Brief Definitive Report

H-2-LINKED CONTROL OF CYTOTOXIC T-CELL RESPONSIVENESS TO ALPHAVIRUS INFECTION

Presence of H-2D^k during Differentiation and Stimulation Converts Stem Cells of Low Responder Genotype to T Cells of Responder Phenotype

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The alphaviruses are a globally distributed viral genus; they are transmitted by insect vectors (1), and in appropriate animal hosts they cause a variety of disease syndromes, frequently involving the central nervous system (2, 3). In recent studies of the murine cytotoxic T cell (T_c cell) response to three different alphaviruses, Sindbis (SIN), Semliki Forest (SFV) and Bebaru (BEB), we found that only mice carrying the D^k region of the *H-2* gene complex were high responders (k and o haplotypes) whereas other haplotypes (d, s, q, and b) were low responders (4).

Two general classes of low or nonresponder, with respect to H-2 restricted T_c-cell responses, have been reported thus far. One class is characterized simply by an apparent inability of certain self H-2K or H-2D molecules to associate with a particular foreign antigen (X) in a manner immunogenic for self (4–8) T_c cells. The other class is more complicated in that genes elsewhere in the H-2 complex influence in some way the immunogenicity of a particular combination of self H-2K or H-2D plus X (9–15). We report here evidence that low responders to alphavirus infection may be examples of the former, simple class of low responder outlined above.

Materials and Methods

Animals. Mice were bred in the John Curtin School, and only females were used in experiments at 5–11-wk old.

Viruses and Immunization. SIN and BEB viruses were grown and titrated as described before (4). Mice were immunized i.p. with either 5×10^5 plaque-forming units (PFU) of SIN or BEB.

In Vitro Secondary Cultures (Memory Culture). The method has been described in detail elsewhere (4). In brief, 8×10^7 spleen cells of mice primed with SIN or BEB 2–10 wk previously were cultured with 8×10^6 syngeneic SIN or BEB-infected stimulator spleen cells (1–2 PFU/cell at 2×10^7 cells/ml) for 5 d at 37°C.

Cytotoxicity Assay with Macrophage Target Cells. The method has been described in detail elsewhere (16). The multiplicity of infection was 50 PFU/cell SIN or BEB (2×10^6 macrophages/ml). Data given have had spontaneous release subtracted and are the means of triplicates for assays run at 37°C for 6 h. SEM were $< \pm$ 5% and are omitted for clarity. Significance was determined by Student's *t* test.

Preparation of Chimeras. C3H.OH mice were irradiated with 950 rads from a ^{60}Co source 24 h before reconstitution i.v. with 4×10^7 BALB/c fetal liver cells (14 to 15-d embryos). Chimeric mice were primed with either BEB or SIN 5–9 wk postreconstitution as described above.

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TABLE I

H-2D^k Restricted Cytotoxic T-Cell Response to Sindbis Virus

Secondary‡ T_c cells	Killer:target ratio	Percent specific lysis of macrophage targets*									
		CBA/H§ (kkkkk)		BALB/c (ddddd)		C3H. OH (ddddk)		B10. (4R) (kkbbb)		(CBA/H × BALB/c)F_1 $\left(\frac{kkkkk}{ddddd}\right)$	
		SIN	Normal	SIN	Normal	SIN	Normal	SIN	Normal	SIN	Normal
CBA/H	3:1	42.2	0	7.9	5.2	39.2	7.2	9.1	6.2	40.4	11.1
	1:1	20.6	0	6.1	5.1	22.9	4.1	1.0	0	19.3	11.0
BALB/c	3:1	8.6	9.2	28.3	25.3	8.3	8.1	NT‖	NT	26.4	25.6
	1:1	5.3	2.1	10.8	14.3	4.2	3.7	NT	NT	12.7	14.4
C3H.OH	3:1	46.6	7.9	10.2	8.1	47.0	12.2	NT	NT	40.6	13.7
	1:1	19.5	4.9	5.3	5.1	18.0	0.1	NT	NT	28.7	0
(CBA/H × BALB/c)	3:1	53.5	6.3	22.5	17.7	49.2	7.9	NT	NT	49.9	11.3
	1:1	25.9	3.4	10.2	9.1	29.8	3.7	NT	NT	27.8	2.1

* Percent ^{51}Cr release of targets over a 6-h period with spontaneous release subtracted (<20%). Means of triplicate given with SEM never >3.1%.

