Ir-GENES IN *H-2* REGULATE GENERATION OF ANTI-VIRAL CYTOTOXIC T CELLS

Mapping to *K* or *D* and Dominance of Unresponsiveness*

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Immune response (*Ir-1*)[1] genes clearly participate in generating cytotoxic T lymphocytes (CTL) in response to H-Y antigens (1–4) and possibly to trinitrophenyl (TNP) modified cells (5, 6). Like the *Ir-1* gene effects that control humoral responsiveness to antigens (7–9), the H-Y CTL response shows allelic complementation of *H-2I* genes, and this immune responsiveness is dominant over unresponsiveness (7–9). However, both types of responses – T helper-B cell and cytotoxic T cells – are difficult to interpret: it is unclear whether *Ir-1* gene effects are expressed solely at the helper T-cell level or also by macrophages (10, 11) and B cells (8); and anti-H-Y CTL responses are associated with unresponsiveness both to *H-2K* plus Y and to *H-2D* plus Y, and may therefore be explained by *Ir* effects during the generation of either T helper cells or T killer cells (1–4).

So far, no *Ir-1* gene effect has been found for the generation of virus-specific *H-2K* or *D* restricted cytotoxic T cells (12, 13). Here, we describe the generation of *Ir* gene controlled, *H-2D* restricted cytotoxic T cells in the primary virus-specific immune response in vivo. We establish that: (*a*) responder-nonresponder alleles exist for *D* restricted cytotoxic T cells in various virus models (vaccinia virus, Sendai virus, lymphocytic choriomeningitis virus [LCMV]), (*b*) the genes map to *K* in one case and probably to *D* in another, under conditions in which *H-2K* allele restricted responses are always present so as to rule out generalized *Ir* defects at the T helper cell level, (*c*) surprisingly, unresponsiveness has dominant character.

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[1] *Abbreviations used in this paper:* CTL, cytotoxic T lymphocytes; Ir, immune response; LCMV, lymphocytic choriomeningitis virus; HU, hemagglutinating units; PFU, plaque-forming units; TNP, trinitrophenyl.

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Materials and Methods

Mice. The following mouse strains were used (14). B10A, B10.A (2R), B10.D2, B10.A (5R), B10.BR, C57BL/10, and C57BL/6 were from The Jackson Laboratory, Bar Harbor, Maine; A, BALB/c and C3H were from Strong Research Foundation, San Diego, Calif.; B10.A (1R) and B10A (15R) were from Dr. J. Stimpfling; B10.AM were from Dr. D. Shreffler; B10.BYR, B10.D2 (R101), R(103), B10.HTG, B10.D2 (R107), and Hzl were from Dr. J. Klein. Also from Dr. J. Klein were the B10.BUA19, a new inbred strain of mice that derives its *H-2* type from a wildtype mouse; this strain has a new *K* specificity, but its private *D* specificity is indistinguishable from the known D^k allele.[2] B10.A (4R), B10.BYR, A.TH, BALB.K, BALB.B were kindly donated by Dr. M. Cohn and R. Epstein, Salk Institute, La Jolla, Calif. B6.AK1 ($H\text{-}2^{ozl}$) were from Dr. L. Flaherty (personal communication, Mouse Newsletters, issue 58, p. 32, 1978). C3H.OL, C3H.OH and all the F_1 hybrids used were bred at Scripps. Original breeding stocks of C3H.OL, C3H.OH and A.TL, were obtained from Dr. D. Shreffler, and of the other strains as listed above.

Virus and Immunization. Vaccinia virus WR, lymphocytic choriomeningitis virus (LCMV), and immunization procedures have been described in detail elsewhere (reviewed in 15–17). Egg-grown Sendai virus (5–10,000 hemagglutinating U (HU/ml) of the Harris strain was injected (0.2 ml) intravenously into mice. Mice were killed on day 6 after injection of vaccinia or Sendai virus or 8 days for LCMV and their spleen cells tested for cytotoxicity as described (15). Vaccinia 6-day immune spleens contain about three to six times as many lymphocytes; this increase of spleen size served as a control for adequate immunization e.g., in the case of B10.BUA19.

Target Cells. L929 ($H\text{-}2^k$) from C3H mice, MC57G cells ($H\text{-}2^b$) from C57BL/6 mice, P815 cells ($H\text{-}2^d$) from DBA/2 mice, EL4 cells ($H\text{-}2^b$) from C57BL/6 mice (15, 16), and methylcholanthrene induced fibrosarcoma cell lines D2 ($H\text{-}2^d$) from B10.D2 mice, 2R cells ($H\text{-}2^{k/b}$) from B10.A (2R) mice and 5R cells ($H\text{-}2^{b/d}$) from B10.A (5R) mice (18) have been described as noted. These target cell lines are permissive to infection as follows: L929, MC57G, D2, 2R and 5R, by vaccinia virus; L929, EL4, P815, 2R and 5R, by Sendai virus; L929, MC57G, D2, 2R, 5R, by LCMV as described previously (15–17). In some studies peritoneal macrophages were used, either unstimulated or 2 days after mice were injected with 2 ml of thioglycolate intraperitoneally (19). All these target cells were assayed for expression of *H-2K* and *D* products by testing them with alloreactive T cells; all expected and no unexpected *K* and *D* specificites were found. In particular, in mixed lymphocyte culture generated cytotoxic T cells (20) specific for D^b lysed both $H\text{-}2^b$ targets (MC57G and EL4), and those specific for D^k lysed the $H\text{-}2^k$ (L929) target cell line (data not shown).

