DELAYED-TYPE HYPERSENSITIVITY TO ALLOGENEIC CELLS IN MICE

III. Sensitivity to Cell-Surface Antigens Coded by the Major Histocompatibility Complex and by Other Genes*

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Different subpopulations of T lymphocytes respond predominantly to different types of cell-surface antigens. For example, cytotoxic T lymphocytes (T_C)[1] respond primarily to antigens controlled by the H-2K and H-2D regions of the mouse major histocompatibility complex (MHC) (1, 2), whereas the population of T cells involved in active proliferation in mixed leucocyte reactions (MLR) respond to H-2I-region-associated determinants (3, 4). Differences between non-H-2-coded antigens usually generate much smaller responses in both these T-cell populations, a notable exception being a difference across the Mls locus, which gives strong MLR (5). This high reactivity of T cells to H-2-coded antigens is thought to reflect a greater number of alloreactive precursors (6, 7) and has important implications when considering mechanisms of generation of the T-cell repertoire.

It was of interest to determine whether the T cells involved in delayed-type hypersensitivity (DTH) also respond preferentially to certain alloantigens. We have demonstrated previously that DTH could be induced to allogeneic cells which differ at many gene loci (8, 9). The conditions for the response have been optimized and the reaction has been extensively characterized. It exhibited all the hallmarks of a DTH reaction. In the present study, we have used congenic strains of mice to determine whether DTH can be induced across both non-H-2 (background) and H-2 differences, and whether there is any preferential response to antigens coded by certain H-2 regions.

H-2I restriction of transfer of DTH to protein antigens has been interpreted as reflecting recognition of these antigens in association with H-2I-coded products on host-derived macrophages (10). We investigated the requirement for host macrophage presentation to allogeneic cell-surface antigens by studying the conditions required for sensitization and transfer. Results obtained suggest different requirements for different types of antigens.

Materials and Methods

Mice. The congenic strains used in this study and their MHC haplotypes are given in Table I. All mice were derived from the specific pathogen-free colony of the Walter and Eliza Hall

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[1] *Abbreviations used in this paper:* DTH, delayed-type hypersensitivity; EBSS, Eisen's balanced salt solution; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; T_C and T_D, T lymphocytes responsible for cytotoxicity and DTH, respectively; UdR, deoxyuridine.

Institute where strict sib mating is maintained.

Cell Suspensions. Suspensions of cells from spleen and from cervical, axillary, and inguinal lymph nodes were prepared by teasing with fine forceps through an 80-mesh stainless steel sieve into cold Eisen's balanced salt solution (EBSS). Clumps were allowed to settle by incubation on ice for 2 min. The single cell suspension was then routinely washed twice in EBSS by centrifugation (400 *g* for 7 min) and resuspended in EBSS to the desired concentration. Cell viability was estimated by the dye exclusion technique.

Sensitization Procedures. 5×10^7 viable spleen cells were given subcutaneously 2 d after a subcutaneous injection of 200 mg/kg cyclophosphamide as described previously (8). In some experiments suspensions of viable spleen cells were sonicated to disrupt the cells, emulsified in an equal volume of complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, Mich.), and injected into the hind footpads. Every experimental group contained five mice.

Adoptive Transfers. Cell suspensions from sensitized mice were injected intravenously into appropriate recipients as indicated in the text.

Isotopes. 5-iodo-2′-deoxyuridine-^{125}I([^{125}I]UdR, sp act 4–6 μCi/μg) and ^{51}Cr (sodium chromate, sp act 50–200 μCi/μg) were used as described previously (8).

Test for DTH. The radioisotopic ear method developed in this laboratory was used (11). Briefly, 4×10^6 viable spleen cells were injected intradermally in a 10-μl volume into the left (L) ear of the mouse. The right (R) ear remained uninjected. 10^{-7} mol of 5-fluorodeoxyuridine was injected intraperitoneally, followed 20 min later by an intraperitoneal injection of 1.5 μCi ^{125}I-UdR. 24 h later (48 h for adoptive transfer), the ears were cut off and the ratio of the radioactivity in the L/R ears was taken to be a measure of the extent of DTH. The magnitude of ratios obtained in naive mice differed between experiments and should only be compared with ratios obtained in sensitized mice within the same group.

