Immunology 1993 **78** 482–490

Two distinct types of cellular mechanisms in the development of delayed hypersensitivity in mice: requirement of either mast cells or macrophages for elicitation of the response

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Accepted for publication 11 October 1992

SUMMARY

Using mast cell-deficient mutant W/W^v mice and their normal counterpart we re-evaluated the significance of participation of mast cells in allergic inflammatory response. W/W^v mice developed immediate hypersensitivity (IH) footpad reaction (FPR) to a somewhat lesser degree than the normal mice, suggesting that the mast cell might amplify the response. To exert classical tuberculin (tbc) delayed-type hypersensitivity (DTH) mast cells were not an essential cellular component. Vasoactive amines were essential to develop the response, but it did not necessarily originate from mast cells. When mice were immunized with methylated human serum albumin (MHSA) emulsified in incomplete Freund's adjuvant (IFA), mast cells were required to elicit DTH FPR. This was confirmed by the lack of the response in W/W^v mice, and the restoration of FPR by local transplantation of mature mast cells into mutant mice. This mast cell-dependent (MD) DTH was different from tbc DTH as follows: mast cell dependency, macrophage dependency as revealed by ferritin sensitivity, kinetics of sensitization, effect of host's age and histopathology. Thus we concluded that there are two types of DTH in mice; one is macrophage-dependent tbc and the other is mast cell-dependent DTH. The correspondence of the DTH to the Jones-Mote (JM) DTH is discussed, although the dominance of mast cells in MD DTH lesion was not observed.

INTRODUCTION

Delayed-type hypersensitivity (DTH) skin reactions are mediated by circulating sensitized CD4$^+$ T cells[1,2] that cause inflammatory skin lesions with a characteristic infiltration of inflammatory cells.[3] For the elicitation of inflammatory lesions following T-cell activation, the essential involvement of vasoactive amines in DTH response has been suggested by previous investigators, based on observations of the blocking effect of reserpine premedication on the development of the response[4,5] and on the depletion of radioactive serotonin from the cytoplasmic granules of mast cells located in DTH skin lesions.[6] With regard to the participation of mast cells in DTH response, Askenase and his colleagues reported that the DTH response in contact-sensitized mice is characterized by two subsequent T-cell activities;[7-9] an early skin swelling reaction and an ordinary delayed-type skin reaction, peaking at 2 hr and 24–48 hr after challenge, respectively. They suggested that both T-cell-dependent inflammatory reactivities require mast cells to elicit the response.[7,10] On the other hand, employing the same experimental system, some investigators argued that mast cell-deficient mice can evoke DTH skin responses almost as much as normal control mice.[11-14]

In order to determine the mast cell requirements in murine DTH responses, we employed footpad reaction (FPR) to methylated human serum albumin (MHSA) immunized with complete Freund's adjuvant (CFA) and sheep red blood cells (SRBC) immunized i.v. or s.c., which have been proven to induce classical tuberculin (tbc) DTH in mice.[15,16] In the first part of this investigation, we showed that the tbc DTH response did not require mast cells to elicit a response.

With respect to the involvement of mast cells/basophils in DTH, two histopathologically different types of DTH response have been reported in the guinea-pig;[17-18] tbc DTH reactions and cutaneous basophil hypersensitivity (CBH). Both DTH reactions are thought to be mediated by sensitized T cells which recruit bone marrow-derived effector cells via release of lympho-

Abbreviations: CBH, cutaneous basophil hypersensitivity; CFA, complete Freund's adjuvant; DTH, delayed-type hypersensitivity; FPR, footpad reaction; HSA, human serum albumin; ΔHSA, heat-aggregated human serum albumin; IH, immediate hypersensitivity; IFA, incomplete Freund's adjuvant; MD, mast cell dependent; MHSA, methylated human serum albumin; MAO, methylated ovalbumin; +/+, littermate control of W/W^v mice; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; tbc, tuberculin; W/W^v, mast cell-deficient mice, WBB6F$_1$-W/W^v.

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kines upon antigen encounter. CBH skin lesions in the guinea-pig are characterized by a distinctive histopathology, featuring substantial accumulation of basophils in addition to mononuclear cells,[19-20] and this type of hypersensitivity is thought to be related to Jones-Mote reactions in man, where accumulation of basophils has been noted in the skin lesions of contact dermatitis.[21-23] However, the presence of this DTH response in mice is still uncertain. Earlier efforts were made by Crowle and Hu to elucidate this problem, using various sensitizing procedures.[24] However, no Jones-Mote (JM) DTH response was evident in their mice. Since then, there have been few comprehensive reports on JM DTH in mice, in spite of accumulating reports on DTH, such as its role in contact hypersensitivity. In the second part of this investigation, attempts were made to examine the evidence for another type of DTH response, which was expected to require mast cells for the development of DTH FPR.

