THE EFFECT OF ALLOGENEIC PRESENSITIZATION ON H-Y GRAFT SURVIVAL AND IN VITRO CELL-MEDIATED RESPONSES TO H-Y ANTIGEN

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The homograft response to H-Y antigen differs markedly in different strains of mice (reviewed by Gasser and Silvers, 1). Females of $H\text{-}2^b$ haplotype such as C57BL/6 and C57BL/10 (hereafter B6 and B10) regularly reject syngeneic male skin, whereas females of most other *H-2* haplotypes, so far examined, do not. In vitro, the target cell specificity of the secondary cytotoxic response of B10 female cells, sensitized to B10 male, is restricted by the *H-2* major histocompatibility complex (MHC)[1] (2). Furthermore, female responder cells, primed in vivo with a syngeneic male skin graft and challenged in vitro in mixed lymphocyte culture (MLC) with allogeneic male cells, fail to lyse syngeneic male target cells (2). These observations might be taken to indicate that H-Y is not detected on allogeneic stimulating cells or allogeneic target cells and that H-Y is strain specific. This prompts us to publish data from our two laboratories on the capacity of allogeneic male skin grafts to sensitize B6 or B10 female mice to second grafts of male skin from the same (B6 or B10) strain. Additional data demonstrate that B10 females, primed in vivo with allogeneic male skin grafts and subsequently challenged in vitro with syngeneic male cells, generate cytotoxic cells specific for syngeneic male target cells. The evidence presented here indicates that at least some component of H-Y is detected on allogeneic cells in vivo during primary sensitization, and that the second set cell-mediated response to H-Y is not necessarily restricted by the *H-2* haplotype of the sensitizing strain.

Materials and Methods

Animals. B6, B6-$H\text{-}2^k$, A, A.BY, B6-Ly-2^a mice were obtained from stocks maintained by E. A. Boyse at Memorial Sloan Kettering Cancer Center, New York. The congenic B6-Ly-2^a stock

* Surgical scientist trainee, Harvard Medical School, Massachusetts General Hospital, Boston, Mass. Supported by U. S. Public Health Service Grant 5T01 GMO 1710-09.

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§ Supported by NCI-CA-08748 and AI-11982.

‖ Supported by a grant from the Yale University School of Medicine, New Haven, Conn.

[1] *Abbreviations used in this paper:* A:T, attacking cell to target cell ratio; B6, C57BL/6; B10, C57BL/10; FCS, heat-inactivated fetal calf serum; MHC, the *H-2* major histocompatability complex in the mouse; MLC, mixed lymphocyte culture; MST, median survival times; T^C, cytotoxic effector T lymphocyte; T^H, helper (cooperator) T lymphocyte.

used in these experiments was from the eighth backcross generation and differed from B6 by two weak histocompatibility loci *H(Ly-2-N8)* and *H(Ly-2-N16)* (3, 4). B10.A, A/J, and B10.BR mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Other inbred mice used in experiments presented in Tables II-VI were obtained from the Animal Division of the Clinical Research Centre, Harrow, Middlesex, England.

Skin Grafts. Body skin grafts, 1 × 1.5 cm, were grafted to B6 females as described by Billingham (5) with modifications described recently by Wachtel et al. (6). B10 and CBA females were grafted with tail skin, 0.5 x 0.5 cm (5). All grafts were scored visually for signs of rejection. In experiments with B6 females, 3-6 mo after the first grafts had been rejected, B6 male skin was applied to the opposite side of the thorax. In experiments with B10 or CBA female recipients, the second male skin grafts were applied to the opposite side of the thorax 1 mo after acute rejection of allogeneic first grafts. Median survival times (MST) were computed by the graphic method of Litchfield (7). Statistical significance was determined by the Wilcoxon rank sum test (8)