Cytotoxicity Assay. This method has been described in detail by Burton et al. (12). Briefly, spleen cells from mice sensitized 6 d previously were added to ^{51}Cr-labeled target cells. The release of ^{51}Cr into the supernate after incubation for 6 h at 37°C, is proportional to the number of cytotoxic lymphocytes added and to the number of target cells lysed. Percent specific lysis is calculated by the formula,

$$\frac{^{51}\text{Cr release in experiment} - \text{background}}{\text{maximal releasable } ^{51}\text{Cr} - \text{background}} \times 100;$$

where background is the release of ^{51}Cr from target cells in the absence of immune lymphocytes and maximal releasable ^{51}Cr is the amount released when labeled target cells are lysed with detergent. The target cells used in this assay were the P815 mastocytoma (H-2^d, of DBA/2 origin), the EL4 T lymphoma (H-2^b, of C57BL origin), and the C1-18 plasmacytoma (H-2^k, of C3H origin).

Statistics. Calculations of *P* values according to Student's *t* test were performed. Differences between two groups were not considered significant when $P > 0.05$.

Results

Ability of Both H-2 and Non-H-2 Antigens to Elicit DTH. Initial experiments were performed to determine whether both H-2 and background antigens contributed to the DTH response of mice to allogeneic cells. Cyclophosphamide-pretreated mice were injected subcutaneously with 5×10^7 viable spleen cells and challenged 6 d later with 4×10^6 viable spleen cells intradermally. Congenic mice of known genetic backgrounds and H-2 haplotypes were used as described in the Materials and Methods. With these strains it was possible to test for the elicitation of DTH to certain defined cell-surface antigens. As shown in Table II, a difference in either H-2 or background antigens was sufficient to elicit a DTH response.

The strongest antigens recognized by T_C cells on target cells are coded by H-2K and H-2D regions, whereas H-2I and background differences give poor reactions. Results shown in Table III suggest that a different situation exists for recognition by

TABLE I

MHC Haplotypes in Inbred Congenic Mouse Strains Used

Mouse strains	MHC regions*							
	H-2K	I-A	I-B	I-J	I-E	I-C	S	H-2D
B10.BR, CBA, C3H, BALB.K	k	k	k	k	k	k	k	k
B10, C3H.SW, BALB.B	b	b	b	b	b	b	b	b
B10.D2, BALB/c	d	d	d	d	d	d	d	d
B10.A, A/J	k	k	k	k	k	d	d	d
B10.A(2R)	k	k	k	k	k	d	d	b
B10.A(4R)	k	k	b	b	b	b	b	b
B10.AQR	q	k	k	k	k	d	d	d
B10.S(9R)	s	s	‡	k	k	d	d	d
B10.T(6R)	q	q	q	q	q	q	q	d
A.TL	s	k	k	k	k	k	k	d
B10.A(5R)	b	b	b	k	k	d	d	d

* From Klein et al. (27).
‡ Origin of allele not known.

TABLE II

Ability of Both H-2 and Background Antigens to Elicit DTH

Mice for sensitization	Cells given for sensitization and challenge	Region of difference	L/R [^{125}I]UdR uptake in:	
			Naive mice	Sensitized mice*
B10.A	A/J	Background	1.4 ± 0.2	4.9 ± 0.6
B10.BR	CBA	Background	1.3 ± 0.2	3.3 ± 0.6
B10	C3H.SW	Background	1.0 ± 0.1	2.9 ± 0.5
B10.BR	B10.D2	H-2	1.0 ± 0.1	2.3 ± 0.3
BALB.B	BALB.K	H-2	1.5 ± 0.2	2.9 ± 0.3
C3H.SW	C3H	H-2	1.6 ± 0.1	2.5 ± 0.1

* All P values for difference between naive and corresponding sensitized group range from <0.0005 to <0.005.