^{51}Cr Released Assay. The test has been described in detail (15–17). Usually cytotoxicity was measured for 6 h. Spontaneous release did not exceed 30% for any of the targets, and was usually below 20%. Percent specific release and SEM were calculated on a Hewlett-Packard mini-computer using the formula:

$$\frac{\text{experimental} - \text{spontaneous release}}{\text{water} - \text{spontaneous release}} \times 100.$$

Percent relative activity was determined by calculating lytic units as described by Cerottini and Brunner (21); responder strains were given a relative activity of 1 and the other activities were expressed as a percent thereof.

Adoptive Sensitization. Normal or immune spleen cells are transferred (3–5 × 10^7) i.v. to lethally irradiated and virus infected recipients; recipients were killed 5 or 6 days later and spleen cells assayed for cytotoxicity.

Results

Anti-Viral Killing Restricted to the H-2D^k Allele. A wide variety of mouse strains each carrying the *H-2*D^k allele was immunized with vaccinia, Sendai, or LCMV, and their spleen cells were tested for cytotoxicity restricted to the *H-2*D^k allele. In all animals tested, after vaccinia or Sendai infection, the spleen cells accomplished much less D^k restricted killing activity than, *K*-restricted

[2] W. Duncan and J. Klein. Manuscript in preparation.

TABLE I
D^k Restricted Cytotoxic T-Cell Response to Vaccinia, Sendai, or LCM Virus

Experiments	Mouse strain	*H-2* *K*	*I-A I-B I-J I-E I-C*	*D*	Ratio of killer to target cells	Specific ^{51}Cr Release from infected target cells* (L929) K^k D^k	(MC57G) K^b D^b	(D2) K^d D^d	(OH) K^d D^k
						%			
					Vaccinia‡				
A 1	C3H	*k*	*kkkkk*	*k*	40	104	0	2	3
					13	80	0	0	0
					4	38	0	0	2
2	B10.BR	*k*	*kkkkk*	*k*	40	85	NT	NT	3
					13	45			2
					4	22			0
3	C3H.OH	*d*	*ddddd*	*k*	40	6	NT	58	42
					13	0		28	27
					4	0		5	18
4	C3H.OL	*d*	*ddddd*	*k*	40	8	NT	65	NT
					13	3		32	
					4	1		6	
5	B6.AK1	*b*	*bbbbb*	*k*	40	13	30	NT	
					13	9	50		
					4	3	25		
6	B10.BUA19	*x*	*xxxxx*	"*k*"	40	5	0	0	
					13	3	3	0	
					4	0	0	0	
B 1	C3H.OH × C57BL/6	*d*/*b*	*ddddd*/*bbbbb*	*k*/*b*	40	10	NT	100	
					13	6		58	
					4	4		30	
2	C3H.OH × C3H.Q	*d*/*q*	*ddddd*/*qqqqq*	*k*/*q*	40	18	NT	105	
					13	10		76	
					4	4		45	
3	C3H	*k*	*kkkkk*	*k*	40	100	NT	5	
					13	80		4	
					4	51		0	
C 1	C3H.OH × B10.S	*d*/*s*	*ddddd*/*sssss*	*k*/*s*	40	14	NT	59	
					13	12		46	
					4	7		22	

killing; however, LCMV-immune spleen cells were able to lyse in a D^k restricted manner (Table I). Vaccinia-immune C3H ($H\text{-}2^k$) and B10.BR ($H\text{-}2^k$) lymphocytes lysed K^kD^k-infected L929 targets but not OH infected K^dD^k targets (Table I, Exp. A 1, 2). The same low D^k restricted vaccinia specific activity was found

TABLE I—*Continued*

Experiments	Mouse strain	*H-2* *K*	*I-A I-B I-J I-E I-C*	*D*	Ratio of killer to target cells	Specific ^{51}Cr Release from infected target cells* (L929) K^k D^k	(EL4) K^b D^b	(P815) K^d D^d
							%	
			Sendai					
D 1	C3H	*k*	*kkkkk*	*k*	40	100	0	8
					13	91	1	0
					4	37	0	
2	C3H.OH	*d*	*ddddd*	*k*	40	0	0	75
					13	1	2	50
					4	0	0	32
3	B6.AK$_1$	*b*	*bbbbb*	*k*	40	18	100	8
					13	10	72	4
					4	12	11	
						(L929)	(MC57G)	(D2)
			LCMV					
E 1	C3H	*k*	*kkkkk*	*k*	40	100		4
					13	105	NT	2
					4	65		0
2	C3H.OH	*d*	*ddddd*	*k*	40	60		42
					13	28	NT	25
					4	10		5
3	C3H.OL	*d*	*ddddd*	*k*	40	105		100
					13	82	NT	45
					4	48		20
4	BALB/c	*d*	*ddddd*	*d*	40	10		90
					13	5	NT	71
					4	2		33

