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DETECTION OF A *t*-COMPLEX ANTIGEN BY SECONDARY CELL-MEDIATED LYMPHOCYTOTOXICITY

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SUMMARY

Because of the inconsistency in published results concerning the serological detection of cell surface antigens coded for by the *t*-complex, a cell-mediated lymphocytotoxicity (CML) assay, secondary CML, was used in a search for *t*-antigens. By sensitizing C3H.*Ttf* (C3H.Brachyury, tufted) with the congenic strain C3H.*Ttf*/t^{w18} splenic cells, a response against lipopolysaccharide (LPS) stimulated splenic cells from C3H.*Ttf*/t^{w18} mice is obtained. The locus coding for the antigen detected by this reaction lies to the left of *tf* on the murine seventeenth chromosome. The secondary CML response to this antigen is *H-2* restricted and detects an antigen on all *t*-haplotypes tested: t^{w18}, $t^{w18}tf$, t^{12}, t^{6}, $t^{h2}tf$, and t^{w5}.

INTRODUCTION

Much recent effort has been expended on the search for cell surface antigens coded by the *t*-complex of the mouse. Controversy exists over whether such antigens can be found by serological methods. Positive identification has been claimed using an anti-teratocarcinoma (F9) serum Artzt *et al.*, 1974) and anti-sperm sera (Yanagisawa *et al.*, 1974). However, negative results have also been reported with anti-F9 serum (Erickson & Lewis, 1980) and anti-sperm sera (Gable *et al.*, 1979). An alternative approach is described here, using a cell-mediated lymphocytotoxicity (CML) assay, secondary CML, which is capable of detecting minor histocompatibility antigens (Bevan, 1975). Two minor histocompatibility loci have been described within the *t*-region of the seventeenth chromosome: *H-33* (Flaherty, 1975) and H-39 (Artzt *et al.*, 1977). *H-39* was mapped to the left of the tufted (*tf*) hair mutation. An allele of *H-39* was also described for the *t*-haplotype, t^{w18}. Here, a positive reaction is reported for all *t*-haplotypes tested in a secondary CML assay against a t^{w18} antigen. This reaction is *H-2* restricted and the antigen detected is coded for by a locus to the left of *tf*.

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MATERIAL AND METHODS

Mice

Mice bearing the following *t*-haplotypes were used: t^{w18}, $t^{w18}tf$, t^6, $t^{h2}tf$, t^{w5} and t^{12}. The *t*-haplotypes t^{w18} and t^{w5} have been placed on the C3H.*Ttf*/+*tf* background by Dr H. O. McDevitt. The $t^{w18}tf$ haplotype arose as a cross-over event in the C3H.*Ttf*/t^{w18} strain. The other *t*-haplotypes are maintained on their own backgrounds and were outcrossed to C3H/DiSn for these studies. The *H-2* and *t*-haplotypes of the mice are listed in Table 1.

TABLE 1. List of mouse strains used and their *H-2* and *t*-haplotypes

Mouse strain	*t*-haplotype	H-2 haplotype
C3H.*Ttf*/t^{w18}	t^{w18}	$H\text{-}2^k/H\text{-}2^k$ or $H\text{-}2^k/H\text{-}2^d$
C3H.*Ttf*/$t^{w18}tf$	$t^{w18}tf$	$H\text{-}2^k/H\text{-}2^k$
C3H.*Ttf*/+*tf*	+	$H\text{-}2^k/H\text{-}2^k$
C3H.*Ttf*/t^{w5}	t^{w5}	$H\text{-}2^k/H\text{-}2^{w31}$
BALB/cJ	+	$H\text{-}2^d/H\text{-}2^d$
(C3H/DiSn × BALB/cJ)F_1	+	$H\text{-}2^k/H\text{-}2^d$
(C3H/DiSn × *Ttf*/t^6)*F_1	t^6	$H\text{-}2^k/H\text{-}2^{w30}$
Ttf/t^6	t^6	$H\text{-}2^q/H\text{-}2^{w30}$
(C3H/DiSn × $t^{h2}tf/t^{h2}tf$)F_1	$t^{h2}tf$	$H\text{-}2^k/H\text{-}2^{q/k}$
(*T*/t^{12} × C3H/DiSn)*F_1	t^{12}	$H\text{-}2^k/H\text{-}2^{w28}$
T/t^{12}	t^{12}	$H\text{-}2^b/H\text{-}2^{w28}$

* Normal-tail offspring carrying the *t*-haplotype were used.