T cells involved in DTH (T_D). T_D- and T_C-cell induction can occur at the same time, but appear to be directed primarily against different antigens. Thus, although strong cytotoxic responses occurred in combinations exhibiting K- and D-end differences, strong DTH responses resulted from K-end, but not D-end, differences. Also, DTH responses occurred across background differences which did not provoke cytotoxic responses. Further congenic combinations were tested for DTH elicitation (Table IV). From the limited number of combinations available, DTH reactions could be detected to determinants coded by the I region (two out of two combinations tested), K region (one out of two), but not the D region (zero out of two). Although it is tempting to suggest that alloantigens coded by the I region may be more immunogenic in DTH induction than those coded by H-2K and H-2D, more strain combinations are required to confirm this.

DTH reactions to background differences alone were generally greater in magnitude than those to H-2 antigens. Fig. 1 shows dose-response curves for DTH responses in two selected strain combinations, one involving background differences, the other an I-region difference. Both showed similar patterns, the level of sensitization detected

TABLE III

Alloantigens Involved in T_C- and T_D-Cell Activation

Cell donors	Antigens to which elicited response is directed in DTH and cytotoxicity assays	DTH assay		Cytotoxicity assay	
		Challenging cells	L/R [^{125}I]UdR uptake	Target* cells	Percent specific ^{51}Cr-release
					%
B10 mice sensitized to B10.A cells	K end of H-2	B10.BR	2.2 ± 0.2	C1-18	21
B10 mice sensitized to B10.A cells	D end of H-2	B10.D2	1.5 ± 0.2‡	P815	21
B10.BR mice sensitized to B10.A(5R) cells	K end of H-2	B10	1.8 ± 0.1	EL4	22
B10 mice sensitized to B10.A(5R) cells	D end of H-2	B10.D2	1.2 ± 0.1‡	P815	30
B10 mice sensitized to A.TL cells	D region of H-2	B10.D2	1.5 ± 0.2‡	P815	20
B10 mice sensitized to A.TL cells	Background	C3H.SW	2.1 ± 0.2	EL4	0‡

* Effector to target ratio was 100:1.

‡ Difference between this value and value in nonsensitized mice not significant ($P > 0.05$).

TABLE IV

DTH to Distinct H-2 Regions

Mice for sensitization	Cells given for sensitization	Cells given for challenge	Antigens to which elicited response is directed	L/R [^{125}I]UdR uptake in:	
				Naive mice	Sensitized mice
B10.D2	B10.BR	B10.BR	H-2	1.5 ± 0.1	3.1 ± 0.2*
B10.BR	B10.D2	B10.D2	H-2	1.0 ± 0.1	2.3 ± 0.3*
B10.A	B10.D2	B10.D2	K end of H-2	1.3 ± 0.1	2.6 ± 0.3*
B10.A	B10.BR	B10.BR	D end of H-2	1.5 ± 1.0	1.5 ± 0.2§
B10.A	B10.T(6R)	B10.AQR	H-2K	1.5 ± 0.2	2.2 ± 0.1‡
B10.A	B10.S(9R)	A.TL	H-2K	1.4 ± 0.1	1.7 ± 0.3§
B10.A	B10.A(2R)	B10.A(2R)	H-2D	1.3 ± 0.1	1.5 ± 0.3§
B10.A(2R)	B10.A	B10.A	H-2D	1.2 ± 0.1	1.4 ± 0.1§
B10.D2	B10.A	A.TL	H-2I	1.3 ± 0.2	2.5 ± 0.1*
B10.D2	B10.BR	A.TL	H-2I	1.1 ± 0.1	2.1 ± 0.2*

* $P < 0.001$.

‡ $P < 0.01$.

