

CYTOTOXIC T-CELL RESPONSE TO ECTROMELIA
VIRUS-INFECTED CELLS
Different *H-2* Requirements for Triggering
Precursor T-Cell
Induction or Lysis by
Effector T Cells Defined by the
BALB/*c-H-2^{db}* Mutation

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Significant portions of the amino acid sequences of H-2 and HLA antigens correspond, thus suggesting that the basic form of these antigens has been conserved through a considerable part of the evolution of vertebrates (1, 2). Despite this, some antigenic determinants borne on products of the major histocompatibility complex (MHC)¹ are highly polymorphic, and the genes coding for these antigens are apparently mutating at an extraordinary rate (3). Thus, there seems to be evolutionary pressure to change certain antigenic features of MHC products, while conserving their basic form and function. Perhaps the search for T-cell-mediated mechanisms allowing recovery from potentially lethal viral infections, such as ectromelia infection in mice (4) is a part of this evolutionary process. This idea is supported by findings that virus-immune cytotoxic T (T_c) cells that specifically lyse virus-infected target cells in vitro (reviewed in reference 5), and promote recovery from primary viral infection in vivo (4) recognize H-2 antigens together with virus-specific antigens on infected cell surfaces (4-11). The relevant genes in the *H-2* complex map in either the *K* or the *D* region, but not in the *I* region (4, 5, 10, 12, 13). Extensive experiments with five mutants of the *H-2K^b* region and ectromelia, vaccinia, and lymphocytic choriomeningitis viruses (4, 5, 10, 14-16) have shown that a single gene in *H-2K^b*, probably a cistron coding for an H-2 polypeptide antigen (17) makes an essential, direct contribution to the antigenic patterns recognized by T_c cells specific for *H-2K^b* plus virus. Evidence from the *H-2K^b* mutants (16) and recent work with Sendai virus (18) suggests that H-2 antigens become physically associated with viral antigens in infected cell membranes, but the way this happens is unclear. Therefore, another important feature of H-2 antigens may be their capacity to form compound or interaction antigens by associating with viral gene products (5, 6, 16, 19, 20).

The present experiments examine further the role of H-2 molecules in ectromelia virus-induced antigenic changes. We have used a new *H-2* mutant,

¹ *Abbreviations used in this paper:* H, histocompatibility; i.v., intravenous; MHC, major histocompatibility complex; NIAID, National Institute of Allergy and Infectious Diseases; PFU, plaque-forming unit; T cell, thymus-derived lymphocyte; T_c cell, cytotoxic T cell.

BALB/c-*H-2^{db}*, that is unique among *H-2* mutants characterized thus far (16, 21). It is carried in an inbred strain coisogenic with the parental strain (BALB/cKh). The mutation is of the loss type since BALB/c-*H-2^{db}* reject BALB/cKh skin grafts, but not vice versa. Complementation studies have localized the mutation to the same locus in *H-2D* as B10.D2-*H-2^{da}* (M504, see references 16, 22), a different gain and loss mutation. Serological analysis indicates that structure(s) identified by antibodies against the H-2.28 family of public specificities have been lost in BALB/c-*H-2^{db}*, all other H-2 and Ia specificities being intact (21). Biochemical studies (23) show that the *H-2D^d* region apparently codes for two similar proteins, each of about 45,000 mol wt. One of these bears serologically-defined private H-2 specificities; the other does not, but reacts with certain antibodies contained in anti-H-2.28 sera. It is the latter molecule which is not expressed in BALB/c-*H-2^{db}*. This molecule has been designated D' by Hansen et al. (23). We report here evidence which suggests that D' may be involved in a physical association between *H-2D^d*-coded and viral antigens that is in turn required for efficient induction of precursors of T_c cells. In contrast, D' is apparently nonessential for target cell recognition and lysis by effector T_c cells.

Materials and Methods

Mice. A.TH, A.TL, A.TFR2, A.TFR5, B10.A(2R), DBA/1, DBA/2, BALB/c, SJL/J, D2.GD, CBA/H, C3H.OH, C3H.OL, C57BL/6, and various F₁ hybrids were bred at The John Curtin School. BALB/c-*H-2^{db}* (mutant) and the coisogenic BALB/cKh (wild type) mice were bred at the Austin Hospital and shipped to the John Curtin School. All mice were used at 6-12 wk of age.