‡ Secondary responses generated in vitro 2 wk after priming as described in Materials and Methods.

§ H-2 map corresponding to *K*, *IA*, *IB*, *IC*, and *D* region.

‖ Not tested.

Results

T_c-Cell Response to SIN Is Restricted to the D^k Region of H-2. In our initial studies (4), out of five mouse strains tested (CBA/H, BALB/c, SJL/J, C57Bl/6, and DBA/1) only CBA/H mice gave a significant secondary T_c-cell response to SIN. On mapping of the response, an absolute requirement for D^k in effector and target cells became apparent. Results showing a D^k requirement at the target cell level for lysis to occur are presented in Table 1. CBA/H, C3H.OH, and (CBA/H × BALB/c) F_1 hybrids gave high specific lysis of CBA/H, C3H.OH, or F_1 SIN-infected targets. No lysis of infected targets was associated with K^k as shown by B10.A(4R). BALB/c, a low responder strain to SIN and other alphaviruses, did give lysis of BALB/c and F_1 targets, but uninfected targets were lysed as much as infected targets, a result discussed in detail elsewhere (4).

There was a lack of complementation in F_1 hybrids between high and low responder, even though responsiveness was dominant. Thus, (CBA/H × BALB/c) F_1 T_c cells only lysed targets which carried the D^k region, and did not lyse BALB/c targets.

Stem cells of low responder genotype can given rise to T_c cells which recognise SIN, if allowed to differentiate in high responder, irradiated recipients. BALB/c fetal liver cells were allowed to differentiate in the thymus of irradiated C3H.OH mice. Splenic T_c cells taken 9 wk after reconstitution and 2 wk after priming with SIN were boosted in vitro by coculturing with SIN-infected C3H.OH spleen stimulator cells inactivated with 2,000 rads. The responder cells were tested by complement-mediated lysis with the appropriate anti-H-2 sera and found to be >95% of D^d (i.e. BALB/c) phenotype. Table II shows the results of such an experiment in which T_c cells of BALB/c origin lysed SIN-infected targets bearing D^k antigen, provided D^k was present during differentiation and stimulation of the T_c cells. A similar result was obtained using BEB virus instead of SIN in the same experimental protocol (data not shown).

Discussion

In these experiments, we converted T_c cells of BALB/c origin (low responder genotype) to responder phenotype, by allowing them to differentiate in a responder

TABLE II

Development of Responder Capability in T_c Cells of BALB/c Low Responder Origin in Irradiated C3H.OH High Responder Mice

Secondary‡ T_c cells	Killer: target ratio	Percent specific lysis of macrophage targets*					
		CBA/H		BALB/c		C3H.OH	
		SIN	Normal	SIN	Normal	SIN	Normal
C3H.OH (ddddk)§	9:1	75.8	7.4	10.9	12.3	64.1	13.8
	3:1	68.3	6.5	0	7.5	57.5	8.3
	1:1	50.4	2.1	0	3.0	47.6	2.6
BALB/c (ddddd)	9:1	12.8	10.6	41.5	38.8	10.1	11.9
	3:1	6.1	4.7	25.5	25.2	17.6	8.4
	1:1	7.4	13.0	10.4	15.0	12.4	5.9
(BALB/c → C3H.OH chimera (ddddd)‖	9:1	45.1	13.4	8.5	6.9	41.7	13.4
	3:1	30.5	4.8	0	0	39.0	8.4
	1:1	25.6	3.8	0	0	33.0	12.1

* Percent ^{51}Cr release of targets over a 6-h period with spontaneous release subtracted (<20%). Means of triplicates given with SEM never >4.7%.

‡ Secondary responses generated in vitro 2 wk after priming as described in Materials and Methods.

§ H-2 map corresponding to *K*, *IA*, *IB*, *IC*, and *D* region.

