Brief Definitive Report

IN IRRADIATION CHIMERAS, K OR D REGIONS OF THE CHIMERIC HOST, NOT OF THE DONOR LYMPHOCYTES, DETERMINE IMMUNE RESPONSIVENESS OF ANTIVIRAL CYTOTOXIC T CELLS*

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H-2-linked *Ir* genes, which regulate the virus-specific responsiveness of cytotoxic *H-2K* or *D* restricted T cells, have the following characteristics: they are virus- and *H-2*-specific. They act on cytotoxic T cells directly, but probably not on conventional T helper cells, since in all mouse strains responsiveness restricted to either *K* or *D* is high. Low responsiveness is quantitative, not qualitative unresponsiveness, and it has dominant character; *Ir* genes that regulate responsiveness of cytotoxic *K* or *D* restricted T cells map either to the *K* or *D* region of *H-2* (1).

Two general types of *Ir* gene regulation have been studied: (*a*) the K^k haplotype causes low, but the K^b and K^q cause high cytotoxic T-cell responsiveness to D^b plus vaccinia virus. This Ir effect can be best explained by the immunodominance of a K^k-restricted response to vaccinia over a response to D^b plus vaccinia. This explanation is compatible with the finding that nonresponder lymphocytes of K^kD^b mice respond well to D^b plus vaccinia antigen if they are restimulated selectively in an immunogenic environment expresing D^b plus vaccinia, but not in one expressing K^k plus vaccinia. (*b*) Vaccinia virus-specific cytotoxic T-cell responses that are restricted to D^k apparently cannot be generated, regardless of any tested *K* or *I* region alleles. Therefore, this *Ir* gene seems to map to *D*. Low response may again reflect the fact that all the vaccinia responses restricted to all *K* or *D* alleles tested so far are immunodominant over the response to D^k plus vaccinia. These *Ir* effects can be explained in a dual recognition model of T-cell recognition either by a genetic exclusion mechanism at the level of the genes coding for T-cell receptor variable regions, or by tolerance (1, 2). However, it cannot be excluded that *Ir* effects may be expressed at the level of antigen presentation, determined by the capacity of viral antigen to "complex" more or less immunogenically with the restricting K or D structures (3, 4). These results and models are compatible with Ir effects regulating T-helper cells (4, 5), and T cells specific for the male H-Y antigen (6, 7).

Recently we demonstrated that the restriction specificity of cytotoxic T cells was selected by the *H-2K* and *D* haplotype of the radioresistant portion of the thymus (8,

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9) during T-cell maturation. Since *H-2*-linked *Ir* genes seem to act predominantly on T cells, it is expected that the expression of "*Ir* genes" and Ir effects may also be determined by the *H-2*-region alleles of that thymic haplotype which dictated the restriction specificity.

To demonstrate this point, we formed irradiation bone marrow chimeras by reconstituting lethally irradiated nonresponder or responder parental mouse strains with lymphohemopoietic stem cells from (nonresponder × responder)F_1 mice. The results of these experiments assessing the responsiveness of virus-specific cytotoxic T cells to vaccinia virus are compatible with similar ones by Press and McDevitt (10), who examined T-helper responsiveness in (responder + nonresponder) → F_1 double bone marrow chimeras, by Billings et al. (11), who used *H-2 Ir*-regulated trinitrophenol cross-reactivity (11), and by von Boehmer et al. (12), who worked with the H-Y model. These experiments demonstrate that lymphocytes expressing the low responder *H-2* allele can be converted to a responder phenotype if the thymic major histocompatibility complex lacks and therefore excludes selection of the "immunodominant" T-cell specificity. In contrast, bone marrow stem cells of high responder origin, differentiating in a low responder type host, usually express the low responder phenotype.

Materials and Methods

Mice, Virus, Immunization Procedures, and Cytotoxic Tests. These were identical to those described in (1). HTG targets from B10.HTG were a generous gift of Dr. B. Knowles, The Wistar Institute, Philadelphia, Pa. All target cell lines had spontaneous releases between 10 and 20%/6 h.

