THE T SUPPRESSOR CELL ALLOANTIGEN Tsu^d MAPS NEAR IMMUNOGLOBULIN ALLOTYPE GENES AND MAY BE A HEAVY CHAIN CONSTANT-REGION MARKER ON A T CELL RECEPTOR*

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We have described a mouse T cell alloantigen, Tsu^d,[1] which may be an allotypic or idiotypic determinant on a T cell receptor (1–3). Tsu^d is associated with the T cell receptor for idiotype, apparently on suppressor T cells or their precursors (2). The determinant is defined by antisera made in BALB/c mice against T cell blasts from allotype congenic C.AL-20 mice and can be detected by immunofluorescent staining on subpopulations of splenic Lyt-2^+ T cells, concanavalin A blasts, and mature thymocytes. It cannot be detected on Lyt-1^+ T cells, prethymocytes, or B cells (1). Antiserum against Tsu^d induces the production of T suppressor cells for the antibody responses to various T dependent antigens (3). The expression of Tsu^d is controlled by a gene closely linked to the immunoglobulin heavy chain gene cluster, *Igh*. Only strains possessing the Igh^d or Igh^e heavy chain haplotypes, such as AKR, AL/N, A, and NZB, express Tsu^d, and the linkage suggested by this association is confirmed by the appearance of Tsu^d in the allotype congenic strain C.AL-20 (1), as well as by the findings reported here. The allotype linkage of Tsu^d and its association with the T cell receptor suggested that Tsu^d might be an allotypic or idiotypic determinant of an immunoglobulin heavy chain that forms part of the T cell receptor. Several other studies have reported heavy chain determinants associated with T cell receptors. There are reports of antibody idiotype and specific heavy chain idiotype markers associated with T cell receptors (4–6), and more recently a heavy chain variable-region (Vh)[2] framework determinant has been found on T cells (7). These and other studies have failed to demonstrate immunoglobulin light chain markers or heavy chain constant-region allotypes on T cells, suggesting that a T cell receptor may consist of a heavy chain variable region expressed with a T cell-specific constant region either alone or in combination with a protein other than immunoglobulin light chain. The Tsu^d determinant could then be a Vh idiotype or framework marker.

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[1] The previously used symbol, Ts^d, has been changed to Tsu^d to avoid confusion with the preexisting symbol, Ts, for the Tail-short gene.

[2] *Abbreviations used in this paper:* BSA, bovine serum albumin; PBS, phosphate-buffered saline; PFC, plaque-forming cells; RI, recombinant inbred strains; SRBC, sheep erythrocytes; Vh, variable region.

The present study was undertaken to determine the genetic map position of the *Tsu^d* locus among the heavy chain genes to assist in the identification of Tsu^d. A position among the Vh genes (*Igh-V*) would indicate that Tsu^d is an idiotypic or Vh framework determinant. A location among the constant region allotype genes (*Igh-C*) would suggest that it is an IgT allotype. A position significantly outside either of these gene clusters would suggest that Tsu^d is not an immunoglobulin-related structure and that its genetic proximity to the *Igh* complex is fortuitous, like that of serum prealbumin, *Pre-1* (8), and an allotype-linked histocompatibility antigen, *H(Igh)* (9, 10).

Materials and Methods

Mice. AKXL recombinant inbred (RI) strains (11) were derived from the cross of AKR/J × C57BL/J and are maintained at The Jackson Laboratory, Bar Harbor, Maine. NX8 RI lines were derived from the cross of NZB/NIcr × C58/J (12) at the Institute for Cancer Research, Philadelphia, Pa. The AXC and C.B.AL strain panels are *Igh*-recombinant strains (13, 14), each derived from an independent backcross mouse that had a genetic crossover between the *Igh-Dex* dextran idiotype gene and the *Igh-1* allotype locus. The AXC strains were derived from the cross A/HeNIcr × BALB/cAnNIcr and are similar to recombinant inbred lines. The C.B.AL strains are congenic to BALB/c because they were derived from the cross between two BALB/c allotype congenic strains, BAB/14 × C.AL-9 (14, 15). These strains are maintained at the Institute for Cancer Research. The C.AL-20 and C.B-20 strains were obtained from Dr. Michael Potter, National Cancer Institute, and have been maintained at Tufts University since January 1978. BALB/cAnN animals were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.), and all other strains were purchased from The Jackson Laboratory.

