Proc. Natl. Acad. Sci. USA
Vol. 76, No. 7, pp. 3482–3485, July 1979
Immunology

Reciprocal stimulation of negatively selected high-responder and low-responder T cells in virus-infected recipients

(major histocompatibility complex/*Ir* genes/H-2 restriction/vaccinia virus)

JACK R. BENNINK AND PETER C. DOHERTY

The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Communicated by Hilary Koprowski, May 7, 1979

ABSTRACT **After depletion of alloreactive potential, immunologically naive T cells from C57BL/6J (K^b-D^b) mice (B6) can be induced to respond to vaccinia virus in the context of both *H-2K*^{k} and *H-2D*^{b} when stimulated in B10.A(4R) (K^k-D^b) recipients. However, negatively selected B10.A(2R) (K^k-D^b) T cells respond to *H-2D*^{b}–vaccinia virus but not to *H-2K*^{b}–vaccinia virus when primed in an irradiated B6 environment. The B6 mouse strain is a high responder to vaccinia virus associated with *H-2D*^{b}, whereas the B10.A(2R) and B10.A(4R) recombinants are low responders. Responsiveness in the context of *H-2D*^{b} is thus recognized when the only homology between T cell and recipient is at the *H-2D* locus and is suppressed when *H-2K*^{k} is also present in both situations. The fact that negatively selected *H-2K*^{b}-*D*^{b} T cells can be induced to recognize *H-2K*^{k}–vaccinia virus may reflect the existence of an "altered self" complex which is recognized via a single receptor, perhaps drawn from an alloreactive T-cell repertoire. At least in some instances, patterns of T-cell responsiveness are not totally constrained by the spectrum of H-2 antigens encountered in thymus.**

Recent experiments indicate that events occurring during T-cell differentiation in thymus are the central factor determining subsequent capacity to generate potent H-2-restricted cytotoxic responses (1–3). Genetic mapping studies have defined high- and low-responder situations for vaccinia virus presented in the context of the *H-2D*^{b} allele. Mice of the *H-2K*^{k}-*D*^{b} genotype are low responders to *H-2D*^{b}–vaccinia virus, whereas those expressing *H-2K*^{b} (but not *H-2K*^{k}) and *H-2D*^{b} are high responders (4, 5). Strong responses are recognized for both *H-2K*^{k} and *H-2K*^{b}. Chimeras made by reconstituting irradiated mice with F_1 bone marrow cells (*H-2K*^{k}-*D*^{b} × *H-2K*^{b}-*D*^{b}) are high responders to vaccinia virus–*H-2D*^{b} if physiological differentiation occurs in an *H-2K*^{b}-*D*^{b}–thymus, but low responders are generated if an *H-2K*^{k}-*D*^{b} mouse is used instead. Are patterns of responsiveness immutably fixed by the H-2 phenotype of the radiation-resistant thymic environment (6) encountered during T-cell ontogeny?

The present paper examines the problem of levels of responsiveness to *H-2D*^{b}–vaccinia virus in a different way. Acute negative selection procedures (7, 8) are used so that T-cell populations can then be exposed to *H-2D*^{b}–vaccinia virus in the absence of *H-2K*^{k} or *H-2K*^{b} antigens encountered during ontogeny. Our results show that there is no inherent defect in the capacity of the low-responder *H-2K*^{k}-*D*^{b}-mice to stimulate a vaccinia-specific cytotoxic T-cell response in the context of *H-2D*^{b}. Furthermore, T cells from these low-responder *H-2K*^{k}-*D*^{b} mice can be induced to recognize *H-2D*^{b}–vaccinia virus when sensitized in *H-2K*^{b}-*D*^{b} recipients.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Mice and Target Cells. The mice used were bred at the Wistar Institute (Philadelphia, PA) or Jackson Laboratory or were supplied by the Division of Cancer Treatment of the National Cancer Institute. The H-2 types of all strains used have been reported (9), and the *H-2K* and *H-2D* alleles are shown in the tables [for instance, CBA/J (*kk*)]. The same convention is used to identify the target cells, some of which (designated K, SV) were of kidney origin and were transformed with simian virus 40 (4).