§ Not significant ($P > 0.05$).

falling off rapidly at doses below 10^7 cells per mouse and plateauing above this. The higher sensitization levels reached by background than by I-region differences may simply reflect the greater number of background antigens involved.

Lack of Evidence for a Helper T Cell in DTH. In MLR, T_C cells are generated which primarily recognize H-2K and H-2D antigens (1, 2). In this reaction, however, a maximum cytotoxic response required the simultaneous stimulation of the responding cells with both these antigens and H-2I antigens (13). Without this helper effect, a less intense cytotoxic response resulted. Thus, it was possible that low DTH responses to H-2K and H-2D antigens may have been a result of the lack of a helper effect. Table III, however, shows that low DTH responses were obtained to antigens coded by the D end of the H-2 complex even when an H-2I-region difference was present during sensitization. Further results given in Table V show that there was no significant

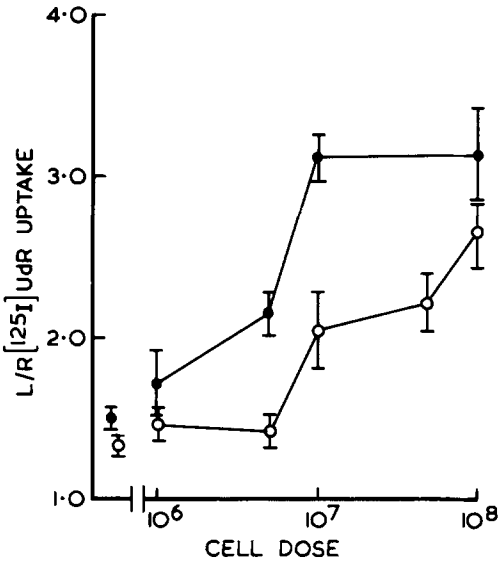

FIG. 1. Dose-response curve of DTH reaction to cell-surface alloantigens after sensitization with increasing doses of viable spleen cells. ●, response to background antigens of B10.A mice sensitized to A/J cells and challenged with A/J cells. ○, response to I-region antigens of B10.D2 mice sensitized to B10.BR cells and challenged with A.TL cells.

increase in the DTH response to H-2K or H-2D antigens when an I-region difference was present during induction.

Individual Processing of Cell-surface Antigens Coded by H-2 and Background Genes. Experiments were performed to determine whether H-2 and background antigens were coprocessed in the immune system. Cyclophosphamide-pretreated C3H.SW mice were injected subcutaneously with 5×10^7 viable B10.BR spleen cells, thus exposing T cells to foreign cells differing at H-2 and background antigens. 6 d later, the sensitized mice were ear challenged with 4×10^6 viable spleen cells from B10.BR, B10, or C3H mice. Challenge with B10.BR cells measured the response to both H-2 and background antigens, B10 challenge measured the response to background antigens alone and C3H challenge measured the response to H-2 antigens alone. The results are given in Table VI. To detect a DTH response to a background antigen, it was not necessary for this to exist on the surface of a cell bearing the same H-2 haplotype as that of cells given for sensitization (cf. groups 1 and 2, Table VI). The DTH response to foreign H-2 antigens was also independent of the presence of foreign background antigens (cf. groups 1 and 3, Table VI). The results therefore suggest that H-2 and background antigens may be individually processed before sensitization.

Sensitization with Disrupted Cells. Because T_D cells are H-2 restricted in their response to protein antigens (10), they may also be restricted in their response to background antigens. Thus, two types of interaction could result in T_D-cell sensitization after immunization with allogeneic cells. Firstly, direct T_D cell-allogeneic cell interaction could occur, the T_D cell recognizing foreign H-2 antigens, or background antigens in association with foreign H-2 antigens, on the surface of the allogeneic cell. It is not known whether T cells can recognize background antigens in association with foreign H-2 antigens, and we cannot test for the existence of these cells in the

