

HISTOCOMPATIBILITY LINKED IMMUNE
RESPONSIVENESS AND RESTRICTIONS
IMPOSED ON SENSITIZED LYMPHOCYTES*

By J. F. A. P. MILLER, M. A. VADAS, ALISON WHITELAW, JENNIFER GAMBLE,
AND C. BERNARD

(From the Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital,
Melbourne 3050, Australia)

In the last few years, it has become clear that products of the major histocompatibility complex (MHC) play a critical role in restricting the activities of immune T cells. Thus, helper T cells (1, 2) and T cells involved in delayed-type hypersensitivity (DTH) to protein antigens (3) are restricted by the *I-A* region of the MHC, whereas cytotoxic T cells are restricted by the *K* or *D* regions (4). Recently, we showed that T cells transferring DTH to protein antigens were sensitized to both the antigen and to the MHC product responsible for restriction (5). A similar conclusion was reached in experiments with helper T cells (6, 7).

Certain individuals within a species fail to respond to a specific antigen and, in many, the deficiency maps in the *I* region of the MHC (1). Responsiveness is generally manifest in T-cell functions, not in B-cell potential. For this reason, it was at one time suggested that the MHC-linked *Ir* genes may code for the antigen recognition unit on T cells (1). On the other hand, the studies reported here on the MHC-imposed constraints on DTH transfer to a synthetic polypeptide antigen, suggest an alternative mechanism of *Ir* gene control of T-cell-dependent immune responses.

Materials and Methods

Mice. 2-to-3-mo old female mice were used for sensitization and as recipients of sensitized cells. The highly inbred strains A.TH, A.TL, A.SW, A.QR, B10.A, B10.A(4R), B10.A(5R), BALB/c, C57BL, CBA, and SJL were obtained from the Walter and Eliza Hall Institute stock. Their origins and maintenance have been described elsewhere (3, 5).

Antigens and Sensitization. 2 days before antigen administration, mice were given 200 mg/kg cyclophosphamide (Endoxan, Asta; Mead Johnson, Crows Nest, Australia) subcutaneously. The random terpolymer of L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) was a generous gift from Professor Baruj Benacerraf. It was emulsified in complete Freund's adjuvant and 10 μ g in 0.1 ml injected in the four footpads and subcutaneously in the abdomen. For transfer of sensitivity, peripheral lymph node or spleen cells were taken from mice 5 days after the primary or secondary sensitization and injected intravenously into naive mice.

Test for DTH. This has been described in detail elsewhere (8). Briefly, the assay measures the influx of cells labeled with ¹²⁵I-5-iodo-2'-deoxyuridine (¹²⁵I-UdR) (sp act 90-110 μ Ci/ μ g, Radiochemical Centre, Amersham, England) into DTH lesions. Antigen is deposited in the left (L)

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pinna and the right (R) remains uninjected. 24–48 h later the ears are cut off and the ratio of the radioactivity in the L/R ears (L/R ^{125}I -UdR uptake) reflects the extent of DTH. To increase labeling efficiency, 10^{-7} mol of 5-fluorodeoxyuridine is injected intraperitoneally 20–30 min before the intraperitoneal injection of 2 μCi ^{125}I -UdR. Values obtained in nonsensitized mice or in naive mice not given sensitized cells are usually ≈ 1.2 .

Results

Mice of the $H\text{-}2^{a, b, d, f, j, k, r, u, v}$ haplotypes are responders (R) to GAT because they can produce specific anti-GAT antibodies whereas mice of the $H\text{-}2^{n, p, q, s}$ haplotypes are nonresponders (NR) as they form no detectable antibodies (9). It was therefore of interest to determine whether DTH could be elicited to GAT in NR strains. As shown in Fig. 1, good responses were obtained in A.TL and (A.TL \times A.TH) F_1 mice, but at no time could a detectable response be elicited after primary or secondary sensitization in A.TH mice. DTH to GAT could not be elicited in mice of $H\text{-}2^s$ or $H\text{-}2^q$ haplotypes although it could readily be produced in other strains (Table I).