Chimeras. The methods for making irradiation bone marrow chimeras have been described (8, 9, 10, 12). Briefly, recipient mice were irradiated with 950–1,000 rads and reconstituted with 2×10^7 bone marrow cells that were depleted of T cells by two anti-θ plus complement treatments. Chimeras were infected 3–4 mo after reconstitution. Each individual chimera was typed for *H-2*, and all were reconstituted to 90–95% with donor type lymphohemopoietic cells.

Results and Discussion

Chimeras Used to Investigate K-Region-Regulated, D^b-Restricted Responsiveness to Vaccinia Virus. Irradiation bone marrow chimeras were of two general types—(nonresponder K^k × responder K^b)F_1 bone marrow stem cells were used to reconstitute lethally irradiated responder parents (Table I, Group 1), or nonresponder parents (Table I, group 3). These chimeras generated vaccinia-immune cytotoxic activity corresponding to the responsiveness of the chimeric host. Thus, immune cells from chimeras [C57BL/6 (K^b) × C3H (K^k)] → C57BL/6 (K^b responder to D^b) (group 1) lysed infected D^b targets as well as did immune spleen cells from responder B10 (group 6). In contrast, spleen cells from chimeras [B10.BR (K^k) × B10 (K^b)] → B10.A (4R) (K^k nonresponder D^b) lysed infected $H\text{-}2^k$ targets, but lysed D^b infected targets much less. Spleen cells from B10.A (4R) or (C3H × C57BL/6)F_1 mice gave virtually identical results (groups 4 and 5). Similarly, C3H → C3H × C57BL/6 chimeric spleen cells that were adoptively sensitized in acutely irradiated and infected (C3H × C57BL/6)F_1 sensitizing recipients (group 10) lysed $H\text{-}2^k$ and $H\text{-}2^b$ targets, but not infected D^b targets (9).

These experiments demonstrate that the irradiated chimeric host determines the responder phenotype of the T cells derived from the reconstituting stem cell pool. They indicate that the mere presence of immunogenic K^k plus vaccinia, which cannot

TABLE I

Response to D^b plus Vaccinia Virus in Irradiation Bone Marrow Chimeras of the Type F_1 (K^k Nonresponder × K^b Responder) into Responder or Nonresponder Parents

Group	Chimera*	Sensitizing‡ recipient	Spleen to target cell ratio	Specific ^{51}Cr release from targets§					
				L($H\text{-}2^k$)		MC57G ($H\text{-}2^b$)		HTG (K^dD^b)	OH (K^dD^k)
				Vacc.	Nor.	Vacc	Nor.	Vacc.	Vacc.
				%					
Experiment A									
1	C57BL/6 × C3H →	C57BL/6	40:1	10	6	80‖	4	73	
	(K^kD^k) × (K^kD^k)		13:1	5	4	42	3	51	
			4:1	1	0	17	1	26	
2	B10.A (2R)	B10.A (2R)	40:1	54	2	17	2	22	
	(K^kD^b)		13:1	32	2	8	0	8	
			4:1	22	0	4	0	7	
3	(B10.BR × B10) →	B10.A (4R)	40:1	95	2	15	2	6	
	(K^kD^k × K^bD^b)	(K^kD^b)	13:1	88	1	2	0	5	
			4:1	41	0	0	1	4	
4	B10.A (4R)		40:1	92	2	15	3	12	
	(K^kD^b)		13:1	81	2	3	2	8	
			4:1	44	2	1	0	5	
5	(C3H × C57BL/6)		40:1	86	5	75	4	30	
	(K^kD^k × K^bD^b)		13:1	69	4	52	2	17	
			4:1	32	2	24	0	10	
6	C57BL/6		40:1	9	3	73	0	100	
	(K^bD^b)		13:1	9	2	50	0	52	
			4:1	6	0	20	0	28	
Experiment B									
7	C3H → C3H × C57BL/6 →	C3H × C57BL/6	40:1	53	N.T.¶	53	N.T.	15	0
	K^kD^k → (K^kD^k × K^bD^b)		13:1	41		41		10	−2
			4:1	27		20		8	0
8	C57BL/6		40:1	5	N.T.	90	N.T.	70	
	(K^bD^b)		13:1	0		57		43	
			4:1	0		27		25	
9	C3H		40:1	94	N.T.	4	N.T.	0	4
	(K^kD^k)		13:1	68		0		6	0
			4:1	43		0		0	0
10	BALB/c		40:1	3	N.T.	N.T.	N.T.	N.T.	52
	(K^dD^d)		13:1	0					37
			4:1	0					10

* Chimeras were formed by reconstituting lethally irradiated (950–1,000 rads) recipient mice with T-cell-depleted bone marrow cells. Chimeras were typed individually for H-2 and found to be >90–95% of donor type. Chimeras were infected with 2–5 × 10^6 plaque-forming units of vaccinia virus and killed 6 days later. Spleen cells were tested at the indicated ratios.