TABLE V

Lack of Potentiation of DTH Response to K- or D-region-coded Antigens by Presence of I-region Incompatibility

Group	Mice for sensitization	Cells given for sensitization	Cells given for challenge	Antigens to which elicited response is directed	L/R [^{125}I]UdR uptake in:	
					Naive mice	Sensitized mice
1.	B10.A	B10.AQR	B10.AQR	H-2K	1.5 ± 0.2	2.2 ± 0.2‡
	B10.A	B10.T(6R)	B10.AQR	H-2K*	1.5 ± 0.2	2.2 ± 0.1‡
2.	B10.A	B10.A(2R)	B10.A(2R)	H-2D	1.2 ± 0.1	1.4 ± 0.1§
	B10.A	B10.	B10.A(2R)	H-2D*	1.2 ± 0.1	1.5 ± 0.1§
3.	B10.A(2R)	B10.A	B10.A	H-2D	1.2 ± 0.1	1.3 ± 0.2‖
	B10.A(2R)	B10.D2	B10.A	H-2D*	1.2 ± 0.1	1.6 ± 0.1‖

* H-2I difference also present during induction of response (but not during elicitation).
‡ Difference between these two values not significant ($P > 0.05$).
§ Difference between these two values not significant ($P > 0.05$).
‖ Difference between these two values not significant ($P > 0.05$).

TABLE VI

DTH Response to Background Antigens is Independent of That to H-2 Antigens

Group	Mice for sensitization	Cells given for sensitization	Cells given for challenge	Antigens to which elicited response is directed	L/R [^{125}I]UdR uptake in:	
					Naive mice	Sensitized mice*
1.	C3H.SW	B10.BR	B10.BR	H-2 and background	1.4 ± 0.1	3.2 ± 0.2
2.	C3H.SW	B10.BR	B10	background	1.4 ± 0.1	2.8 ± 0.2
3.	C3H.SW	B10.BR	C3H	H-2	1.5 ± 0.1	2.1 ± 0.1

* All P values for difference between naive and corresponding sensitized group range from <0.0005 to <0.005.

present experimental system as the reaction to foreign H-2 antigens alone would mask their response. However, adoptive transfer experiments can determine whether T_D cells can interact directly with foreign H-2 antigens. Secondly, sensitization could result from T_D cells recognizing macrophage processed H-2 or background antigens which would then be seen in association with the macrophage's own H-2 antigens. To determine whether macrophage processing of allogeneic cells could result in sensitization, mice were immunized with disrupted cells, which presumably must be processed by host macrophages for sensitization to occur (14, 15). Cyclophosphamide-pretreated C3H.SW mice were sensitized with B10.BR cells which had been disrupted by sonication. 6 d later they were challenged with 4×10^6 viable spleen cells from B10.BR, B10.D2, B10, or C3H mice. Results in Table VII show that sensitization to both H-2 and background antigens was achieved. The H-2 haplotype of the challenging cells was irrelevant to the level of sensitization obtained with background antigens. This would be expected if the background antigens were recognized in association with host macrophage H-2 antigens.

It was hoped to determine the importance of this type of sensitization by adoptive transfer studies. If the majority of T_D cells had become sensitized to the antigens on the surface of host macrophages, transfer would be self H-2 restricted. However, if the

TABLE VII

Elicitation of DTH to Cell-surface Antigens after Sensitization with Disrupted Cells

Group	Mice for sensitization	Sonicated cells given for sensitization	Viable cells given for challenge	Antigens to which elicited response is directed	L/R [^{125}I]UdR uptake	
					Naive mice	Sensitized mice*
1	C3H.SW	B10.BR	B10.BR	background and H-2	1.4 ± 0.1	2.6 ± 0.3
2	C3H.SW	B10.BR	B10.D2	background	1.4 ± 0.1	2.9 ± 0.1
3	C3H.SW	B10.BR	B10	background	1.6 ± 0.2	3.3 ± 0.3
4	C3H.SW	B10.BR	C3H	H-2	1.6 ± 0.1	2.2 ± 0.1

* All P values for difference between naive and corresponding sensitized group range from <0.0005 to <0.005.