Antisera. The production and T cell specificity of anti-Tsu^d has been described previously (1, 3). Briefly, BALB/cAnN females were immunized with concanavalin A blasts from selected C.AL-20 animals. The blast population was fractionated on bovine serum albumin (BSA) discontinuous gradients in an ultracentrifuge, and only the least dense population floating on 26% BSA was used for immunization. This constituted 20% of the total blast population. Antiserum was collected and pooled from 20 donors. Antiserum used for this study was produced using normal, unimmunized C.AL-20 animals as donors in contrast to the first reports of this serum (1, 2).

Tsu^d Typing by Immunofluorescence. All cells used for typing in fluorescence assays were splenic T lymphocytes eluted from nylon wool by the method of Julius et al. (16). Cells (5×10^5) were incubated for 1 h at 4°C in 50 μl of 2% horse serum in 0.04 M NaN_3 in phosphate-buffered saline (PBS) with anti-Tsu^d serum. After three washes, cells were incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgG_1 antibody for 45 min at 4°C. Cells were washed and examined in a Leitz fluorescent microscope (E. Leitz, Inc., Rockleigh, N. J.). Details of labeling and organ specificity of the sera have been described previously (1).

Assay for Suppression of an IgM Plaque-forming Cells (PFC) Response to Sheep Erythrocytes (SRBC). Briefly, 2 μl of anti-Tsu^d serum in 200 μl PBS was injected on day −4 into the tail vein. On day 0, 10^8 SRBC were injected into the tail vein. The number of direct anti-SRBC PFC was evaluated on day 3 of the primary response using the Cunningham slide-chamber assay (17). It was found that the antisera induced a three- to fivefold reduction in the IgM PFC response without accelerating the IgG response. It was determined by adsorption that T and not B cells remove the suppressive activity of the serum. Details of the in vivo assay are described in reference 2. We had previously shown that the in vitro properties of the antiserum were primarily directed against T cells (1).

Results

Igh Linkage of In Vivo Anti-Tsu^d Activity. The allotype congenic partners, BALB/c (*H-2^d, Igh-1^a*) and C.AL-20 (*H-2^d, Igh-1^d*) were used to produce the antisera used in this study. Therefore, it seemed probable that anti-Tsu^d would be allotype-restricted

in its specificity. We injected anti-Tsu^d in vivo into a variety of mouse strains. Table I shows that the IgM PFC response to SRBC of C.AL-20 animals was inhibited 75%, whereas C.B-20 (*Igh-1*^{b}, *H-2*^{d}) and BALB/c responses were not inhibited. C.AL-20 and C.B-20 are both allotype-congenic strains on a BALB/cAnN background. In all strains having allotypes *Igh-1*^{d} (AKR/J and C.AL-20) and *Igh-1*^{e} (A/J, A/HeJ, and A.BY), the IgM PFC response was inhibited by BALB/c anti-C.AL-20 Tsu^d. Each strain was inhibited ~70% with individual animal variations ranging from 50 to 95% suppression. In contrast, mice with *Igh-1*^{j} (CBA/J and C3H/HeJ) and *Igh-1*^{b} (C57BL/10J) allotypes were not inhibited. This is in agreement with our earlier in vitro fluorescent studies (1) used to evaluate the strain distribution of Tsu^d. The reduction in the number of IgM PFC had been previously shown to reflect stimulation of suppressor T cells and not direct cytotoxicity of B cells (2).

