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Genes required for cytotoxicity against virus-infected target cells in *K* and *D* regions of H-2 complex

EVIDENCE is mounting that in mice, certain specific immunological effector functions of thymus-derived (T) lymphocytes are efficient only when donors of T cells and the cells with which they interact have at least a part of the H-2 gene complex in common[1-8]. Examples include T helper function *in vivo* and *in vitro*[1-3], cytotoxicity mediated by T cells against virus-infected[4-6] or TNP-modified[7] target cells *in vitro*, immunopathology mediated by T cells[4], or protection against bacterial[8] and viral infection[6] *in vivo*. This requirement for H-2 compatibility has been studied in detail by measuring the cytotoxicity of T cells from donors immunised with either lymphocytic choriomeningitis (LCM) or ectromelia virus using target cells infected with the homologus virus.

These two viruses are very different, LCM being a non-cytopathic, single-stranded RNA virus which acquires an envelope by budding from infected cell membranes, whereas ectromelia is a cytopathic DNA virus which is assembled completely within infected cells[9]. Evidence described in detail elsewhere[4,10,11] indicates that immune T cells kill virus-infected target cells directly by contact without production of detectable soluble factors or any requirement for ancillary cells such as mononuclear phagocytes or thymus-independent (B) cells. Experiments with target cells of different H-2 haplotypes and a repertoire of inbred mouse strains, including congenic pairs, have shown that the non-H-2 genetic background, the M locus and H-2 public specificities are irrelevant[4,11]. Maximal cytolysis only occurs when immune T cells and virus-infected target cells share the same H-2 haplotype[4,11].

These findings suggest that the genes required are either the H-2 private specificities, which code for the major H-2 antigens on which the haplotype classification is based, or genes closely linked to them in the *K* and *D* regions of the H-2 complex. Identity of *Ir* genes, which map in the *I* region between *K* and *D* (Table 1) and which are common to many different H-2 haplotypes, should not be sufficient[12]. This reasoning was tested directly using mouse strains bearing recombinant H-2 haplotypes (Table 1). Immune spleen cells were obtained from donor mice immunised with either LCM virus or ectromelia virus as

Table 1 H-2 composition of mouse strains used

Strain	H-2 regions					
	K	*I*			*S*	*D*
		A	*B*	*C*		
B10.A	k	k	k	d	d	d
B10.A(2R)	k	k	k	d	d	b
B10.A(4R)	k	k	b	b	b	b
A.TL	s	k	k	k	k	d
A.TH	s	s	s	s	s	d
C3H.OH	d	d	d	d	d	k
C3H.OL	d	d	d	d	k	k
AQR	q	k	k	d	d	d

Table 2 Activity of splenic cytotoxic T cells* from LCM-immunised mice against target cells infected with LCM virus

Mouse strain†	Spleen cell status	L929 targets (H-2^k)		P-815 targets (H-2^d)		Mouse embryo cells (H-2^b)	
		Uninfected	Infected	Uninfected	Infected	Uninfected	Infected
B10.A	Normal	15.9 ± 1.0	19.4 ± 1.3	21.4 ± 1.4	30.1 ± 3.2	36.8 ± 1.6	45.1 ± 1.4
	Immune	17.2 ± 1.7	*72.6 ± 0.9*	31.2 ± 2.3	*86.5 ± 1.2*	38.5 ± 2.9	45.9 ± 1.8
B10.A(2R)	Normal	24.9 ± 0.7	20.9 ± 1.8	22.7 ± 1.1	29.0 ± 3.7	40.1 ± 0.7	48.2 ± 0.8
	Immune	21.9 ± 2.0	*80.9 ± 2.6*	41.7 ± 0.9	*49.0 ± 1.3*	42.7 ± 2.1	*61.9 ± 1.8*
B10.A(4R)	Normal	17.0 ± 0.8	18.5 ± 2.1	32.1 ± 3.2	26.0 ± 4.5	39.6 ± 1.0	45.7 ± 2.0
	Immune	18.9 ± 1.3	*74.3 ± 1.6*	40.7 ± 1.9	35.9 ± 1.7	44.4 ± 1.4	*59.7 ± 1.5*
						Mouse macrophages, SJL (H-2^s)	
A.TL	Normal	14.1 ± 0.6	16.5 ± 0.9	14.3 ± 0.9	14.1 ± 1.1	40.7 ± 4.2	39.1 ± 3.7
	Immune	17.6 ± 1.7	17.6 ± 1.7	16.2 ± 1.1	*64.4 ± 2.4*	39.6 ± 2.4	*76.1 ± 2.9*
A.TH	Normal	12.3 ± 0.7	13.0 ± 0.9	30.5 ± 4.5	28.6 ± 2.1	ND	ND
	Immune	13.2 ± 1.6	16.3 ± 1.9	30.1 ± 4.8	*81.0 ± 1.2*	ND	ND
C3H.OH	Normal	20.8 ± 0.6	15.4 ± 1.4	14.1 ± 0.5	27.8 ± 1.1	ND	ND
	Immune	22.4 ± 1.1	*56.5 ± 2.0*	23.7 ± 0.5	*74.5 ± 2.8*	ND	ND
C3H.OL	Normal	23.2 ± 0.6	27.4 ± 0.6	15.3 ± 0.4	17.2 ± 0.8	ND	ND
	Immune	52.8 ± 2.2	*80.5 ± 1.3*	18.0 ± 0.3	*49.0 ± 0.8*	ND	ND
						Mouse macrophages, DBA/1 (H-2^q)	
AQR	Normal	ND	ND	16.1 ± 0.5	16.9 ± 0.6	39.1 ± 3.5	36.9 ± 2.6
	Immune	ND	ND	28.0 ± 0.5	*92.6 ± 0.6*	44.8 ± 2.0	*62.6 ± 2.3*