‡ Chimeric spleen cells were transferred to acutely irradiated and infected recipients for 6 days and then tested as usual.

§ The test duration was for 6 h, spontaneous release was from 10 to 21%. Vacc., plus vaccinia; Nor., normal.

‖ Statistically significant results are in boxes.

¶ N.T., not tested.

be recognized by T cells, easily allows D^b plus vaccinia-restricted responses to vaccinia D^b to arise. For example, (C3H × C57BL/6) → C57BL/6 chimeras (group 1) generate T cells that express restriction specificity for K^b and D^b, but not for K^k; since the immunogenic lymphoreticular system of the chimera is of donor ($H\text{-}2^k$ × $H\text{-}2^b$) type (9), immunogenic K^k plus vaccinia is expressed on macrophages, but cannot be recognized by the T cells of this chimera.

Chimeras to Study Responsiveness to D^k plus Vaccinia. Responsiveness to D^k plus vaccinia appears to be low, independent of any K or I region haplotype tested.

$H\text{-}2^d$ mice are high responders both to K^d plus vaccinia and to D^d plus vaccinia. The question is, will stem cells of $H\text{-}2^d$ type when selected for restriction specificity D^k in a chimeric host of D^k type respond or not respond to D^k plus vaccinia? Irradiation bone marrow chimeras BALB/c ($K^dI^dD^d$) → C3H.OH ($K^dI^dD^k$) were formed. 3 mo later, after sensitization in an acutely irradiated and infected sensitizing host (BALB/c × C3H.OH)F_1, the potential of their lymphocytes to respond to D^k plus vaccinia was assessed (Table II, group 1). Responsiveness was high to $H\text{-}2^d$ plus vaccinia (presumably K^d restricted), but low to D^k plus vaccinia. Thus, under the conditions tested, stem cells of $H\text{-}2^d$ (K^dD^d vaccinia responders) that matured in a host and thymus of $H\text{-}2K^dD^k$ type did not respond to D^k plus vaccinia. When chimeric lymphocytes from (C3H → C3H × C57BL/6) chimeras were adoptively sensitized in an acutely irradiated and infected sensitizing host (C3H × C57BL/6)F_1, responsiveness was high to K^k or $H\text{-}2^b$ plus vaccinia, but was low for D^k plus vaccinia (Table I, group 7). Thus, stem cells expressing D^k cannot respond to D^k plus vaccinia whether selected in a thymus of $H\text{-}2D^k$ or $H\text{-}2^b$ type as was shown previously (8, 9).

In contrast, in C3H.OL → BALB/c chimeras, stem cells from D^k plus vaccinia nonresponder C3H.OL ($K^dI^dD^k$) were selected for D^d restriction specificity in a D^d thymus. When sensitized in the appropriate acutely irradiated and infected $H\text{-}2^d$ (B10.D2) recipients, these chimeric lymphocytes were responsive to D^d plus vaccinia (Table II, group 5). This illustrates again that the D or K allele expressed by the host, and probably by the thymus, not the D or K genetically coded for and expressed by the precursor or mature T cells, determines whether responsiveness to a particular virus is high or low.

These data are compatible with the results from similar experimental analyses of Ir gene effects by Billings et al. (11), von Boehmer et al. (12), and Press and McDevitt (10). For example, the latter authors demonstrated that (low responder + responder) → F_1 chimeras only produced antibodies to (Tyr_1Glu)-Ala-Lys (TGAL) of the responder Ig allotype. This result can be explained by arguing, as in the previous section, that T-cell precursors of both responder and nonresponder types can be selected in the thymus so as to acquire restriction to the high responder I-region structure. Thereafter, both responder and nonresponder T cells can help responder B cells. T cells of both responder and nonresponder $H\text{-}2$ type that are selected for restriction to the low responder I region structures apparently cannot help B cells of the nonresponder $H\text{-}2$ type. This result suggests, therefore, that expression of restriction specificity for nonresponder $H\text{-}2I$ excludes expression of a receptor for TGAL resulting in the absence of help for B cells of the nonresponder $H\text{-}2$ type.