In Vitro Sensitization and Cytoxicity Assay. The materials and methods employed for MLC and the microcytotoxicity assay have been previously described (2, 9). Briefly, for MLC, spleen cell suspensions from primed B10 females were adjusted to 5×10^6 cells per ml in RPMI medium with 10% fetal calf serum (FCS). For antigen, a similarly prepared suspension of male spleen cells was given 2,000 R from a cobalt 60 source. Responder cells, 10 ml (5×10^7), and irradiated stimulating cells, 10 ml (5×10^7), were dispensed into 25 cm^2 plastic tissue culture flasks (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.) and incubated, with flasks standing upright, at 37°C in a humidified 10% CO_2 atmosphere. After 5 days, responder cells from the MLC were harvested as a source of attacking cells for the microcytotoxicity assay. These cells were adjusted to 2×10^6 per ml in Eagle's minimal essential medium with 10% FCS, and twofold serial dilutions were performed. 0.2 ml of each of these attacking cell suspensions was added to wells of a flat-bottomed Microtiter plate (Cooke, Division of Dynatech Laboratories, Ltd., Billingshurst, England) allowing 3 or 4 replicates for each attacking cell to target cell (A:T) ratio. 0.05 ml (1×10^5) of ^{51}Cr-labeled concanavalin A blast spleen target cells was dispensed into each well. Maximum lysis was determined by adding 0.05 ml of target cells and 0.2 ml 5% Triton to a set of wells. Spontaneous release of ^{51}Cr was measured from target cells incubated with medium alone. After 5-min centrifugation at 500 rpm, plates were incubated for 3 h at 37°C in a humidified 10% CO_2 atmosphere. Plates were then centrifuged at 1,000 rpm for 10 min and 0.1-ml samples of supernate removed for gamma counting. The corrected percent lysis was computed according to the formula of Wunderlich et al. (10), and linear regression was used for analysis of dose-response data.

Results

In Vivo Second Set Responses to H-Y. Responder female mice, B6 or B10, which usually reject male skin grafts, were grafted with male donor skin incompatible for *H-2* and/or non-*H-2* antigens. Syngeneic male skin grafts, applied after rejection of the first male allografts, were rejected in an accelerated manner (Tables I and II). The second set response was observed only when first grafts were male, not in mice previously ungrafted or grafted with female skin of otherwise equivalent genotype. The effect of male first grafts from congenic *H-2* resistant mice (e.g., B6-$H\text{-}2^k$ and B10.A) was statistically significant, indicating that at least some component of H-Y is detected in vivo on primary stimulating cells which are incompatible for *H-2* with the responder female. However, A.BY ($H\text{-}2^b$) male grafts were more effective than A ($H\text{-}2^a$) male grafts (Table I), and B10.A ($H\text{-}2^a$) male grafts were more effective than A/J ($H\text{-}2^a$) male grafts (Table II). Although both A and A/J male skin grafts appeared to shorten the median survival time of subsequent syngeneic male grafts, the effect was not statistically significant.

Table III presents data obtained when nonresponder CBA females were grafted first with allogeneic male skin and then with syngeneic male skin. This procedure did not convert these mice to H-Y responders.

TABLE I
Survival Times of Male Skin Grafts on B6 Females

First skin graft			Survival times of second (B6 male) skin grafts (days)		
From:	Histocompatibility differences	Survival time (days)	Individual values	MST	*P*
B6-*H-2*^{k}♂	*H-2* + H-Y	10–12	11, 13, 13, 15, 15, 17, 18	13.5	<0.01
B6-*H-2*^{k}♀	*H-2*	8–11	19, 20, 28, 28, 28, 30, 34, 34, 53, 55	28	
A♂	*H-2* + non-*H-2* + H-Y	8–12	10, 14, 14, 18, 26, 28, 32	17	<0.1
A♀	*H-2* + non-*H-2*	9	15, 20, 25, 30, 30, 35, 40, 55	26	
A.BY♂*	Non-*H-2* + H-Y	10–14	11, 11, 12, 12, 12, 12, 12, 13, 16	11.5	<0.01
A.BY♀	Non-*H-2*	10–14	15, 18, 23, 23, 25, 28, 28, 38	22	
B6-Ly-2^{a}♂	Non-*H-2* + H-Y	20–34	10, 10, 10, 10, 11, 11, 11, 12	10	<0.01
B6-Ly-2^{a}♀	Non-*H-2*	20–71	11, 15, 16, 18, 20, 23, 28, 28, 38, 38	20	
B6♂	H-Y	13–50‡	9, 9, 10, 11, 11, 12, 15, 16	10.5	<0.01‡

* A.BY mice express the H-2^{b} phenotype.
‡ MST, 26 days for 42 recipients grafted in the same week as the allogeneic grafts. Eight of these females were selected at random for second set grafts done in parallel with the allogeneic grafted animals. The survival times of the second graft were compared with the survival times of the first graft for the eight animals presented here as well as the survival times of the first grafts for the entire group.