DTH responsiveness to MHSA in mice sensitized with the antigen emulsified in incomplete Freund's adjuvant (IFA), but not in CFA, is shown to require mast cells for the elicitation of DTH response, based on observations in parallel experiments done on mast cell-deficient and mast cell-reconstituted W/W^v mice.[25] The results of this study revealed the presence of a mast cell-dependent (MD) DTH response in mice in addition to the tbc DTH. Identification of this type of DTH in mice as JM DTH is discussed.

MATERIALS AND METHODS

Mice

C57BL/6Cr were purchased from the Experimental Animal Cooperative Association of Shizuoka (Hamamatsu, Japan) and bred in the Animal Institute of Shimane Medical University (Izumo, Japan). Mast cell-deficient mice, $WBB6F_1$-W/W^v, and their normal littermates, $WBB6F_1$-$+/+$, were raised and maintained in the Animal Center of the Cancer Research Department of Osaka University (Osaka, Japan). Mice from 3 to 4 months old were age and sex matched for each experiment.

Antigen

Crystallized human serum albumin (HSA) and crystallized ovalbumin (OVA) were purchased from Nutritional Biochemical Co. (Cleveland, OH). MHSA and methylated OVA (MOA) were prepared by methylating the carboxyl group by the methanol–hydrochloric acid method of Crowle *et al.*[26] Heat-aggregated HSA (ΔHSA) was prepared by heating HSA solution at 85° for 25 min. SRBC in Alsever's solution were washed three times and suspended in phosphate-buffered saline (PBS) (pH 7·2).

Sensitization

Sensitization was performed as previously described.[15,27] Briefly, a protein antigen preparation, diluted or suspended to 5 mg/ml in PBS, was emulsified with an equal volume of CFA containing 3 mg/ml of killed *Mycobacteria* H37Rv, or IFA. 0·05 ml of emulsion was s.c. injected into the left hind footpad of mice. Mice were sensitized with SRBC suspended in PBS by an i.v. administration into the retro-ocular plexus of a suspension of 3×10^6 SRBC in 0·2 ml of PBS[28] or by s.c. injection of an emulsion of cell suspensions of varying concentrations with CFA or IFA.

For adoptive transfer experiments, donor mice were sensitized s.c. with MHSA emulsified in CFA or IFA, in both hind footpads.

Assay for footpad reaction

The footpad (FP) thickness was measured under a dissecting microscope before and after challenge injection. Footpad reaction (FPR) was the increment of thickness at 3 hr [immediate hypersensitivity (IH)] or 24 hr (DTH) as expressed in 1/10 mm.[15]

Pretreatment of mice

Reserpine treatment. Mice were injected with reserpine (Ciba-Geigy, Co., Zurich, Switzerland) i.p. at 2·5 mg/kg body weight 16 hr before challenge injection according to the method of Gershon *et al.*[4]

Thymectomy. Mice were thymectomized at 2 months of age, and given a total body irradiation of 800 rads 15 days later. These mice were then reconstituted with a given number of lymphoid cells on the same day of irradiation.[27]

Ferritin treatment.[16,28] Six times crystallized, cadmium-free horse spleen ferritin obtained from Pentex Biochemicals (Kankakee, MI) was dialysed extensively against distilled water and PBS successively before use. The mice were injected i.p. with 0·2 ml of 0·1% ferritin solution before receiving challenge injection.

Mast cell preparations

Bone marrow-derived connective tissue-type mast cells were obtained by the method of Kitamura *et al.*[29] Briefly, the bone marrow cells obtained from C57BL/6 were cultured with Iscove's modified medium containing 10% foetal calf serum (FCS) and 20% pokeweed mitogen (PWM)-stimulated spleen cell culture supernatant. Half of the culture medium was changed once a week. One month later, the cultured bone marrow cells were harvested. At the time of harvest, almost all cells had differentiated into mature mast cells containing typical granules in their cytoplasms.

T-cell depletion

The lymphoid cells from C57BL/6 mice sensitized with MHSA and IFA were treated with rabbit anti-thymocyte antisera (ATS) and rabbit complement (Cedarlane Lab., Ontario, Canada).[27]

Adoptive transfer of immunization

Sensitized lymphoid cells, a mixture of spleen and popliteal lymph node cells (4:1) prepared from immunized mice, were adoptively transferred i.v. into naive C57BL/6 mice. After an interval of a few hours recipient animals were i.d. challenged with MHSA in the right FP.

Histology

The skin lesion of FPR was examined by routine histopathology of haematoxylin and eosin stain or by toluidine blue or Bismark Brown staining to detect mast cells.

Statistics

Standard errors and the means and *P*-values, if necessary, were calculated using the Student's *t*-test.

