PATTERNS OF VIRUS-IMMUNE T-CELL RESPONSIVENESS

Comparison of (H-2^k × H-2^b) → H-2^b Radiation Chimeras and Negatively Selected H-2^b Lymphocytes*

By PETER C. DOHERTY AND JACK R. BENNINK

From The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

The vaccinia-immune cytotoxic T lymphocyte (CTL)[1] response associated with the H-$2D^b$ allele offers one of the few examples of an immune response (Ir) gene effect in the virus systems (1, 2). A strong virus-specific CTL response in the context of H-$2D^b$ is seen in C57BL/6 (B6) or B10 mice (H-$2K^b$I-A^bD^b), but the B10.A(2R) and B10.A(4R) strains (H-$2K^k$I-A^kD^b) are low responders in this regard. The Ir gene effect apparently maps to H-$2K^k$ rather than to I-A^k, as the B10.BYR recombinant (H-$2K^q$I-A^kD^b) is also a high responder (2). Furthermore, low responsiveness to H-$2D^b$-vaccinia virus is apparently dominant in the (H-$2K^k$ I-A^kD^b × H-$2K^b$I-A^bD^b)F_1 situation. Does this mean that the virus-immune CTL response associated with H-$2K^k$ is in some way suppressing that occuring at H-$2D^b$?

One approach to the further analysis of this problem has been to first filter (3) high responder B6 T cells through an irradiated low responder B10.A(4R) environment, and to then stimulate these negatively selected (to H-$2K^k$ and I-A^k alloantigen) thoracic duct lymphocytes (TDL) with vaccinia virus in a further set of irradiated B10.A(4R) recipients. The result of this procedure is that the B6 TDL respond strongly to vaccinia virus presented in the context of both H-$2K^k$ and H-$2D^b$ (4). Apparently, the aberrant response of the B6 TDL to H-$2K^k$-vaccinia virus (5), which has obviously not been determined by physiological differentiation (6) in the context of H-$2K^k$ antigens encountered in thymus, does not suppress the generation of CTL that is specific for H-$2D^b$-vaccinia virus.

The present paper describes attempts at suppressing the stimulation of negatively selected (3, 5) high responder TDL by mixing them with low responder [F_1]T cells, before priming with H-$2D^b$-vaccinia virus in a low responder environment. Evidence is also presented that the virus-specific responder phenotype of an F_1 → parent radiation chimera (6) may not always be equivalent to that associated with the H-2 type of the irradiated parent.

Materials and Methods

Mice, Viruses, Negative Selection, Immunization, Anti-H-2 Treatment, and Cytotoxic Assay. All materials and procedures were identical to those used previously (1, 3, 5). Recipient mice were

* Supported by U. S. Public Health Service grants AI-14162, AI-15412, and NS-11036.

[1] *Abbreviations used in this paper*: B6, C57BL/6J mice; C′, guinea pig complement; CTL, cytotoxic thymus-derived lymphocyte; Ir gene, immune response gene; N, lymph node; S, spleen; SV, SV40 transformed target cell; TDL, thoracic duct lymphocyte.

TABLE I

Response of Negatively Selected Parental and F_1 T Cells to Vaccinia Virus Presented in the Context of H-2K^k and H-2D^b

Exp.	Group	No. of TDL ($\times 10^6$)		850 rad recipient	Percent specific ^{51}Cr release*			
		B6 – B10.A(4R)	[B10 × B10.A(4R)]F$_1$		L cells (kk)		MC57G (bb)	
					Vacc.	N	Vacc.	N
1	A	17	0	B10.A(4R)	57	7	40	0
	B	17	17	B10.A(4R)	95	18	34	0
	C	0	17	B10.A(4R)	75	4	15	2
	D	0	17	B6 (bb)	5	5	58	0
2	E	20	20	B10.A(4R)	87	25	28	0
	F			‡	53	—	25	0
Unirradiated controls:								
1	G	B10 (bb)			10	4	48	0
	H	B10.Br (kk)			52	3	0	0
2	I	B6			17	29	68	0
	J	B10.Br			76	12	0	0
	K	B6 + B10.Br		‡	7	18	77	0

Vacc., cells infected with vaccinia virus; N, normal cells.