TABLE II
Survival Times of Male Skin Grafts on B10 Females

First skin grafts		Survival times of second (B10 male) skin grafts (days)		
Donor	Histocompatibility differences	Individual values	Median	*P*
B10♂	H-Y	16, 16, 16, 16, 23, 23, 31, 31, 40+*, 40+	23	0.05
B10♀	None	27, 27, 27, 27, 38, 40+, 40+, 40+, 40+, 40+	39	
B10.A♂	*H-2* + H-Y	18, 18, 18, 18, 18, 18, 23, 23, 27, 27	18	<0.01
B10.A♀	*H-2*	23, 27, 31, 38, 38, 40+, 40+, 40+, 40+	38	
A/J♂	Non-*H-2* + *H-2* + H-Y	16, 16, 18, 23, 23, 27, 27, 31, 31, 31	25	0.1
A/J♀	Non-*H-2* + *H-2*	23, 23, 27, 38, 38, 38, 40+, 40+	38	
A/θAKR♂	Thy 1 + non-*H-2* + *H-2* + H-Y	23, 23, 23, 23, 23, 23, 23, 27, 27, 27, 27, 27	23	<0.01
A/θAKR♀	Thy 1 + non-*H-2* + *H-2*	27, 27, 27, 31, 31, 38, 38, 38, 38, 40+, 40+, 40+	38	

* Grafts were not scored from 41 to 59 days after grafting. By 60 days all second grafts had been rejected by their female hosts.

In Vitro Secondary Responses to H-Y. To determine whether the secondary response to H-Y, after in vivo allogeneic primary sensitization, is restricted by the *H-2* complex, a series of in vitro secondary sensitizations and cytotoxicity assays was performed. B10 females were primed in vivo by grafting with either B10, BALB/c, or CBA male skin. After 2 or more wk, primed B10 female spleen cells were placed in MLC with B10, BALB/c, or CBA male spleen cells for 5 days and then assayed against a panel of B10 and BALB/c or CBA male and female target cells. The results are given in Table IV. B10 females, primed in vivo and challenged in vitro with B10 male, gave the expected cytotoxic response restricted to B10 male target cells. If primed and challenged with allogeneic (BALB/c or CBA) male, the cytotoxic response was restricted to allogeneic male and female target cells (anti-MHC response). However, B10 females, primed in vivo with an allogeneic (BALB/c or CBA) male graft and challenged in MLC with B10 male cells, gave cytotoxic responses restricted to B10 male targets. Similar results were obtained using the B10 congenic *H-2* resistant strain

TABLE III
Survival Times of Male Skin Grafts on CBA Females

First skin grafts		Survival times of second (CBA male) skin grafts (days)	
Donor	Histocompatibility differences	Individual values	Median
CBA♂	H-Y	100+, 100+, 100+, 100+, 100+, 100+, 100+, 100+	100+
CBA♀	None	100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10.BR♂	Non-H-2 + H-Y	100+, 100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10.BR♀	Non-H-2	100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10.A♂	Non-H-2 + H-2(K, IA, IB) + H-Y	18, 18, 100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10.A♀	Non-*H-2* + *H-2*(*K*, *IA*, *IB*)	100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10♂	Non-*H-2* + *H-2* + H-Y	100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+
B10♀	Non-*H-2* + *H-2*	48, 100+, 100+, 100+, 100+, 100+, 100+, 100+, 100+,	100+

B10.BR, as shown in Table V. Furthermore, B10 females, primed in vivo with a B10.BR female skin graft and challenged in MLC with B10 male, failed to give cytotoxic responses to either B10 or B10.BR male or female target cells. Allogeneic presensitization to H-Y, therefore, requires the use of male cells and is not a nonspecific adjuvant effect of allogeneic priming.

F_1 females produced from a responder (B10) parent and a nonresponder (such as CBA) parent, when sensitized to a male of one of the parental haplotypes, give cytotoxic responses restricted to that male parental haplotype (11, footnote 2). Thus, (CBA × B10)F_1 females, primed with a CBA male graft and challenged in MLC with CBA male cells, will lyse CBA but not B10 male targets. Similarly, if primed and challenged with B10 male, the F_1 responder cells will lyse B10 male but not CBA male targets (Table VI). However, if the F_1 female is grafted with male skin of one parental haplotype (B10 or CBA) and then challenged in MLC with male cells of the other parental haplotype (CBA or B10), no cytotoxicity is seen against either male parental target cell (B10 or CBA), as shown in Table VI.