Virus. Stocks of attenuated (Hampstead egg) and virulent (Moscow) strains of ectromelia virus were grown, titrated, and stored as described (24).

Immunization. Mice were injected intravenously (i.v.) with 10³-10⁴ plaque-forming units (PFU) of attenuated virus; the T_c-cell response was not affected by virus dose within this range (25). Spleens were taken for T_c cell assay on day 6, at the peak of the primary response (24, 25), or after at least 3 wk when they were used as a source of memory T cells for secondary responses in vitro (26, 27).

Secondary T-Cell Response In Vitro to Virus-Infected Cells. The basic methods used have been described in detail (26, 27). When the genotype of responder and stimulator cells was different (as in Table IV), infected macrophages were used as stimulators with procedures designed to minimize escape of virus particles, or virus-specified antigens from the nominated stimulators into the responder population, which could in turn act as stimulators. Therefore, macrophage stimulators were infected in suspension with γ -irradiated Moscow virus for 40 min at 37°C (10⁸ cells/ml; 1 PFU/cell). They were then washed three times in cold medium and dispensed into tissue culture flasks. The flasks were incubated at 37°C for 2-3 h followed by vigorous washing (with warm medium) to remove nonadherent cells and residual virus before culture with memory T cells (responders). In addition, carbonyl iron-adherent (phagocytic) cells were removed from the responder population before culture. Responder:stimulator ratio was 8:1 and the cultures were held at 39°C (nonpermissive for viral replication) for 5 days before harvesting and assaying for T_c-cell activity.

When spleen stimulator cells were used (as in Table VI), the cells were infected with γ -irradiated Moscow virus, washed three times with warm medium, left for 1.5-2 h at 37°C, and then washed three more times before culturing for 5 days at 39°C with memory T cells at a responder:stimulator ratio of 10:1.

Response to Minor Histocompatibility (H) Antigens. The method used was similar to that of Bevan (20). DBA/2 mice were primed by i.v. injection of 10⁷ spleen cells from either BALB/c or BALB/c-*H-2^{db}*. After 14-16 days spleen cells from the primed mice were stimulated a second time in vitro by coculturing for 5 days with irradiated BALB/c or mutant stimulator cells, and T_c-cell activity against minor H antigens was then assayed against the appropriate macrophage target cells.

Cytotoxicity Assay with Macrophage Target Cells. The method has been described in detail elsewhere (15). Data given have had spontaneous release subtracted and are the means of

TABLE I
Effect of the BALB/c-H-2^{db} Mutation on T-Cell-Mediated Lysis of Virus-Infected Macrophages

Exp.	Donors of virus-immune T cells*							Specific lysis of infected targets†			
	Strain	H-2 map						A.TH	A.TFR5	BALB/c-H-2 ^{db}	BALB/c-Kh
		K	I-A	I-B	I-C	S	D				
										%	
1	A.TH	s	s	s	s	s	d	24.0	13.5	32.7	20.4
	A.TFR5	f	f	f	f	k	d	35.6	34.0	33.0	41.2
	BALB/c-H-2 ^{db}	d	d	d	d	d	db	3.2‡	0‡	47.7	43.6
	BALB/c-Kh	d	d	d	d	d	d	36.8	31.3	46.7	42.1
							A.TL	A.TFR2	BALB/c-H-2 ^{db}	BALB/c-Kh	
2	A.TL	s	k	k	k	k	d	41.3	27.8	24.7	34.7
	A.TFR2	f	f	f	f	s	d	36.0	33.9	42.9	35.2

* T cells obtained from pools of two spleens at the peak of the primary response in vivo 6 days after i.v. immunization with attenuated virus.

† Percent ⁵¹Cr release from ectromelia-infected targets over a 16-h period with spontaneous release and release from uninfected controls subtracted. Killer:target ratio was 30:1. Means of triplicates are given. SE of means were never larger than 2.9 (exp. 1) and 2.5 (exp. 2).

‡ Not significantly greater lysis than uninfected control targets. All other killer:infected target combinations gave significant lysis ($P < 0.01$).

triplicates \pm SE of the mean (in some Tables, SE are omitted for the sake of clarity). Statistical significance was determined by Student's *t* test.

