

SUPPRESSION OF T CELLS SPECIFIC FOR THE  
NONTHYMIC PARENTAL  
H-2 HAPLOTYPE IN THYMUS-GRAFTED CHIMERAS\*

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The activation of various subsets of T lymphocytes by antigen is controlled by gene products of the major histocompatibility complex (MHC). There is a requirement for H-2K or H-2D matching between cytotoxic T cells and their targets (1), for H-2I matching between helper T cells and B cells (2), and for H-2I matching between T cells involved in delayed-type hypersensitivity (DTH) and antigen-presenting macrophages (Mph) (3). These results are most easily interpreted by postulating two receptors on the T cell, one directed against an H-2-coded component and the other directed against any other antigen. Recent experiments suggest that T cells are committed in the thymus to the recognition of antigen in association with the MHC-coded components displayed on the thymus epithelium (1). Thus ( $P_1 \times P_2$ )F<sub>1</sub> stem cells differentiated within a P<sub>1</sub> thymus can only transfer DTH to naive recipients whose cells express components coded by the H-2I region of the P<sub>1</sub>, not P<sub>2</sub>, haplotype (3). Hence, although these chimeras are tolerant of P<sub>2</sub>-haplotype-bearing cells, as their reticuloendothelial system is completely repopulated by such cells, they are not able to recognize foreign antigens in association with P<sub>2</sub> H-2-coded components.

Two main models have been suggested to explain these findings. One model proposes that precursor T cells consist of many subsets, each bearing two receptors, both of which are complementary to a given H-2-coded component of the species. Precursor T cells, able to recognize the H-2-coded components expressed on the thymus, are driven to divide and differentiate, but only those that mutate one of their receptors away from self-reactivity will be allowed to mature, and these cells eventually give rise to the entire mature T-cell population (4). Because the T-cell repertoire would thus be initially restricted to T cells that recognize the thymic H-2, T cells should not be able to recognize antigen in association with allogeneic H-2 components. Some investigations support this prediction (5, 6), but others have found the reverse (7-9). This model would also predict that T and B cells would use a different anti-X V-gene pool, and that T-cell idiotypes would be H-2 linked. The sharing of idiotypes between anti-X receptors on T and B cells, and the linkage of such idiotypes to allotype and not to MHC, do not favor this model (10).

An alternative model proposes that an anti-X specificity of precursor T cells is determined independently of the thymic H-2, and that each precursor T-cell subset is potentially capable of recognizing antigen in association with a different H-2

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haplotype (11, 12). The thymus exerts its influence on already committed precursors. Those that bind to thymic H-2 strongly enough to be activated will be eliminated, leaving only those that must recognize a foreign antigen as well as the thymic H-2 for activation. All other subsets of T cells will be allowed to mature. This model predicts that T cells are able to recognize antigen in association with allogeneic MHC components and is compatible with idiotypic sharing between T and B cells.

The restriction of T-cell activities by only P<sub>1</sub>, not P<sub>2</sub>, haplotype in (P<sub>1</sub> × P<sub>2</sub>)F<sub>1</sub> → P<sub>1</sub> chimeras is easily explained by the first model. The second model explains this observation by invoking a suppressor mechanism directed against all cells that recognize antigen in association with P<sub>2</sub> (12). For example, any cell which recognizes P<sub>2</sub> or non-H2 antigens in association with P<sub>2</sub>, which are not expressed on the thymus but are expressed on all F<sub>1</sub>-derived cells, will be stimulated to proliferate and may then activate an anti-idiotypic suppressor system directed against the anti-P<sub>2</sub> receptor.

We have carried out the following studies to determine whether such an anti-P<sub>2</sub> receptor suppressive mechanism exists. Our results suggest that it does.

### Materials and Methods

*Mice.* 2- to 3-mo-old female mice were used for sensitization, as recipients of chimeric cells, and as lymph node cell donors. All mice used were obtained from the specific pathogen-free breeding unit of the Walter and Eliza Hall Institute, Melbourne, Australia.