Discussion

Previous experimental evidence has indicated that male cells from low responder strains and/or allogeneic male grafts can sensitize responder strain females to syngeneic male grafts. In an early report of growth inhibition of a tumor of male origin, immunization with cells from males of low responder strains, C3H and ST, was shown to prolong the survival of C57BL hybrid females subsequently challenged with C57BL male tumor (12). Billingham and Silvers (13) demonstrated that bone marrow from males of several low responder strains (A, C3H, CBA, and AU) was capable of inducing H-Y-specific tolerance in newborn B6 females. It has also been reported that CBA ($H\text{-}2^k$) male lymph node cells or male lymph node cells from rats can sensitize B6 females ($H\text{-}2^b$) to give a second set response to B6 male skin (14). Finally rejection of B6 or B10 ($H\text{-}2^b$) male skin by B6 or B10 females is accelerated by contralateral grafting of $H\text{-}2^k$ male skin (15, 16). Therefore it is not surprising that allogeneic male skin grafts are capable of eliciting a second set response to syngeneic male skin.

[2] Gordon, R. D., L. E. Samelson, and E. Simpson. 1976. Further studies on the specificity of T-cell mediated cytotoxic responses to H-Y antigen in mice. In preparation.

TABLE IV
Specificity of Cell-Mediated Cytotoxic Responses by B10♀ Primed with BALB/c♂ or CBA♂ Skin

	Responding cell	Antigen		Target cell	Corrected percent lysis (A:T = 4:1)
		In vivo	In vitro		
A	B10♀	BALB/c♂	BALB/c♂	B10♂	−0.55 ± 0.32
				B10♀	1.55 ± 1.32
				BALB/c♂	29.90 ± 2.57
				BALB/c♀	28.70 ± 2.62
	B10♀	BALB/c♂	B10♂	B10♂	20.84 ± 1.46
				B10♀	2.26 ± 0.33
				BALB/c♂	2.57 ± 0.14
				BALB/c♀	1.23 ± 0.13
	B10♀	B10♂	B10♂	B10♂	27.56 ± 1.52
				B10♀	2.79 ± 0.37
				BALB/c♂	3.70 ± 0.75
				BALB/c♀	2.70 ± 0.13
B	B10♀	CBA♂	CBA♂	B10♂	−1.56 ± 0.80
				B10♀	−0.96 ± 0.96
				CBA♂	8.02 ± 1.70
				CBA♀	9.62 ± 1.05
	B10♀	CBA♂	B10♂	B10♂	17.73 ± 1.35
				B10♀	0.90 ± 0.76
				CBA♂	2.14 ± 0.79
				CBA♀	0.62 ± 0.53
	B10♀	B10♂	B10♂	B10♂	29.65 ± 2.05
				B10♀	1.81 ± 1.08
				CBA♂	0.78 ± 1.15
				CBA♀	2.00 ± 0.24

B10 female spleen cells from mice primed in vivo with a BALB/c, CBA, or B10 male skin graft and challenged in vitro with BALB/c, CBA, or B10 male spleen cells, were assayed in quadruplicate for 3 h with ^{51}Cr-labeled target cells at A:T = 0.5:1, 1:1, 2:1, and 4:1. Corrected percent lysis is the percent killing of target cells (corrected for background) at A:T = 4:1 as determined from a four point linear regression fit ± 1 SE. Background (spontaneous) ^{51}Cr release was less than 12%, and SE were less than 3% of mean counts.

In Tables I and II, male grafts from mice incompatible with the responder female for *H-2* and/or non-*H-2* antigens were as effective as syngeneic male grafts in their ability to presensitize B6 or B10 females to a second syngeneic male graft, with the curious exception of A and A/J male first grafts. This might be an effect of the more acute first set rejection by B6 and B10 seen with A and A/J grafts and/or, since B10.A male grafts did presensitize responders to H-Y, this might be an effect of non-*H-2* antigens in the A and A/J strains.