*Expressed as percentage ^{51}Cr released (mean of four replicates ± s. e. m.) over 16 h at a killer–target ratio of 30:1 (corrected for water lysis). Significant specific lysis ($P < 0.05$) is indicated by italics.

†Data given in this table were derived from several separate experiments in which the various mouse strains were tested. CBA.H (H-2^k), BALB/c (H-2^d), C57BL (H-2^b), SJL (H-2^s) and DBA/1 (H-2^q) mile were always included in the experiment as controls where necessary. They gave specific lysis only with H-2-compatible infected target cells (see refs 4 and 9) (data not shown).

ND, not determined.

Table 3 Activity of splenic cytotoxic T cells* from ectromelia-immunised mice against target cells infected with ectromelia virus

Mouse strain†	Spleen cell status	L929 targets (H-2^k) Uninfected	L929 targets (H-2^k) Infected	P-815 targets (H-2^d) Uninfected	P-815 targets (H-2^d) Infected	Mouse embryo cells (H-2^b) Uninfected	Mouse embryo cells (H-2^b) Infected
B10.A	Normal	28.2 ± 0.5	28.3 ± 0.5	17.2 ± 0.6	29.6 ± 0.3	34.7 ± 1.5	34.1 ± 1.0
	Immune	40.5 ± 0.3	*95.5 ± 1.3*	24.6 ± 1.1	*59.4 ± 1.2*	34.1 ± 1.6	32.9 ± 1.0
B10.A(2R)	Normal	25.6 ± 2.3	26.7 ± 1.7	22.0 ± 1.5	24.0 ± 1.6	38.1 ± 1.8	34.5 ± 1.7
	Immune	29.7 ± 1.2	*83.4 ± 1.9*	27.0 ± 1.6	*37.8 ± 3.1*	41.7 ± 1.2	*67.1 ± 1.3*
B10.A(4R)	Normal	25.5 ± 1.2	27.4 ± 2.3	28.6 ± 3.0	23.6 ± 0.8	45.5 ± 2.0	34.7 ± 1.1
	Immune	30.1 ± 1.2	*78.5 ± 2.7*	31.1 ± 4.0	30.5 ± 3.5	40.3 ± 1.9	*58.8 ± 0.9*
A.TL	Normal	35.6 ± 1.3	31.5 ± 1.4	19.5 ± 0.2	23.7 ± 0.5	ND	
	Immune	42.3 ± 1.7	44.0 ± 1.5	32.3 ± 0.7	*78.4 ± 0.6*	ND	
A.TH	Normal	36.1 ± 1.2	38.0 ± 2.1	20.1 ± 0.3	22.1 ± 0.4	ND	
	Immune	39.0 ± 0.8	37.4 ± 1.2	21.6 ± 0.5	*63.1 ± 1.3*	ND	
C3H.OH	Normal	21.3 ± 1.5	21.2 ± 1.1	17.4 ± 0.6	27.4 ± 1.2	ND	
	Immune	33.2 ± 2.0	*47.0 ± 2.2*	37.1 ± 0.7	*71.5 ± 3.8*	ND	
C3H.OL	Normal	32.0 ± 2.9	33.1 ± 2.8	20.6 ± 2.6	22.5 ± 2.1	ND	
	Immune	27.2 ± 2.1	*50.9 ± 1.7*	39.0 ± 1.5	*48.0 ± 2.8*		
						Mouse macrophages, DBA/1 (H-2^q)	
AQR	Normal	25.8 ± 2.2	20.8 ± 1.0	15.4 ± 0.8	20.9 ± 1.4	39.3 ± 1.8	42.5 ± 3.7
	Immune	39.9 ± 2.1	24.8 ± 0.8	31.5 ± 1.2	*58.9 ± 4.1*	43.4 ± 3.6	*65.0 ± 4.4*