*Chimeras.* Thymectomized (ATXBM) and thymus-grafted chimeras were produced as described previously (13). The constitution of the chimeras is denoted in the following way: the letters before the arrow refer to the strain that provides bone marrow, those after the arrow to the strain that provides the thymus graft. P<sub>1</sub> and P<sub>2</sub> denote two MHC-incompatible strains used to provide F<sub>1</sub> hybrids.

*Antigens and Sensitization.* 2 d before sensitization mice were injected subcutaneously with 200 mg/kg cyclophosphamide (CY) (Endoxan, Asta, Mead Johnson, Crows Nest, Australia). Keyhole limpet hemocyanin (KLH) was emulsified in complete Freund's adjuvant (CFA) and 100 µg in 0.1 ml was injected into the two hind footpads. KLH-pulsed Mph were prepared by *in vivo* antigen pulsing a Mph-rich peritoneal exudate as described previously (13).

*Cell Suspensions.* Lymph node cell suspensions from both naive and sensitized mice were prepared from regional lymph nodes. All cell suspensions were injected intravenously into naive recipients.

*DTH Assay.* The radioisotopic ear assay of Vadas et al. (14) was used.

*Statistical Analysis.* Arithmetic means ± SE are given. Statistical significance was determined by Student's *t* test.

### Results

*Suppression of Induction of DTH in Naive Cells.* If a suppressive component were present, it would be expected to suppress stimulation of adoptively transferred naive cells known to bear the functionally suppressed receptor. Both allogeneic and semiallogeneic chimeras were used in these experiments. 10<sup>8</sup> lymph node cells from naive (CBA × C57BL)F<sub>1</sub> mice, which contain subsets of T cells able to recognize antigen in association with C57BL H-2 components, were adoptively transferred into C57BL → CBA, F<sub>1</sub> → CBA, or ATXBM F<sub>1</sub> mice. All three types of mice present antigen on the surface of cells bearing C57BL H-2 components, but are not able to generate a response to such specificities (13). On adoptive transfer of naive T cells known to be able to recognize such specificities, followed by CY and KLH-CFA immunization, one would predict that a DTH response would be generated by these transferred T

TABLE I  
*Suppression of DTH Induction in Naive Cells Adoptively Transferred into Chimeras*

Group	Cell donor*	Naive recipients	Form of challenge	L/R <sup>125</sup> I-UdR uptake in:‡	
				Naive mice	Sensitized mice
I	—	CBA	KLH	1.1 ± 0.1	2.7 ± 0.2
	—	ATXBM CBA	KLH	—	1.3 ± 0.1§
	(CBA × C57BL) <sub>F</sub> <sub>1</sub>	ATXBM CBA	KLH	—	2.5 ± 0.3
	(CBA × C57BL) <sub>F</sub> <sub>1</sub>	C57BL → CBA	KLH	—	1.3 ± 0.1§
II	—	(CBA × C57BL) <sub>F</sub> <sub>1</sub>	KLH-C57BL Mph	1.6 ± 0.2	2.6 ± 0.1
	—	ATXBM <sub>F</sub> <sub>1</sub>	KLH-C57BL Mph	—	1.6 ± 0.2§
	(CBA × C57BL) <sub>F</sub> <sub>1</sub>	ATXBM <sub>F</sub> <sub>1</sub>	KLH-C57BL Mph	—	3.1 ± 0.2
	—	<sub>F</sub> <sub>1</sub> → CBA	KLH-C57BL Mph	—	1.7 ± 0.2§
	(CBA × C57BL) <sub>F</sub> <sub>1</sub>	<sub>F</sub> <sub>1</sub> → CBA	KLH-C57BL Mph	—	1.7 ± 0.2§

\*  $10^8$  lymph node cells injected intravenously into naive recipients 3 d before sensitization.

‡ Ratio of radioactivity of left (L) to right (R) ear 24 h after intradermal challenge in left ear and intraperitoneal injection of <sup>125</sup>I-5-iodo-2'-deoxyuridine.

§ No significant difference between this value and the value in nonsensitized mice ( $P > 0.05$ ).