* Exp. 1 was assayed at a ratio of 20:1, Exp. 2 at 40:1.

‡ Treated with antiserum to H-2^k + complement, the unirradiated control cells were mixed in equal numbers. The treatment killed 72% of cells in group F and 65% in group K.

injected with TDL and vaccinia virus on the same day, and spleen populations were assayed 6 d later. The assays were incubated for 10 h at 37°C, and the results were expressed as specific ^{51}Cr release relative to the detergent and medium controls.

Chimeras. The chimeras were made following the procedures of Zinkernagel et al. (6), with the exception that a single treatment with monoclonal anti-Thy 1.2 reagent (provided by Dr. J. Sprent [J. Sprent and T. McKearn. Manuscript in preparation.]) and guinea pig complement (C') was used to remove T cells from the transferred (CBA × C57)F$_1$ bone marrow populations. The C57BL/6J (B6) mice were given 950 rads 24 h before reconstitution with 1.7×10^6 F$_1$ bone marrow cells, and held for 12 wk before use. At least 90% of the spleen and lymph node cells from these mice bore the H-2K^k alloantigen.

Results

Vaccinia-immune T-Cell Response in the Context of H-2D^b. Negatively selected B6 (K^{b}I-A^{b}D^b) T cells mediate a strong virus-immune CTL response in the context of H-2D^b (4) when sensitized in 850 rads B10.A(4R) (K^{k}I-A^{k}D^b) recipients (group A, Table I, MC57G target; group L, Table II, HTGSV target). Considerably less effector function is seen when [B10 × B10.A(4R)]F$_1$ T cells are stimulated in the same way (group C, Table I, MC57G target). Both lymphocyte populations also generate high responses to H-2K^k-vaccinia virus (groups A, C, and F, Table I, L-cell target).

Mixing the high (B6) and low (F$_1$) responder populations together before stimulation does not result in any significant diminution in the level of CTL generation associated with H-2D^b-vaccinia virus (groups A and B, Table I, MC57G target; groups L and M, Table II, HTGSV target). In fact, removal of the low responder F$_1$ population with antiserum and complement may enrich for the B6 T cells reacting to virus in the context of H-2D^b (groups M and N, Table II, HTGSV and MC57G targets). This failure to show suppression could reflect that the suppressor T cells are

TABLE II

Concurrent Stimulation of Negatively Selected B6 and [B10 × B10.A(4R)]F_1 TDL in 850 rads B10.A(4R) Recipients

Group	Population* stimulated	Percent specific ^{51}Cr release (30:1)					
		2RSV (kb)		HTGSV (db)		MC57G (bb)	
		Vacc.	N	Vacc.	N	Vacc.	N
L	B6 − B10.A(4R)	65	0	79	4	41	2
M	L + [B10 × B10.A(4R)]F_1‡	79	0	75	0	20	12
N	M + anti-H-2^k + C′	79	0	100	0	36	7
Unirradiated controls:							
O	BALB/c (dd)	0	0	55	17	4	2
P	B6 (bb)	53	0	39	0	63	5
Q	C3H (kk)	65	0	0	0	14	3
R	[B10 × B10.A(4R)]F_1 (bb × kb)	93	0	1	0	45	8
	P + R + anti-H-2^k + C′§	60	0	44	8	67	4
	P + R + C′‡§	69	0	20	0	52	5

Vacc., cells infected with vaccinia virus; N, normal cells.

* 15 × 10^6 negatively selected TDL and 20 × 10^6 F_1 TDL.

‡ Equivalent to an effector:target ratio of 15:1 for each lymphocyte population.

§ The level of lysis caused by the P + R population on L cells (kk) infected with vaccinia virus was 16% after treatment with antiserum + C′, and 38% after incubation with C′ alone. Normal L cells were lysed 7% in each case.

restricted to the H-2K^k or I-A^k of the CTL and cannot, therefore, modulate the response of the B6 TDL. We thought that we might circumvent this problem by using the appropriate F_1 → parent radiation chimera.

