

T CELLS THAT ENCOUNTER VIRUS IN THE COMPLETE
ABSENCE OF A PARTICULAR H-2 ANTIGEN ARE
NONRESPONSIVE WHEN STIMULATED AGAIN IN THE
CONTEXT OF THAT H-2 ANTIGEN*

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Thoracic duct lymphocyte (TDL)¹ populations from both H-2K^dD^d (BALB/c and B10.D2) and H-2K^bD^b (C57BL/6J, [B6]) mice can, after removal of alloreactive T cells by filtration through irradiated recipients (1), be induced to respond to vaccinia virus presented in the context of H-2K^k (2-5). This capacity to recognize virus in association with an allogeneic H-2 determinant allows us to examine the consequences of priming with virus in the complete absence of the H-2 antigen in question. Obviously, there is no chance that H-2K^dD^d cytotoxic T lymphocytes (CTL) stimulated in an H-2K^dD^d environment will ever encounter virus presented in the context of the private H-2K^k specificities that determine H-2 restriction (6).

Several different models may be argued. One possibility is that the clone of H-2^d T cells which recognizes H-2K^k-vaccinia virus is a component of the self-H-2-restricted T-cell repertoire for vaccinia virus. An alternative proposition is that exposure to virus in the absence of the H-2 antigen associated with the response may be sufficient to prime, but not to allow the development of effector function. The consequence of both these ideas would be that prior sensitization of H-2^d T cells with virus presented in the context of self-H-2^d should result in an enhanced response, after appropriate filtration and stimulation with H-2K^k-vaccinia virus.

The converse idea is that an encounter with virus in the absence of a particular H-2 antigen may tolerize the T cells that could respond in the context of that H-2 antigen. According to the Cohn and Epstein (7) version of the Bretscher and Cohn (8) model for lymphocyte activation, binding of virus alone may paralyze the T cell, whereas interaction with both virus and H-2 antigen leads to stimulation and differentiation to effector function. Exposure of H-2^d or H-2^b T cells to vaccinia virus presented in the normal self situation would thus be expected to result in tolerization of those lymphocytes capable of interacting with H-2K^k-vaccinia virus. Our results support this concept.

Materials and Methods

Mice. The CBA/J (H-2^b), B6 (H-2^b), C57BL/10Sn (H-2^b), CBA/J × B6 F₁, A/J (H-2^a), BALB/c × B6 F₁, C3H × DBA/2 F₁, B10.A (H-2^a), B10.A(2R) (H-2^{k-b}), and B10.Br (H-2^k)

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¹ Abbreviations used in this paper: B6, C57BL/6J mice; CTL, cytotoxic thymus-derived lymphocytes; GAT, L-glutamic acid⁹⁰-L-alanine³⁰-L-tyrosine¹⁰; PFU, plaque-forming units; TDL, thoracic duct lymphocyte(s).

strains were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c (H-2^d) and C3H/He (H-2^k) mice were from The Institute for Cancer Research Fox Chase, Philadelphia, Pa. CBA/J × BALB/c F₁ mice were bred in our own animal facility at The Wistar Institute. The B10.A(4R) mice were obtained through the Division of Cancer Treatment of the National Cancer Institute.

Virus. The vaccinia virus (WR isolate) was obtained from Dr. R. Zinkernagel, Scripps Clinic and Research Foundation, La Jolla, Calif., and was propagated in L929 cells. Stock virus contains 4×10^7 plaque-forming units (PFU) per milliliter. Mice were infected intravenously with 0.5×10^7 PFU and target cells were infected with 10^7 PFU per $5-10 \times 10^6$ cells.

Cytotoxic Assay. The assay has been described previously (9). Briefly, L929 fibroblasts (L cells, C3H, H-2^k), MC57G methylchol-anthrene-induced tumor (B6, H-2^b) and BALB/c SV (SV40-transformed kidney fibroblasts provided by Dr. B. Knowles, The Wistar Institute) were infected with virus subsequent to labeling with Na₂⁵¹CrO₄. The assays were incubated for 10 h at 37°C, and results were expressed as mean percent specific ⁵¹Cr release for replicates of four wells. The formula used for calculating percent specific ⁵¹Cr release is $(It - MT) \times 100/DT - Mt$, where D is detergent lysis, t is the target, I is immune lymphocytes, and M is spontaneous release for incubation in medium alone.