TABLE II  
*Failure to Suppress DTH Expression by Sensitized Cells Transferred into Chimeras*

Group	Donor of sensitized cells*	Naive recipients	L/R <sup>125</sup> I-UdR uptake in:‡	
			Naive mice	Recipients of sensitized cells
I	C57BL	ATXBM <sub>F</sub> <sub>1</sub>	1.2 ± 0.1	2.7 ± 0.2
		<sub>F</sub> <sub>1</sub> → CBA	—	2.3 ± 0.1
II	CBA	<sub>F</sub> <sub>1</sub> → CBA	1.3 ± 0.2	1.8 ± 0.2
		<sub>F</sub> <sub>1</sub> → C57BL	—	2.3 ± 0.2

\*  $4 \times 10^7$  lymph node cells from mice sensitized to KLH in CFA 5 d previously were injected intravenously into naive recipients.

‡ See Table I footnote.

cells if no suppression mechanism existed. Results in Table I (group I) show that DTH was not elicited on KLH challenge in C57BL → CBA chimeras that had received the <sub>F</sub><sub>1</sub> cells, although good responses were obtained in ATXBM <sub>F</sub><sub>1</sub> cell recipients. Because <sub>F</sub><sub>1</sub> → CBA chimeras are able to give a DTH response to antigen seen in association with CBA H-2 components, these mice had to be challenged with antigen-pulsed C57BL Mph. Results (group II) show that DTH could not be elicited in <sub>F</sub><sub>1</sub> → CBA recipients of naive <sub>F</sub><sub>1</sub> cells on challenge with KLH-pulsed C57BL Mph, even though ATXBM <sub>F</sub><sub>1</sub> cell recipients gave good responses to this challenge. Thus, any potential to recognize C57BL H-2 components in association with antigen is not expressed in these chimeras.

*Failure of Suppression of Expression of DTH by Sensitized Cells.* The experiments described above for allogeneic chimeras were repeated transferring sensitized C57BL cells instead of naive cells to look for suppression of their function.  $4 \times 10^7$  KLH-sensitized C57BL lymph node cells were transferred into either <sub>F</sub><sub>1</sub> → CBA or ATXBM <sub>F</sub><sub>1</sub> mice, which were immediately ear challenged with KLH and examined for evidence of a DTH response 48 h later. Results in Table II (group I) show that <sub>F</sub><sub>1</sub> → CBA and ATXBM <sub>F</sub><sub>1</sub> mice act as equally good recipients of sensitized cells, both allowing high DTH responses. Positive DTH transfers were also obtained by putting KLH-sensitized CBA cells into either <sub>F</sub><sub>1</sub> → CBA or <sub>F</sub><sub>1</sub> → C57BL chimeras (group II). Thus there is no apparent suppression of sensitized T-cell activity.

TABLE III  
*Failure to Suppress DTH Response in Normal Mice by Transferred Chimeric Cells*

Donor of cells*	Naive recipients	L/R <sup>125</sup> I-UdR uptake in:‡	
		Naive mice	Sensitized mice
—	C57BL	1.2 ± 0.1	3.4 ± 0.2
C57BL → CBA	C57BL	—	4.0 ± 0.4

\* 10<sup>8</sup> spleen and lymph node cells from naive allogeneic chimeras were injected intravenously into naive recipients 1 mo before sensitization.

‡ See Table I footnote.

*Failure of Suppression of Induction of DTH in Naive Mice.* Because it was possible to suppress DTH induction in naive cells that had been adoptively transferred into allogeneic chimeras (Table I), it may be predicted that C57BL → CBA cells, when adoptively transferred into a naive C57BL recipient, would suppress DTH induction in this recipient. Thus, 10<sup>8</sup> spleen and lymph node C57BL → CBA cells were adoptively transferred into naive C57BL recipients. 1 mo later the mice were tested for their ability to mount a DTH response (Table III). Their capacity to give a good DTH response was unimpaired when compared with a normal C57BL mouse. Thus, no suppressive effect as a result of the presence of chimeric cells was observed.