* For the determination of percent specific activity see Materials and Methods. The actual ^{51}Cr release test conditions were as follows: Exp. A–C test duration 6 h, spontaneous releases <20%, SEM <4%, Exp. D test duration 14 h, spontaneous releases <25%, SEM <5%, Exp. E test duration 6 h, spontaneous releases <25%, SEM <4%. Multiple positive and negative controls with complete H-2 compatible or incompatible immune spleen cells were included in each test in addition to normal cells and medium release. Results were compared with negative controls and statistically significant results are boxed.

‡ Mice were infected i.v. with vaccinia virus (2×10^7 plaque-forming units (PFU)) or with Sendai virus (2,000 H.U.) 6 days before assay of the spleen cells, or with LCMV (10^3 PFU) 8 days before assay.

in C3H.OH (K^dI^d), C3H.OL (K^dI^d), B6.AKI (K^bI^b), and B10.BUA19 (K^xI^x) in Exp. A3-6 and in three types of F$_1$ heterozygotes—C3H.OH × C57BL/6 (*H-2^b*), C3H.OH × C3H.Q (*H-2^q*), and C3H.OH × B10.S (*H-2^s*) (Exp. B, C). Similarly, Sendai virus-immune C3H.OH (K^dI^d) or B6AKl (K^bI^b) lysed at least 30 times

less D^k infected targets than the relevant K-target cells (Exp. D). In contrast, LCMV-immune C3H.OH or C3H.OL (K^dI^d) lysed infected K^kD^k (L929) cells and C3H ($K^kI^kD^k$) lysed infected K^dD^k OH-macrophage targets (Exp. E). Therefore, it seems that during vaccinia or Sendai viral infections, $H\text{-}2D^k$ restricted, specific cytotoxic T cells are not being generated, yet the K-restricted response is great.

Anti-Viral Cytotoxic T-Cell Activity Restricted to H-2D^b. Cells from vaccinia-immune C57BL/6 mice ($H\text{-}2^b$) or the mutant Hzl mice ($H\text{-}2^{ba}$) lysed infected D^b targets efficiently (Table II, Exp. A1, 2). In contrast, B10.A (4R), 2R and AM cells, all possessing K^k and various portions of the k allele of the I-region, failed to lyse infected D^b targets (MC57G) (Exp. A 3–5). The same was true for Sendai-immune D^b restricted activity (Exp. B2, 3; C2). However, B10.BYR ($K^qI^kD^b$) vaccinia-immune spleen cells lysed D^b infected targets indicating that the presence of K^q (or K^bI^b in the case of C57BL/6 or $K^{ba}I^b$ for Hzl) allowed generation of D^b restricted vaccinia specific killing. This maps the low responder influence to K^k since this is the only region which differs between B10.A (2R) and B10.BYR. The negative influence of K^k on D restricted vaccinia responses is specific for D^b, not general, since B10.A ($K^kI^kD^d$) responds equally well to infected K^k as to infected D^d targets (Table II, Exp. A7). The regulatory role of K^k and D^b restricted responses is virus-specific; thus, LCMV-immune spleen cells from B10.A (2R) or B10.A (4R) lysed infected D^b targets very efficiently.

$H\text{-}2K^d$ exerts a somewhat similar but much smaller effect on D^b restricted responses. The responses of vaccinia or Sendai-immune spleen cells from B10.HTG (K^dI^{d}D^b) or D2.GD ($K^dI^{d/b}D^b$) were about 4 to 10 times lower when tested on D^b targets than on K^d restricted targets (Table II, Exp. A9, 10; C4). No such effect was seen in the LCMV model. However, LCMV-immune spleen cells were found to be generally of lower activity on K^k infected targets compared to their activities on the relevant D-specific targets. Examples are given in Table II, Exp. D3 and 4.

Responsiveness of F$_1$ Hybrids: Evidence for Dominant Unresponsiveness. F$_1$ heterozygous mice that were crosses between responders for D^b vaccinia ($H\text{-}2$ K^b I^b or $H\text{-}2K^q$) and D^b-nonresponder (K^kI^k) mice, responded only weakly to D^b plus virus when compared with responder C57BL/6 ($H\text{-}2^b$) Hzl or B10.BYR (K^qD^b) mice. This suggests the dominance of unresponsiveness with some variation in penetrance (Table III, Exp. A-D). (Hzl × 2R)F$_1$, (4R × 5R)F$_1$, (2R × 3R)F$_1$, and (5R × 2R)F$_1$ hybrids all give > 10 times lower cytotoxic activity against vaccinia infected D^b cells than against infected K^k cells (compare relative numbers of D^b targets killed shown in the first or last columns to numbers of K^k targets killed shown in the second column). The D^b restricted responses were low in these hybrids' spleen cells when compared with responder C57BL/6 despite the fact that they possessed the responder alleles K^b or K^{ba} plus $I\text{-}A^b$ or the entire K through I regions of b and ba in cis or in trans-complementation. In all examples the presence of K^k was constant and therefore may have been responsible for the low responses. The only exceptions were hybrids whose cells sometimes showed only a three to five times lower degree of response to vaccinia D^b when compared to K^k: (B6.AK × B10.AM)F$_1$, (B10 × B10.BR)F$_1$. Similarly, (2R × C3H.Q)F$_1$ vaccinia-immune spleen cells lysed