Negative Selection and Stimulation. A total of 25 mice were irradiated [950 rads (9.5 J/kg)] and then each was injected intravenously 24 hr later with $2.5–3.0 \times 10^8$ lymph node cells or $4.0–5.0 \times 10^8$ spleen cells, derived from 70 donor mice. Cannulae were inserted into the cisterna chylae on the following morning, and thoracic duct lymphocytes (TDL) were collected 15–42 hr after cell transfer (7). Yields of $3.5–8.0 \times 10^7$ TDL were generally obtained, depending on the mouse strains used. The identity of the TDL was tested in some experiments by complement-mediated lysis (9), using an anti-*H-2K*^{k} antiserum supplied by D. Götze (Max Planck Institute for Biology, Tübingen, Germany). Significant numbers (>5%) of lymphocytes originating from the filter mouse were not found in any case, which has also been the experience of Sprent and von Boehmer (7) in numerous experiments. Recipient mice were injected intravenously with $1.2–1.5 \times 10^7$ cells at 24 hr after irradiation (950 rads) and with vaccinia virus after a further 3 hr; spleen cells were assayed 6 days later.

Cytotoxic Assay. Lymphocytes were assayed for effector function as described (1, 4, 9). The T cells and targets were incubated together for 14–15 hr at 37°C, and results are expressed as specific ^{51}Cr release relative to medium and detergent lysis controls (4). The spontaneous release was generally between 25 and 35%.

RESULTS

Unprimed T cells from C57BL/6J (B6) (K^bI-A^bD^b) mice were filtered through an irradiated B10.A(4R) (K^kI-A^kD^b) environment and then exposed to vaccinia virus in further irradiated B6 and B10.A(4R) recipients. Spleen cells assayed after a further 6 days were strongly lytic for virus-infected, but not for normal, target cells expressing the H-2D^b alloantigen (MC57G or HTGSV, Table 1). The responder status of the recipient is apparently irrevelant in this situation, as the B6 is a high responder to vaccinia virus presented in the context of *H-2D*^{b} and the B10.A(4R) is a low responder (4, 5).

Abbreviations: B6, C57BL/6J mouse strain; SV, simian virus 40-transformed; TDL, thoracic duct lymphocytes.

Table 1. Response of negatively selected $H\text{-}2K^b\text{-}D^b$ T cells to $H\text{-}2K^k$-vaccinia virus and $H\text{-}2D^b$-vaccinia virus

Exp.	Population stimulated	Recipient	% specific ^{51}Cr release (40:1) MC57G (*bb*) Vacc.	N	L cell (*kk*) Vacc.	N	KHTGSV (*db*) Vacc.	N	K2RSV (*kb*) Vacc.	N		
1	$B6_{\text{-B10.A(4R)}}$	B10.A(4R)	35	0	78	23	32	0	71	0		
	Unirradiated controls:											
	(A/J × B6)F_1 (*kd* × *bb*)		52	2	81	17	4	9	74	11		
	BALB/c (*dd*)		0	1	8	8	32	12	5	5		
	CBA/J (*kk*)		7	4	91	9	2	8	71	0		
	(CBA × B6)F_1 (*kk* × *bb*)		60	4	83	13	11	9	67	0		
			MC57G		L cell		K2RSV		KB/cSV (*dd*)		K5RSV (*bd*)	
			Vacc.	N	Vacc.	N	Vacc.	N	Vacc.	N	Vacc.	N
2	$B6_{\text{-B10.A(4R)}}$	B6	81	11	18	24	28	0	16	17	91	23
		B10.A(4R)	48	0	71	9	—	—	16	13	5	24
	Unirradiated controls:											
	B10.A(4R) (*kb*)		11	0	74	9	42	1	10	9	12	13
	B6 (*bb*)		74	0	24	26	22	4	17	16	75	20
	B10.D2 (*dd*)		11	0	17	17	11	12	65	10	52	19
	B10.Br (*kk*)		4	0	82	7	40	9	11	11	10	10

TDL (1.5×10^7) were transferred to each of two irradiated (950 rads) recipients per group, which were injected with vaccinia virus 3 hr later. Spleen cells from these mice were assayed after a further 6 days. Serological analysis (9) at the time of cell transfer revealed that few (<5%), if any, of the TDL bore the recipient H-2K^k alloantigen. Vacc., virus-infected target cells; N, normal target cells.

The converse experiment was done using B10.A(2R) rather than B10.A(4R) T cells because, for some reason that we do not understand, three separate attempts at filtering B10.A(4R) lymphocytes through B6 mice gave insufficient TDL. The B10.A(2R) strain is also $H\text{-}2K^kI\text{-}A^kD^b$ and a low responder to vaccinia virus presented in the context of $H\text{-}2D^b$ (4, 5). Negatively selected B10.A(2R) cells, when stimulated in an irradiated B6 recipient, gave a strong virus-immune cytotoxic T-cell response at $H\text{-}2D^b$ (MC57G target, Table 2). Thus, the low-responder status of the lymphocytes can be reversed by priming in a "high-responder" environment.