Table 1. Immediate and delayed footpad response of W/W^v and control mice on day 12 after immunization with protein antigen emulsified in CFA

Mice	Antigen		Reserpine injection*	FPR†	
	Sensitization	Test		3 hr	24 hr
W/W^v	MHSA	MHSA	−	3·9±0·7 [$P>0·5$]	11·2±1·2 [$0·4>P>0·2$]
	MHSA	MHSA	+	1·6±0·3	2·5±0·8 ($P<0·001$)
	MHSA	MOA	−	1·9±0·6	0·5±0·3
	ΔHSA	HSA	−	14·3±2·0 [$0·05>P>0·025$]	8·4±1·9 [$0·2>P>0·1$]
	ΔHSA	OA	−	1·0±0	1·0±0·3
+/+	MHSA	MHSA	−	3·6±0·5	13·0±1·2
	MHSA	MHSA	+	2·4±0·2	3·5±1·1 ($P<0·001$)
	MHSA	MOA	−	2·9±0·6	1·2±0·3
	ΔHSA	HSA	−	19·9±1·0	10·6±1·5
	ΔHSA	OA	−	2·0±0·6	1·0±0·3

* Reserpine was injected i.p. at a dose of 2·5 mg/kg body weight 16 hr before antigen challenge.
† Each value represents an average of five or six mice±SE.
[ ] Comparison between W/W^v and control mice.
( ) Effect of reserpine pretreatment.

Table 2. DTH to SRBC on day 4 of sensitization in W/W^v mice and control mice

Mice	Sensitization*	Reserpine†	FPR (mean±SE)	
			3 hr	24 hr
W/W^v	+	−	3·7±0·6	6·0±0·6 [$0·2>P>0·1$]
	+	+	3·0±0·6	2·4±0·4 ($0·005>P>0·001$)
	−	−	4·2±0·9	2·0±1·0
+/+	+	−	5·1±0·5	8·6±1·3
	+	+	6·0±0·5	1·6±0·5 ($P<0·001$)
	−	−	4·0±0·4	2·3±0·3

* Mice were immunized by i.v. injection of 3×10^6 SRBC.
† Mice were pretreated by i.p. injection with reserpine at 2·5 mg/kg body weight 16 hr before challenge.
[ ] Comparison between W/W^v and control mice.
( ) Effects of reserpine.

RESULTS

Mast cell independence in the induction and expression of allergic mouse footpad reaction

Mast cell-deficient W/W^v mouse could mount both delayed and immediate FPR to soluble protein antigens

As shown in Table 1 W/W^v mice immunized with MHSA emulsified in CFA fully developed delayed FPR similar to that of +/+ mice. These mice were equally sensitive to reserpine which was injected i.p. 16 hr before antigen challenge to delete tissue vasoactive amines. The expression of immediate FPR in W/W^v mice induced by immunization with heat-aggregated HSA and CFA was slightly weaker than those in +/+ mice. These results indicate that W/W^v as well as +/+ mice can mount cell-mediated and humoral immune responses and express delayed and immediate FPR to MHSA and HSA, respectively.

W/W^v mice could also develop DTH against particulate antigen, SRBC, by either i.v. or s.c. injection with CFA or IFA

We further confirmed that immediate and DTH responses could be induced against different forms of antigen and by different methods of immunization. Table 2 shows that both W/W^v and +/+ mice could effectively develop DTH to SRBC by i.v. administration of low doses of the antigen. Reserpine again inhibited the expression of delayed FPR.

Both immediate and delayed FPR were assessed in the groups of mice immunized with s.c. injections of varying doses of SRBC emulsified with either CFA or IFA. No difference in the pattern of response was observed by the use of different forms of adjuvant (Fig. 1). Also no difference in the degree of delayed FPR was seen between the two groups of mice, although there was a difference in the optimal dose that gave the highest response. Histopathological examination demonstrated that essentially the same feature of DTH inflammation was observed in the skin lesion of both groups of mice except the absence of