### Discussion

Previous results obtained in this laboratory and by others (1, 3, 4) suggest that the H-2 type of the thymus determines the specificity of T cells. Thus, (C57BL × CBA)F<sub>1</sub> T cells maturing in a CBA thymus will be restricted to recognizing antigen in association with CBA H-2-coded components. We initiated this series of experiments to determine whether these constraints imposed on T-cell specificity were a result of a suppressive mechanism. For example, peripheral T cells in a (C57BL × CBA)F<sub>1</sub> mouse with a CBA thymus may be very similar to those in a normal F<sub>1</sub>, except that the cells with receptors that recognize C57BL H-2 components are prevented from responding by a suppressive mechanism directed against the idotype of the anti-C57BL receptor. Our results support the existence of such a suppressive mechanism in both allogeneic (C57BL → CBA) and semiallogeneic [(C57BL × CBA)F<sub>1</sub> → CBA] chimeras.

We were unable to induce DTH directed against antigens seen in association with C57BL components in naive (C57BL × CBA)F<sub>1</sub> lymphoid cells that were adoptively transferred into either (C57BL × CBA)F<sub>1</sub> → CBA or C57BL → CBA chimeras. However, good DTH reactions were obtained in ATXBM F<sub>1</sub> recipients of the same batch of cells. These results suggest the existence of an active in vivo suppressive mechanism directed against cells bearing anti-C57BL receptors. An alternative explanation for lack of sensitization of cells in the chimera is one of lack of T-cell space. We are unable to show directly that the cells can be sensitized to antigen in association with H-2 components expressed on the thymus, because the chimera itself makes a response restricted to this specificity. However, preliminary experiments indicate that there is no release of suppression when chimeras are lightly irradiated to create space before transfer of cells.

Although it was possible to suppress the induction of DTH in transferred naive cells (Table I), it was not possible to suppress the expression of DTH in transferred

cells that had been sensitized to antigen 5 d previously (Table II). Thus, the suppressive mechanism appears only capable of preventing induction, not expression, of DTH by these cells. There have been other reports that different mechanisms may be involved in suppression of induction and expression of DTH (15, 16). However, most mechanisms suppressing induction of DTH have been shown to be CY sensitive, whereas the phenomenon described in this paper is observed after CY treatment. Thus, once the suppressive mechanism has been induced (which must be very soon after reconstitution of chimeras) it is unable to be abrogated by CY. The suppression observed is most likely mediated by  $T_S$  cells rather than by antibody, as it is present in allogeneic chimeras that have been shown by other workers to be immunologically impotent in responses requiring helper cell interactions (1).

Attempts by other workers to demonstrate suppression in chimeric mice have failed so far (17, 18). These workers have looked for suppression of sensitization of normal cells after mixing with chimeric spleen or bone marrow cells, and transfer into irradiated recipients. Although we were able to show suppression of induction of DTH in naive cells that were transferred into chimeras, we were unable to transfer suppression into naive mice by injection of chimeric cells. Thus, the suppressive mechanism may be short lived, or may be successfully mediated endogenously by only a small number of cells of which not enough can be transferred to give effective suppression.

We feel that the existence of a suppressive mechanism in  $F_1 \rightarrow P_1$  mice directed against cells that are able to recognize antigen in association with  $P_2$  favors a negative selection role for the thymus as outlined in the second model discussed in the introduction. However, until it can be shown that the anti- $P_2$  receptor present on cells, which must recognize a foreign antigen in association with  $P_2$  for activation, is different from that on alloreactive cells directed against  $P_2$ , it is not possible to say that T cells that can recognize antigen in association with  $P_2$  are formed in a  $P_1$  thymus.

### Summary

The mechanism of restriction of T-cell specificity by the genotype of the thymus in allogeneic and semiallogeneic chimeras was investigated. Lack of induction of delayed-type hypersensitivity (DTH) directed against antigen in association with the non-thymic parental haplotype in naive cells adoptively transferred into chimeras suggests the existence of an *in vivo* suppressive mechanism. However, it was not possible to suppress the expression of DTH in sensitized cells transferred into chimeras, or to transfer this suppression to normal naive recipients.

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