TABLE II
D^b Restricted Cytotoxic T-Cell Responses to Vaccinia, Sendai, or LCM Virus

Experiments	Mouse strain	*H-2* *K*	*I-A I-B I-J I-E I-C*	*D*	Ratio of killer to target cells	Specific ^{51}Cr Release from infected target cells* (L929) K^k D^k	(MC57G) K^b D^b	(D2) K^d D^d	(2R) K^d D^b
						%			
					Vaccinia‡				
A 1	C57BL/6	*b*	*bbbbb*	*b*	40		93	8	43
					13	NT	67	4	22
					4		27	2	8
2	Hzl	*ba*	*bbbbb*	*b*	40	8	70		40
					13	7	50	NT	21
					4	6	22		10
3	B10.A(4R)	*k*	*kbbbb*	*b*	40	106	33		
					13	72	23	NT	NT
					4	61	11		
4	B10.A(2R)	*k*	*kkkkd*	*b*	40	83	15		50
					13	75	5	NT	32
					4	37	6		13
5	B10.AM	*k*	*kkkkk*	*b*	40	103	34		
					13	104	20	NT	NT
					4	68	8		
6	B10.BYR	*q*	*kkkkd*	*b*	40	0	111		
					13	6	71	NT	NT
					4	4	50		
7	B10.A	*k*	*kkkkd*	*d*	40	74		59	
					13	40	NT	20	NT
					4	18		8	
8	B10.D2	*d*	*ddddd*	*d*	40		6	109	5
					13	NT	5	93	2
					4		4	33	1
9	B10.HTG	*d*	*ddddd*	*b*	40		43	93	
					13	NT	27	72	NT
					4		9	35	
10	D2.GD	*d*	*dbbbb*	*b*	40		21	74	
					13	NT	12	42	NT
					4		3	11	
11	B10.A(5R)	*b*	*bbkkd*	*d*	40		71	62	
					13	NT	34	39	NT
					4		16	18	

TABLE II—*Continued*

Experiments	Mouse Strain	*H-2* *K*	*I-A I-B I-J I-E I-C*	*D*	Ratio of killer to target cells	Specific ^{51}Cr Release from infected target cells* (L929) K^k D^k	(EL4) K^b D^b	(P815) K^d D^d
							%	
					Sendai			
B 1	C57BL/6	*b*	*bbbbb*	*b*	40	10	100	NT
					13	8	62	
					4	0	18	
2	B10.A(2R)	*k*	*kkkkd*	*b*	40	100	0	NT
					13	60	0	
					4	29	2	
3	B10.AM	*k*	*kkkkk*	*b*	40	60	5	NT
					13	40	0	
					4	17	0	
C 1	C57BL/6	*b*	*bbbbb*	*b*	40	7	112	11
					13	7	92	8
					4	8	35	10
2	B10.A(4R)	*k*	*kbbbb*	*b*	40	86	10	NT
					13	23	9	
					4	10	2	
3	B10.A	*k*	*kkkkd*	*d*	40	76	8	61
					13	30	9	36
					4	13	10	12
4	B10.HTG	*d*	*ddddd*	*b*	40	NT	20	65
					13		14	30
					4		9	13
						(L929)	(MC57G)	
					LCVM			
D 1	C57BL/6	*b*	*bbbbb*	*b*	40	7	76	
					13	5	58	
					4	3	28	
2	Hzl	*ba*	*bbbbb*	*b*	40	6	63	
					13	6	40	
					4	5	18	
3	4R	*k*	*kbbbb*	*b*	40	60	65	
					13	45	47	
					4	17	21	
4	2R	*k*	*kkkkd*	*b*	40	37	74	
					13	24	49	
					4	17	29	

* The ^{51}Cr release test conditions were: Exp. A, D: test duration 6 h, spontaneous release <20%, SEM <5%. Exp. B, C: test duration 14 h, spontaneous release <25%, SEM <5%. Multiple positive and negative controls of completely H-2 compatible and H-2 incompatible immune and normal cells were included in each test; only tests in which negative controls were <10% were included for the analysis. Results were compared with negative controls and statistically different data are boxed.

‡ Mice were infected i.v. with vaccinia virus (2×10^7 PFU), or with Sendai virus (2,000 H.U.) 6 days before assay of the spleen cells, or with LCMV (10^3 PFU) 8 days before assay.