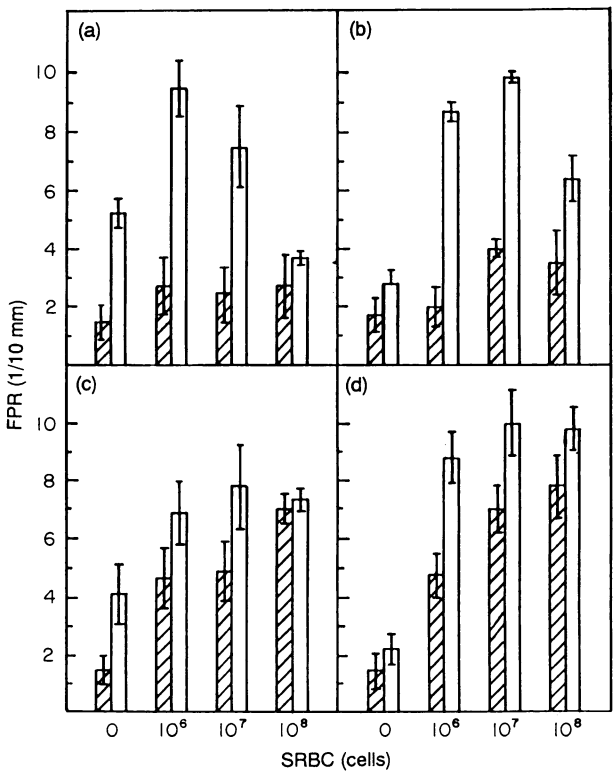

Figure 1. IH (▨) and DTH (□) to SRBC of W/W^v (a, b) and +/+ (c, d) mice sensitized s.c. with varying doses of SRBC emulsified in either CFA (b, d) or IFA (a, c) 12 days previously. All mice were challenged with 10^8 SRBC suspended in 0·05 ml of PBS i.d. Each group consists of five or six mice. Vertical bars are SE.

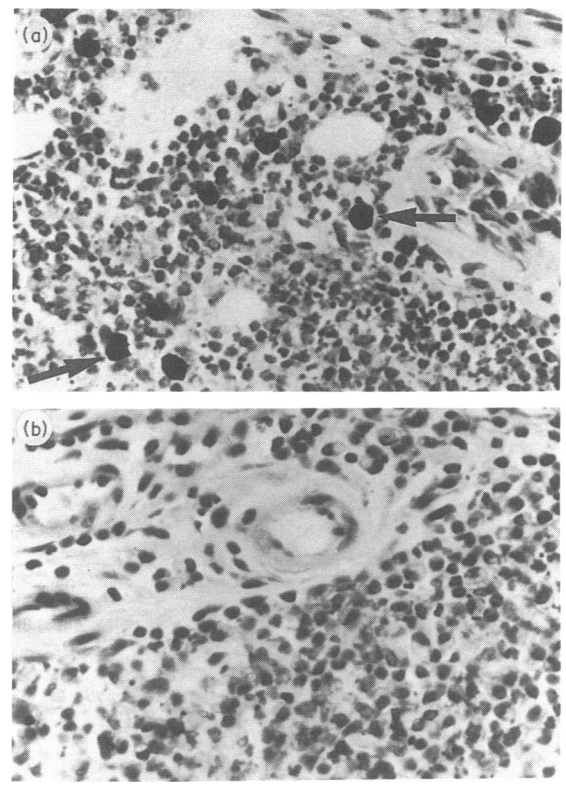

Figure 2. Histopathology of the DTH response to SRBC in +/+ (a) and W/W^v (b) mouse sensitized s.c. with 10^6 SRBC emulsified in IFA 13 days previously. FP skin lesions were taken at 24 hr after challenge with 10^8 SRBC. Sections were stained by May-Grünwald-Giemsa stain. Massive mononuclear cells and granulocyte infiltrations were observed equally in both W/W^v and +/+ mice lesions except for the appearance of basophilic granule-positive cells (arrow) in the lesion of the latter group. Macrophages with SRBC debris in their cytoplasm around the SRBC deposition in the lesions appeared equally in both groups.

mast cells in the lesion of W/W^v mice (Fig. 2). On the other hand more prominent immediate FPR was induced in +/+ mice than in W/W^v mice immunized with higher doses of the antigen regardless of the form of the adjuvant.

Presence of MD delayed FPR in mice

Immunopathologically two different kinds of DTH are recognized, i.e. tbc and JM in humans or cutaneous basophil hypersensitivity (CBH) in guinea-pigs. DTH FPR examined hitherto in the present paper had been shown to be of the tbc type.[27,28] On the other hand, no particular models for JM hypersensitivity response have been reported in experimental mice systems. Since CBH, which is considered to correspond to JM responses in humans, can be induced by immunizing guinea-pigs with proteins antigen emulsified in IFA, we further examined the hypersensitivity response of W/W^v and +/+ mice immunized with MHSA along with IFA.

W/W^v mice could not express DTH FPR at 3 to 4 weeks post-immunization with MHSA and IFA

Figure 3 shows the kinetics of sensitization of +/+ and W/W^v mice when immunized with MHSA and IFA. A group of +/+ mice gradually sensitized and developed moderate IH and DTH FPR to the antigen at days 21–28 after immunization. W/W^v mice, however, showed only minimal IH FPR and moreover their DTH FPR was almost 0 at and after day 21 of sensitization. Although the 24-hr FPR of +/+ mice was a feeble

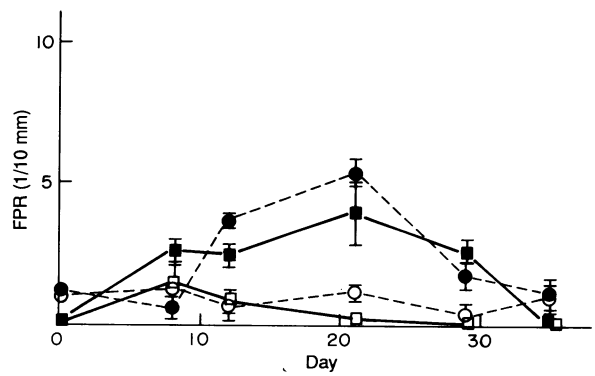

Figure 3. Kinetics of footpad reaction to MHSA in W/W^v (○, □) and +/+ (●, ■) mice sensitized with the antigen emulsified in IFA. Immediate hypersensitivity (– – – –) assessed 3 hr after challenge and delayed hypersensitivity (——) at 24 hr are shown.