We were unable to convert nonresponder CBA females to responders for H-Y by presensitizing CBA females with allogeneic male skin grafts. A further experiment with a total of 20 mice, 10 in each group, indicates that A female mice grafted with contralateral A male and A.BY male grafts do not reject A

TABLE V

Specificity of Cell-Mediated Cytotoxic Responses by B10♀ Primed with B10.BR♂ or B10 BR♀ Skin

Responding cell	Antigen		Target cell	Corrected percent lysis (A:T = 4:1)
	In vivo	In vitro		
B10♀	B10.BR♂	B10.BR♂	B10♂	−3.04 ± 1.00
			B10♀	−3.61 ± 0.61
			B10.BR♂	17.38 ± 0.14
			B10.BR♀	16.49 ± 0.11
B10♀	B10.BR♂	B10♂	B10♂	18.42 ± 2.85
			B10♀	−1.01 ± 0.58
			B10.BR♂	0.55 ± 0.51
			B10.BR♀	0.31 ± 1.30
B10♀	B10.BR♀	B10.BR♂	B10♂	−1.46 ± 1.33
			B10♀	−1.89 ± 0.93
			B10.BR♂	24.88 ± 1.44
			B10.BR♀	25.46 ± 2.81
B10♀	B10.BR♀	B10♂	B10♂	−0.32 ± 0.36
			B10♀	−1.61 ± 0.85
			B10.BR♂	−0.76 ± 0.55
			B10.BR♀	−0.46 ± 1.31
B10♀	B10♂	B10.BR♂	B10♂	3.06 ± 1.31
			B10♀	1.71 ± 0.82
			B10.BR♂	44.01 ± 2.93
			B10.BR♀	47.65 ± 0.41
B10♀	B10♂	B10♂	B10♂	57.31 ± 1.09
			B10♀	5.20 ± 1.57

B10 female spleen cells from mice primed in vivo with B10.BR male or female or B10 male skin grafts and challenged in vitro with B10.BR or B10 male spleen cells were assayed in triplicate for 3 h with ^{51}Cr-labeled target cells at A:T = 1:1, 2:1, 4:1, and 8:1. Corrected percent lysis is the percent kill of target cells at A:T = 4:1 as determined from a four point linear regression fit ± 1 SE. Background (spontaneous) ^{51}Cr release was less than 13% and SE were less than 5% of mean counts.

male grafts better than A females grafted with contralateral A male grafts.[3] However, others, using sequential body skin graft timing similar to the experiments reported here, have reported that allogeneic A.BY or B10 male grafts sensitized A females to subsequent A male grafts (17). Since contralateral male skin grafts have shown different effects than sequential grafts in other H-Y reports (see 15, 16) and body skin grafts are more sensitive to rejection than tail skin in weak histocompatibility systems including H-Y (4, 18), these results are not in conflict.

In vitro *H-2*-restricted cell-mediated cytotoxicity was first demonstrated for responses to virus-induced and hapten-modified cell surface antigens (reviewed by Doherty et al. and Shearer et al., 19, 20). To these examples may now be added responses to syngeneic fibroblasts (21) and minor H antigens (22, 23), as well as H-Y (2). In nearly all these systems it has been established that F_1 cytotoxic responder cells sensitized to parental cells of one *H-2* haplotype will lyse only target cells bearing that parental *H-2* haplotype at least *H-2K* or *H-*

[3] Mathieson, B. J., Unpublished observations.

TABLE VI
Specificity of Cell-Mediated Cytotoxic Responses by (CBA × B10)F_1♀ Primed and Challenged with CBA♂ and/or B10♂ Cells

Responding cell	Antigen		Target cell	Corrected percent lysis (A:T = 4:1)
	In vivo	In vitro		
(CBA × B10)F_1♀	CBA♂	CBA♂	B10♂	−0.11 ± 0.20
			B10♀	0.49 ± 0.27
			CBA♂	21.73 ± 1.38
			CBA♀	5.37 ± 0.30
(CBA × B10)F_1♀	CBA♂	B10♂	B10♂	1.91 ± 0.62
			B10♀	1.73 ± 0.55
			CBA♂	1.10 ± 0.67
			CBA♀	3.98 ± 0.43
(CBA × B10)F_1♀	B10♂	B10♂	B10♂	26.34 ± 2.34
			B10♀	2.78 ± 1.02
			CBA♂	0.92 ± 1.07
			CBA♀	1.89 ± 0.20
(CBA × B10)F_1♀	B10♂	CBA♂	B10♂	−0.59 ± 0.80
			B10♀	2.86 ± 1.23
			CBA♂	0.55 ± 0.04
			CBA♀	1.65 ± 0.33