TABLE III
K or D Plus Virus Specific Activity of Vaccinia Virus Infected F_1 Hybrid Mice

Mouse strain	K	I ABJEC	D	Relative activity on target cells*											
				R103/D2.GD(K^dD^b) Experiments				L(K^kD^k) Experiments				MC57B(K^bD^b) Experiments			
				A	B	C	D	A	B	C	D	A	B	C	D
									%						
C57BL/6‡	*b*	*bbbbb*	*b*	100	100	100	100	<1	<1	–	<1	–	100	100	100
Hzl	*ba*	*bbbbb*	*b*	100	100	–	–	–	<1	–	–	–	90	–	–
Hzl × 2R	*ba*/*k*	*bbbbb*/*kkkkd*	*b*/*b*	–	<1	–	–	–	100	–	100	–	40	–	20
Hzl × 4R	*ba*/*k*	*bbbbb*/*kbbbb*	*b*/*b*	–	25	–	10	–	100	–	80	–	20	–	<10
B10 × B10.BR	*b*/*k*	*bbbbb*/*kkkkk*	*b*/*k*	–	35	–	10	–	100	–	100	–	150	–	100
C3H×C57BL/6				–	1	1	<20	–	70	100	>100	–	75	50	90
B6.AK × AM	*b*/*k*	*bbbbb*/*kkkkk*	*k*/*b*	10	–	20	10	100	–	100	>100	–	–	40	90
4R × 5R	*k*/*b*	*kbbbb*/*bbkkd*	*b*/*d*	2	<1	–	<10	100	70	–	90	–	90	–	100
5R × 2R	*b*/*k*	*bbkkd*/*kkkkd*	*d*/*b*	<1	<1	1	20	25	30	100	80	–	70	30	70
2R × C3H.Q	*k*/*q*	*kkkkd*/*qqqqq*	*b*/*q*	–	<1	–	<10	–	50	–	80	–	<1	–	<5
2R × 3R	*b*/*k*	*bbkkd*/*kkkkd*	*b*/*d*	–	–	<1	<5	–	–	35	40	–	–	<100	40
2R × C57BL/6	*k*/*b*	*kkkkd*/*bbbbb*	*b*/*b*	–	–	5	<1	–	–	80	10	–	–	100	10

* Determination of percent relative activity, see Materials and Methods. The ^{51}Cr release test conditions were: test duration 6 h, spontaneous release for R103 and D2.Gd macrophages <32% for L and MC57G <20%; SEM were smaller than 5%.
‡ Mice were infected with 2×10^7 PFU of vaccinia virus; spleen cells were assayed 6 days later.

about 10 times less D^b infected targets than the relevant K^k targets and much less than responder B10.BYR (K^qD^b) lymphocytes. Again this indicates that the presence of K^q (a responder for D^b in B10.BYR) is not sufficient to promote responsiveness and that generally it is the presence of K^k that inhibits generation of vaccinia specific D^b restricted responses.

Adoptive Sensitization of F_1 in Responder or Nonresponder Parents. Lymphocytes from F_1 (responder K^b × nonresponder K^k) were triggered in lethally irradiated and vaccinia infected responder, nonresponder or F_1 recipients and tested for D^b restricted vaccinia specific cytotoxicity (Table IV). When low responder F_1 was sensitized in an environment expressing vaccinia antigen plus D^b and alleles of responder K^b or K^q, they responded well to vaccinia D^b. In contrast, the same F_1 cells triggered in a vaccinia infected irradiated D^b host expressing the nonresponder allele K^k (i.e., nonresponder parent or F_1) response to vaccinia D^b was low. This result cannot be explained simply by the possible influence of an allogeneic effect; since in both B10.A (2R) × C3H.Q boosted in 2R or BYR a parental allogeneic effect is exerted. Thus, absence of K^k at the time of immunization or boosting will allow high D^b vaccinia responses.

Discussion

H-2 dependent and virus specific *Ir* genes that regulate the generation of virus-specific *K*, or *D* restricted cytotoxic T cells in primary responses during virus infections in vivo have the following characteristics: (*a*) they operate at

TABLE IV

Adoptive Sensitization of Lymphocytes From F_1 (Responder K^qD^b × Nonresponder K^kD^b) in Irradiated Parental Responder or Nonresponder Mice

Donor*	Sensitizing* recipient	Ratio of lymphocytes to target cells	Specific ^{51}Cr release from vaccinia infected targets‡ (L929) K^k D^k	(MC57G) K^b D^b	(R101) K^d D^b
				%	
B10. × B10.BR	C57BL/6	13	−2	50	63
$\frac{b}{k}$ $\frac{bbbbb}{kkkkk}$ $\frac{b}{k}$	*b* *bbbbb* *b*	4	1	47	40
	B10.A(2R)	13	81	7	8
	k *kkkkd* *b*	4	54	8	10
B10.A(2R) × C3H.Q	B10.A(2R)	13	91	15	
$\frac{k}{q}$ $\frac{kkkkd}{qqqqq}$ $\frac{b}{q}$	*k* *kkkkd* *b*	4	65	7	NT
	B10.BYR	13	0	66	
	q *kkkkd* *b*	4	0	49	NT
	B10.A(2R) × C3H.Q	13	80	12	
	$\frac{k}{q}$ $\frac{kkkkd}{qqqqq}$ $\frac{b}{q}$	4	62	6	NT

* Normal or 6 days vaccinia immune spleen cells were transferred (4 × 10^7) to lethally irradiated and vaccinia virus infected recipients; recipients were killed 6 days later and spleen cells assayed for cytotoxicity.