Table 3. Delayed and immediate footpad response in W/W^v and control mice on day 28 of sensitization; effect of adjuvant on sensitization

Exp.	Sensitization	Challenge	Mouse	No. of mice	FPR (mean ± SE) 3 hr	24 hr	
1	MHSA+IFA	MHSA	+/+	5	1·8±0·5	2·6±0·5	
	MHSA+IFA	MHSA	W/W^v	5	0·4±0·2	0	
	MOA+IFA	MHSA	+/+	5	1·2±0·5	0	
	MOA+IFA	MHSA	W/W^v	5	1·0±0·5	0	
	MHSA+CFA	MHSA	+/+	5	2·8±0·6	7·2±1·3	($P<0·1$)
	MHSA+CFA	MHSA	W/W^v	5	2·2±0·7	3·8±0·5	
	ΔHSA+IFA	HSA	+/+	5	13·6±1·5	1·2±0·4	
	ΔHSA+IFA	HSA	W/W^v	4	2·8±1·2	0	
2	MHSA+IFA	MHSA	+/+	7	ND	4·6±0·6	($P<0·001$)
	MHSA+IFA	MHSA	W/W^v	6	ND	0·7±0·3	
	MHSA+IFA	MHSA	C57BL/6	7	4·3±0·8	8·8±0·7	($P<0·01$)*

* Comparison between 3 and 24 hr FPR.
ND, not determined.

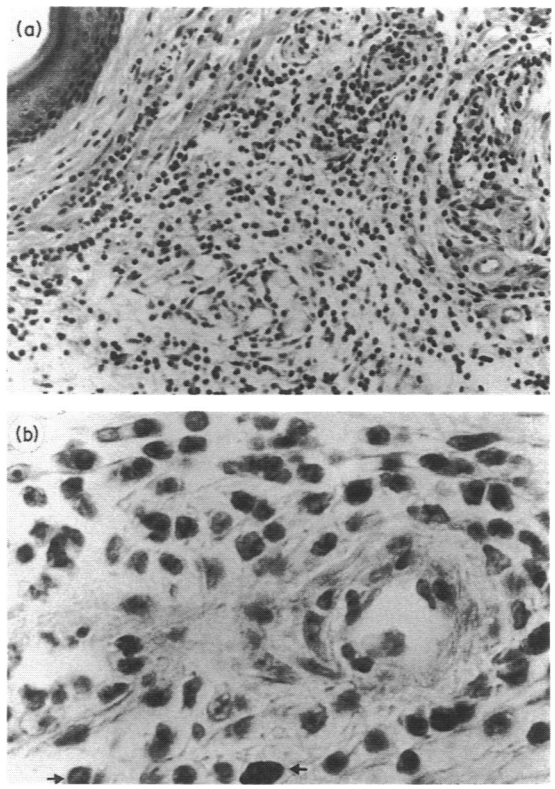

Figure 4. Histopathology of MD DTH in C57BL/6 mice sensitized with MHSA and IFA 28 days previously. FP skin lesions were taken at 24 hr after challenge. Sections were stained by Bismark Brown to detect mast cells. Dense mast cell infiltrations were not observed in the lesions but sparsely distributed mast cells (arrow) were observed.

Table 4. Delayed footpad reaction in W/W^v mice locally transferred with mast cells before sensitization with MHSA and IFA

Mouse	Mast cell inoculation*	No. of mice	FPR at 24 hr (mean ± SE)
+/+	No	6	1·7±0·7
W/W^v	No	5	0·6±0·2
W/W^v	Yes	6	2·5±0·2†

* W/W^v mice were injected with cultured mast cell to right hind footpad intradermally 2 weeks before MHSA and IFA sensitization and received challenge injection into the same footpad 28 days later.

† FPR is not significantly different from that of +/+ mice and significantly different from that of mast cell uninoculated W/W^v mice ($P<0·001$).

response, the difference between these two groups in their ability to mount the response was clearly shown (Table 3, exps 1 and 2). Furthermore C57BL/6 mice developed substantially larger 24-hr FPR than 3-hr FPR (Table 3, exp. 2), suggesting this 24-hr FPR was a true DTH response and not a residual inflammation of an IH response. Since this DTH response contrasted with tbc DTH, i.e. W/W^v mice immunized to the same antigen along with CFA developed moderate DTH FPR (the difference was not statistically significant compared to +/+ mice) even a long time (day 28) after sensitization (Table 3); we further looked into the possibility that it might be MD DTH of mice.