The transfer of DTH to a protein antigen was shown to require identity at the *I*-A region of the MHC (3). It can be seen from Table II that identity between donors and recipients at the *I*-A region alone (A.TL \rightarrow B10.A[4R] and B10.A[4R] \rightarrow A.TL) was sufficient to allow DTH transfer to GAT. Identity at *I*-B alone (B10.A[4R] \rightarrow B10.A[5R]) or at *D* alone (A.TL \rightarrow BALB/c) did not allow transfer. Whether *K* identity alone would have allowed transfer could not be determined with the strains available, since in combinations identical only at *K*, one member of the pair was a NR and transfer could not be achieved from R to NR recipients (e.g. A.TL \rightarrow A.SW).

We have previously demonstrated that primed lymphoid cells from F_1 mice could successfully transfer DTH to both parental strains as well as to the F_1 (3). We therefore examined whether sensitivity could be transferred to naive mice of the parental strains from F_1 mice between R and NR strains, which themselves showed high levels of DTH to GAT (Fig. 1). As can be seen from Table III, when sensitized (R \times NR) F_1 lymphoid cells were transferred to naive parental strain recipients and to F_1 , sensitivity was detected only in the F_1 and in parental strain recipients of the R haplotype, never in parental strain recipients of the NR haplotype.

Discussion

No detectable DTH could be elicited in mice known to be NR to GAT on the basis of antibody responses, even after cyclophosphamide pretreatment (Fig. 1). This is unlike the situation we observed with the isozyme B of lactic dehydrogenase in which a short period of sensitivity was obtained after cyclophosphamide (5). There may thus be a difference in the mechanism of nonresponsiveness to these antigens, possibly reflecting differential activation of cyclophosphamide sensitive suppressor effects. This is currently under investigation.

DTH transfer to GAT was possible if sensitized donors and naive recipients were identical at the *I*-A region of the MHC (Table II), just as had previously been observed with a protein antigen (3). One implication is that DTH transfer to all protein and polypeptide antigens requires identity at *I*-A. Alternatively, the MHC region which imposes constraints on DTH transfer may also control

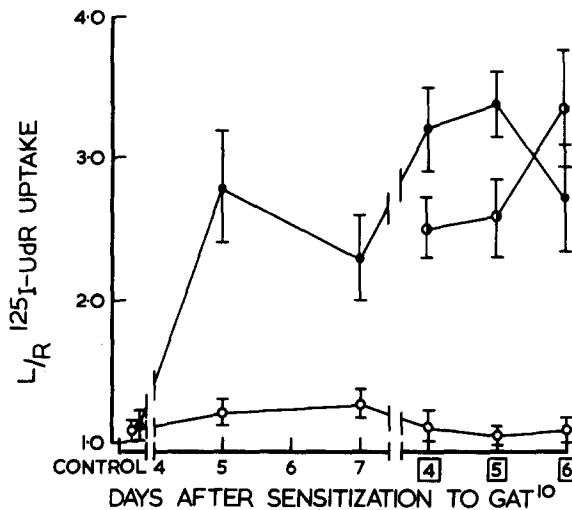


FIG. 1. Time-course of the DTH response to GAT in A.TL (●), A.TH (○), and (A.TL × A.TH)_{F1} (◐) mice at various days after a first, or a second (days in squares) sensitization. The mice received cyclophosphamide 2 days before sensitization. The second sensitization was performed 22 days after the first. Control mice were not sensitized. Vertical bars span 2 SEM. Five mice per point.

TABLE I
Mouse Strain Differences in DTH Responsiveness to GAT

Strain of mice sensitized to GAT	MHC*	L/R ¹²⁵ I-UdR uptake†
CBA	k k k k k k k	3.4 ± 0.4
BALB/c	d d d d d d d	2.2 ± 0.2
A.TL	s k k k k k k d	2.2 ± 0.2
A.TH	s s s s s s s d	1.1 ± 0.1
A.SW	s s s s s s s s	1.2 ± 0.1
SJL	s s s s s s s s	0.8 ± 0.2
DBA/1	q q q q q q q q	1.0 ± 0.1

* Regions indicated are K, I-A, I-B, I-J, I-E, I-C, S, and D.

† Values are arithmetic means ± 1 SEM. Five to six mice per group.

responsiveness to that antigen. If this is so, restrictions with certain antigens should be imposed by different *I* subregions corresponding to those in which have been mapped the *Ir* genes governing responsiveness to those antigens. This is also under investigation.