Negative Selection. Lymphocyte populations (A) specifically depleted of alloreactivity (A-B) for particular major histocompatibility complex antigens (B) were prepared according to procedures fully described elsewhere (1). Briefly, this involved acute filtration of $\sim 1.0 \times 10^8$ TDL, or $2.0 \times 10^8-5.0 \times 10^8$ spleen and lymph node cells, through irradiated (950 rads) recipients that are at least partly allogeneic. Memory vaccinia lymphocytes were obtained from mice primed from 14 d to 6 wk before use with $\sim 10^5$ TCID₅₀ of vaccinia virus. TDL were then collected from the recipients over the period 18-42 h after cell transfer.

Generation of Cytotoxic T Cells. Lymphocytes were injected intravenously at various levels into irradiated (850 rads) recipients. All lymphocyte populations were stimulated with virus 3 h after cell transfer. Spleen cells from the irradiated recipients were assayed 4-6 d later. Control, unirradiated mice were also injected with virus, and spleen cells were assayed concurrently.

Results

We confirmed our earlier finding (2, 3) that naive BALB/c T cells can, after appropriate filtration to remove alloreactive precursors, be induced to respond to H-2K^k-vaccinia virus when stimulated in either H-2-recombinant (A/J, H-2K^kD^d) or F₁ (C3H × DBA/2) recipients (L-cell target, Table I). Lysis of the normal L-cell target by negatively selected T cells is high in one experiment (Exp. 2, Table I), but this is

TABLE I
Naive BALB/c TDL are Able to Recognize H-2K^k-Vaccinia Virus

Experiment	Filtered TDL (1.5×10^7)	950-rad* recipients	Percent specific ⁵¹ Cr release (20:1)					
			L cells (kk)		BALB/c SV (dd)		MC57G (bb)	
			Vaccinia	N‡	Vaccinia	N	Vaccinia	N
1	BALB/c - (CBA × BALB/c)F ₁	A/J	48	7	62	19	8	12
2	BALB/c - (C3H × DBA/2)F ₁	(C3H × DBA/2)F ₁	49	21	32	6	8	19
Unirradiated controls								
Experiment 1								
	CBA (kk)		64	6	13	3	1	10
	BALB/c (dd)		8	15	63	19	6	12
	(CBA × BALB/c)F ₁		21	9	44	13	11	9
	B6 (bb)		6	7	12	11	57	1
Experiment 2								
	A/J (kd)		65	14	33	0	12	19
	(C3H × DBA/2)F ₁ (k/d × k/d)		46	10	27	4	17	11
	BALB/c		9	9	32	4	8	4
	B6		19	30	4	16	74	26

* Mice irradiated 24 h previously were injected with TDL, dosed with vaccinia virus 3 h later, and spleens taken for assay after a further 6 d.

‡ N = normal; not infected with vaccinia virus. dd = H-2K^dD^d; bb = H-2K^bD^b; kk = H-2K^kD^k; kd = H-2K^kD^d

TABLE II
Vaccinia-primed BALB/c (K^d-D^d) TDL Cannot be Sensitized to H-2K^k-Vaccinia Virus

Experiment	Filter (950 rad)	No. of TDL ($\times 10^7$)	Recipient (850 rad)	Assay		Percent specific ⁵¹ Cr release					
				Day	Ratio	L cells (kk)		BALB/c SV (dd)		MC57G (bb)	
						Vaccinia	N*	Vaccinia	N	Vaccinia	N
1	(C3H \times DBA/2)F ₁	1.1	(C3H \times DBA/2)F ₁	4	20:1	12	5	84	1	3	0
2	(C3H \times DBA/2)F ₁ kk \times dd	1.1	(C3H \times DBA/2)F ₁	6	20:1 40:1	0 7	0 0	67 78	11 —	20 —	0 —
3	(CBA \times B6)F ₁ kk \times bb	2.0	B10.A kd	6	12:1 25:1	2 3	0 0	52 61	6 5	0 0	— 0
Unirradiated controls											
Experiment 1			CBA/J (kk)	6	20:1	70	11	8	0	22	0
Experiment 2			B6 (bb)	6	20:1	18	14	25	15	74	0
Experiment 3			C3H (kk)	6	20:1	67	0	15	23	0	17
			B6	6	20:1	7	0	18	24	60	0
			(BALB/c \times C57)F ₁	6	25:1	20	8	44	19	13	6
			bb \times dd								
			B10.A (kd)	6	25:1	36	0	44	6	3	1

* See Table I footnote.

also true for the unirradiated control populations. Vaccinia-primed BALB/c T cells could not, however, be induced to recognize H-2K^k-vaccinia virus (L-cell target, Table II), although a strong virus-specific response was seen in the context of H-2^d (BALB/c SV target, Table II). This was maximal at 4 d after inoculation (Exp. 1, Table II), whereas primary responses to vaccinia virus are not generally apparent before day 5 (10).