The negatively selected B6 lymphocytes that were stimulated in B10.A(4R) recipients gave a strong virus-immune T-cell response at $H\text{-}2K^k$ (L cells, Table 1), which is apparently equivalent to the aberrant recognition (9) that we described previously for $H\text{-}2K^d\text{-}D^d$ T cells exposed to vaccinia virus in the context of $H\text{-}2K^k$ and $H\text{-}2K^s$. This aberrant recognition phenomenon thus seems to be nonreciprocal, because neither $H\text{-}2K^k\text{-}D^b$ (Table 2) nor $H\text{-}2K^k\text{-}D^k$ T cells (Table 3) can be induced to recognize vaccinia virus presented in the context of either $H\text{-}2K^b$ or $H\text{-}2D^b$. Also, lack of the aberrant response is not modified by varying the non-*H-2* genetic background: experiments using B10.Br, C3H (Table 3), or CBA/J (10) T cells were negative.

DISCUSSION

After removal of alloreactive precursor lymphocytes, high-responder ($H\text{-}2K^b\text{-}D^b$) T cells can be sensitized to vaccinia virus presented in association with $H\text{-}2D^b$ when primed in irradiated "low-responder" ($H\text{-}2K^k\text{-}D^b$) recipients. Similarly, low-responder ($H\text{-}2K^k\text{-}D^b$) T cells can be stimulated at $H\text{-}2D^b$ in a "high-responder" ($H\text{-}2K^b\text{-}D^b$) environment. The capacity to generate vaccinia-specific cytotoxic T cells in the context of $H\text{-}2D^b$ is thus independent of whether $H\text{-}2K^b$, $H\text{-}2K^k$, $I\text{-}A^b$, or $I\text{-}A^k$ is encountered during either physiological differentiation in thymus or on virus-infected stimulator cells at the time of immunization. The only obvious constraint is that the lymphocytes must have "seen" $H\text{-}2D^b$ in both situations (but not $H\text{-}2K^k$). A response is possible when the sole major histocompatibility complex region shared by the thymus, the T cells, and the stimulator environment is the one ($H\text{-}2D^b$) with which antigenicity is identified in the cytotoxic assay.

Table 2. Sensitization of negatively selected B10.A(2R) T cells in B6 recipients

Exp.	Population stimulated	Recipient*	% specific ^{51}Cr release† MC57G (*bb*) Vacc.	N	L cell (*kk*) Vacc.	N	K5RSV (*bd*) Vacc.	N	K2RSV (*kb*) Vacc.	N	KB/cSV (*dd*) Vacc.	N
1	$B10.A(2R)_{\text{-B6}}$	B6	40	0	4	2	0	0	22	8	—	—
2	$B10.A(2R)_{\text{-B6}}$	B6	52	0	—	—	—	2	53	0	22	16
	Unirradiated controls:											
1	BALB/c (*dd*)		0	3	4	7	20	8	12	0	39	0
	B6 (*bb*)		68	1	12	—	49	16	53	3	24	0
	B10.A(2R) (*kb*)		4	2	53	16	8	9	56	0	10	0
	C3H (*kk*)		0	4	52	17	18	10	54	6	12	0
2	CBA (*kk*)		22	0	70	11	0	0	65	7	8	0
	BALB/c		12	0	12	7	3	0	20	18	37	0
	B6		74	0	18	14	31	2	62	12	25	15
	B10.A(2R)		18	0	65	3	0	0	58	1	7	0

*Irradiated with 950 rads.
†A ratio of 40:1 was used in Exp. 1 and 20:1 in Exp. 2. Abbreviations as in Table 1.

Table 3. Negatively selected $H\text{-}2K^k\text{-}D^k$ T cells cannot be induced to recognize vaccinia virus presented in the context of either $H\text{-}2K^b$ or $H\text{-}2D^b$

Exp.	Population stimulated	Recipient*	% specific ^{51}Cr release (25:1)† MC57G (*bb*) Vacc.	N	L cell (*kk*) Vacc.	N	K2RSV (*kb*) Vacc.	N	K5RSV (*bd*) Vacc.	N
1	B10.Br (*kk*)$_{(CBA\times B6)F_1}$	(CBA × B6)F_1	0	4	82	2	29	0	0	1
	Unirradiated controls:									
	CBA/J (*kk*)		0	2	64	6	38	1	15	9
	B6 (*bb*)		60	0	16	22	27	12	48	12
	(CBA × B6)F_1		38	10	64	7	45	0	33	3
	C3H$_{(CBA\times B6)F_1}$	B6	0	0	0	0	11	0	—	—
		(CBA × B6)F_1	2	0	77	14	81	1	11	8
2	Unirradiated controls:									
	C3H (*kk*)		6	0	64	9	76	4	14	19
	B6 (*bb*)		42	1	10	7	64	13	54	9
	(CBA × B6)F_1 (*kk* × *bb*)		35	3	75	15	81	11	65	18

*Irradiated with 950 rads.
†Abbreviations as in Table 1.

