H-2 Compatibility Required for Tolerance Induction in Contact Sensitivity to DNFB in Allogeneic Bone Marrow Murine Chimeras*

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SUMMARY **Using irradiated bone marrow murine chimeras, we analysed the H-2 compatibility required for tolerance induction to the contact sensitiser DNFB (2, 4-dinitrofluorobenzene). In our previous reports, we showed that a previous injection of DNBS (2, 4-dinitrofluorobenzene sulfonic acid sodium salt) failed to induce tolerance to the relevant antigen (DNFB) in H-2-incompatible bone marrow chimeras, but it succeeded in H-2-compatible chimeras. In the present study, we employed a number of chimeras constructed from various combinations of marrow cells from B10 H-2 recombinant strains and AKR recipient mice to evaluate fine subregion compatibility at the H-2 complex required for the induction of tolerance. We found that total H-2 compatibility was required for tolerance induction in these chimeras.**

ASIAN PACIFIC J ALLERG IMMUN 1984; 2 : 207-211.

Bone marrow transplantation is an important manoeuvre in the field of fundamental research on immunology[1-4] as well as in clinical applications.[5] This "cellular engineering" manoeuver[6] has been used with mice for the last decade; irradiated recipient mice have been reconstituted with bone marrow cells from either syngeneic, semi-allogeneic or allogeneic strains. In our laboratory, several immunological characteristics of bone marrow chimeric mice have been studied.[7-11] These characteristics include humoral immune response,[7-9] contact sensitivity,[7,10] cytotoxic T-lymphocyte (CTL) activity,[7] natural killer (NK) cell activity,[7] and granuloma formation to BCG cell-wall vaccine.[11]

The experimental system for the study of contact sensitivity in mice is well established.[12,13] Hypersensitivity, specific for a relevant hapten group, can easily be evoked following sensitisation with 2, 4-dinitrofluorobenzene (DNFB). On the other hand, when mice are injected intravenously with 2, 4-dinitrobenzene sulphonic acid sodium salt (DNBS), which shares antigenic moieties with DNFB, the mice will not show any manifestations of hypersensitivity following sensitisation with DNFB.[14] This phenomenon has been studied extensively as one of the experimental models for "immunological tolerance" and it is well established that suppressor T cells are responsible for the tolerance induction.[15]

We previously reported that H-2-incompatible bone marrow chimeras were unable to induce immunological tolerance to DNFB following intravenous injection with DNBS.[7,10] From these data, we postulated that some of the pathways involved in the induction of tolerance did not work in these animals as had been shown in the system of primary antibody response to sheep erythrocytes.[7,8] These pathways may include macrophage-T cell interaction, T-T cell interaction(s) and soluble factor(s)-T cell interaction(s); some of these interactions have been demonstrated to work under the control of the H-2 gene (H-2 restriction).[16-18] In the present study, we

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This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 57770279, 58480184, 58770365).

prepared a number of chimeras constructed from various combinations of marrow cells from B10 H-2-recombinant mice and AKR recipients to evaluate fine histocompatibility between donor and recipient animals required for the induction of tolerance to DNFB. The results strongly suggest that in this experimental system bone marrow recipients must share the total H-2 region with bone marrow donors in order to show immunological tolerance following intravenous injection with DNBS.

MATERIALS AND METHODS

Mice

Female B10. A(2R), B10. A(5R), B10. AKM and AKR were purchased from Jackson Laboratory, Bar Harbor, Maine, U.S.A. Female B10. A(3R), B10. A(4R) and B10. AQR were produced in our animal faculty. First, two strains were originated from the breeding pairs kindly previded by Dr. T. Hamaoka of Osaka University and the B10. AQR mice were provided by Dr. K. Okuda of Yokohama City University. Mice 8-12 weeks old were used as irradiated recipients; mice 7-10 weeks old, as bone marrow cell donors. All mice were maintained on sterilised water and a conventional diet *ad libitum*.