One possible explanation for the divergence in the capacity of primed and naive T cells to recognize H-2K^k-vaccinia virus (Tables I and II) is that the secondary T cells operating in the context of H-2^d are generated much more rapidly (Exp. 1, Table II), and eliminate the virus-infected stimulator populations before the primary response to H-2K^k-vaccinia has progressed sufficiently (9). To test this we mixed primed, negatively selected H-2^d TDL with naive F₁ (C3H \times DBA/2) lymphocytes and stimulated the two populations concurrently. Again, the primed H-2^d TDL made no response to H-2K^k-vaccinia virus (groups B and C, Table III). Furthermore, presence of these secondary T-cell populations did not obviously suppress the capacity of the H-2^k \times ^dF₁ T cells to respond to H-2K^k-vaccinia virus (groups D, E, and F, Table III). This experiment was repeated, with identical results, using primed B10.Br T cells and naive (CBA \times B6)F₁ TDL. In this case, the secondary response to vaccinia virus presented in the context of H-2K^k had little, if any, effect on generation of CTL associated with H-2^b (groups H, I, and J, MC57G target, Table III).

Priming with vaccinia virus thus removed the capacity of negatively selected BALB/c T cells to recognize H-2K^k-vaccinia virus. Is the same true for the B6 (K^b-D^b)? Naive B6 T cells can, after filtration through B10.A(2R) or B10.A(4R) (K^k-D^b) recipients be stimulated with vaccinia virus presented in the context of both H-2K^k and H-2D^b (Exps. 1 and 2, Table IV). Contrary to the findings for the BALB/c TDL, lymphocytes from the B6 mice that had been primed with vaccinia virus 6 wk previously were able to respond to both H-2K^k-vaccinia virus and to H-2D^b-vaccinia virus (Exp. 3, Table IV). However, lysis of the H-2^k target was very much lower than that for the H-2^b target (25:1 ratio L cell and MC57G, Exp. 3, Table IV), whereas the

TABLE III
Concurrent Stimulation of Primed Parental and Naive F₁ TDL in Homologous 850-rad F₁ Recipients

Experiment	Group	No. of TDL ($\times 10^7$)		Percent specific ⁵¹ Cr release							
		Primed parent*	Naive F ₁	L cell (kk)		BALB/c SV (dd)		MC57G (bb)			
				Vaccinia	N	Vaccinia	N	Vaccinia	N		
1‡		BALB/c (dd) - (C3H \times DBA/2)F ₁	(C3H \times DBA/2)F ₁ kk \times dd§								
	A	0	0	2	4	4	1	—	—		
	B	0.5	0	2	7	53	4	0	7		
	C	1.0	0	6	14	50	5	1	2		
	D	0	1.5	73	11	52	2	0	2		
	E	0.5	1.5	56	10	48	6	2	8		
	F	1.0	1.5	53	8	54	4	0	2		
		Unirradiated controls: C3H (kk)		66	9	19	12	8	12		
		B6 (bb)		7	5	10	7	36	5		
				L cell				MC57G			
				Vaccinia		N		Vaccinia		N	
				20:1	40:1	20:1	40:1	20:1	40:1	20:1	40:1
2		B10.Br (kk) - (CBA \times B6)F ₁	(CBA \times B6)F ₁ kk \times bb								
	G	1.0	0	73	90	3	0	3	6	1	0
	H	1.0	2.0	88	86	8	11	35	57	0	0
	I	0	2.0	86	86	22	28	57	64	7	6
	J	0	1.0	86	93	15	19	53	67	4	7
		Unirradiated controls: (CBA \times B6)F ₁		64	79	12	17	43	47	14	20
		BALB/c		6	7	12	13	0	2	2	8

* BALB/c and B10.Br mice were injected with vaccinia virus 6-8 wk previously.