(CBA × B10)F_1 female spleen cells from mice primed in vivo with a CBA or B10 male skin graft and challenged in vitro with CBA or B10 male spleen cells were assayed in triplicate for 3 h with ^{51}Cr-labeled target cells at A:T = 1:1, 2:1, 4:1, and 0.5:1 or 8:1. Corrected percent lysis is the percent kill of target cells at A:T = 4:1 as determined from a four point linear regression fit ±1 SE. Background (spontaneous) ^{51}Cr release was less than 15% and SE were less than 5% of mean counts. Maternal parents are listed first in describing the origins of F_1 mice, e.g., (CBA × B10)F_1 means (CBA female × B10 male)F_1.

2D. As shown in Table VI and as documented more extensively elsewhere (11, footnote 2), this has also been shown for T-cell-mediated responses to H-Y. Thus, *H-2* compatibility appears to be required between stimulating cell and target cell, not just between responder cell and target cell.

Zinkernagel and Doherty (24) originally proposed two explanations for *H-2*-restricted cytotoxicity. The first hypothesis, the intimacy or dual recognition hypothesis, requires matching of *H-2* determinants on responding T cell and target cell as a prerequisite for recognition of target cell antigen. The second hypothesis, the altered-self or interaction hypothesis, postulates recognition of a new antigenic determinant or neoantigen resulting from the interaction of self-*H-2* gene products and the antigen. The finding that *H-2* compatibility is required between stimulating cell and target cell has been interpreted as better supporting the altered-self hypothesis, but has not ruled out a dual recognition mechanism.

We here report that responder females, primed in vivo with an allogeneic male skin graft and challenged in MLC with syngeneic male cells, generate cytotoxic cells able to lyse only syngeneic male cells. In addition, we have found that F_1 female responders, primed in vivo with one male parental haplotype and challenged in MLC with the other male parental haplotype, fail to give cytotoxic responses against either male parental target cell. Allogeneic female cells did not prime in vivo or in vitro cytotoxic responses to H-Y. Thus, heterogeneous

immunization, involving stimulating cells of different *H-2* haplotypes in primary and secondary sensitization, is only successful in generating an H-Y-specific response if (*a*) the haplotype of the primary stimulating cell in vivo is male and alloantigenic to the MHC of the responder, and (*b*) the secondary stimulating cell in MLC is male and syngeneic with the responder.

On the basis of present information it is difficult to reconcile these observations with either the intimacy or the altered-self hypothesis. We have been able to achieve in vivo primary sensitization of female responder cells to H-Y using a male stimulating cell with *H-2* haplotype different from the responder cell. Thus, primary sensitization to H-Y does not require dual recognition of self-*H-2* determinants and H-Y antigen. Furthermore, F_1 females, primed with a skin graft bearing one male parental haplotype and challenged in MLC with stimulating cells bearing the other male parental haplotype, fail to give cytotoxic responses against either male parental target cell despite the fact that both parental male cells share *H-2* determinants with the responder cell.

The altered-self hypothesis predicts that the H-Y antigen for T-cell cytotoxic responses is the product of an interaction between *H-2* and H-Y gene products and is therefore unique for each *H-2* haplotype, but this seems inconsistent with the observation that it is possible to presensitize female responder cells in vivo with male stimulating cells of *H-2* haplotype different from the stimulating cell in secondary MLC and target cell in cytotoxicity assay. Perhaps the stimulating cell antigen and the target cell antigen involve different components of H-Y or, as has been previously suggested (2), a helper determinant, important during primary sensitization, is shared by inbred strains. However, the data demonstrating that F_1 females, primed with one male parental haplotype and challenged in MLC with the other male parental haplotype, fail to generate cytotoxic activity against either male parental haplotype argue against a shared helper determinant.