Bone marrow chimeras

Chimeric mice were prepared as described previously.[7,10,19] Briefly, recipient mice were irradiated with 880-900 R of X-radiation and injected with 25 x 10^6 bone marrow cells pretreated with monoclonal anti-Thy 1.1 (clone T11D7e, Olac Ltd., England) or anti-Thy 1.2 (clone F7D5, Olac). All chimeric mice were allowed to rest more than eight weeks prior to analyses for chimerism and contact sensitivity. (For simplicity, an irradiated AKR chimera reconstituted with B10.A(2R) cells is referred to herein as [B10.A(2R) → AKR]; other chimeras established with different combinations are referred to according to this nomenclature.)

Cytotoxic test

All chimeric mice were analysed for chimerism by the cytotoxic test as described earlier.[7] Thymocytes were incubated with anti-Thy 1.1, anti-Thy 1.2 or medium 199 followed by incubation with rabbit complement. The percentage of dead cells was evaluated by using the dye-exclusion method with 0.2% trypan blue; the cytotoxic index (C.I.) was determined according to the following formula:

$$\text{C.I.} = \frac{(\%\text{ of dead cells in experiment}) - (\%\text{ of dead cells in complement control})}{100 - (\%\text{ of dead cells in complement control})} \times 100$$

Proliferative response to mitogen

Spleen cells (5 x 10^5) were cultured with 1 μl/ml of phytohaemagglutinin (PHA) (Difco Laboratories, Detroit, U.S.A.), 5 μg/ml of Con A (Sigma Chemical Co., St. Louis, U.S.A.) or 25 μg/ml of *E. coli* LPS (Difco Laboratories) in a 96-well round-bottom plate (A/S Nunc, Denmark) in 200 μl of RPMI 1640 (Gibco Laboratories, Grand Island, N.Y., U.S.A.) supplemented with 10% foetal calf serum (Gibco Laboratories), 100 U/ml of penicillin and 100 μg/ml of streptomycin. The plate was cultured in a humidified 5% CO_2 atmosphere at 37°C for 72 hours with 0.5 μCi [methyl-^{3}H] thymidine (NET-027, New England Nuclear, Boston, U.S.A.) 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 counts per minute (cpm).

Contact sensitivity and tolerance induction to DNFB

DNFB and DNBS were obtained from Wako-Junyaku Co., Tokyo. Sensitisation with DNFB and tolerance induction by DNBS were based on the method of Phanuphak *et al.*[14] The mice were sensitised by two daily paintings on the shaved abdomen using 25 μl of 0.5% DNFB in a 4:1 acetone:olive oil vehicle. Five days after the first painting, the mice were challenged with 20 μl of 0.2% DNFB on the right ear and with 20 μl of vehicle on the left ear as control. Twenty-four hours later, ear thickness was measured with a dial thickness gauge (Model G, Peacock C, Tokyo). The responsiveness of the mice to contact sensitisation was calculated by subtracting the thickness of the left ear from the thickness of the right ear and expressed in units of 10^{-2} mm. Tolerance to DNFB was induced by intravenous injection of DNBS (750 mg/kg) seven days before sensitisation with DNFB.

RESULTS

Chimerism by allogeneic marrow transplantation

All the chimeric mice were evaluated for chimerism when they were sacrificed for experimentation following 8-12 weeks of rest after irradiation. Thymocytes of the chimeric mice were estimated on

Table 1 Thy 1 phenotype of chimeric mice

Strain	Cytotoxic Index (%)	
	Thy 1.1	Thy 1.2
[B10. A(2R)→AKR] *	4	79
[B10. A(3R→AKR]	3	81
[B10. A(4R)→AKR]	8	76
[B10. A(5R)→AKR]	6	85
[B10.AKM→AKR]	7	74
[B10.AQR→AKR]	15	82
[AKR→AKR]	60	11
C57BL/6	0	81

*[B10. A(2R)→AKR] indicates irradiated ARK mice reconstituted with bone marrow cells of the B10. (22R) stain.

Thy 1 phenotype by means of cytotoxic test. As shown in Table 1, thymocytes obtained from AKR mice (Thy 1.1) reconstituted with B10 congeneic mice (Thy 1.2) showed a Thy 1.2 phenotype as did those from normal B6 mice. These data demonstrate that all mice were reconstituted with cells of the donor phenotype, thus were full chimeras.