‡ All populations assayed at an effector:target ratio of 40:1, with the exception of group A (20:1).

§ See Table I footnote.

converse is true for every other experiment that we have done with this system (Exp. 1 and 2, Table IV; [4, 5]). Perhaps the relatively low response to H-2K^k-vaccinia virus was mediated by T cells that had emerged from thymus, or differentiated as a result of extrathymic stimulation with other antigens (11), over the 6 wk since priming with vaccinia virus.

Spleen and lymph node T-cell populations from mice injected with vaccinia virus cannot be induced to filter through irradiated recipients for at least 4 wk after exposure to virus (J. R. Bennink and P. C. Doherty. Unpublished data.). We thus drained TDL from mice given virus 14 or 18 d previously, and then selected them through 950-rad B10.A(4R) recipients. The results obtained by this procedure were identical to those found for the BALB/c TDL (Table II). A strong response was recognized for vaccinia virus presented in the context of H-2D^b but not of H-2K^k (Exps. 4 and 5, Table IV), although naive B6 TDL generated potent CTL populations in the context of both H-2 determinants (Exp. 2, Table IV).

Discussion

Our results are consistent with the idea that T cells that are exposed to virus in the absence of the relevant H-2 antigen are eventually either paralyzed or deleted. A similar interpretation may be made of the finding of Pierce et al. (12) that helper T

TABLE IV
Sensitization of Negatively Selected Naive and Primed B6 (K^b - D^b) TDL in the Context of H-2K^k-
Vaccinia Virus

Experiment	Population stimulated*	Irradiated recipients	Ratio	Percent specific ⁵¹ Cr release			
				L cells (kk)		MC57G (bb)	
				Vaccinia	N‡	Vaccinia	N
1	Naive B6 S + N – B10.A(2R)	B10.A(2R) (kb)	40:1	31	3	22	0
2	Naive B6 S + N – B10.A(4R)	B10.A(4R) (kb)	20:1 40:1	28 43	8 7	22 33	0 0
	Naive B6 TDL – B10.A(4R)	B10.A(4R)	20:1 40:1	26 41	5 6	17 31	1 0
	Primed B6 S + N – B10.A(2R)	B10.A(2R)	25:1 50:1	17 34	7 7	53 66	— —
4	Primed B6 TDL – B10.A(4R)	B10.A(4R)	20:1 40:1	7 6	1 3	40 57	0 0
	Primed B6 TDL – B10.A(4R)	B10.A(4R)	20:1 40:1	1 1	2 1	36 50	2 0
Unirradiated controls:							
1		B10 (bb)	40:1	8	9	38	0
		C3H (kk)	40:1	48	8	5	0
2		B6 (bb)	40:1	10	9	46	0
		CBA/J (kk)	40:1	67	9	9	3
		BALB/c (dd)	40:1	15	11	9	4
3		B10 (bb)	50:1	17	11	60	—
		B10.A(2R)	50:1	57	5	11	—
4		B6	40:1	9	9	43	2
		C3H	40:1	68	5	4	3
5		B6	40:1	7	5	29	4
		CBA/J	40:1	45	5	7	2
		BALB/c	40:1	5	8	2	3

* Either TDL or spleen and lymph node (S + N) cells were first filtered through irradiated B10.A(2R) or B10.A(4R) recipients and then stimulated with virus for 6 d in a further set of irradiated recipients. The primed S + N populations were from mice given vaccinia virus 6 wk previously and the primed TDL were from mice stimulated for 17–18 d (Exp. 4) or 13–14 d (Exp. 5).

‡ See Table I footnote.

cells sensitized with antigen L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) presented on macrophages expressing one set of I-region determinants subsequently show a greatly diminished primary response to GAT associated with different I-region determinants.

No support was found for the concept that suppression is involved. Concurrent stimulation of negatively selected, memory-parental T cells and naive F₁ TDL did not obviously depress the capacity of the F₁ lymphocytes to generate a primary cytotoxic response, even though potent secondary CTL effectors were present for at least 1 or 2 d longer. This presumably does not reflect elimination of the parental T cells by an F₁ anti-parent response, as the necessary feedback stimulation would not be mediated by cell populations present in the filtered TDL (13). Furthermore, B6

lymphocytes taken at 6 wk, but not at 18 d, after immunization could be induced to recognize H-2K^k-vaccinia virus, which indicates that the response of T cells emerging from thymus (or newly differentiated after peripheral exposure to other antigens [11]) is not suppressed by the presence of the secondary CTL. The fact that the BALB/c T cells taken at 6 wk after immunization could not be stimulated with H-2K^k-vaccinia, whereas the B6 T cells could, probably reflects a difference in precursor pool size, as the latter response is generally stronger and more predictable (2-5).