Histopathologically, the lesion was more exudative than that of the tbc type, and the inflammatory cells were composed of mononuclear cells and granulocytes. Mast cells were, however, sparsely distributed in the lesion and were not a major constituent (Fig. 4).

Table 5. Adoptive transfer of DTH by lymphoid cells from mice immunized with MHSA and IFA

		Footpad swelling†	
Donor*	Recipient	FPR (mean ± SE)	No. of mice with positive inflammation/ no. of mice tested‡
+/+	+/+	1·4 ± 0·2	5/5
W/W^v	+/+	1·6 ± 0·2	5/5
+/+	W/W^v	0	0/4
W/W^v	W/W^v	0	0/5

* Donor female mice aged 3·5 months were sensitized with MHSA and IFA 28 days before harvest of popliteal lymph node and spleen cells.

† Recipients received challenge injection at left hind footpad a few hours after i.v. injection of 10^8 lymphoid cells. Sensitized lymphoid cells were a mixture of 8×10^7 spleen and 2×10^7 lymph node cells. FPR was the difference in thickness between left and right footpads which was estimated 24 hr after test injection.

‡ DTH inflammation was assessed histopathologically.

Restoration of the DTH response in W/W^v mice by a local inoculation of mature mast cells

We next tested whether the DTH response could be restored by supplementing deficient W/W^v mice with mature mast cells. A group of W/W^v mice received 1×10^6 cultured mast cells derived from normal bone marrow in the right hind FP 2 weeks before sensitization with MHSA and IFA. FPR was tested 28 days after sensitization at the same site where the mast cell was inoculated. As shown in Table 4, recipient W/W^v mice could develop the DTH FPR to a similar magnitude to that observed in +/+ mice. Thus the DTH appeared to be MD.

Immune lymphoid cells from W/W^v mice could mediate the DTH responsiveness to naive +/+ mice

To determine whether the inability of W/W^v mice to develop MD DTH was due to a deficiency of afferent or due to a deficiency of efferent limb of the response, passive transfer experiments were performed. As shown in Table 5 lymphoid cells but not sera (data not shown) harvested from either +/+ or W/W^v mice could mediate DTH FPR by adoptive transfer to naive +/+ mice but not to W/W^v mice. The FPR elicited was very small probably because cell transfer was via the i.v. route, not a local transfer, and to naive, not to immune-suppressed mice. Histopathological examination confirmed all mice which received immune cells developed discernible inflammatory lesions. If immune cells were deprived of T cells by *in vitro* treatment with anti-T-lymphocyte serum plus rabbit complement, they were rendered unable to transfer the response to syngeneic mice (Table 6). These results indicate that W/W^v mice can mount cellular immune responses against MHSA when immunized with IFA, but failed to express the T-cell-mediated FPR because of the lack of tissue mast cells.

T cells were limiting cells in the induction of MD DTH

Adult thymectomized and irradiated C57BL/6 mice were reconstituted with syngeneic bone marrow cells along with thymocytes and tested on their ability to mount MD DTH. T-cell dependency of the response was confirmed because B mice which were reconstituted without thymocytes could not develop FPR (Fig. 5). Moreover it was shown that the degree of FPR was dependent on the dose of thymocytes but not on bone marrow cells.

Administration of ferritin, a macrophagetropic agent, could differentiate MD from tbc DTH

Ferritin is a macrophagetropic agent and has been shown to inhibit expression of tbc FPR if administered to mice just before

Table 6. Effect of deletion of T cells on adoptive transfer of DTH to MHSA

	Transferred cell*			FPR
Exp.	Cell source	Dose	Pretreatment	(mean ± SE)†
1	Normal spleen cell	5×10^7	—	0‡ 0/4§
	Sensitized spleen cell	5×10^7	—	1·8 ± 0·3 5/5
	Sensitized spleen cell	5×10^7	ATS and C′	0 0/5
	Sensitized lymph node cell	5×10^7	—	1·3 ± 0·3 4/4
2	None	—	—	0 0/5
	Sensitized lymphoid mixture cell	1×10^8	—	1·4 ± 0·4 4/5
	Sensitized lymphoid mixture cell	1×10^8	—	1·0 ± 0·3 4/5
	Sensitized lymphoid mixture cell	1×10^8	ATS and C′	0 0/5

* Donors (C57BL/6 female, 10 months) were sensitized with MHSA and IFA 28 days before harvest.

† Recipients (C57BL/6 female, 3 months) were challenged by the i.d. injection of MHSA in left hind footpad a few hours after i.v. injection of either lymphoid cells. Sensitized lymphoid cells were a mixture of 8×10^7 spleen cells and 2×10^7 lymph node cells. The difference in thickness between left and right footpads was estimated 24 hr after test injection.