Responses of spleen cells of chimeric mice to mitogens

Next, we observed proliferative responses to three kinds of mitogens (PHA, Con A, LPS) to evaluate the T cell and B cell reconstitution. Representative experiments comparing responses to these mitogens of spleen cells from six kinds of chimeras, [AKR→AKR] syngeneic marrow transplanted mice and normal B6 mice are shown in Table 2. The last group of B6 mice comprised three subgroups, which had been sensitised with DNFB or toleralised with DNBS or left untreated. As the table shows, all groups of chimeras responded at a level comparable to that of normal B6 mice, which result was consistent with previous observations on other chimeric mice.[7,10] Sensitisation with DNFB or desensitisation with DNBS had no influence on their responses.

Based on these results, it was assumed that all irradiated mice had been successfully reconstituted with bone marrow cells of donor origin and that the lymphoid cells of the mice were well developed and sufficiently mature to respond to the mitogens. These mice were then used for contact sensitivity experiments.

Contact sensitivity and tolerance induction to DNFB in chimeras

To investigate interactions of the immuno-competent cells and the host cells or cellular components, an analysis was carried out with regard to the capacity of the H-2-subregion-compatible chimeras to develop and express contact sensitivity to DNFB, using the ear swelling technique of Phanuphak *et al.*[14] As shown in previous papers,[7,10,20] in which H-2-incompatible allogeneic chimeras showed vigorous responses in contact sensitivity expression, a vigorous capacity to develop and express contact sensitivity was also present in the H-2-subregion-compatible chimeras (data not shown).

Analyses to evaluate the capacity to produce specific tolerance to DNFB by intravenous administration of DNBS before the sensitising application of DNFB gave a different result. Ear swelling data on these chimeric mice are presented in Table 3 together with the H-2 map of the donor strains. Underlines in the H-2 map indicate histocompatible regions between donor and recipient strains. As may be seen in the table, tolerance was successfully induced in syngeneic [AKR→AKR] mice. However,

Table 2 Proliferative responses to mitogens in chimeric mice.

Strain	Incorporation of ^{3}H-Thymidine (cpm)			
	Medium	PHA	Con A	LPS
[B10. A(2R)→AKR]*	2,634	9,879	28,174	20,034
[B10. A(3R)→AKR]	2,697	12,220	28,793	17,477
[B10. A(4R)→AKR]	2,459	21,132	31,138	19,843
[B10. A(5R)→AKR]	1,109	5,836	31,205	15,351
[B10. AKM→AKR]	1,289	4,455	32,891	13,730
[B10. AQR→AKR]	1,461	10,257	33,682	13,547
[AKR→AKR]	3,763	9,447	37,145	21,790
C57BL/6 (sensitised)	2,324	14,443	44,386	14,338
C57BL/6 (tolerant)	952	12,936	54,200	16,898
C57BL/6 (normal)	784	9,722	46,388	15,092

*See footnote in Table 1.

Table 3 Tolerance induction requiring total H-2 compatibility between donor and recipient mice.

Strain	H-2*							No.	DNBS	DNFB	Ear swelling (x 10^2 mm)
	K	A	B	J	E	C	D		iv+	sens+	mean ± s.d.
[B10. A(2R)→AKR]†	*k*	*k*	*k*	*k*	*k*	d	b	6	+	+	7.0 ± 3.0
[B10. A(3R)→AKR]	b	b	b	b	*k*	d	d	6	+	+	12.8 ± 3.5
[B10. A(4R)→AKR]	*k*	*k*	b	b	b	b	b	6	+	+	13.0 ± 4.1
[B10. A(5R)→AKR]	b	b	b	*k*	*k*	d	d	7	+	+	7.7 ± 5.3
[B10. AKM→AKR]	*k*	*k*	*k*	*k*	*k*	*k*	q	5	+	+	8.0 ± 1.6
[B10. AQR→AKR]	q	*k*	*k*	*k*	*k*	d	d	5	+	+	9.8 ± 4.1
[AKR→AKR]	*k*	*k*	*k*	*k*	*k*	*k*	*k*	4	+	+	2.0 ± 0.8
C57BL/6 (sensitised)								5	–	+	12.0 ± 2.5
C57BL/6 (tolerant)								5	+	+	1.8 ± 0.8
C57BL/6 (normal)								3	–	–	1.7 ± 0.6

† See footnote in Table 1.