Secondary CML assay

Effector cells were obtained from spleens of mice that had previously been given 2 or 3 i.p. injections of spleen cells (1–3 × 10^7 cells/0·3 ml of PBS) from C3H.*Ttf*/t^{w18} or C3H.*Ttf*/$t^{w18}tf$ animals of the same sex as the recipient strain. A 10-day interval separated the second injection from the first and if a third was given, it was received a week later. Seven days after the last injection, effector cells were sensitized *in vitro* with irradiated (2000 Rad) splenic cells from C3H.*Ttf*/t^{w18} or C3H.*Ttf*/$t^{w18}tf$. These cells were mixed together at a 1:1 ratio (4 × 10^6 cells/ml) in RPMI 1640 supplemented with 2 mM L-glutamine, 10% FCS, 5 × 10^{-5} M 2-mercaptoethanol and 1% penicillin-streptomycin and cultured for 5 days. Target cells or cells used as unlabelled inhibition cells were generated from splenic lymphocytes that had been stimulated with lipopolysaccharide (LPS, Difco) at a concentration of 50 μg/ml for a period of 48 hr. Testicular cells were obtained by digesting the seminiferous tubules with 0·1% collagenase for 20 min at 34°C, then gently pipetting the tubules to free the germ cells. These cells were cultured in the supplemented RPMI for 48 hr and the non-adherent cells used as target cells.

Target cells were labelled on the day of the assay with 100 μCi ^{51}Cr (New England Nuclear). One hundred microlitres of labelled target cells (10^5/well) were added to 100 μl of effector cells at the appropriate ratio in V-bottom 96-well microtitre plates in triplicates and incubated for 4 hr at 37°. The inhibition assay was done by adding 100 μl of labelled target cells (10^5/well) to 50 μl of effector cells and 50 μl of cold blast cells at the appropriate ratio and incubating for 4 hr at 37°C. Maximum release was obtained

by incubating 100 μl of a 1% detergent in H_2O solution with 100 μl target cells for 4 hr at 37°C. After 4 hr, 100 μl of the supernatant was removed and counted in a Beckman gammacounter.

Because of the high spontaneous backgrounds when target cells were cultured with medium alone, it was necessary to run unsensitized lymphocytes of the same strain as the effector cells. Background release values for each experimental effector to target cell ratio were based upon the ^{51}Cr release values from equivalent unsensitized lymphocyte to target cell ratios. Percent specific cytotoxicity was expressed as:

$$\frac{\text{experimental c.p.m.} - \text{background release c.p.m.}}{\text{maximum c.p.m.} - \text{background release c.p.m.}} \times 100.$$

RESULTS

Detection of the t^{w18} antigen on lymphocytes but not testicular cells

Because it was found that C3H.*Tf*/t^{w18} animals were still segregating for the *H-2* haplotype of t^{w18}, *H-2*^{d}, even after fourteen backcrosses to C3H.*Tf*/+*tf*, it was necessary to use the hybrid (C3H/DiSn × BALB/cJ)F_1 as the source of effector cells. These effector cells react with C3H.*Tf*/t^{w18} target cells. The only difference between these two strains is the proximal end of the seventeenth chromosome. However, testicular cells from C3H.*Tf*/t^{w18} males do not react with the effector cells capable of lysing C3H.*Tf*/t^{w18} LPS-stimulated lymphocytes (Table 2).

TABLE 2. Presence of t^{w18} antigen on lymphocytes but not testicular cells

Effector cells	Sensitizing cells	Effector: target cell ratios	Target cells: Lymphocytes C3H.*Tf*/t^{w18}	Lymphocytes C3H.*Tf*/*tf*	Testicular cells C3H.*Tf*/t^{w18}	Testicular cells C3H.*Tf*/*tf*
(C3H/DiSn × BALB/cJ)F_1	C3H.t^{w18}	50:1	25%*	−2%	3%	−4%
		25:1	23%	−2%	−5%	−0·2%
		12:1	19%	−2%	0·3%	−1%
		6:1	16%	−4%	−1%	−3%
		1:1	4%	−12%	−2%	1%

* Percent specific cytotoxicity.

H-2 restriction and strain distribution of reactivity

Table 3 demonstrates that the cytolytic response to the 't^{w18}' antigen is *H-2* restricted, cytolysis is seen with t^{12} and t^6 target cells, only in combination with a *H-2*^{k} haplotype. The 't^{w18}' antigen is present on all *t*-haplotypes tested (t^{12}, t^6, t^{h2}*tf* and t^{w18}*tf*). Its presence on t^{h2}*tf*, a recombinant of the t^6 haplotype which acquired *tf* in the cross-over event, and t^{w18}*tf*, a *t* haplotype derived by a recombination event in the *tf* region, places the 't^{w18}' antigen to the left of *tf*.