‡ Each value represents an average of four or five mice ± SE.

§ Number of positive mouse expressing FPR $\geqslant$ 1 over number of tested mouse.

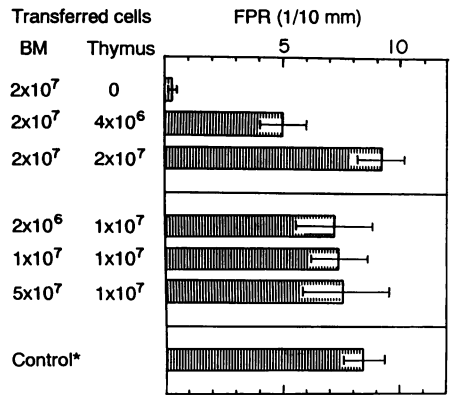

Figure 5. T-cell dependency of MD DTH to MHSA; male C57BL/6 mice were thymectomized at the age of 2·5 months, irradiated with 800 rads and reconstituted with thymus and bone marrow cells from normal syngeneic mice. FPR was tested 28 days after sensitization with MHSA and IFA. Average of five to six mice ± SE. * Non-thymectomized normal mice were sensitized.

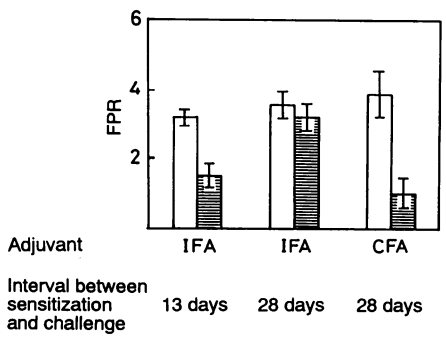

Figure 6. Effects of ferritin on MD DTH to MHSA. Six-week-old male C57BL/6 mice sensitized with MHSA and IFA or CFA received either 0·2 ml ferritin solution (1 mg/ml) (■) or PBS (□) just before challenge injection. FPR was estimated 24 hr after challenge. Average of five to six mice ± SE.

challenge injection of an antigen.[16] This is probably because accumulation of inflammatory monocytes is hampered by the treatment. C57BL/6 mice immunized with either MHSA and CFA or MHSA and IFA were injected i.p. with 0·2 mg ferritin prior to antigen challenge, to see if FPR was inhibited or not. As shown in Fig. 6 MD DTH FPR when tested on day 28 post-sensitization were not affected by ferritin treatment whereas tbc DTH induced by immunization with MHSA and CFA were inhibited. The result indicates that macrophage is not an essential component among inflammatory infiltrates of MD DTH whereas tbc DTH is macrophage dependent for its expression. Inhibition by ferritin of FPR of mice immunized with MHSA and IFA at day 13 of sensitization, however, indicates that cellular constituent might be different from that of day 28 FPR of MD DTH.

DISCUSSION

W is a mutation found on *c-kit*, an oncogene which has been reported to be an important gene in controling proliferation and differentiation of haematopoietic lineage cells.[30] We utilized mast cell deficient W/W^v mice and their normal counterpart +/+ mice to study the significance of the participation of mast cells in the development of allergic FPR.

There has been controversy on the critical role of mast cells, especially in the efferent phase of DTH response.[4,6] In the present study W/W^v mice developed both the IH and DTH responses to the selected antigen with the proper methods of immunization (Tables 1, 2, Figs. 1, 2). In the expression phase of DTH response pretreatment of mice with reserpine, a drug which deletes tissue vasoactive amines, prevented the development of DTH FPR in both groups of mice (Tables 1, 2). This indicates that vasoactive amines are essential chemical mediators to develop DTH inflammation as shown by previous investigators, but they do not necessarily come from mast cells. It is very likely that vascular endothelium and/or platelets produce these chemical mediators in response to the signals from immune competent cells.[31,32]

Discrepancy in the literature on the issues of dependency on mast cells in DTH inflammatory lesions may be a reflection of the nature of antigens and/or methods of immunization applied. The DTH FPR which we revealed to be mast cell-independent for its expression has been known to be classical tbc DTH response.[27,28] In the present paper we found that MD DTH response in mice was another form of DTH, different from the classical tbc type. The finding was dependent upon a proper selection of antigen and method of immunization. Indeed MHSA emulsified in CFA was a preferential sensitizer of cell-mediated immunity and not of humoral immunity (Table 1).[15,16,27,28] The antigen when immunized with IFA induced rather small but distinct DH on day 28 of sensitization as compared with the response observed in mice immunized with CFA (Table 3). The active participation of mast cells to this DTH response was confirmed as follows: (1) absence of FPR in W/W^v mice (Table 3); (2) failure of passive transfer of responsiveness by immune lymphoid cells from +/+ mice to W/W^v mice (Table 5); (3) successful restoration of FPR by local injection of mature mast cells to W/W^v mice (Table 4) (this indicates that inoculated mast cells remained biologically active as suggested by Nakano[29] and the expression of FPR was dependent on these cells); (4) successful transfer of the responsiveness by the immune lymphoid cell from W/W^v mice to +/+ mice (Table 5). This means that the afferent limb of the response was intact and exerted its function on mast cells of +/+ mice.[12] Thus we considered this DTH response to be MD.