T_D cells were able to interact with the antigens on the surface of the allogeneic cell, transfer would be unrestricted.

K-end Restriction of DTH Transfer to Background Antigens. Transfer studies were therefore carried out to investigate further the importance of different sensitization mechanisms in the elicitation of DTH to allogeneic cell antigens. 4×10^7 lymph node cells, from cyclophosphamide-pretreated B10.A mice injected subcutaneously with 5×10^7 viable A/J spleen cells 6 d previously, were injected intravenously into normal nonsensitized congenic recipients. The recipients were then challenged with 4×10^6 viable A/J spleen cells and assayed at 48 h. Because B10.A and A/J mice share the same H-2 haplotype, sensitivity is directed to background antigens only. Results in Table VIII show that successful transfer was possible to B10.A (identical with the lymph node donor), and B10.BR (which shares the K end of MHC with the donor), but not to B10.D2 (which shares the D end of MHC with the donor) or B10 (which does not share any MHC with the donor). This K-end restriction of transfer supports the notion that T_D cells that are activated to background antigens are sensitized to macrophage-processed antigens rather than as a result of a direct interaction with the allogeneic cells. These T_D cells do not appear to be able to recognize such antigens as they occur on the surface of allogeneic cells even if these cells have the same H-2 haplotype as those on the cells of the sensitized donor. It is probable, therefore, that background antigens are altered in some way as a result of macrophage processing and presentation.

Vadas et al. (10) have shown that rejection of transferred cells cannot account for the lack of DTH transfer to H-2 incompatible recipients. It is thus unlikely that the lack of transfer to B10.D2 and B10 mice (Table VIII) could result from rejection. Furthermore, the results given in the next section indicate that the DTH response can be transferred to H-2 incompatible hosts when DTH is induced across H-2 differences.

Lack of Restriction of DTH Transfer to H-2 Antigens. Transfer studies were also carried out to investigate T_D-cell interaction with foreign H-2 antigens. Cyclophosphamide-pretreated B10.D2 mice were sensitized and then challenged with B10 cells which have the same background, but a different H-2 haplotype. Thus, any sensitivity detected would be a result of H-2 differences alone. Transfer of sensitivity with 4×10^7 lymph node cells did not require H-2 compatibility between lymphoid cell donor and recipient (Table IX). These results suggest the T_D cells activated to foreign H-2 antigens do not require macrophage processing of cellular challenge for the elicitation of a DTH response. They must interact directly with the foreign cell.

TABLE VIII

Transfer of Sensitivity to Background Antigens is Restricted by the K End of the H-2 Complex

Recipients of lymph node cells from B10.A mice sensitized to A/J spleen cells*	H-2 regions shared by lymphoid cell donor and recipient	L/R [^{125}I]UdR uptake in:		*P* values
		recipients of sensitized cells	naive mice	
B10.A	All	2.3 ± 0.1	1.4 ± 0.1	<0.005
B10.BR	K end	2.1 ± 0.2	1.3 ± 0.2	<0.05
B10.D2	D end	1.6 ± 0.2	1.2 ± 0.1	NS‡
B10	None	1.2 ± 0.1	1.3 ± 0.2	NS

* A/J cells were used for ear challenge to elicit sensitivity.
‡ Not significant ($P > 0.05$).

TABLE IX

Transfer of Sensitivity to H-2 Antigens Is Unrestricted

Recipients of lymph node cells from B10.D2 mice sensitized to B10 spleen cells*	H-2 regions shared by lymphoid cell donor and recipient	L/R [^{125}I]UdR uptake in:		*P* values
		Recipients of sensitized cells	Naive mice	
B10.D2	All	2.5 ± 0.2	1.5 ± 0.1	<0.001
B10.A(5R)	D end	1.9 ± 0.1	1.5 ± 0.1	<0.05
B10.BR	None	1.9 ± 0.1	1.4 ± 0.1	<0.005

* B10 cells were used for ear challenge to elicit sensitivity.