The induction of suppressor T cells by anti-Tsu^d does not appear to be affected by *H-2* type because A.BY (*H-2*^{b}) animals are as well suppressed as A/J (*H-2*^{a}), C.AL-20 (*H-2*^{d}), or AKR/J (*H-2*^{k}). Of these strains, AKR/J is *Lyt-3*^{a} and *Igk-V*^{a}, whereas the others are *Lyt-3*^{b} and *Igk-V*^{b}. The strong suppression observed in AKR/J indicates that neither *Lyt-3* type nor kappa light chain type affects the expression of Tsu^d.

TABLE I

Association of Anti-Tsu^d Activity In Vivo with Igh Allotype

Strain	Igh-1 Allotype	H-2 Haplotype	Lyt-2 Genotype	ARS Cross-reactive idiotype	Anti-Tsu^d Fluoroscein isothiocyanate intravenously*		IgM PFC/10^8 Spleen cells ± log SEM‡	Suppression factor	Significance
								%	r§
C.AL-20	*d*	*d*	*b*	+	+	NMS	56,000 ± 3.4	0	
						αTsu^d	14,000 ± 3.0	75	0.007
C.B-20	*b*	*d*	*b*	−	−	NMS	22,000 ± 3.6	0	
						αTsu^d	23,000 ± 3.4		
BALB/cAnN	*a*	*d*	*b*	−	−	NMS	414,000 ± 4.8	0	
						αTsu^d	317,000 ± 5.1	23	0.61
A/J	*e*	*a*	*b*	+	+	NMS	46,100 ± 3.9	0	
						αTsu^d	11,000 ± 3.4	77	0.022
A.BY	*e*	*b*	*b*	+	+	NMS	97,000 ± 3.4	0	
						αTsu^d	29,000 ± 3.0	71	0.036
A/HeJ	*e*	*a*	*b*	+	+	NMS	96,900 ± 4.1	0	
						αTsu^d	28,000 ± 3.6	71	0.049
AKR/J	*d*	*k*	*a*	−	+	NMS	19,900 ± 3.6	0	
						αTsu^d	4,000 ± 3.0	80	0.07
CBA/J	*j*	*k*	*a*	−	−	NMS	31,000 ± 3.4	0	
						αTsu^d	38,000 ± 3.7		
C57BL/10J	*b*	*b*	*b*	−	−	NMS	95,000 ± 4.4	0	
						αTsu^d	83,000 ± 4.2	13	0.93
C3H/HeJ	*j*	*k*	*a*	−	−	NMS	172,000 ± 3.9	0	
						αTsu^d	132,000 ± 4.6	24	0.79

* Antibody was injected into the tail vein in 200 μl PBS on day −4. On day 0, 10^8 SRBC were injected intravenously in 200 μl PBS.

‡ Animals were killed on day 3 after antigen injection. PFC values represent the average of duplicate determinations on three animals ± log SEM.

§ Student's *t* test was used to calculate *r*, the probability of random occurrence of the difference between the two groups, NMS- or Ab-treated mice.

TABLE II

Tsu^d Typing of Igh Recombinant Strains

Strain	Tsu^d		Igh-Dex	Igh-C	Pre-1	Dex	Igh	Tsu^d	Pre-1
	Suppression*	Fluorescence‡							
	%								
C.AL-9	75	+	−	*d*	*a*				
BAB/14	0	−	+	*b*	*c*				
C.B.AL/2	5	−	−	*b*	*c*				
C.B.AL/4	0	−	−	*b*	*c*				
C.B.AL/5	0	−	−	*b*	*c*				
C.B.AL/1	7	−	−	*b*	*a*				
A/HeNIcr	71	+	−	*e*	*c*				
BALB/cAnNIcr	12	−	+	*a*	*a*				
AXC-1	51	+	+	*e*	*c*				
AXC-2	62	+	+	*e*	*c*				
AXC-3	73	+	+	*e*	*c*				
AXC-4	72	+	+	*e*	*c*				
AXC-5	66	+	+	*e*	*c*				
AXC-6	70	+	+	*e*	*c*				
AXC-8	15	−	−	*a*	*c*				

* Average percent suppression of PFC for at least six mice per strain.
‡ Immunofluorescent typing done on splenic T cells from at least two mice per strain.