Anti-H-2 Sera. The sera used were either obtained from the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Md., or prepared and titrated as described previously (28).

(a) Anti-H-2.4: this serum was prepared as (B10.AKM \times 129)F₁ anti-B10.A; it contains antibodies against the D^d private specificity (H-2.4) and cytotoxic titer against BALB/c cells was 1/256.

(b) Anti H-2.28: this was serum D28b obtained from NIAID and is described in the Catalogue of mouse alloantisera. It was prepared as (B10.Br \times LP.RIII)F₁ anti-B10.A(2R) and contains antibodies against the H-2.28 family of public specificities. Cytotoxic titer against BALB/c cells was 1/256. This serum will be referred to as anti-H-2.28 for the sake of simplicity. It reacts with the molecule designated D' that is coded in the H-2^d region (23, 29).

(c) Anti-H-2.31: this serum was prepared as (C57BL/6 \times A)F₁ anti-B10.D2; it contains antibodies against the K^d private specificity H-2.31 and cytotoxic titer against BALB/c cells was 1/512.

Method for Antisera-Blocking of T-Cell-Mediated Lysis of Macrophage Targets. Target macrophages were infected with virus for 1 h as described (15), then washed and left for 1 h at 37°C to allow virus-induced antigens to be expressed. Medium was then removed, and anti-H-2 sera diluted one-half in complete medium containing 10% fetal calf serum were immediately added to each well of target cells in a vol of 100 μ l and left for 30 min at 37°C before addition of T_c cells in a further 100 μ l vol. Thus, antisera were present at a one-quarter dilution throughout the 4-h of the T_c-cell assay. Data presented are representative of three experiments. It should be noted that blocking was demonstrable in this system only if a short assay time and potent secondary T_c cells were used.

Results

Effect of the BALB/c-H-2^{db} Mutation on Virus-Specific T-Cell-Mediated Lysis. BALB/c-H-2^{db}, BALB/cKh, and a number of other strains with recombinant H-2 haplotypes were immunized with ectromelia virus. Virus-immune T_c cells from A.TH, A.TFR5, A.TL, and A.TFR2 mice, which share only the D region with BALB/cKh (wild type) or BALB/c-H-2^{db} (mutant) lysed infected targets of either mutant or wild type with similar efficiency (Table I). In contrast, virus-immune T_c cells from BALB/c-H-2^{db} mice gave little or no significant lysis of infected A.TH or A.TFR5 targets (Tables I and II), but did kill infected self or infected BALB/cKh targets, presumably through H-2K^d sharing (Table I). Thus the mutation did not seem to directly affect the virus-induced

TABLE II
Effect of the BALB/c-H-2^{db} Mutation in Heterozygotes Containing a Gene for H-2.28

Exp.	Donors of virus-immune T cells*							Specific lysis of infected targets†			
	Strain	H-2 map						A.TH	A.TFR5	BALB/cKh (dddddd)	C57BL/6 (bbbbbb)
		K	I-A	I-B	I-C	S	D	%			
1	A.TFR5	f	f	f	f	k	d	38.8	43.8	39.8	0
	BALB/c-H-2 ^{db}	d	d	d	d	d	db	7.4§	11.8§	39.9	1.3
	B10.A(2R)	k	k	k	d	d	b	0.5	0	0	44.9
	(B10.A(2R) × BALB/c-H-2 ^{db})F ₁							0§	0§	44.0	28.5
								A.TFR5	BALB/cKh	DBA/1	SJL/J
2	A.TFR5	f	f	f	f	k	d	35.6	35.4	ND	ND
	DBA/1	q	q	q	q	q	q	0	0	39.2	ND
	(DBA/1 × BALB/c-H-2 ^{db})F ₁							10.8§	38.1	ND	ND
	SJL/J	s	s	s	s	s	s	0	0	ND	26.9
								9.0§	27.6	ND	ND

* As for Table I.

† As for Table I except that SE of means were never larger than 3.3 (exp. 1) and 3.1 (exp. 2).

§ All cases where BALB/c-H-2^{db} or F₁ T cells were placed on infected targets from recombinants (A.TH and A.TFR5) sharing only D region with BALB/c gave significantly less lysis ($P < 0.001$) than where the same T cells were placed on infected BALB/c Kh targets.

|| Significantly more lysis than uninfected control targets ($P < 0.05$).

antigenic change(s), dependent upon the H-2D^d region, which are required for recognition and lysis by virus-immune T_c cells. But these same antigenic change(s) apparently induced only a very weak T_c-cell response in BALB/c-H-2^{db} mice.