Discussion

We have induced DTH to allogeneic cells. Higher levels of sensitization were reached when background rather than MHC differences were involved. This may simply reflect the greater number of background antigens involved. Using congenic B10 mice, DTH reactions could be detected to determinants coded by the I region (two out of two combinations tested), K region (one out of two) but not the D region (zero out of two). T_C cells which show K and D restriction in target lysis, produced a greater response to foreign cells differing at the K or D regions of the MHC than to those differing at the I region (16). By analogy with the T_C-cell situation, T_D cells, being usually I restricted, may be expected to respond more readily to antigens coded by the I region than by the K or D regions. Although our results suggest this may be so, more strain combinations need to be tested to confirm this.

Properties attributable to the T cell involved in DTH parallel those of T cells involved in MRL, graft-versus-host reactions, and tissue graft rejection (liver slice-to-kidney bed) (17, 18). In all these systems, multiple background differences may give as strong a response as H-2 differences and within the H-2 complex, the I region plays a dominant role. Whether K and D regions stimulate strongly depends on the given combination. Thus, had more D-region combinations been tested, a DTH response might have been observed. However, some differences exist between the antigens that provoke MLR and those that provoke DTH, as a strain combination able to produce an MLR in only one direction was able to produce a DTH reaction using either strain as the host (8). The differences may, however, only reflect the sensitivity of the method employed.

There was no significant increase in the DTH response to H-2K or H-2D antigens when an H-2I difference was present during induction. This lack of potentiation may indicate that there are sufficient helper determinants coded for within the K or D regions, such that the low response obtained is already at a maximum, or that helper cells are not required for maximal DTH expression. Previous results obtained with allogeneic chimeras support the latter alternative (20). Lack of T_C-cell induction in cells derived from totally allogeneic chimeras, even when placed in an environment expressing the chimera's thymic H-2 haplotype, has been explained as a result of defective helper T-cell cooperation (19). The fact, however, that we could sensitize totally allogeneic chimeras to antigens given in association with appropriate macrophages suggests that no such T cell – T-cell interactions occurred during the induction of DTH reactions (20). Thus, we have not yet found any evidence for a helper T cell involved in DTH induction.

The predominant mechanism of sensitization to background antigens appears to require macrophage processing. Studies with disrupted cells showed that macrophage processing alone could lead to high levels of sensitivity. Sensitization with disrupted cells presumably required macrophage processing because, according to Lafferty and Woolnough (14) and Batchelor et al. (15), for sensitization to occur T cells must not only recognize the appropriate antigen, but must receive a second signal from an intact nucleated cell. In our experiments, the response to background antigens was independent of the H-2 haplotype of the sensitizing or challenging cell and transfer of sensitivity to these antigens was restricted by the H-2 of the host. These results would be expected if the majority of T_D cells were sensitized to the antigens on the surface of host macrophages.

Observations made in other systems also point to the greater stimulating ability of macrophage-processed background antigens. Bevan and Matzinger (21) immunized F_1 (BALB/c × BALB.B) ($H\text{-}2^{d/b}$) mice with B10.D2 ($H\text{-}2^{d}$) cells and found equal numbers of T_C cells directed against background antigens in association with either $H\text{-}2^{b}$ or $H\text{-}2^{d}$, even though they were injected on a cell bearing $H\text{-}2^{d}$. This observation infers that T cells were sensitized to the background antigens on the surface of host macrophages rather than by direct interaction with the allogeneic cells. In our system, the T_D cells sensitized to background antigens did not appear to be able to recognize and interact with these as they occurred on the surface of allogeneic cells, even if these cells had the same H-2 haplotype as those on the cells of the sensitized donor. There are several possible explanations for this observation. Background antigens may be altered as a result of macrophage processing and presentation; new determinants, perhaps buried within the lipid bilayer of the allogeneic cell, may be exposed; much of the DTH response may be directed to molecules found within the allogeneic cell, not on its surface; or the antigenic determinants may be present on the allogeneic cells but may not associate with the relevant MHC-coded molecules (presumably I-region-coded molecules) in high enough concentration or in correct conformation for T_D-cell recognition. The possibility that background antigens are altered receives some support from evidence obtained in other systems that new antigenic determinants may be created following macrophage processing. In guinea pigs, DNA synthesis (22) and the production of macrophage inhibition factor (23) by T cells that are stimulated by antigen-pulsed macrophages, are blocked by addition of anti-Ia antibody to the macrophage-antigen complex in contrast to the lack of inhibition after similar treatment with antibody to exogenous antigen (24).