TABLE 3. H-2 restriction and strain distribution of the response to the t^{w18} antigen

Effector cells	Sensitizing cell	Effector: target cell ratio	Target cell	Percent specific cytotoxicity
(C3H/DiSn × BALB/cJ)F_1	C3H.t^{w18}	40:1	C3H.*Ttf*/t^{w18}	31%
		40:1	C3H.*Ttf*/+*tf*	6%
		40:1	*Ttf*/t^6	−3%
		50:1	(C3H/DiSn × *Ttf*/t^6)$_{NT}$	27%
		40:1	(C3H/DiSn × t^{h2}*tf*/t^{h2}*tf*)F_1	32%
C3H.*Ttf*/+*tf*	C3H.*Ttf*/t^{w18}*tf*	50:1	C3H.*Ttf*/t^{w18}*tf*	29%
		50:1	C3H.*Ttf*/+*tf*	−1%
		50:1	BALB/cJ	−1%
		50:1	(*T*/t^{12} × C3H/DiSn)$_{NT}$	25%
		50:1	*T*/t^{12}	−3%

Inhibition by competing cells

Unlabelled LPS-stimulated blast cells from various *t*-haplotype inhibit the lysis of t^{w18}*tf* target cells (Table 4). This inhibition is *H-2* restricted as *H-2*^{k}/t^{12} is capable of inhibition, whereas *H-2*^{b}/t^{12} is not. Inhibition by *t*-haplotypes can be divided into two groups: t^{w18}*tf*, t^6 and t^{h2}*tf* all show strong inhibition, while t^{12} and t^{w5} are able to inhibit to a lesser degree. Whether this represents variation in the assay or presence of two specificities is not known.

DISCUSSION

An antigen coded within the *t*-complex (the t^{w18} haplotype was the immunogen) is detected by a secondary CML assay using LPS-stimulated splenic lymphocytes as target cells. The locus responsible for this antigen maps to the left of *tf*. Within this region of the *t*-complex, lie two genetic loci which could be responsible for the antigen described here: (1) *Tcp*-1, which codes for p63/6·9a, a cell surface protein (Silver *et al.*, 1979) detected by two-dimensional gel electrophoresis and (2) *H-39* (Artzt *et al.*, 1977), a minor histocompatibility locus. Both of these loci are located to the left of *tf* and each has been reported to have a *t*-variant.

The strain distribution of the *t*-variant of p63/6·9, p63/6·9a (Silver *et al.*, 1979) is quite similar to the distribution of the 't^{w18}' antigen, suggesting similarity or linkage of the two. Because p63/6·9 is found on germ cells, testicular cells were used as target cells in the secondary CML assay. However, testicular cells were not lysed by sensitized effector cells. Since both germ and somatic cells from the testes express *H-2* antigens (Erickson, 1977), the absence of reactivity with testicular cells, suggest an absence of the 't^{w18}' antigen from testicular cells.

Skin grafts between (C3H/DiSn × BALB/cJ)F_1 and C3H.*Ttf*/t^{w18}*tf* (C. Hammerberg, unpublished results) are rejected (mean survival time, 22 days) within the range of published results (Artzt *et al.*, 1977). Thus, an allelic variant at the *H-39* locus is being carried by C3H.*Ttf*/t^{w18}*tf*. The response in the secondary CML assay to the 't^{w18}' antigen follows the usual pattern of *H-2* restricted responses to minor histocompatibility antigens (Bevan, 1975). It could, therefore, be argued that the 't^{w18}' antigen is coded for by the

TABLE 4. Inhibition of the secondary CML response to the t^{w18} antigen by unlabelled cells from various *t*-haplotypes

Effector cells*	Sensitizing cell	C:T†	Inhibiting cells‡						
			C3H.*Ttf*/t^{w18}*tf*	C3H.*Ttf*/+*tf*	C3H.*Ttf*/t^{w5}	(C3H/DiSn × *Ttf*/t^{6})$_{NT}$	(C3H/DiSn × t^{h2}*tf*/t^{h2}*tf*)$_{NT}$	(C3H/DiSn × *T*/t^{12})$_{NT}$	*Tf*/t^{12}
(C3H/DiSn × BALB/cJ)F_1	C3H.t^{w18}	20:1	57%	9%	36%	66%	59%	36%	2%
		10:1	36%	7%	36%	45%	48%	27%	20%
		5:1	27%	9%	16%	36%	39%	7%	9%
		1:1	14%	18%	9%	34%	7%	11%	5%

* Effector to target cell ratio used was 20:1. At this ratio specific ^{51}Cr release was *44*% for C3H.*Ttf*/t^{w18}*tf* target cells and *3*% for C3H.*Ttf*/+*tf* target cells.

† C:T is the ratio of unlabelled blast cells to labelled C3H.*Ttf*/t^{w18}*tf* target cells.

‡ % Inhibition of ^{51}Cr release from labelled C3H.*Ttf*/t^{w18}*tf* target cells by addition of the unlabelled blasts cells from the indicated mouse strain.

H-39 locus and is linked to *Tcp*-1. If so, the *H-39* locus is probably not involved in the abnormalities associated with the *t*-complex (i.e. transmission ratio distortion) because of the apparent absence of its antigen from testicular cells. The presence of the 't^{w18}' antigen on all *t*-haplotypes tested, which represent four complementation groups (t^{w18}, t^{6}, t^{12} and t^{w5}) would be additional evidence for a few ancestral *t*-haplotypes for all *t*-haplotypes (Hammerberg & Klein, 1975; Silver *et al.*, 1979).

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