TABLE III

Tsu^d Typing of NX8 RI Strains

Strain	Tsu^d		Igh-Dex	Igh-C	Pre-1	Dex	Igh	Tsu^d	Pre-1
	Suppression	Fluorescence							
	%								
NZB/NIcr	77	+	−	*e*	*a*				
C58/J	0	−	+	*a*	*c*				
NX8-6	72	+	−	*e*	*a*				
NX8-19	51	NT*	−	*e*	*a*				
NX8-3	0	+‡	+	*e*	*a*				
NX8-15	66	+	−	*e*	*c*				
NX8-18	55	+	−	*e*	*c*				
NX8-13A	68	+	+	*a*	*c*				
NX8-13B	55	+	+	*a*	*c*				
NX8-16	0	−	+	*a*	*a*				
NX8-20	0	NT	+	*a*	*a*				
NX8-4	0	−	+	*a*	*c*				
NX8-5	0	−	+	*a*	*c*				
NX8-9	0	−	+	*a*	*c*				
NX8-17	NT	−	+	*a*	*c*				

* Not tested.
‡ Five of 12 mice tested show positive Tsu^d staining, three strongly and two weakly. This suggests that NX8-3 has the *Tsu^d* gene but for unknown reasons expresses it poorly.

Immunofluorescent staining of T cells from the various strains with anti-Tsu^d serum as previously determined (1) and shown in Table I is in complete agreement with the suppression results. Mice with either *Igh-1*^{a} or *Igh-1*^{e} allotype have T cells that stain with the antiserum and have diminished anti-sheep cell responses after antibody treatment. Mice with other allotypes do not express the Tsu^d antigen as detected in either assay.

Tsu^d Typing the Igh Recombinant Strains. The AXC and C.B.AL *Igh* recombinant strains were constructed to preserve genetic crossovers between *Igh-Dex*, the antidextran idiotype locus, and *Igh-C*, the heavy chain allotype gene cluster (13, 14). These strains were examined to determine whether the *Tsu^d* gene is located among the heavy chain variable-region genes, *Igh-V*, or among or near the constant region, or allotype, loci, *Igh-C*. The typing data are presented in Table II and include schematic representations of the recombinant chromosome segments. The expression of Tsu^d was assayed by both immunofluorescence and suppression of the primary PFC response to SRBC. In all 11 *Igh* recombinant strains tested, the two assays agreed, and in every case the Tsu^d phenotype matched the allotype of the strain and not the idiotype. Of the identified *Igh-V* genes, the *Dex* locus is among those nearest the constant-region genes (14). Thus, in the *Igh* recombinant strains, most, and in some cases perhaps all, *Igh-V* genes were inherited from the parent other than the source of the allotype loci. It is therefore very unlikely that Tsu^d could be a variable-region framework or idiotype determinant coded by an *Igh-V* gene. As indicated, in strains C.B.AL/1 and AXC-8

TABLE IV

Tsu^d Typing of AKXL RI Strains

Strain	Tsu^d		Igh-Dex	Igh-C	Pre-1	Dex	Igh	Tsu^d	Pre-1
	Suppression	Fluorescence							
	%								
AKR/J	80	+	−	*d*	*a*				
C57L/J	−	−	+	*a*	*b*				
AKXL-5	69	NT*	NT	*d*	*a*	NT			
AKXL-8	60	+	−	*d*	*a*				
AKXL-12	64	NT	−	*d*	*a*				
AKXL-17	81	NT	−	*d*	*a*				
AKXL-28	72	NT	−	*d*	*a*				
AKXL-37	60	NT	−	*d*	*a*				
AKXL-38	75	+	−	*d*	*a*				
AKXL-9	‡	+	NT	*d*	*a*	NT			
AKXL-25	0	+	−	*d*	*a*				
AKXL-24	0	−	−	*d*	*b*			×	
AKXL-14	0	−	+	*a*	*a*				×
AKXL-13	12	−	+	*a*	*a*				×
AKXL-29	0	NT	+	*a*	*a*				×
AKXL-6	0	NT	+	*a*	*b*				
AKXL-16	0	NT	+	*a*	*b*				
AKXL-19	0	NT	+	*a*	*b*				
AKXL-21	0	NT	+	*a*	*b*				

* Not tested.