This could be thought to indicate that *I-A*-restricted T–T help does not operate in these responses (11). However, there are at least two possible alternative explanations. The first is that T–T help functions directly between the transferred T-cell subsets (8) and does not require stimulation by the host environment. The second is that there is some form of allogeneic effect (12, 13) mediated via radiation-resistant lymphocytes in the recipients. This cannot be discounted, although numerous attempts at demonstrating such an allogeneic effect have failed (unpublished data). Also ($K^kI\text{-}A^kD^d \times K^bI\text{-}A^bD^b)F_1$ T cells respond at $H\text{-}2D^b$ when primed in virus-infected $K^bI\text{-}A^bD^b$ but not in $K^kI\text{-}A^kD^b$ irradiated mice (4). An allogeneic effect of the type proposed above should be possible in both cases, although it may be that there is overriding suppression in the nonresponder situation.

Perhaps, as proposed by Zinkernagel *et al.* (5), the low response found for $H\text{-}2D^b$-vaccinia virus in B10.A(2R) and B10.A(4R) mice in some way reflects a suppressive influence associated with $H\text{-}2K^k$. $I\text{-}A^k$ apparently is not involved (5). How could this suppression operate? One possibility that could be argued is that the relative affinities of the H-2K^k and H-2D^b antigens for vaccinia virus proteins may be such that there is not a sufficient association on the cell surface between vaccinia virus glycoprotein and the H-2D^b antigen to produce stimulation (14). The necessary patterns of molecular association at the cell membrane (15) may differ for stimulation and for lysis (16), as cytotoxicity specific for $H\text{-}2D^b$–vaccinia virus is recognized when $H\text{-}2K^k\text{-}D^b$ target cells are exposed to virus-immune T cells from $H\text{-}2K^b\text{-}D^b$ mice. However, this idea does not seem to apply to the present situation. There is apparently no inherent defect, insofar as $H\text{-}2D^b$ is concerned, in the stimulator populations encountered by B6 T cells in irradiated B10.A(4R) recipients.

Another possibility is that the virus-specific cytotoxic T-cell response occurring at $H\text{-}2K^k$ in some way suppresses that at $H\text{-}2D^b$. The simplest means by which this could operate is if the T cells functioning at $H\text{-}2K^k$ are generated earlier and eliminate the virus-infected stimulator populations before the response at $H\text{-}2D^b$ has progressed sufficiently (17). This could reflect a difference in precursor pool size between the two T-cell subsets. Alternatively, some form of suppression may be mediated via direct interaction between the T cells, although requiring stimulation by the irradiated recipient [otherwise the negatively selected B10.A(2R) T cells could not respond in the B6]. Any such effect is specific for $H\text{-}2D^b$, because ($H\text{-}2K^k\text{-}D^k \times K^b\text{-}D^b)F_1$ mice respond to vaccinia virus in the context of both $H\text{-}2K^k$ and $H\text{-}2K^b$, although not of $H\text{-}2D^b$. Furthermore, negatively selected B6 lymphocyte populations sensitized in a B10.A(4R) environment generate cytotoxic T cells specific for both $H\text{-}2K^k$–vaccinia virus and for $H\text{-}2D^b$–vaccinia virus. The "aberrant" cytotoxic response (9) at $H\text{-}2K^k$ is thus not suppressive. Is the apparent suppression found in B10.A(2R) and B10.A(4R) mice mediated by cytotoxic T cells or by another subset of lymphocytes mapping to $H\text{-}2K^k$?

Both B6 ($H\text{-}2K^b\text{-}D^b$) and BALB/c ($H\text{-}2K^k\text{-}D^d$) T cells (9), after appropriate filtration and stimulation, are able to recognize vaccinia virus presented in the context of $H\text{-}2K^k$. Thus, the capacity of T-cell populations to mediate this "aberrant" (9) cytotoxic response is apparently independent of both *H-2* type and of non-*H-2* genetic background. Possible explanations for this phenomenon have been stated (9). Another alternative not previously discussed, but equally feasible, is that this may reflect recognition of a vaccinia virus–$H\text{-}2K^k$ "altered self" complex, mediated via a highly conserved alloreactive T-cell repertoire (14, 18, 19). The fact that the converse does not occur (neither $H\text{-}2K^k\text{-}D^b$ nor $H\text{-}2K^k\text{-}D^k$ T cells of three different non-*H-2* genetic backgrounds can be induced to interact with vaccinia virus presented in the context of $H\text{-}2K^b$) is also consistent with this idea.