*Expressed as percentage ^{51}Cr released (mean of four replicates ± s. e. m.) over 16 h at a killer–target ratio of 60:1 (corrected for water lysis). Significant specific lysis ($P < 0.05$) is indicated by italics.
†Data given in this table were derived from several separate experiments which included control strains listed in the footnote to Table 2.
ND, not determined.

described elsewhere[5,11], and specific cytotoxicity mediated by T cells was measured by ^{51}Cr release from virus-infected target cells of various H-2 haplotypes using optimal spleen cell–target cell ratios[5,11] (Tables 2 and 3). Immune cells almost invariably caused more lysis than normal cells, irrespective of target cell type, but significant specific lysis was defined as occurring only when combinations of immune cells and infected targets gave ^{51}Cr release which was significantly higher ($P < 0.05$) than all three control combinations, such as immune cells with uninfected targets, or normal cells with either infected or uninfected targets. Comparison of Tables 1 and 2 shows that specific lysis of LCM-infected H-2^k target cells required immune T cell donors to be of H-2^k type only in the *K* or *D* regions of the gene complex. It was not sufficient for all of the *I* and *S* region to be k (as in A.TL mice). Lysis of LCM-infected H-2^d or H-2^b targets also required only *D* region homology. The use of H-2^s and H-2^q macrophages as target cells confirmed that *K* region homology was sufficient.

Results obtained with ectromelia virus (Table 3) were essentially similar to the LCM system, with one exception. Lysis of infected H-2^k target cells by ectromelia-immune cells from C3H.OH or C3H.OL mice (which are of H-2^k type in the *D* region) was not as reproducible or powerful as with LCM. Ectromelia provoked a significant response against infected H-2^k targets (Table 3) in only one out of three experiments with C3H.OH, and one out of two experiments with C3H.OL, whereas a significant response always occurred against infected H-2^d targets. Thus major gene(s) active in the LCM system seemed less active in the ectromelia system. The factors responsible for this variation are not known, but *Ir* genes are candidates for further investigation.

With both viruses, B10.A (2R) gave a small but statistically significant response against H-2^d target cells in one out of two experiments (Tables 2 and 3), suggesting that genes outside the *K* or *D* regions (for example, in *IC* or *S* regions in this case) may sometimes exert a minor influence.

In summary, these data support the concept that the major genes required for cytolysis mediated by T cells, of virus-infected target cells are located in the *K* or *D* regions of the H-2 complex. In most cases, these genes are sufficient, without the requirement for *I* region homology. Whether *Ir* genes play a secondary, regulatory role remains to be determined. This is consistent with the hypothesis proposed[4,5] that the H-2-dependent restriction of lysis, mediated by T cells, of virus-infected target cells results from T cell recognition of altered self antigens (possibly H-2 private specificities) on the surfaces of virus-infected cells.

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Evolutionary conservation of H-Y ('male') antigen

THE male specific (H-Y) antigen of mice was discovered with the observation that within certain inbred strains, females reject male skin grafts, whereas skin grafts exchanged between all other sex combinations are accepted[1] (reviewed in ref. 2). It is now established that females sensitised with male skin grafts (or immunised with male spleen cells) produce antibody which is cytotoxic for sperm[3] and dissociated male epidermal cells[4]. Using the sperm cytotoxicity test and the mixed haemadsorption-hybrid antibody (MHA.HA) test, we demonstrated earlier[5] that the H-Y antigen of mice is cross reactive or identical with antigen