‡ The PFC responses of AKXL-9 were widely scattered and did not permit meaningful analysis.

a second crossover occurred in the 10 map-unit interval between *Igh-C* and the prealbumin locus, *Pre-1*. Again, in these cases the Tsu^d type matches the allotype, thus placing the *Tsu^d* locus in or near the *Igh-C* cluster.

Tsu^d Typing the NX8 RI Strains. The NX8 RI lines were bred from the cross of NZB (*Igh-1^e*, Tsu^d positive) by C58 (*Igh-1^a*, Tsu^d negative). They are typical RI lines, derived by inbreeding from randomly chosen F2 pairs without selection for any characteristics. Thus, this strain panel contains only a single crossover between *Dex* and *Igh-C* and five independent crossovers between *Igh-C* and *Pre-1*. In this strain panel, the Tsu^d type again matches the allotype, with the exception of NX8-13, and in every strain with a crossover among these genes, the Tsu^d type disagrees with the *Dex* or *Pre-1* type, as shown in Table III. The one discordancy of allotype and Tsu^d in these strains is represented by NX8-13A and 13B, two sublines that were separated at the F7 generation and differ little from each other. The *Tsu^d* gene carried by NX8-13 is the positive allele from NZB, although the *Igh-V*, *Igh-C*, and *Pre-1* genes are all of the C58 type. If the *Tsu^d* is located between *Igh-C* and *Pre-1* as indicated, the double crossover necessary to produce this arrangement is not unlikely considering the 10 map-unit interval involved and the large number of recombinant strains tested in these studies. This crossover does separate the *Tsu^d* gene from the *Igh-C* cluster but does not help to define the map position of *Tsu^d*.

NX8-3 presents an unusual Tsu^d phenotype. As indicated in Table III, only a minority of mice of this strain express Tsu^d strongly enough to be detectable by immunofluorescence, and in a different group of NX8-3 mice injected with anti-Tsu^d serum, none showed the suppression of PFC characteristic of the presence of the Tsu^d antigen. Our interpretation is that these mice do possess the NZB allele at the Tsu^d locus consistent with their allotype and prealbumin phenotype, but for unknown reasons the antigen is expressed at variable levels, usually below our ability to detect it. Other interpretations can be entertained, such as a different map position for the *Tsu^d* locus in NZB mice, or a more complex model postulating a T cell alloantigen located between *Igh-C* and *Pre-1* and detected by staining, and whose expression is required in combination with a particular *Igh-V* to produce the target for anti-Tsu^d, resulting in suppression. These models are not more satisfactory at explaining the NX8 data and disagree with the data from the other strains tested.

Tsu^d Typing of AKXL RI Strains. Table IV shows the typing data for the AKXL RI lines derived from the cross of AKR/J (*Igh-1^d*, Tsu^d positive) and C57L/J (*Igh-1^a*, Tsu^d negative). In this strain panel, no crossovers between *Dex* and *Igh-C* were fixed, but four strains have recombinant genotypes for *Igh-C* and *Pre-1*. In three of these, the Tsu^d phenotype matches the allotype, and in AKXL-24 it matches the prealbumin type. These four crossovers map the *Tsu^d* gene to a position between *Igh-C* and *Pre-1*, and near to, but separable from, *Igh-C*.

In one strain, AKXL-25, the Tsu^d antigen was detected by immunofluorescence, but anti-Tsu^d administration did not suppress the response to sheep cells. In this strain, as in NX8-3, the expression of Tsu^d may vary among individual mice or may be too low to result in suppression, or in these strains a step in the suppression process subsequent to the binding of anti-Tsu^d may be deficient. Cells from AKXL-9 stained brightly, but wide variations in both the control and suppressed PFC responses rendered the suppression data uninterpretable.