It should be recognized that both the chimera and negative selection procedures have possible limitations as probes for examining questions concerning T-cell repertoire and responder patterns. The (A × B)F_1 → A bone marrow chimeras that respond to virus in the context of A but not of B may be considered to reflect positive selection of a self-monitoring T-cell repertoire restricted to A. However, it is by no means excluded that the effect is mediated via deletion, or suppression in thymus, of the repertoire concerned with B. The negative selection procedure [A filtered through (A × B)F_1] suffers from the potential problem that T cells of type A that might be able to recognize B + virus may also have low affinity for B alloantigen and could thus be removed in the filter environment. The fact that both $H\text{-}2^b$ and $H\text{-}2^d$ T cells can be induced to recognize $H\text{-}2K^k$–vaccinia virus may simply reflect that the tip of this particular iceberg is visible. Are these procedures concerned more with defining the nature of tolerance, whether developmental (chimera) or acute (filtration), rather than with assessing the ontogeny of the T-cell repertoire? This is not a simple problem, given the emerging complexity of lymphocyte interactions in any immune response.

We thank Janet La Martina and Michael Melino for excellent technical assistance and Dr. D. Götze for the gift of antiserum. This work was supported by U.S. Public Health Service Research Grants AI 14162 from the National Institute of Allergy and Infectious Diseases and CA 09140 and CA 20833 from the National Cancer Institute. The B10.A(4R) mice were supplied by the Division of Cancer Treatment, Animal Program of the National Cancer Institute.

1. Zinkernagel, R. M., Althage, A., Cooper, S., Callahan, G. & Klein, J. (1978) *J. Exp. Med.* **148,** 805–810.
2. von Boehmer, H., Haas, W. & Jerne, N. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2439–2442.
3. Billings, P., Burakoff, S. J., Dorf, M. E. & Benacerraf, B. (1978) *J. Exp. Med.* **148,** 352–360.
4. Doherty, P. C., Biddison, W. E., Bennink, J. R. & Knowles, B. B. (1978) *J. Exp. Med.* **148,** 534–543.
5. Zinkernagel, R. M., Althage, A., Cooper, S., Kreeb, G., Klein, P. A., Sefton, B., Flaherty, L., Stimpling, J., Shreffler, D. & Klein, J. (1978) *J. Exp. Med.* **148,** 592–606.
6. Zinkernagel, R. M., Callahan, G. N., Althage, A., Cooper, S., Klein, P. A. & Klein, J. (1978) *J. Exp. Med.* **147,** 882–896.
7. Sprent, J. & von Boehmer, H. (1976) *J. Exp. Med.* **144,** 617–626.
8. Bennink, J. R. & Doherty, P. C. (1978) *Nature (London)* **276,** 829–831.
9. Doherty, P. C. & Bennink, J. R. (1979) *J. Exp. Med.* **149,** 150–157.
10. Bennink, J. R. & Doherty, P. C. (1978) *J. Exp. Med.* **148,** 128–135.
11. Zinkernagel, R. M., Callahan, G. N., Althage, A., Cooper, S., Streilein, J. W. & Klein, J. (1978) *J. Exp. Med.* **147,** 897–911.
12. Katz, D. H. (1972) *Transplant Rev.* **12,** 141–179.
13. Corley, R. B., Kindred, B. & Lefkovits, I. (1978) *J. Immunol.* **121,** 1082–1089.
14. Zinkernagel, R. M. & Doherty, P. C. (1974) *Nature (London)* **251,** 547–548.
15. Cohen, R. T. & Eisen, H. N. (1977) *Cell. Immunol.* **32,** 1–9.
16. Blanden, R. V., McKenzie, I. F. C., Kees, U., Melvold, R. W. & Kohn, H. I. (1977) *J. Exp. Med.* **146,** 869–880.
17. Pang, T. & Blanden, R. V. (1976) *J. Exp. Med.* **143,** 469–481.
18. Jerne, N. K. (1971) *Eur. J. Immunol.* **1,** 1–9.
19. Bellgrau, D. & Wilson, D. B. (1979) *J. Exp. Med.* **149,** 234–243.