‡ Percent specific release after 6 h; means of triplicate determination; SEM were smaller than 4%; spontaneous release was smaller than 20%.

the level of generating cytotoxic T cells, and not obviously at the level of T helper cells; (*b*) they seem to map the *K* or *D* region of *H-2*; (*c*) nonresponsiveness seems to be dominant. These conclusions derive from experiments with various *H-2* congenic, *H-2* recombinant, and F_1 heterozygous mice that were infected systemically with virus; their T-cell-mediated immune response was assessed by measuring cytotoxic T-cell activity against virus and *H-2K* or *H-2D* allele specific target cells in ^{51}Cr release assays. Similar results have been obtained by Dr. P. Doherty and co-workers (*J. Exp. Med.* 148:000).

D^k restricted cytotoxic T-cell responses to infection of mice by vaccinia and Sendai viruses are at least 30 times lower than *K* restricted responses in all mice tested so far, representing K^kI^k, K^bI^b, K^dI^d, and other *K*, *I*-regions (*q*, *s*) in F_1 hybrids. In contrast, LCMV induces high responses to D^k. The observed Ir-effect could not be mapped, but the data are consistent with it mapping to D^k for vaccinia- or Sendai-specific cytotoxicity.

D^b restricted responses to vaccinia or Sendai virus are more than 10 to 30 times lower than the *K* region and relatively diminished in mice with K^dI^d. In turn, $K^{ba}I^b$, K^bI^b, and K^q allow high responsiveness (i.e., activity to D^b and K^b or K^{ba} is about equal). This *Ir*-effect maps to the *K* region since B10.BYR (K^q)

mice were responders, whereas B10.A (2R) mice, which differ only for K^k were nonresponders. The negative effect of K^k on *D* restricted responses to vaccinia is not general, since K^k allows high responses to D^d vaccinia. Besides being allele-specific, the *Ir* effect is also virus-specific. In the case of cytotoxic T-cell-immune responsiveness to LCMV, K^k and K^b are both associated with high responsiveness to LCMV-D^b.

Thus, *Ir* effects on the generation of virus-specific cytotoxic T cells can be exerted by both *K* and *D* region genes for *D*-restricted killer T cells; they are virus-specific and haplotype specific. At the moment it is unexplained why Sendai and vaccinia virus are under quite similar *H-2 Ir* gene regulation (Table I and II) in the examples tested, despite the fact that the two viruses do not show antigenic cross-reactivity by known immunological criteria (15, 17). For all other K^k, K^d, and K^b haplotypes tested responsiveness was high to all three viruses; however, since in most cases only one (the homologous) *I* and *D* region haplotypes was tested, poossibly low responsiveness could exist in association with other *D* (or *I*) region haplotypes.

Our results are compatible with the findings of Koszinowski and Ertl (22, 23) who noted that C3H (H-2^k) vaccinia immune T cells failed to lyse infected L cells (H-2^k) when an anti-K^k alloantiserum was added; one would not expect this result if killing was directed against D^k vaccinia infected cells as well. Similarly, the response of C3H.OH (K^dD^k) mice against ectromelia (mouse pox) virus infected D^k targets was variable (24). Relatively greater activity for LCMV-*D* than for LCMV-K^k has also been documented (25).

H-2 associated *Ir* phenomena in the generation of cytotoxic T-cell responses were described previously. Schmitt-Verhulst and Shearer found that the generation of TNP-specific D^d restricted cytotoxic T cells mapped to the left of I-A (5, 6). Simpson and Gordon, Hurme et al., (1, 2) and von Boehmer and co-workers (3, 4) observed that generation of H-Y male antigen-specific cytotoxic T cells was subject to the following two kinds of *H-2 Ir* effects: (*a*) many *H-2* haplotypes tested were unable to generate measurable *K* and *D* restricted cytotoxicity against H-Y antigens in association with certain *I*-subregions. Since many nonresponsive samples on which the analysis was based failed to respond to either *K* and *D*, this *Ir* defect could have operated at the level of induction of cytotoxic T cells or that of helper T cells, assuming that restricted helper T cells are involved in H-Y specific cytotoxic T-cell response as they are postulated to be in virus-specific T-cell responses (26). The latter conclusion may in fact be supported by the finding that the *Ir* genes map to I-A or I-C, or both (2). If conventional *I*-restricted helper T cells are involved in triggering virus-specific cytotoxic T cells (26), our examples of *Ir* genes' activities suggest that these genes operate at the level of induction of cytotoxic T cells, not at the level of T helper cells, because strong virus-specific cytotoxic activity was always generated for at least one, the *K* or the *D* region.

(*b*) Other examples in which nonresponsiveness to H-Y was restricted to D^d (and maybe to K^k) resemble the vaccinia (and Sendai) - D^k nonresponsiveness; lack of response could not be restored by any homozygous *K*, *I* region nor any *H-2* haplotype introduced in heterozygotes.