‖ 7 wk postrecognition chimera; phenotype of spleen cells tested by complement-mediated lysis and anti-H-2D^d serum to be >95% of BALB/c phenotype.

thymus, and then stimulating them with alphavirus infected responder-type cells. This is a similar observation in principle to that reported for the vaccinia system by Zinkernagel et al. (14). Paradoxically, however, *H-2D^k*, which is the only *K* or *D* region out of the 10 tested thus far which gives a high response to alphaviruses (4), is associated with low responsiveness to ectromelia (5), vaccinia, and Sendai viruses (12, 13). Our results, and those of others (11, 12, 14, 15) exclude the possibility that low or nonresponder genotypes are absolutely unable to produce T_c cells which can recognise or respond to particular viral antigens. They emphasize the point that the phenotype (low or high responder) is dependent upon the effects of a particular *H-2K*- or *H-2D*-region gene product either at the level of T-cell ontogeny (11, 14, 15) and/or at the level of stimulation of the response during viral infection (12).

The latter of these two possibilities is the simplest. It implies that in low responders there may be a failure of self H-2K or H-2D molecules to associate (or form a complex) with an alphavirus antigen molecule in the same infected cell membrane; thus, there would be no antigenic moiety available to stimulate alphavirus-specific H-2-restricted T_c cells. Recent evidence shows that H-2 antigens bind to alphavirus particles (17), i.e. H-2 and viral molecules can form complexes when present in different membranes, but this may not be relevant to their relationship when adjacent to each other in the same membrane, because the orientations of the molecules in these two cases would be different.

The other possibility is that even if H-2K or H-2D and alphavirus antigens can form an appropriate complex, the T_c-cell repertoire lacks antigen-receptor(s) which recognize this complex. von Boehmer et al. (11) have recently suggested an explicit dual recognition hypotheses to account for this. Their hypothesis states that: a T_c cell uses two receptors to recognize H-2K (or H-2D) plus foreign antigen (X); the gene coding for the anti-X variable (V) region is derived by mutation from the germ-line anti-H-2 V region gene expressed by the same T_c cell; and a particular anti-H-2 V

gene gives rise to a limited repertoire of mutants, and thus, may not generate a mutant V gene appropriate for a certain X.

Our results are compatible with both of the possibilities outlined above i.e., either H-2D^k is the only H-2 antigen of those tested able to form a complex with alphavirus-coded protein(s), or an anti-H-2D^k V gene is the only V gene which mutates to give an anti-alphavirus V gene. At present we cannot resolve between them.

Similar findings to those reported here have also been obtained with the H-Y system by Matsunaga and Simpson (15) and by von Boehmer et al. (11), except in the latter case, the low, or nonresponders were not defective in the cytotoxic class of T cells, but may have been lacking in helper T cells. This seems less likely to be involved in low responsiveness to alphaviruses. Available evidence suggests that induction and/or expression of T help requires certain permissive *I*-region genes (8–11), but we obtained high alphavirus-specific T_c-cell responses in C3H.OH mice (I^d, D^k) though they possess the *I* region of a low responder haplotype (e.g. BALB/c, H-2^d). Furthermore, if one argues that help is essential for T_c-cell responses, then it must have been present in F_1 hybrids between high and low responders, because these mice always gave high responses. However, their responding T_c cells invariably recognized only alphavirus-infected targets bearing H-2D^k. Therefore, if a lack of T help were the cause of low responses associated with all other *K* and *D* regions then it must be that T_c cells which recognize H-2D^k plus alphavirus antigen are exempt from this defect for some reason. The same problem is posed by other cases in which the antiviral T_c-cell response in a particular mouse strain is limited to either a *K* or a *D* region (5–7, 12–14). Because the only identifiable difference between T_c cells restricted to H-2K plus X and others restricted to H-2D plus X is the idiotype of their antigen-receptors, an hypotheses involving a helper T-cell defect in these cases of T_c-cell low responsiveness implies that helper T cells recognize T_c-cell idiotypes, or some unknown correlate of idiotype. We are presently investigating this possibility.

Summary

Secondary T_c cells generated against Sindbis virus (SIN) are restricted to D^k. All other H-2*K* or *D* regions tested show low specific responsiveness. F_1 hybrids between low and high responders show dominance of responsiveness but lack complementation. When BALB/c ($K^dI^dD^d$) low responder fetal liver stem cells were allowed to mature in irradiated high responder recipients C3H.OH ($K^dI^dD^k$) a response to D^k plus SIN could be generated with T_c cells of BALB/c origin. This result, together with the failure of complementation in the F_1 hybrids, implies that the lesion of low responsiveness is in the inability of viral antigen to stimulate a T_c-cell response in association with any self H-2K or H-2D molecule (of those tested) other than H-2D^k. Hypotheses compatible with these data are discussed.

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