It has recently been established that there are several functional subsets of T lymphocytes (reviewed by Medawar and Simpson, 25) including cytotoxic effector cells (T^C), and cooperator (helper) cells (T^H). The T^H cell is required for optimum responses by T^C cells. Blanden et al. (26) have suggested that the antigen-receptor dictionaries of these two subsets may be different from each other and that in the mouse the T^C subset is restricted to responding to variations in antigenic patterns coded for by *H-2K* and *H-2D* region genes, and the T^H cell is restricted to variations in antigenic patterns dictated by genes in the *H-2I* region (Ia antigens?). T-cell responses may be limited to either non-self (allogeneic or xenogeneic) MHC antigens or altered-self antigens which result from the interaction of self-*H-2* antigens and foreign antigens (or a non-*H-2* gene product such as an H-Y product). This might imply a complex H-Y antigen with *H-2K/D* and possibly *H-2 I*-region determinants. It may be that the T^H subset generated during in vivo primary sensitization to non-self (allogeneic) male antigen is capable of helping secondary cytotoxic responses to altered-self (syngeneic) male antigen by the *H-2*-restricted T^C cells generated in secondary MLC. Thus, $H\text{-}2^b$ (B10 female) T^H cells generated in vivo in response to $H\text{-}2(Y)^k$ (CBA male) may be able to help subsequent responses by $H\text{-}2^b$ (B10 female) T^C cells generated in MLC in response to $H\text{-}2(Y)^b$ (B10 male). We have no definitive evidence that this is the case but such an explanation might reconcile the

observations reported here with the altered-self hypothesis and has the virtue of being amenable to experimental testing.

The data discussed here do not necessarily conflict with the view that the different inbred strains of mice express the same H-Y gene product. Gordon et al. (2) have shown that B10 female cells, primed and challenged with B10 male cells, will lyse F_1 targets produced by reciprocal matings of B10 males and females with females and males of nonresponder strains (BALB/c, CBA, and A). These F_1 targets were lysed regardless of whether the H-Y gene was inherited from a responder or nonresponder strain male parent and whether the required *H-2* compatible haplotype ($H\text{-}2^b$) came from a male or female B10 parent. *H-2*-restricted T-cell cytotoxic responses to H-Y can thus be explained on the basis of the interaction of an H-Y gene product common to all strains and *H-2* gene products unique to each strain. The H-Y gene confers the sex specificity and the *H-2* complex the strain specificity of the response. It is not necessary to invoke multiple H-Y alleles to explain strain specificity.

H-Y antigen has demonstrated a high degree of cross-reactivity serologically, not only between inbred strains of mice, but amongst species as well (27, 28). The graft rejection data presented as well as the literature discussed above (12–16), also supports the concept of cross-reactivity between strains for the homograft response. Furthermore, Silvers and Yang have demonstrated that in vivo xenogeneic sensitization of female mice with male rat cells results in accelerated syngeneic, mouse, male skin rejection (14). It is likely that the antigen-receptor dictionary for the B cell is different from that for the T^C cell, and that B cells are not restricted to responding to variations in antigenic patterns dictated by MHC gene products. Since an in vitro stimulation system requires cells to (*a*) recognize a specific antigenic difference (H-Y), (*b*) survive and proliferate in the MLC culture, and (*c*) subsequently respond as T^C cells in the cytotoxicity assay, this highly selected effector population may not reflect the total population of effector cells responsible for the H-Y in vivo homograft response. However this highly selected system may allow us to examine the contribution of subsets of immunologically reactive cells at specific points in the immune response.

Summary

C57BL/6 and C57BL/10 female mice were grafted with skin from male or female donors incompatible for *H-2* and/or non-*H-2* antigens. Syngeneic male grafts applied after the rejection of primary allografts or syngeneic male grafts were rejected in accelerated (second set) fashion, whereas male grafts applied after primary female grafts were not. In addition, C57BL/10 female spleen cells, primed in vivo with an allogeneic (BALB/c, CBA, or B10.BR) male graft and challenged in vitro in mixed lymphocyte culture with syngeneic (C57BL/10) male cells, produced cytotoxic cells specific for syngeneic male target cells.

We conclude that at least some component of H-Y is detected by female responder cells on allogeneic male cells, and that the second set cell mediated response to H-Y is not necessarily restricted by the *H-2* haplotype of the primary sensitizing strain. Moreover, (CBA × B10) F_1 females, primed in vivo with male cells of one parental haplotype (B10 or CBA) and challenged in vitro with male cells of the other parental haplotype (CBA or B10), fail to lyse male target cells of either parental haplotype. It therefore seems unlikely that a helper determi-

nant shared between B10 and CBA is sufficient to explain the ability of CBA male cells to prime *H-2*-restricted T-cell cytotoxic responses by B10 females.

Received for publication 19 May 1976.

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