F₁ hybrids between BALB/c-H-2^{db} and B10.A(2R), DBA/1, or SJL were also tested; these last three strains all possess a gene for members of the H-2.28 family of specificities (21). After immunization with virus, the F₁ hybrids gave little or no significant T_c-cell-mediated lysis of infected A.TH or A.TFR5 target cells (Table II). Thus, introducing genes for other versions of H-2.28 was insufficient to overcome the effect of the BALB/c-H-2^{db} mutation.

Capacity of BALB/c-H-2^{db} Mice to Respond to Virus-Induced Antigenic Changes Associated with Their Own H-2D Region. Although BALB/c-H-2^{db} mice generated few virus-immune T_c cells that recognized wild-type H-2D^d determinants plus virus-specific antigen, they may have responded to virus-induced changes specific for mutant H-2D antigenic patterns. This was tested by taking pools of BALB/c-H-2^{db} and D2.GD virus-immune T_c cells and assaying them for a short time at various killer:target ratios against BALB/c-H-2^{db} and C3H.OH macrophage targets. Lysis of infected C3H.OH targets by these two T_c cell pools was similar at 10:1 (BALB/c-H-2^{db}) and 5:1 (D2.GD) (Table III). This lysis would be caused by T_c-cell clones recognizing virus-specific antigenic changes associated with H-2K^d (12, 13). If the BALB/c-H-2^{db} T_c-cell pool contained additional clones recognizing virus-induced changes specific for the mutant H-2D region, then it should cause more lysis of infected BALB/c-H-2^{db} targets than the D2.GD T_c-cell population. This was not the case; killing was again similar (Table III), thus suggesting a poor response to mutant H-2D region-associated, virus-induced antigenic patterns. This is not definitive, however, since in our hands, killer:target dose-response relationships are not reproducible with macrophage target cells (15). Attempts to resolve the issue with the cold target competition approach (14) were also unsuccessful.

TABLE III
Relative Efficiency of Lysis of Infected Self or Infected H-2K-Compatible Targets by BALB/c-H-2^{db} Virus-Immune T Cells

Donors of virus-immune T cells*	Killer:target ratio	Specific lysis of macrophage targets [§]					
		BALB/c-H-2 ^{db} (d ddd d ^{db})		C3H.OH (d ddd dk)		A.TH (s sss sd)	
		Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
BALB/c-H-2 ^{db} (dddd ^{db})	10:1	26.0 ± 1.7	2.5 ± 0.1	21.6 ± 3.1	0	2.5 ± 2.1	1.2 ± 0.5
D2.GD (ddbddd)	5:1	29.6 ± 1.7	0	24.4 ± 2.6	0	0	0

* As for Table I.

† H-2 map regions as for Table I.

§ Assay run for 4 h. Data given are means of triplicates ± SE of the mean with spontaneous release subtracted.

TABLE IV
Capacity of Infected BALB/c-H-2^{db} Macrophages to Stimulate Cytotoxic T-Cell Clones that Recognize Virus-Induced Antigenic Patterns Associated with Either H-2K or H-2D

Exp.	Responder T cells*	Infected macrophage stimulator cells	Yield [†]	Killer:target ratio	Specific lysis of infected targets			
					C3H.OL (ddddkk) [§]	A.TH (ssssd)	CBA/H (kkkkkk)	
1	(CBA/H × BALB/c)F ₁	BALB/c (dddddd)	32	5:1	31.6 ± 2.8	19.7 ± 1.3	4.7 ± 1.5	
				2:1	26.2 ± 0.9	16.8 ± 1.9	2.1 ± 1.3	
	(CBA/H × BALB/c)F ₁	BALB/c-H-2 ^{db} (dddd ^{db})	32	5:1	35.1 ± 6.2	6.9 ± 0.9 ^e	4.9 ± 1.6	
				2:1	23.7 ± 2.2	3.5 ± 1.7 ^e	0	
	2	(C57BL/6 × BALB/c)F ₁	BALB/c (dddddd)	44	8:1	27.1 ± 1.1	23.5 ± 1.0	0
					2:1	15.7 ± 1.3	13.0 ± 1.7	0
(C57/BL/6 × BALB/c)F ₁		BALB/c-H-2 ^{db} (dddd ^{db})	38	8:1	26.6 ± 1.6	10.1 ± 0.9 ^e	0	
				2:1	27.8 ± 1.8	0.7 ± 0.5 ^e	0	

* Cytotoxic T cells were generated in secondary responses in vitro (see Materials and Methods).