The Situation for (CBA × B6)F_1 → B6 Chimeras. We know, from the studies of Zinkernagel and colleagues (7), that such chimeras respond to H-2D^b-vaccinia virus, but not to H-2K^k-vaccinia virus. This presumably reflects sensitization with virus presented on both H-2^b and (H-2^k × H-2^b)F_1 stimulator cells, and latter originating from the transferred bone marrow. Pooled spleen and lymph node or TDL populations from individual chimeras were divided into equal parts and injected into one B6 (K^bI-A^bD^b) or one B10.A(4R) (K^kI-A^kD^b) recipient. Strong virus-immune CTL responses were seen in the context of H-2^b after priming in the B6 recipients (Table III, MC57G target). However, little, if any, specific lysis was recognized for vaccinia virus associated with either H-2K^k or H-2D^b for T cells from 10 of the 11 [(CBA × B6)F_1 → B6] chimeras sensitized in irradiated B10.A(4R) recipients (Tables III and IV). The exception (chimera 11, Table IV) probably reflects carry over of T cells from the bone marrow donor, as only one anti-θ treatment was used rather than the two deemed necessary by Zinkernagel et al. (6).

Discussion

We describe here one instance where an F_1 → parent radiation chimera does not assume the complete responder phenotype of the irradiated parent (7–10). Negatively selected B6 (K^bI-A^bD^b) T cells can respond to vaccinia virus presented in the context of both H-2K^k and H-2D^b when stimulated in an 850 rads B10.A(4R) (K^kI-A^kD^b) recipient. However, lymphocytes from [(CBA × B6)F_1 → B6] radiation chimeras

TABLE III

Stimulation of T cells from [(CBA × B6)F_1 → B6] Bone Marrow Chimeras in Irradiated Recipients

Chimera*		850 rads‡ recipient	Cells§ yield ($\times 10^6$)	Percent specific ^{51}Cr release							
				L cells (kk)				MC57G (bb)			
				Vaccinia		Normal		Vaccinia		Normal	
No.	T cells			20:1	40:1	20:1	40:1	20:1	40:1	20:1	40:1
1	S + N	B10.A(4R)	48	13	25	2	10	6	13	2	4
		B6	6	5	—	0	—	54	—	8	—
2	S + N	B10.A(4R)	74	7	16	5	6	5	8	3	4
		B6	51	2	1	1	5	49	62	3	4
3	S + N	B10.A(4R)	31	9	10	4	1	1	4	2	7
		B6	20	—	2	—	0	—	66	—	4
4	S + N	B10.A(4R)	63	8	16	6	7	4	13	5	7
		B6	22	—	0	—	0	—	62	—	0
5	S + N	B10.A(4R)	65	5	9	6	2	7	17	4	10
		B6	23	0	1	5	5	43	53	1	2
6	TDL	B10.A(4R)	10	—	15	—	3	—	17	—	5
		B6	23	—	4	—	0	—	49	—	3
7	TDL	B10.A(4R)	20	—	10	—	3	—	10	—	5
		B6	15	—	8	—	0	—	58	—	4
8	TDL	B10.A(4R)	3	10	—	1	—	12	—	2	—
Unirradiated Controls:											
Chimeras 1–5		C3H (kk)		35	44	10	13	3	10	1	2
		B6 (bb)		8	9	6	7	38	53	9	0
Chimeras 6–8		C3H		—	50	—	6	—	15	—	9
		B6		8	14	3	7	29	43	7	7
		BALB/c (dd)		10	13	1	4	7	13	4	6

* Greater than 90% of lymphocytes from each chimera were shown to bear the H-2^k alloantigen using antibody + complement treatment.

‡ Spleen and lymph nodes were pooled for individual chimeras, and equal numbers of spleen and lymph node cells (S + N, at least 4.0×10^7) or TDL (2.0×10^7) were given to one B6 and one B10.A(4R) (kb) recipient. Insufficient TDL were obtained from chimera 8 to allow stimulation in a B6 recipient.