Discussion

Previous work identified a T cell differentiation alloantigen in mice that was controlled by a gene linked to the immunoglobulin allotype loci (1). This antigen, Tsu^d, was identified by immunofluorescence using a BALB/c antiserum to C.AL-20 T cell blasts. Inbred strains that possessed the *Igh-1*^{a} or *Igh-1*^{e} allotypes expressed the Tsu^d antigen. Allotype-linked inheritance was indicated by the appearance of Tsu^d in the allotype-congenic strain C.AL-20 (BALB/c.AL/N-*Igh-1*^{a}), but not in the partner strain BALB/c. The antigen was detectable on a minor population of mature Lyt-2^+ T cells, and subsequent work showed that administration of anti-Tsu^d serum in vivo could activate Lyt-2^+ suppressor cells and suppress primary immune responses to T-dependent antigens (3). The present study demonstrates that this in vivo activity of anti-Tsu^d serum has the same strain distribution as the appearance of Tsu^d in conventional inbred strains (Table I). In the mapping studies in *Igh* recombinant strains and RI strains, concordance of these two traits was nearly complete (Tables II–IV). Disagreement was observed in 2 of 42 strains tested (NX8-3, AKXL-25), and was always seen as the failure of the antiserum to induce T suppressor cells in Tsu^+ strains. The coincidence of immunofluorescence staining and induction of suppressor cells by the anti-Tsu^d serum indicates that these two activities result from interaction of antibodies with the same cellular determinants, i.e., the Tsu^d antigen. The discordant strains in which the current protocol of anti-Tsu^d treatment failed to induce suppression, although positive staining was seen, could be regarded as demonstrating the existence of two antigens, one staining and the other suppressing, controlled by closely linked but separable genes. Identification of a mouse strain with reciprocal properties, in which anti-Tsu^d serum induced suppression but did not stain, would argue persuasively for this interpretation. Such a crossover has not been observed, and we favor the simpler, one-antigen interpretation and assume that the two discordant strains did not exhibit suppression for another reason. For example, they may require a stronger suppression-inducing stimulus than was employed in these experiments, or T suppressor cells could be more stringently regulated in these strains.

These genetic mapping experiments were done primarily to assist in the identification of the Tsu^d antigen. Previous work has shown that the Tsu^d antigen is associated with the T cell receptor for idiotype because anti-Tsu^d serum can block the binding of ARS cross-reactive idiotype-bearing Fab fragments by T suppressor cells (1). The genetic linkage of the *Tsu*^{d} locus to heavy chain allotype genes suggested that *Tsu*^{d} might in fact be an antibody gene, either a heavy chain variable-region gene expressed in B cells to make antibodies and in T cells to make their surface receptors, or perhaps a constant-region gene expressed only in T cells, i.e., an IgT isotype. The genetic mapping data, summarized in Fig. 1, excludes the former possibility, and is consistent with, but does not prove, the latter. In all 12 strains carrying genetic crossovers between variable-region and constant-region genes, the Tsu^d type was the same as the constant-region allotype. In strains with recombinations in the region between *Igh-C* allotype and prealbumin, the Tsu^d type again matched the allotype, except in the two instances shown as the two lowest recombinant chromosomes in Fig. 1. These two crossovers clearly separate the *Tsu*^{d} gene from the constant-region genes expressed in B cells and map it just to the right of them. The two crossovers among 30 RI lines tested give a recombination frequency of 1.9% with approximate 95% confidence limits of 0.22–6.4%.

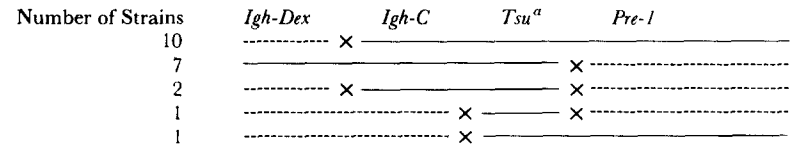

FIG. 1. Mapping *Tsu^d* with *Igh* recombinant strains and RI strains. The *Igh* to *Pre-1* regions of the recombinant chromosomes which are analyzed in the tables are schematically represented. The solid line represents the portion of the recombinant chromosome derived from one parent and the dotted line indicates the segment from the other parent. The crossover point is shown as ×.