Previous results showed that both $Ly1^+$ and $Ly2^+3^+$ cells were involved in transfer of sensitivity to completely allogeneic cells to a naive recipient (9). Many workers have shown that $Ly1^+$ cells are stimulated by antigens coded for by the H-2I region, whereas $Ly2^+3^+$ cells are stimulated by antigens coded for by the H-2K or H-2D regions (25, 26). Therefore, it would be expected that the $Ly2^+3^+$ cells were responsible for sensitivity to allogeneic H-2K- and H-2D-coded antigens, whereas the $Ly1^+$ cells were responsible for sensitivity to H-2I-coded antigens and to background antigens seen in association with host-2I-coded antigens. Unfortunately, levels of sensitivity obtained across K and D differences were never large enough to allow successful transfer of sensitivity. However, studies to determine the Ly phenotype of the cells involved in transfer of sensitivity to I region and background antigens are in progress.

DTH elicitation to H-2 antigens was also examined in more detail. Sensitization to H-2 antigens was achieved using disrupted cells, which infers that macrophage-processed H-2 antigen was immunogenic. Only transfer studies could have established whether this processing altered the way in which the T_D cells perceived the foreign H-2 antigens. Unfortunately the level of sensitivity achieved across H-2 differences using disrupted cells was generally lower than that with viable cells, and it was not possible to obtain a successful transfer. Transfer of sensitivity to H-2 antigens was, however, obtained after sensitization with viable cells and was shown to be unrestricted. These results imply that when sensitization occurs to H-2 antigens given on allogeneic cells, macrophage processing either plays a minor role, or contributes significantly but without altering the way in which the H-2 antigens are perceived. Thus, the sensitized T_D cell can recognize and interact directly with the H-2 antigens as they appear on the allogeneic cells used for challenge.

For T lymphocytes to be activated, the recognition of a presenting structure involving an MHC component must occur to allow the passage of an inductive signal. Thus, the T cells involved in DTH can receive this signal on binding directly to allogeneic H-2 antigens on the surface of allogeneic cells. On the other hand, background antigens must first interact successfully with a presenting structure before T-cell activation can occur. Our results suggest that background antigens and MHC components interact poorly to form presentation complexes on the surface of allogeneic cells. It thus appears that for background antigens to be strongly immunogenic, they must be processed by macrophages, altered in some way, and represented on the macrophage surface in combination with the appropriate MHC component.

Summary

DTH could be induced to cell-surface antigens coded by either H-2 or non-H-2 genes. Sensitivity was more readily induced across I region than across K- or D-region differences. The presence of an I-region difference during sensitization did not significantly increase the DTH response to K- or D-region-coded antigens. Macrophage processing appeared to be the major route of sensitization to background antigens. Thus, high levels of sensitivity were achieved equally well using viable or disrupted cells, the response was independent of the H-2 haplotype of the allogeneic cells, and transfer was restricted to the K end of the host H-2 complex. Although sensitization to H-2 antigens was obtained with disrupted cells, transfer of sensitivity

against viable cells was unrestricted. This suggests a minor role for macrophage processing in sensitization to H-2 antigens.

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