† Number of viable responder cells after 5 days in vitro expressed as a percentage of the original responder number set up.

§ H-2 map regions as for Table I.

|| Assay run for 6 h at killer:target ratios indicated; data given are means of triplicates ± SE of the mean; otherwise as for Table I.

^e Significantly less lysis than that caused by T cells stimulated by infected BALB/c macrophages and assayed on infected A.TH or A.TL targets at the same killer target ratio ($P < 0.001$).

Capacity of Infected BALB/c-H-2^{db} Macrophages to Stimulate T_c-Cell Responses. While infected BALB/c-H-2^{db} macrophages display virus-induced antigens recognizable by virus-immune T_c cells from wild-type H-2D^d mice (Table I), the T_c-cell response against these antigens in BALB/c-H-2^{db} mice is weak or absent. The ability of infected BALB/c-H-2^{db} macrophages to stimulate wild-type H-2D^d T_c cells was therefore tested. Memory T_c cells from virus-primed (CBA/H × BALB/c)F₁ mice were used as responders, to detect any escape of virus-specific antigen from the nominated stimulators into some responder cells.

BALB/c and BALB/c-H-2^{db}-infected macrophages stimulated similar responses against infected C3H.OL targets, where lysis is caused by T_c cells recognizing virus-specific plus H-2K^d antigens (Table IV). But BALB/c-H-2^{db} stimulators were significantly inferior to BALB/c in stimulating T_c cells that

recognize virus-specific plus H-2D^d antigens on infected A.TH targets (Table IV, Exp. 1). Minor escape of virus-specific antigen into the (CBA/H × BALB/c)_{F₁} responder cells may have occurred, since slight lysis of infected CBA/H targets was seen (Table IV, exp. 1). A second, similar experiment with (C57BL/6 × BALB/c)_{F₁} responders gave similar results (Table IV, exp. 2) except that no escape of antigen into responder cells was detected. Thus, it seems that infected BALB/c-*H-2^{db}* cells are significantly inferior to wild type in terms of stimulating a T_c-cell response against virus-specific plus *H-2D^d*-coded determinants. These data suggest the possibility that the physical relationships between virus-specific and H-2D^d molecules that may be required for efficient induction of virus-immune T_c cells could be impaired by the absence of D' (23) from the surfaces of BALB/c-*H-2^{db}* cells.

Capacity of Uninfected BALB/c-H-2^{db} Cells to Stimulate T_c-Cell Responses to Minor H Antigens. The T_c-cell response to minor H antigens exhibits H-2 restriction (20). Thus, it offers a means to test whether the stimulation defect of BALB/c-*H-2^{db}* reflects an intrinsic failure of *D* region-coded molecule(s) to provide the antigenic configurations needed for T_c-cell stimulation, or whether the defect lies in a failure of BALB/c-*H-2^{db}* molecule(s) to associate adequately with virus-specific antigen.

Spleen cells from DBA/2 (H-2^d) mice primed with either BALB/c or BALB/c-*H-2^{db}* cells, were stimulated a second time in vitro with irradiated BALB/c or BALB/c-*H-2^{db}* cells, respectively. T_c-cell activity was then assayed against C3H.OL (K^d, D^k) and A.TL (K^s, D^d) macrophage targets that would display minor H antigens shared with the BALB/c background in association with either H-2K^d (C3H.OL) or H-2D^d (A.TL) determinants. At a given killer:target ratio, C3H.OL and A.TL targets were lysed to a similar extent, regardless of whether stimulation was from BALB/c or BALB/c-*H-2^{db}* cells (Table V). CBA/H (K^k, D^k) targets were not lysed, and SJL/J (K^s, D^s) targets were lysed slightly. These data confirm that the T_c-cell response to minor H antigens is H-2 restricted to some extent (20) and also indicate that little, if any, of the lysis of C3H.OL and A.TL targets is due to nonspecifically activated T_c cells that recognize H-2K or H-2D antigens alone. Apparently, therefore, minor H antigens are as immunogenic in association with *H-2D*-coded antigens of BALB/c-*H-2^{db}* as they are with wild type. Similar results have been obtained by using trinitrophenyl-modified cells (data not shown). These results reinforce the possibility that the defect in the T_c-cell response to ectromelia infection in BALB/c-*H-2^{db}* mice may be caused by inefficient physical association between mutant *H-2D*-coded and virus-specific molecules.