§ Numbers of cells recovered from spleen at 6 d after i.v. inoculation of lymphocytes and vaccinia virus.

generally seem not to recognize vaccinia virus when primed in the same way. In fact, the only correspondence between the two T-cell populations is that both are tolerant to the H-$2K^k$ and I-A^k alloantigens: the negatively selected B6 lymphocytes by virtue of acute deletion in the filter environment, the chimera cells as a result of physiological mechanisms operating during ontogeny. The chimeras are, however, also tolerant to H-$2K^k$-vaccinia virus.

The failure of the chimera T cells to respond to H-$2D^b$-vaccinia virus when primed in an H-$2K^k$I-A^kD^b environment might be thought to reflect an absence of T-cell help originating at the H-2K end (8, 9, 11). It is possible that the response of the negatively selected B6 T cells to H-$2K^k$-vaccinia virus in some way helps the generation of virus-immune CTL in the context of H-$2D^b$. However, we have shown previously (4, 5) that filtered B10.A(2R) [K^kI-A^kD^b] T cells can respond to H-$2D^b$-vaccinia virus when primed in B6 recipients, and that B10.D2 [K^dI-A^dD^d] lymphocytes recognize H-$2D^d$-vaccinia virus when stimulated in B10.A(5R) [K^bI-A^bD^d] mice: in neither case is any CTL activity detected for H-$2K^b$-vaccinia virus. The idea that an allogeneic effect

TABLE IV

Responder Patterns of Chimera T Cells to H-2K^k-Vaccinia Virus in 850 rads B10.A(4R) Recipients

Chimera*	Chimera		Percent specific ^{51}Cr release from L cells			
			Vaccinia		Normal	
	No.	T cells‡	20:1	40:1	20:1	40:1
[(CBA × B6)F$_1$ → B6]	9	TDL	13	—	5	—
		S + N	4	5	1	1
	10	TDL	16	23	6	14
		S + N	—	3	—	0
	11	TDL	—	32	—	0
		S + N	—	16	—	6
[(CBA × B6)F$_1$ → B10.Br]	12	TDL	—	54	—	1
		S + N	36	45	0	0
	13	TDL	25	37	2	3
		S + N	47	61	5	8
(CBA × B6)F$_1$ (kk × bb)		S	75	80	6	13
Unirradiated B10 (bb)			13	22	10	16

* None of the chimera populations caused >12% specific lysis of the vaccinia-infected MC57G (bb) target. However, we are uncertain of the status of the MC57G target in this assay, as the one positive (B10) control caused (40:1) only 21% specific lysis on the vaccinia-infected and 16% lysis on the normal target.
‡ 2.0 × 10^7 TDL, or 4.0 × 10^7 mixed spleen (S) and lymph node (N) cells.

(12, 13) mediated by radiation-resistant recipient T cells replaces help in these experiments has also been considered (4, 5, 14), but an identical situation should apply for the [(CBA × B6)F$_1$ → B6] T cells stimulated in the B10.A(4R) recipients. The same is true for arguments that help functions directly between T-cell subsets (14), and is thus independent of the H-2 phenotype of the irradiated mouse, or that help associated with I-A^k and I-A^b is cross-reactive.

The concept that suppression operates in the case where (H-2^k × H-2^b)F$_1$ T cells can respond to vaccinia virus associated with H-2D^b when primed in a B6, but not in a B10.A(4R) recipient, may have some validity (2). However, we have not been able to formally demonstrate such suppression by mixing negatively selected high responder (B6) T cells with excess low responder [B10 × B10.A(4R)]F$_1$ TDL. A possible explanation for this failure to show suppression is that the suppressor T cells are restricted by the H-2K^k or I-A^k antigens on the F$_1$ CTL, and thus do not interact with the B6 responder lymphocytes. Are we to consider, despite experiments to the contrary for a variety of systems (10, 15, 16), that such suppressors are also generated in the [(CBA × B6) F$_1$ → B6] chimeras? Perhaps we are dealing with complex heirarchies of help and suppression, that vary depending on the experience of T cells during physiological differentiation.

The chimera and negative selection experiments both approach the same, broad question: in what way does the major histocompatibility complex determine patterns of T-cell effector function? Conceptual problems arise when we try to reconcile the phenomena, and models, derived from these two approaches. It may be that the negatively selected TDL are a very atypical population. However, though as many as 95% of transferred T cells are lost in the filter environment (whether syngeneic or allogeneic, 17), we have not yet found a divergence of self-H-2-restricted responsiveness

for negatively selected and normal TDL. Predicted T-cell specificities seem neither to be enriched for nor depleted (4, 5, 18).