The induction of immunological paralysis may require prolonged exposure. Korngold and Sprent² found that filtration of T cells (for a maximum of 42 h) through allogeneic mice expressing a particular set of minor histocompatibility antigens did not prevent a subsequent response to these same minor histocompatibility antigens presented in the context of self H-2. However, this experiment is not absolutely equivalent to those described here. The H-2 antigen defining the minor histocompatibility response is self, which is obviously present on the transferred lymphocyte populations throughout the period of initial exposure.² In our experiments the T cells could not possibly see H-2K^k during their first encounter with virus.

The inescapable implication of the concept that exposure to virus alone leads to tolerization is that, contrary to previous speculation (14), the T cells in question have receptors with sufficient affinity for virion components to make some interaction with virus in the absence of H-2 antigen. The idea that triggering for T-cell function is solely a reflection of the capacity to bind antigen is thus not acceptable. One possibility is that the T cell has two receptors, one with affinity for virus and the other with low affinity for self-H-2 determinants. The function of the receptor for virus would be to focus the lymphocyte onto the infected cell, so that the triggering signal is delivered as a result of the second receptor interacting with H-2 antigen. Failure to encounter the relevant H-2 component would lead, in time, to tolerization.

The single-receptor version of the paralysis argument would be that the triggering signal is only delivered via the H-2 molecule on the stimulator cell. Thus the receptor that binds virus would also need to bind H-2 antigen if lymphocyte proliferation and triggering to effector function is to occur. Whereas the two receptor model implies that the H-2-specific component comprised the on-switch, the single receptor argument is consistent with the idea that the T-cell receptor functions primarily as an off-switch, unless a signal is delivered via the H-2 antigen of the stimulator cell to which it binds.

There is no obvious teleological reason why, if generation of a T-cell repertoire in thymus is accepted (15), negatively selected B6 and BALB/c T cells should possess a second receptor specific for H-2K^k-vaccinia virus. The second receptor presumably does not have low affinity (16) for self (H-2^b or H-2^d) H-2 components, otherwise the response would be cross-reactive with that occurring in the normal physiological situation. This does not seem to be the case (2-5, 9). Experiments with H-2 mutant mice have shown that the H-2^b-restricted T-cell response is very specific, allowing discrimination between the H-2K^b and H-2K^{bm1} mutant phenotypes, which differ by as few as 1 or 2 amino acids (17-19). Again, as discussed previously at length (3), the best way to retain the idea of a thymus-selected T-cell repertoire is to argue for the existence of a single receptor, even though it may be made up of two separate

² Korngold, J., and J. Sprent. Negative selection of T cells inducing lethal graft-versus-host disease across minor H barriers: role of the H-2 complex. Manuscript submitted for publication.

components. Cohn and Epstein (7) would consider that the latter is, in effect, dual recognition. The basic alternative is that the T cells that recognize H-2K^k-vaccinia virus are, like the alloreactive set postulated by Jerne (20), an exceptional population that is completely unaffected by events (other than those involving self tolerance) occurring in thymus (5).

Summary

Immunologically naive BALB/c (H-2^d) and C57BL/6J (B6) (H-2^b) T-cell populations can, after filtration to remove alloreactive precursor lymphocytes, be induced to respond to vaccinia virus presented in the context of H-2K^k when stimulated in an appropriate recipient. Exposure to vaccinia virus 6 wk previously completely abrogated the capacity of BALB/c T cells to interact with H-2K^k-vaccinia virus. This is also true for negatively selected B6 thoracic duct lymphocytes taken at 14 or 18 d, but not at 6 wk after immunization: the discrepancy is thought to reflect the progressive emergence of new T cells in the latter group. No evidence could be found for the operation of suppression, and the results are considered to indicate that T cells that interact with virus in the absence of the relevant H-2 antigen are tolerized. Whereas stimulation to effector function is H-2 restricted, induction of immune paralysis may be unrestricted. The capacity of T-cell populations to respond to virus presented in the context of allogeneic H-2 determinants thus depends upon previous antigenic experience.

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