T- and B-cell mitogens and alloantigens except for the donor and recipient type.

As shown in Tables 4 and 5, [B6→C3H] chimeric mice manifested contact sensitivity to DNFB *in vivo* and *in vitro*. Since sensitisation by painting with DNFB on the skin is known to evoke activation of several subgroups of T lymphocytes in addition to T_{DH}, i.e. $T_{s\text{-}aux}$[14] and T_{prlf},[15] it was indicated that at least T_{DH} and T_{prlf} were activated in the [B6→C3H] chimeric mice. In contrast to the successful sensitisation with DNFB, [B6→C3H] mice were not tolerant to DNFB following an injection of DNBS, which is known to render mice unresponsive to subsequent sensitisation with DNFB owing to the action of suppressor T cells.[16] Failure to induce a tolerant state was also observed in [B6→AKR] mice as reported earlier.[5]

From these findings, three problems remain to be answered. Firstly, are these features, i.e. successful sensitisation and the failure of intravenous DNBS to induce tolerance with regard to contact sensitivity to DNFB, common in allogeneic bone marrow chimera in mice? Secondly, why were the allogeneic [B6→C3H] chimeric mice sensitised successfully? And thirdly, why was tolerance not induced in the [B6→C3H] mice?

Until contact sensitivity is estimated for many kinds of allogeneic chimeric mice, the first question will remain unanswered. However, preliminary studies have shown that [BALB/c→B6] and [BALB/c→C3H] chimeric mice become tolerant after injection of DNBS. Therefore, it is possible that contact sensitivity response differs in chimeric mice depending on the combination of strains in a bone marrow transplant.

T lymphocytes in chimeric mice are thought to differentiate adaptively in the host micro-environment and to differ in any sense from T cells of normal donor strains.[3,4,17] On the other hand, T cells which mediate delayed-type hypersensitivity were divided into H-2K-, D- and I-restricted subpopulations on DNP[18] and NP.[19] Nevertheless, it must be determined whether this is a general rule in cellular immunity; the second problem may have to be resolved from such a point of view. At present, the possibility that H-2K- and D-restricted T cells are activated by DNP-modified keratinocytes in [B6→C3H] chimeric mice is argued without any evidence, because the chimera T cell is thought to recognise the $H\text{-}2^k$ phenotype as self and the $H\text{-}2^k$ phenotype is manifested on keratinocytes but not on Langerhans' cells in the skin of [B6→C3H] mice. Further studies will clarify whether or not this is the case.

The earlier observation concerning [B6→AKR] chimeric mice[5] partially answers the third question, in which the failure of tolerance induction was cleared by the employment of E^k mice (B6-$H\text{-}2^k$ congeneic mice) as bone marrow donors instead of B6 mice. For tolerance induction in [B6→AKR] mice, it was suggested that H-2 compatibility between the donor and recipient would be necessary, which may explain the observation in the case of [B6→C3H] mice. Since suppressor T cells are believed to represent their function through several interactions with other cells under the control of the H-2 gene, it is reasonable to assume that H-2 compatibility is required for tolerance induction in such allogeneic chimeric mice. However, the findings in [BALB/c→B6] and [BALB/c→C3H] mice again confuse the issue because H-2-incompatible [BALB/c→B6 or C3H] chimeric mice were tolerant following the injection of DNBS; furthermore, the tolerance was transferred adaptively with T lymphocytes (data not shown). These discrepancies will have to be explained by future studies.

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