The alternative is that the debate concerning the physiological differentiation of T cells in [(A × B)F_1 → A] radiation chimeras needs to take more account of H-2 antigens (B) present throughout ontogeny on other than radiation-resistant cells in the recipient thymus (A). Specific interaction, even of low affinity, between a developing thymocyte and any antigen (A or B) encountered in thymus may lead eventually to irreversible tolerization. Contact with the same antigen (A) on a stimulator cell (radiation-resistant thymic epithelium) may result in the delivery of a signal which prevents tolerance for low, but not for high, affinity binding. Tolerance in the case of high affinity for A could reflect the delivery of excess signal at a developmental stage before the emergence of T-cell effector function, or operate via some form of positive suppression.

The implication of this model is that the B6 thymocyte which has the potential to recognize H-2K^k-vaccinia virus does not encounter H-2K^k during the process of physiological development in the B6 thymus, and would thus not be deleted as a result of low affinity binding to the alloantigen. Thymocytes in the [(CBA × B6)F_1 → B6] radiation chimera could, however, interact with the H-2K^k alloantigen on adjacent F_1 thymocytes, but not on radiation-resistant B6 thymic epithelium. The existence of a specific hole (19) in the T-cell repertoire of the (H-$2^{k \times b}F_1$ → H-2^b) chimera for H-2K^k-vaccinia virus (compared with the H-2^b parent) offers experimental evidence that this deletion model is worth considering. Instances of lack of complete restriction to A in [(A × B)F_1 → A] chimeras (20, 21) may reflect that the affinity of the particular thymocytes for B is insufficient to result in tolerization. Even so, the consequence of the present findings for the (H-$2^{k \times b}F_1$ → H-2^b) chimeras is that tolerization of the developing thymocytes in the chimera operates at a lower level of affinity than that seen for the recruitment of mature B6 T cells in irradiated B10.A(4R) recipients, which results in removal during the filtration procedure.

Summary

Negatively selected H-2$K^b$$D^b$ TDL can be induced to respond strongly to vaccinia virus presented in the context of both H-2K^k and H-2D^b when stimulated in irradiated H-2$K^k$$D^b$ recipients. Addition of excess (H-2$K^k$$D^b$ × H-2$K^b$$D^b$)F1 TDL, which are low responders to H-2D^b-vaccinia virus, does not obviously suppress the reactivity pattern of the H-2$K^b$$D^b$ T cells. However, lymphocytes from chimeras made by reconstituting H-2$K^b$$D^b$ mice with (H-2$K^k$$D^k$ × H-2$K^b$$D^b$)$F_1$ bone marrow cells make little, if any, cytotoxic T-cell response to vaccinia virus when sensitized in H-2$K^k$$D^b$ recipients. We have thus documented one instance where the responder phenotype of T cells from an F_1 → parent chimera is not equivalent to that associated with the H-2 type of the parental thymus. Lymphocytes from both the chimera and the H-2$K^b$$D^b$ parent (after negative selection) are tolerant to the H-2K^k and I-A^k alloantigens encountered in the recipient, but the chimera T cells are also defective in their response to a neoantigen (vaccinia virus) presented in the context of H-2K^k which the parental T cells invariably recognize. It is thus possible that at least part of the phenomenology associated with the F_1 → parent radiation chimeras reflects deletion of repertoire in the context of H-2 antigens present during thymocyte ontogeny on other than radiation-resistant thymic epithelium.

We thank Janet LaMartina, Michael Melino, and Kathy Perlman for their capable technical assistance, and Dr. J. Sprent for the anti-θ ascitic fluid.

Received for publication 25 June 1979.