* H-2 maps of bone marrow donor strain are presented. Underlined regions are histocompatible between donor and recipient (AKR, H-2^k).

+ Mice were injected with DNBS seven days before DNFB sensitisation.

neither group of chimeric mice reconstituted with B10 congenic strains showed a manifestation of tolerance to DNFB, even if recipient AKR mice shared almost all H-2 regions (except for the H-2D region) with the donor strain B10. AKM, [B10.AKM→AKR].

DISCUSSION

In our previous reports,[7,10] it was demonstrated that [B6→C3H] and [B6→AKR] H-2- incompatible chimeras developed and expressed contact sensitivity to DNFB. However, those mice appeared to be completely unable to develop the specific unresponsiveness to stimulation with DNFB by the intravenous administration of DNBS. By contrast, AKR mice reconstituted with H-2-compatible bone marrow cells from E^k mice (B6 congenic mice with H-2^k) showed successful induction of tolerance.[7] Since expression of tolerance to DNFB by the intravenous route is mediated by suppressor T cells,[12,13,15] it was suggested that the AKR recipient mice had to share the H-2 region with the donor strain for the suppressor T cells to inhibit the response.

In the present study, several groups of bone marrow chimeras were prepared to analyse the precise requirements of histocompatibility within the H-2 complex for tolerance-induction to DNFB. B10 congenic and B10. A intra-H-2 recombinant strains were introduced as bone marrow donors to establish such chimeras. These chimeric mice had contributed to the elucidation of fine genetic restriction in primary humoral response as described elsewhere.[8,21]

It was clearly demonstrated that tolerance was induced by DNBS only when the recipient mice were reconstituted with marrow cells from a syngeneic donor. In [B10. AKM→AKR] chimeric mice, where AKR recipients shared the greater part of the H-2 region (except for H-2D) with the B10. AKM donor, tolerance could not be demonstrated. This finding suggests that total H-2 identity between donor and recipient is required for tolerance induction. These results are in striking contrast to the findings regarding primary humoral responses to sheep erythrocytes,[8,9,21] where compatibility at the left one half of the H-2 region was shown to be sufficient to produce antibody. It seems that the apparent discrepancy is due to the difference in cell types involved in these experimental systems.

Suppressor T cell (Ts) mechanisms exerting "in contact" sensitivity to DNFB have been intensively investigated using several experimental systems, viz. Ts induced by DNBS,[14-17] Ts induced by DNP-modified spleen cells (reviewed in references 12 and 13) or Ts induced by TNBS.[18] However, there is a marked heterogeneity with regard to the nature of Ts involved in these systems, i.e. intravenous administration of DNBS induces Ts functioning in the afferent phase and TNBS activates efferent phase-acting Ts.[22] In the former system, Moorhead[15,16,23] proposed that the suppression of the sensitivity was mediated by a factor (soluble suppressor factor, SSF). Following activation of SSF-producing cells (Ts?) by DNBS, these cells release SSF after stimulation by DNFB painting. Thereafter, SSF, with H-2 antigenic moiety and binding capacity to DNP, interacts with DNFB-immune T cells resulting in a failure of effector T cells develop. For the interaction between the factor and the immune T cells, compatibility at the H-2K and/or H-2D region is required.

On acceptance of this illustration, our findings suggest that the chimeric mice in the present study carried some defects in these interactions. It seems to us that compatibility at the H-2K and H-2D regions between donor and recipient strains is required for the interactions to occur efficiently. However, the suppressor T-cell mechanism is made up of several steps at different stages. Furthermore, when irradiation bone marrow chimeras are employed to analyse immune functions, sometimes wide variations in responsiveness are observed; these are probably influenced by the differences in the strain combinations to establish the chimeras and in the nature of antigens used in the assay systems.[20,24] Further analyses remain to be performed.

ACKNOWLEDGEMENT

We wish to thank Miss Michiyo Konishi for her excellent technical assistance.

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