The results presented here differ from *H-2 Ir* phenomena regulating antibody responses (7-9). Thus, contrary to what has so far been described in the *H-2 Ir*

regulated antibody responses, it seems that virus-specific K or D restricted T-cell responsiveness maps to K, D (and not to I), is recessive and is determined by the absence of a particular gene (or gene product) rather than by the presence of one; e.g., D^b vaccinia response is high when there is no K^k, irrespective of whether homozygous of heterozygous or whether in cis or trans configuration. Despite these apparent contradictions, two sets of data are compatible with the interpretation that *H-2I* regulated unresponsiveness is dominant at the level of T helper cells: Katz described for T-cell help in vivo (27), and Shevach and Rosenthal for antigen-pulsed, macrophage-induced T-cell proliferation in vitro (11) that T cells from F_1 (non-responder × responder) could only cooperate or proliferate with target B cells or target macrophages of the responder *H-2I* type. If one accepts that *Ir* effects are expressed via T cells only (and not in B cells or macrophages), their results are apparently identical to the present results in which cytotoxic F_1 T cells only lyse virus infected targets of responder type.

The following mechanisms could explain our examples of *H-2* linked *Ir* phenomena that regulate generation of virus-specific cytotoxic T cells. The apparent D^k dependent response for D^k-vaccinia and D^k-Sendai may reflect that neither vaccinia nor Sendai virus antigens can associate immunogenically with D^k to form a complex antigen that is immunogenic and/or recognizable by a single T-cell receptor for altered D^k (28). In contrast, LCMV can modify D^k immunogenically. This mechanism has been evoked to explain nonresponsiveness in the Friend virus model (29) and we discussed this model earlier in an attempt to explain *H-2* polymorphism (28). However, this simple speculation, which would be compatible with dominance of nonresponsiveness, does not readily explain why the presence of K^k but not of K^b or K^q should prevent vaccinia antigens from associating immunogenically with D^b. Thus, the altered self or complex antigen model could explain only some low responsiveness (e.g., in D^k vaccinia D^k Sendai, D^d-H-Y or low responsiveness in the Friend virus model [29]) but not others (e.g., D^b – vaccinia). To explain the latter case, one could argue that vaccinia-altered D^b mimicks K^k, and therefore tolerance would prevent generation of the D^b restricted vaccinia virus-specific response. However, tolerance cannot be the correct explanation since low responder lymphocytes from K^k and D^b mice do respond very well if sensitized or boosted in an environment favoring the generation of the D^b restricted nonresponder specificity (Table IV).

Within a dual recognition model one could propose that T-cell self-recognition of D^k excludes or precludes expression of an anti-vaccinia or anti-Sendai specific receptor, but not expression of anti-LCMV specific receptors. To state this another way, if as proposed by Langman (30), anti-self D^k is selected out of the gene pool for recognition of self D^k during differentiation of T cells in the thymus (16), the receptors for vaccinia antigen that otherwise would normally arise from this particular (germ line?) gene by somatic mutation do not develop. Since the receptors for self-K or D are expressed clonally (15), this rule is compatible with the dominance of nonresponsiveness. However, this preclusion rule does not readily explain why (a) despite their antigenic unrelatedness both Sendai and vaccinia virus failed to trigger a response to D^k, or (b) why K^k but

not K^b or K^q caused low responses for vaccinia-D^b. In this latter case, K^k allows strong responses to vaccinia K^k, therefore recognition of K^k does not preclude recognition of cell membrane associated vaccinia antigens. Consequently, as argued for altered self or a mimicking tolerance model, a preclusion rule may, explain only a few examples of *Ir*-phenomena. Since the preclusion rule implies that preclusion must be an exception, (because otherwise immune responses would be the exception and not the rule), this rule may, despite lack of generality, be valid to explain rare examples of *Ir* gene phenomena.

The dominance of unresponsiveness indicates an active process that overrides normal responsiveness. Suppression that acts virus specifically on D^b restricted recognition is difficult to envisage; e.g., an anti-idiotype suppression against receptors for self-D^b would be generated only in vaccinia or Sendai infection, not in LCMV infection.