Specific Blocking of T_c-Cell-Mediated Lysis with Anti-H-2K and Anti-H-2D Sera. Specific anti-H-2 sera against the determinants H-2.4 (D^d, private) H-2.28 (D^d, public, borne on D') and H-2.31 (K^d, private) were used. Virus-infected BALB/c or BALB/c-*H-2^{db}* macrophages were attacked by two separate subsets of virus-immune T_c cells, one subset from D2.GD mice that recognized virus-induced plus H-2K^d antigens (12, 13) and the second subset from A.TH mice that recognized virus-induced plus H-2D^d antigens (12, 13). Lysis of both BALB/c and BALB/c-*H-2^{db}* targets by D2.GD T_c cells was blocked significantly more by anti-H-2.31 than by anti-H-2.4 and anti-H-2.28 (Table VI). Conversely, lysis of BALB/c targets by A.TH T_c cells was blocked more by anti-H-2.4 and anti-H-

TABLE V
Cytotoxic T-Cell Response to Minor H Antigens Stimulated by BALB/c and BALB/c-H-2^{db} Cells

Responder* T cells	Irradiated stim- ulator cells	Yield‡	Killer: target ratio	Specific lysis of macrophage targets			
				C3H.OL (dddkk)§	A.TL (skkkd)	CBA.H (kkkkk)	SJL/J (sssss)
		%			%		
DBA/2 (ddddd)	BALB/c (ddddd)	51	7.5:1 2.5:1	30.9 ± 2.6 10.5 ± 2.3	32.0 ± 4.4 13.0 ± 3.0	0 0	15.0 ± 0.8 6.1 ± 4.6
DBA/2 (ddddd)	BALB/c-H-2 ^{db} (ddddd ^{db})	90	2.5:1 0.8:1	35.5 ± 1.9 21.8 ± 2.4	32.2 ± 2.0 19.5 ± 1.8	0 0	10.8 ± 1.6 3.7 ± 3.1

* Cytotoxic T cells were generated in secondary responses to minor H antigens in vitro (see Materials and Methods).

‡ As for Table IV.

§ H-2 regions as for Table I.

|| Assay run for 16 h; data given are means of triplicates ± SE of mean. Since the culture with BALB/c-H-2^{db} stimulators gave a higher yield and more powerful cytotoxicity, data presented are from killer:target ratios where lysis caused by both T-cell populations is similar.

TABLE VI
Blocking of T-Cell-Mediated Lysis of Virus-Infected Macrophages by "Monospecific" Anti-H-2 Sera

Donors of vi- rus-specific T cells*	H-2 region shared with target	Specificity of blocking anti- serum‡	Specific lysis of infected targets§	
			BALB/cKh	BALB/c-H-2 ^{db}
			%	
D2.GD (ddbddd)	K ^d	H-2.4 (D ^d) H-2.28 (D ^d) H-2.31 (K ^d) Nil	25.9 ± 2.0 25.1 ± 2.4 19.4 ± 1.7¶ 35.2 ± 0.6	41.9 ± 1.5 40.7 ± 1.8 32.7 ± 2.6¶ 43.8 ± 2.2
A.TH (sssssd)	D ^d	H-2.4 (D ^d) H-2.28 (D ^d) H-2.31 (K ^d) Nil	7.3 ± 2.4** 9.6 ± 1.5** 18.0 ± 2.9 17.4 ± 0.9	8.2 ± 1.5‡‡ 19.1 ± 1.7 20.2 ± 0.9 16.9 ± 1.6

* Cytotoxic T cells were generated in secondary responses in vitro to syngeneic ectromelia-infected cells (see Materials and Methods).

|| H-2 map regions as for Table I.