At the moment it cannot be excluded that, for example, D^k fails to form immunogenic complexes with vaccinia, but this may be testable. Understanding the role of tolerance mechanisms, which is obvious but not yet analyzed, is probably crucial for

TABLE II

Response to D^k plus Vaccinia in Irradiation Bone Marrow Chimeras

Group	Chimera*	Sensitizing‡ recipients	Specific ^{51}Cr release from vaccinia targets§		
			L ($H\text{-}2^k$)	D2 ($H\text{-}2^d$)	OH (K^dD^k)
				%	
Experiment A					
1	BALB/c → C3H.OH → (K^dD^d) (K^dD^k)	BALB/c × C3H.OH	0	41	N.T.¶
			0	20	
			1	6	
2	C3H.OH (K^dD^k)		2	60	N.T.
			0	42	
			0	17	
3	C3H (K^kD^b)		94	2	2
			68	0	4
			30	0	3
4	BALB/c (K^dD^d)		0	N.T.	52
			2		37
			0		10
			L ($H\text{-}2^k$)	D2 ($H\text{-}2^d$)	5R (K^bD^d)
Experiment B					
5	C3H.OL → B10.D2 → (K^dD^k) (K^dD^d)	B10.D2	4	40	32
			4	35	22
			3	19	8
6	B10.D2 (K^dD^d)		9	63	55
			6	59	41
			2	23	21
7	C57BL/6 (K^bD^b)		10	10	60
			8	6	48
			6	4	18
8	C3H (K^kD^k)		79	6	6
			71	6	4
			31	0	2

*, ‡, §, ‖, ¶, as in Table I.

explaining many Ir effects. Within the framework of the dual recognition theory, the low response to D^k vaccinia is interesting because this Ir defect seems to represent (at least quantitatively) a defect in the repetoir of antigen recognition. Whether this Ir effect is quantitative or qualitative probably reflects the size or the number of the antigenic determinants and the number of antigen-specific T-cell precursors (1, 2, 12).

In conclusion, expression in the thymus of a particular *H-2* allele, e.g. D^k, and coupled with it, selection of the restriction specificity for D^k results in low response to D^k plus vaccinia, regardless of whether the T cells possess high or low responder *H-2 Ir* genes. The *Ir* gene and the gene coding for the restricting element are thus functionally linked or identical. The *Ir* gene phenotype is a direct consequence of a particular *H-2* allele being selected as a restricting self-marker as dictated by the thymus' *H-2*, but it is (in chimeras) independent of the T cell's own *H-2* type.

Summary

The *H-2* haplotype of the chimeric host determines the responder phenotype of maturing T cells. Spleen cells of chimeric mice formed when (K^k nonresponder to D^b × K^b responder to D^b plus vaccinia)F_1 bone marrow cells were used to reconstitute K^bD^b (C57BL/6 D^b responder) irradiated recipients generated high levels of D^b plus vaccinia virus-specific cytotoxic T cells. The same stem cells used to reconstitute K^kD^b (B10.A (2R) D^b nonresponder) irradiated recipients resulted in spleen cells that responded well to *K* plus vaccinia, but responsiveness to D^b was low. A generally low

response to D^k plus vaccinia, which seems to be regulated by D^k, was confirmed in chimeras. Thus, K^dD^d (D^d plus vaccinia responder) stem cells differentiating in a K^dD^k chimeric host failed to generate a measurable response to D^k plus vaccinia. In contrast, stem cells from K^dD^k (D^k plus vaccinia low responders) differentiating in a K^dD^d (K^d and D^d high responders to vaccinia) host do generate responsiveness to D^d plus vaccinia. These results indicate that in chimeras, the *Ir* phenotype is independent of the donor T cell's *Ir* genotype, and that thymic selection of a T cell's restriction specificity for a particular *H-2* allele of the chimeric host also defines that T cell's *Ir* phenotype.

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