An alternative model to explain dominance of nonresponsiveness could be termed immunodominance and would require in our system, for example, that K^k-vaccinia be a better or stronger immunogen than D^b-vaccinia, in the case of (B10.A (2R) × C57BL/6)F_1 (K^kD^b nonresponder × K^bD^b responder)F_1. This proposition can be discussed within an altered-self or single recognition model (10, 28) as well as within a dual recognition model. Dr. Melvin Cohn pointed out that this argument can be based on receptor affinities for altered *K* and altered *D* or for anti-*K* or *D* and anti-viral antigen. The over-riding influence of K^k on D^b vaccinia specific responses could thus be compared with the dominant idiotype clones in anti-phosphorylcholine or anti-arsonate responses (31, 32). If average anti-LCMV affinity is high, either high or low affinity of anti-K^k or anti-D^b would suffice to trigger the response; in contrast, low average affinity of anti-vaccinia receptors, together with low affinity of anti-D^b, would result in preferential triggering of high affinity anti-K^k plus anti-vaccinia specific T cells. D^k restricted low responsiveness could be similarly explained by arguing that anti-self-D^k is of very low affinity when compared with all other *D* and *K* specificities tested so far. This seems to be an ad hoc assumption since there is no apparent reason why the average anti-vaccinia affinity should be lower than that for anti-LCMV recognition. This argument is however readily compatible with the finding that F_1 (responder × nonresponder) lymphocytes which in the F_1 are of nonresponder phenotype do show high response when triggered or boosted in an immunogen responder environment which expresses D^b plus vaccinia but not the immunodominant determinants, K^k plus vaccinia. If this argument of immunodominance or affinities were relevant one should find a hierarchy of affinities of anti-self *K* and *D* receptors which should show up most readily in models studying weak antigens. Results obtained in the H-Y model and ours fail to give clear picture as yet, e.g. dominance of H-Y responses to K^k over D^b is found in some examples: (C57BL/6 × A/J)F_1 ♀ immunized with F_1 ♂ and then boosted with B10.A (2R) ♂ responds preferentially to K^k-H-Y (3), but not in others: (B10.A (2R) × B10.G)F_1 ♀ immunized with F_1 ♂ and boosted with B10.A (2R) ♂ reacts to D^b-H-Y and not to K^k (2).

A similar affinity argument can be made for the anti-viral recognition if average anti-self recognition affinity is comparable; e.g. affinity or average number of reactive clones is greater for K^k than for D^b (or D^k) restricted vac-

cinia specific responses. Further analysis of the various models is needed not only with respect to this proposition but also to evaluate further whether several mechanisms may in fact be responsible for various *Ir* effects.

Our data support the view that polymorphism of *H-2* may be linked to cellular immunity. Responsiveness against LCMV is high in association with D^b in the presence of the K^k allele but is low for vaccinia in the same combination. In contrast, K^b or K^q alleles confer a high response to vaccinia virus. D^k itself seems to cause low responsiveness against vaccinia D^k or Sendai D^k, whereas D^k is associated with high responsiveness to LCMV. Thus, for a given species and for the individual polymorphism may be an advantage since, based as it is on a multiloci gene system, polymorphism minimizes over-all incidence of nonresponsiveness against various viruses. *H-2* polymorphism may thus improve resistance of the species against intracellular infectious agents (probably a major selective factor) and is therefore of selective advantage (33, 28). Balanced polymorphism may be explained by the influence of a great variety of intracellualr parasites each favoring one or the other *H-2* allele. Our results may also explain some aspects of the apparent association of certain disease susceptibilities with particular major transplantation antigens; the presence of one self marker (like K^k with respect to vaccinia D^b or D^k with respect to D^k vaccinia or Sendai responses) may contribute overall to a defect in protection against a particular parasite.

Summary

H-2 dependent and virus-specific *Ir* genes regulate the generation of primary virus-specific *K* or *D* restricted cytotoxic T-cell responses in vivo. The following examples have been analyzed in some detail: first, D^k restricted responses to vaccinia in Sendai viruses are at least 30 times lower than the corresponding *K*-restricted responses irrespective of the *H-2* haplotypes (*k, b, d, dxs, dxq*) of *K* and *I* regions; in contrast, LCMV infection generates high responses to D^k. These findings are consistent with but do not prove that this *Ir* gene maps to *D*. Second, D^b restricted responses to vaccinia and Sendai viruses are high in strains possessing the K^q or K^bI^b, $K^{ba}I^b$ haplotype, are very low in strains with K^k, and relatively low in mouse strains of the $K^dI\text{-}A^d$ haplotype; LCMV generates high D^b restricted response in the presence of K^k. This *Ir* gene for the response to vaccinia and Sendai viruses maps to *K* since B10.BYR ($K^qI^{kd}D^b$) is a responder and B10.A (2R) is a nonresponder ($K^kI^{kd}D^b$). Third, virus and *K* or *D* allele specific nonresponsiveness is dominant with variable penetrance; in heterozygous mice the nonresponder K^k allele over-rides responsiveness normally found in K^bD^b or K^qD^b combinations. Fourth, when (responder × nonresponder)F_1 lymphocytes are stimulated in an environment expressing vaccinia virus plus only a high responder K^b or K^q allele and D^b, response to vaccinia D^b is high; in contrast when the same F_1 cells are stimulated in an environment expressing the low responder allele K^k, response to vaccinia D^b is low. Thus absence of K^k during immunization allows generation of high responsive D^b restricted vaccinia specific cytotoxic T cells. The D^k dependent low response to vaccinia D^k can be explained by a preclusion rule or by failure of vaccinia to complex with D^b; however the analysis of K^k dependent low response to vaccinia D^b does not support these explanations or that self-

tolerance is responsible for this *Ir* effect but is compatible with the interpretation that K^k vaccinia is immunodominant over D^b vaccinia.

These results are discussed with respect to (*a*) possible mechanisms of regulation by *Ir* genes and (*b*) *H-2* polymorphism and HLA-disease association.

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