‡ Blocking antiserum was added to targets before addition of T cells. Full procedure given in Materials and Methods. Nil, no serum.

§ Assay run for 4 h at a killer target ratio of 2:1. Data given are means of triplicates ± SE of mean with spontaneous release subtracted. Lysis of uninfected controls was negligible.

¶ Significantly less lysis than with anti-H-2.4 and anti-H-2.28 sera ($P < 0.05$).

** Significantly less lysis than with anti-H-2.31 serum ($P < 0.05$).

‡‡ Significantly less lysis than with anti-H-2.31 and anti-H-2.28 sera ($P < 0.01$).

2.28 than by anti-H-2-2.31 (Table VI). Most importantly, anti-H-2.28 failed to block lysis of BALB/c-H-2^{db} targets by A.TH T_c cells while anti-H-2.4 was active (Table VI). This is consistent with the loss of D' from the surfaces of mutant BALB/c-H-2^{db} cells (21, 23), and also suggests that the blocking activity of the sera was directed at target cells, not T_c cells, since A.TH T_c cells possess D'.

The specificity of blocking (Table VI) indicated that T_c cells recognized virus-induced antigenic changes which incorporated, or were physically close to,

either H-2K or H-2D molecules bearing serologically defined determinants. In the case of H-2D^d antigens on BALB/c cells, a molecule with private determinants (H-2.4) and the D' molecule were apparently both involved.

Discussion

The present experiments showed that ectromelia-infected cells of the BALB/c-*H-2^{ab}* line display virus-induced antigenic patterns associated with the *H-2D* region, but BALB/c-*H-2^{ab}* T_c cell precursors seem to respond weakly, if at all, to these antigenic patterns. BALB/c-*H-2^{ab}* is a loss mutation apparently in a gene required for production or expression on cell membranes of a 45,000 mol wt molecule, designated D' by Hansen et al. (23), that bears certain H-2 public specificities (21). No other H-2 or Ia antigens seem to be affected (21). Hypotheses to account for the defective response to ectromelia virus infection were considered in this context. First, has there been deletion or suppression of a gene for a T_c-cell receptor needed for recognition of H-2D^d antigenic determinants on virus-infected cells? This seems unlikely, since variable regions of T_c-cell receptors defined thus far are not coded by MHC-linked genes (8, 30). T_c-cell recognition structures physically associated with D' also seem to be disqualified, since anti-H-2 antibody blocked T_c-cell-mediated lysis by binding to target cells, not to T_c cells (Table VI).

Second, does the absence of the D' molecule in BALB/c-*H-2^{ab}* expose on infected cells other H-2D antigenic determinants, recognizable by T_c cells, that are not normally exposed on infected wild-type cells? These exposed determinants, plus virus-specific determinants, could then be the predominant inducers of virus-immune T_c cells in BALB/c-*H-2^{ab}* mice. There are several points of evidence against this hypothesis, though none of them are definitive. It seems unlikely that new H-2D determinants unique to BALB/c-*H-2^{ab}* should be present on infected cells, in view of the findings that uninfected BALB/c-*H-2^{ab}* cells show no such determinants on the basis of serological or skin graft criteria (21). Also, this hypothesis predicts that BALB/c-*H-2^{ab}* mice should generate a normal level of virus-immune T_c-cell response against their own H-2D determinants plus viral antigen; such a response is not evident (Table III). Finally, and most importantly, it does not account for the demonstrated defect in the capacity of infected BALB/c-*H-2^{ab}* macrophages to stimulate virus-immune memory T_c cells that recognize H-2D^d determinants (Table IV), despite observations that such infected macrophages are lysed to the same extent as wild-type targets by T_c cells recognizing wild-type H-2D antigens plus virus-specific determinants, and that BALB/c-*H-2^{ab}*-infected macrophages are not defective in stimulating virus-immune T_c cells that recognize H-2K^d determinants.