Comparison of characteristics of MD DTH with those of tbc DTH makes it clear that two distinct types of DTH FPR are present in mice. (1) Both tbc and MD DTH are T-cell mediated (Table 6), but the former was sensitive to ferritin treatment and thus macrophage dependent[16] and the latter was not (Fig. 6, day 21). (2) Kinetics of sensitization was quite different (Fig. 3). In contrast to the FPR of tbc DTH which peaks at 8–12 days after sensitization, that of MD peaked later. (3) Mice in a wide range of ages, i.e. 2–24 months, showed a constant level of MD DTH FPR (data not shown), whereas aged mice (10–12 months) showed conspicuously high level of tbc DTH as compared with younger mice.[27] (4) Histopathological features were also different. Inflammatory cells of the former are mainly composed of mononuclear cells (Fig. 2), whereas those of the latter were a mixture of mononuclear cells and neutrophils (Fig. 4). Distribution of mast cells in the skin lesion, however, was not different.

It is not yet clear whether mice can develop mast cell dominant inflammatory lesions, although comprehensive

studies have been made by Crowel *et al.*[26] It is worthwhile to compare some features of MD DTH in mice described in the present paper with those of CBH in guinea-pigs because both are MD.[17,18] The DTH responses were induced by immunizing animals with protein antigens using IFA as an adjuvant. The CBH in guinea-pigs was not affected by pretreatment with carrageenan[17] but the tbc DTH was. Carrageenan exerts the same effect as ferritin on FPR,[16] and the above finding was parallel with differential effects of ferritin on MD and tbc DTH in mice (Fig. 2). Bast *et al.*[33] observed differences in the production of macrophage migration inhibitory activity *in vitro*, i.e. it was not produced by cells from animals sensitized to an antigen with IFA but produced by cells from those immunized with CFA. This might well coincide with macrophage independence of MD DTH response. On the other hand, there is a big difference in histological properties; although mast cells are dominated in the skin lesion of guinea-pigs as Dvorak named it basophil cutaneous hypersensitivity,[17-20] the lesion of the mice is composed of mononuclear cells and neutrophils (Fig. 4). These differences may reflect the extremely rare distribution of basophil leucocyte in the peripheral blood of mice;[34] hence failure to recruit such cells into the skin lesion. Another prominent difference was kinetics of sensitization; it has been shown that CBH in guinea-pigs was elicited early after immunization,[17,19,33] but MD DTH in mice was seen after 4 weeks (Fig. 3). In spite of some inconsistency at present we prefer to consider that MD DTH in mice may belong to the category of JM DTH because of its mast cell dependency. The conclusive evidence to settle this issue shall come from more precise analysis of effector cells and chemical mediators involved in the response.

$CD4^+$ T cells are believed to be effector cells which mediate DTH response[1,2,8,34,35] but some investigators have also suggested that DTH to allogeneic cells and to influenza virus[36,37] are mediated by the $CD5^+$ and $CD8^+$ T cells. In order to analyse the nature of T cells that mediate MD DTH in mice immunohistological studies were carried out using monoclonal antibodies. It was discovered that $CD4^+$ T cells were present in the skin lesion as early as 2 hr after antigen challenge. No difference from tbc DTH was so far recognized in the pattern of T-cell infiltration (I. Torii, S. Morikawa, T. Harada and Y. Kitamura, unpublished observation).

The actual role of mast cells and the nature of their interaction with T cells in MD DTH is still uncertain. T-cell dependency of the inflammatory response and immunopathology of this DTH suggest the involvement of T-cell factors, such as histamine-releasing factors,[38-40] IL-3,[40,41] interferon-γ (IFN-γ),[40,41] working on mast cells and stimulating them to release chemical mediators to evoke local inflammations. Recently, Askenase and his colleagues suggested[10,42,43] that sensitized T cells are capable of releasing factor(s) possessing bidirectional binding activity to antigens and to putative receptors on the mast cell membrane. Factor-stimulated mast cells work for the induction of further inflammatory processes such as increased vascular permeability and local oedema, and the attraction, immobilization and activation of leucocytes,[3] finally producing skin lesions resembling those of the late-phase reaction of type I hypersensitivity.[43,44] Besides allergic lesions in the skin, bronchi and nasal mucosa, MD DTH responses may possibly be involved in the defence mechanisms of some parasitic diseases, especially those in the digestive tract, as well as in the respiratory organs.

ACKNOWLEDGMENTS

We thank Ms B. Stein for help in preparation of the English manuscript.

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