DTH to GAT was transferrable from sensitized (R × NR)_{F1} mice to naive recipients of the R but not of the NR haplotypes. In fact, the situation here is analogous to that already observed in (CBA × BALB/c)_{F1} mice sensitized to antigen-pulsed macrophages from one parental strain: transfer was possible but only to naive recipients of the parental strain of the same genotype as that from which the macrophages used for sensitization were obtained (5). In the NR, therefore, the antigen under *Ir* gene control may not associate effectively with a particular *I* region gene product as we had suggested before (3). It would then be unable to form a product immunogenic for T cells in DTH. There is no reason not to consider the *Ir* gene product itself as being the relevant *I* region molecule involved in restriction. On the other hand, one could imagine *Ir* genes coding for

TABLE II
MHC Restriction of the Transfer of DTH to GAT

Donor of sensitized lymphoid cells	Recipient strain	MHC of recipients*	L/R ¹²⁵ I-UdR uptake in recipient mice†
CBA	CBA	k k k k k k k k	1.7 ± 0.1
	B10.A	k k k k k d d d	1.7 ± 0.1
	A.TL	s k k k k k k d	1.6 ± 0.1
	AQR	q k k k k d d d	1.8 ± 0.2
	C57BL	b b b b b b b b	1.2 ± 0.1
BALB/c	BALB/c	d d d d d d d d	2.6 ± 0.2
	B10.A	k k k k k d d d	1.2 ± 0.1
A.TL	A.TL	s k k k k k k d	2.1 ± 0.2
	B10.A(4R)	k k b b b b b b	1.6 ± 0.2
	BALB/c	d d d d d d d d	0.9 ± 0.2
	A.SW	s s s s s s s s	1.1 ± 0.1
B10.A(4R)	B10.A(4R)	k k b b b b b b	1.5 ± 0.04
	A.TL	s k k k k k k d	1.6 ± 0.2
	B10.A(5R)	b b b k k d d d	1.0 ± 0.02
	CBA	k k k k k k k k	1.5 ± 0.1

* Regions indicated are *K*, *I-A*, *I-B*, *I-J*, *I-E*, *I-C*, *S*, and *D*; letters in italics point to differences in regions between donor and recipient mice.

† Arithmetic means ± SE. Five mice per group.

TABLE III
DTH Transfer to GAT by Sensitized F₁ (Responder × Nonresponder) Cells*

Naive recipients of 5 × 10 ⁷ F ₁ cells	L/R ¹²⁵ I-UdR uptake†
(BALB/c × SJL)F ₁	2.2 ± 0.2
BALB/c	2.6 ± 0.1
SJL	1.2 ± 0.1
(A.TL × A.TH)F ₁	1.9 ± 0.1
A.TL	1.8 ± 0.2
A.TH	1.2 ± 0.1

* Sensitivity in the donors was as follows: (BALB/c × SJL)F₁, 9.3 ± 0.9 and (A.TL × A.TH)F₁, 2.5 ± 0.3.

† Values are arithmetic means ± SE. Five to six mice per group.

components which allow interactions between particular *I* region products and the antigen to form stable structures. These ideas are in general agreement with notions recently expressed by others (10, 11) and based on different experimental systems.

A model based on interactions between *I* region gene products and antigen leads to certain predictions among which are the following. Slight physicochemical alterations of an antigen under *I*_r gene control may lead to a more stable association with the relevant *I* region component, the result being to convert an NR to an R for that antigen (cf. 12). Another prediction would be that antigen unable to associate effectively with the *I* region gene product on the macrophage should come off and be available as soluble antigen to stimulate suppressor T cells. If this is true, antigen-specific suppressor T cells should be found in NR mice and this indeed was demonstrated in one system (9) and implicated in another (5). A further prediction is concerned with the regulation of cytotoxic T cells. Since *K* and *D* regions restrict their activities, the *I*_r genes controlling their reactions may be found in the *K* and *D* regions. It is interesting to note that

the only mapped *Ir*-like gene which controls the generation of cytotoxic effector T cells has been localized between *K* and *I-A* (13).

Summary

Delayed-type hypersensitivity (DTH) transfer to GAT was restricted by the *I-A* region of the major histocompatibility complex (MHC). Sensitized cells from F_1 hybrid mice between responder and nonresponder strains transferred DTH to syngeneic F_1 mice and to naive parental strain recipients of the responder but not of the nonresponder haplotypes. These results are interpreted to favor the postulate that the MHC-linked *Ir* genes exert their effects by coding for components which allow interactions between particular *I* region gene products and the region to form stable structures immunogenic for DTH T cells.

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