The possibility exists, therefore, that the minimum requirements for precursor T_c-cell induction (including memory T_c cells) are more rigorous than those for the process of antigen recognition by effector T_c cells that leads to lysis of infected targets. It seems that the presence of the D' molecule, plus the molecule-bearing *H-2D^d*-coded private determinant(s), plus virus-specific antigen on the infected cell surface are all required for precursor T_c-cell induction, but D' is nonessential for effector T_c-cell recognition and lysis. When tested for capacity to stimulate T_c-cell responses against minor H antigens or trinitrophenyl in association with *H-2D^d*-coded determinants, BALB/c-*H-2^{ab}* cells were as effi-

cient as BALB/c cells. Thus, there seems to be no intrinsic defect in the molecule-bearing *H-2D^d*-coded private determinants in terms of T_c-cell induction. This suggests that the poor induction capability of infected BALB/c-*H-2^{ab}* cells with respect to virus-specific T_c cells may reside in the manner in which the viral and the two *H-2D*-coded molecules physically associate in infected cell membranes. The D' molecule may play a crucial role in this association. That a close physical relationship does exist between both of the *H-2D^d*-coded molecules and virus-specific determinants on the surface of infected cells was shown by the specificity of blocking of virus-immune T_c-cell-mediated lysis by monospecific anti-H-2 sera directed against both private and public determinants (Table VI).

The foregoing evidence, taken together with findings from the *H-2K^b* mutations (16) suggests that there are two features of H-2 molecules that are pertinent to the H-2 restriction phenomenon. The first is a structure bearing antigenic determinant(s) that are recognized by receptors on T_c cells; such determinant(s) are affected by the *H-2K^b* mutants (16), but do not seem to be affected in BALB/c-*H-2^{ab}*. The second may be a structure required for physical association between the H-2 determinant(s) and a virus-specified antigen molecule; such association seems necessary for efficient induction of virus-specific T_c cells. This class of structure is unaffected by the *H-2K^b* mutations (16) but appears to be affected in BALB/c-*H-2^{ab}*. In the case of products of the *H-2D^d* region, this structure is apparently embodied in a second molecule (D') that does not bear private H-2 specificities, but can be identified by antibodies in anti-H-2.28 sera (21, 23, 31, 32). The unusual distribution of the genes for the H-2.28 and H-2.1 families of public specificities among murine *H-2K* and *H-2D* regions discussed by Snell et al. (33, 34) and Démant et al. (31) also deserves comment in this connection. It seems that the H-2.28 and H-2.1 specificities are never both coded in the same *H-2K* or *H-2D* region, but either one or the other is invariably present (31, 33, 34). This raises the possibility that an H-2.1-bearing molecule serves a similar function in other mouse strains to that ascribed to D' in the present experiments with BALB/c. Testing this possibility may depend upon the isolation of appropriate loss mutations.

Summary

The T_c-cell response to ectromelia virus infection was studied in BALB/c-*H-2^{ab}* mice which carry a loss mutation in the *H-2D* region that results in the absence from cell surfaces of a molecule (D') bearing certain public H-2 specificities. When infected, these mice showed a poor response of T_c cells that recognize H-2D^d plus virus-specific determinants on infected macrophage targets, but gave a normal response to H-2K^d plus virus-specific antigens. However, their own infected macrophages do display wild-type antigenic patterns involving virus and H-2D^d since they were killed as efficiently as wild-type (BALB/c, H-2^d)-infected cells by T_c cells specific only for H-2D^d plus viral antigens. When tested in vitro, infected BALB/c-*H-2^{ab}* cells stimulated a poor T_c-cell response to H-2D plus virus-specific antigens, but stimulated a normal response (in comparison with infected BALB/c macrophages) to H-2K^d plus viral antigens. Uninfected BALB/c-*H-2^{ab}* cells stimulated a normal T_c-cell response to minor H antigens or trinitrophenyl in association with H-2D^d, thus suggesting that the defective response to infection may reside in a failure of the relevant H-2D^d

antigens of mutant cells to physically associate with viral antigens. Close association of viral and H-2D-coded molecules was also suggested by ability of specific anti-H-2K or -H-2D to partially block T_c-cell-mediated lysis of infected targets.

These results were interpreted to mean that H-2D^d-dependent, virus-immune T_c cells recognized an antigenic pattern consisting of virus-specific and H-2D^d determinants with the latter borne on an H-2D molecule carrying serologically-defined H-2D^d private specificities. A second H-2D^d-coded molecule (D') was not required for recognition and lysis by activated T_c cells, but was apparently necessary for efficient stimulation of precursor T_c cells, perhaps by promoting appropriate physical association of viral and H-2D^d molecules.

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