References

1. Doherty, P. C., W. E. Biddison, J. R. Bennink, and B. B. Knowles. 1978. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. *J. Exp. Med.* **148**:534.
2. Zinkernagel, R. M., A. Althage, S. Cooper, G. Kreeb, P. A. Klein, B. Sefton, L. Flaherty, J. Stimpfling, D. Shreffler, and J. Klein. 1978. Ir-genes in H-2 regulates generation of antiviral cytotoxic T cells. Mapping to K or D and dominance of unresponsiveness. *J. Exp. Med.* **148**:592.
3. Sprent, J., and H. von Boehmer. 1976. Helper function of T cells depleted of alloantigen-reactive lymphocytes by filtration through irradiated F1 hybrid recipients. I. Failure to collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured in vivo. *J. Exp. Med.* **144**:617.
4. Bennink, J. R., and P. C. Doherty. 1979. Reciprocal stimulation of negatively selected high responder and low responder T cells in virus-infected recipients. *Proc. Natl. Acad. Sci. U. S. A.* **76**:3482.
5. Doherty, P. C., and J. R. Bennink. 1979. Vaccinia-specific cytotoxic T cell responses in the context of H-2 antigens not encountered in thymus may reflect aberrant recognition of a virus-H-2 complex. *J. Exp. Med.* **149**:150.
6. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, P. Klein, and J. Klein. 1978. On the thymus in differentiation of "H-2 self recognition" by T cells. Evidence for dual recognition. *J. Exp. Med.* **147**:882.
7. Zinkernagel, R. M., A. Althage, S. Cooper, G. Callahan, and J. Klein. 1978. In irradiation chimeras, K or D regions of the chimeric host, not of the donor lymphocytes, determine immune responsiveness of antiviral cytotoxic T cells. *J. Exp. Med.* **148**:805.
8. von Boehmer, H., W. Haas, and N. Jerne. 1978. Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2439.
9. Matsunaga, T., and E. Simpson. 1978. H-2 complementation in anti-H-Y cytotoxic T cell responses can occur in chimeric mice. *Proc. Natl. Acad. Sci. U. S. A.* **75**:6207.
10. Billings, P., S. J. Burakoff, M. E. Dorf, and B. Bennacerraf. 1978. Genetic control of cytolytic T-lymphocyte responses. II. The role of the host genotype in parental → F1 radiation chimeras in the control of the specificity of cytolytic T-lymphocyte responses to trinitrophenyl-modified syngeneic cells. *J. Exp. Med.* **148**:352.
11. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus-plus-self-specific cytotoxic T cells: evidence for T help? *J. Exp. Med.* **147**:897.
12. Katz, D. H. 1972. The allogenic effect on immune responses: model for regulatory influences of T lymphocytes on the immune system. *Transplant. Rev.* **12**:141.
13. Corley, R. B., B. Kindred, and I. Lefkovits. 1978. Positive and negative allogeneic effects mediated by MLR-primed lymphocytes: quantitation by limiting diluation analysis. *J. Immunol.* **121**:1082.
14. Bennink, J. R., and P. C. Doherty. 1978. Different rules govern help for cytotoxic T cells and B cells. *Nature (Lond.).* **276**:829.
15. Fink, P. J., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* **148**:766.
16. Zinkernagel, R. M., and A. Althage. 1979. Search for suppression of T cells specific for the

second non-host H-2 haplotype in F1 → parent irradiation bone marrow chimeras. *J. Immunol.* **122**:1742.

17. Sprent, J. 1977. Recirculating lymphocytes. *In* The Lymphocyte: Structure Function. J. J. Marchalonis, editor. Marcel Decker, Inc., New York. p. 43.
18. Bennink, J. R., and P. C. Doherty. 1978. T cell populations specifically depleted of alloreactive potential cannot be induced to lyse H-2-different virus-infected target cells. *J. Exp. Med.* **148**:128.
19. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody responses. V. Helper T-cell recognition of H-2 determinants on accessory cells but not B cells. *J. Exp. Med.* **149**:1208.
20. Blanden, R. V., and M. Andrew. 1979. Primary anti-viral responses in semiallogeneic chimeras are not absolutely restricted to host H-2 types. *J. Exp. Med.* **149**:535.
21. Bevan, M. J., and P. J. Fink. 1978. The influence of thymus H-2 antigens on the specificity of maturing killer and helper cells. *Immunol. Rev.* **42**:3.