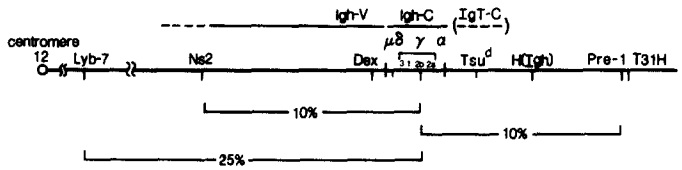

FIG. 2. Genetic map of mouse chromosome 12 showing the position of *Tsu^d* near the *Igh-C* cluster. The map positions and distances were established as follows: *Lyb-7* (18), *Igh-Ns2* (19), *Igh-Dex* (14), the order of *Igh-C* genes (20, 21), *H(Igh)* (9; R. Riblet, unpublished observations), *Pre-1* (8), T31H breakpoint (22; Eicher, E., B. A. Taylor, R. Riblet, unpublished observations).

Our current understanding of chromosome 12 is represented in Fig. 2. The position of *Tsu^d* is shown between the IgA constant-region gene at the end of the *Igh-C* cluster and a minor transplantation antigen, *H(Igh)*. We have found in these experiments that *Tsu^d* is separable from the *Igh-C* cluster. This may mean that Tsu^d, like H(Igh), is a cell-surface alloantigen with no structural relationship to antibodies and allotype linked only by chance. The more interesting alternative is that Tsu^d is one of a series of IgT isotypes, constant regions of T cell receptors of various functions (help, cytotoxicity, suppression), to which *Igh-V* genes are translocated to produce a component of the T cell receptor. This possibility is indicated in Fig. 2 by the suggested *IgT-C* cluster. Our findings do not establish this possibility, nor do they exclude it. If such an *IgT-C* cluster exists, it lies at an appreciably greater distance from the variable-region genes than does the *Igh-C* cluster.

As indicated in Fig. 2, the gene order is *Igh-V—Igh-C—Tsu^d*. Of those V genes whose map positions are determined with some accuracy, the V gene closest to the C-region genes is *Igh-Dex* (14). It is located 0.5 map units from the C genes, and the array of C genes spans a small region, perhaps 0.1 map unit in length (15). An *IgT-C* cluster would then be two map units farther along chromosome 12, and this would require that the translocation of *Igh-V* genes to *IgT-C* would have to operate over greater distances than *Igh-V* to *Igh-C*. This is not a significant objection, however, because the array of *Igh-V* genes is known to be 5–10 map units long (14), and V to C translocation in antibody-producing cells obviously proceeds over these long distances.

Summary

The mouse T cell alloantigen, Tsu^d, is expressed on a minority of mature Lyt-2$^+$ cells, and its expression is controlled by a gene linked to the immunoglobulin heavy chain gene cluster, *Igh*. Tsu^d can be assayed by immunofluorescence staining with an antiserum made in BALB/c mice against C.AL-20 concanavalin A blasts. This antiserum can also be used to induce T suppressor cells in mice expressing Tsu^d. Both

of these assays were used to type several panels of recombinant inbred strains and *Igh* recombinant strains to accurately map the Tsu^d locus. The Tsu^d gene is located very near the heavy chain constant-region genes, *Igh-C*, on the side toward the prealbumin gene, *Pre-1*. Tsu^d is not among the heavy chain variable-region genes, *Igh-V*, and thus is not a variable-region framework allotype, subgroup determinant, or idiotype. The map position suggests that the Tsu^d antigen is a constant-region allotypic determinant on the as yet uncharacterized T cell receptor.

Note added in proof: The variable typing in RI-3 leads to construction of 20 (C58/J × RI-3) F_1 animals which subsequently typed positive for Tsu^d in fluoresence experiments. We conclude that RI-3 is a positive strain with a regulatory defect in expression of the cell surface antigen.

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