MURINE *t* FACTORS: AN ASSOCIATION BETWEEN ALLELES
AT *t* AND AT *H-2**

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The *T/t* system is a complex region of the 17th mouse chromosome which affects embryonic development, sperm function, genetic recombination, and cell surface antigenic structure (1, 2). *t^x* mutations, which are defined by their interaction with the linked mutant, *T* (Brachyury), to produce taillessness, fall into three major categories: homozygous lethal, semiviable, and viable. Originally, it appeared that independently derived homozygous lethal *t* mutations represented alleles at a single genetic locus. However, further study revealed that such lethal mutations occur in six complementing groups, and therefore are probably not allelic (3, 4) (Fig. 1). Crosses between mice which carry *t* mutations of different complementation groups result in offspring which are phenotypically normal except that all males are sterile.

Within the limits of a given complementation group, different *t* mutations can be resolved on three criteria. First, most *t* mutations are associated with a suppression of crossing over in the region of chromosome from Brachyury to the marker tufted, a distance of about seven map units. Different *t* factors can often be shown to differ quantitatively in the degrees to which they suppress crossing over in that region; and so can be separated on that basis. Second, most *t* mutants are associated with a transmission ratio distortion such that males (only) who carry a *t* mutation transmit the *t*-bearing chromosome with non-Mendelian frequencies. Most commonly, the mutant chromosome is transmitted in high proportions, up to 99% for some *t* factors. Because different *t* factors are usually associated with different but consistent degrees of transmission ratio distortion, that degree is a criterion by which to identify separate *t* mutations. Finally, though all *t* factors in a single complementation group are associated with the same gross pattern of mutant morphogenesis seen in the homozygote, it appears that different *t* factors vary in the more subtle characteristics of that pattern.

It has been postulated that the *T/t* system and the linked major histocompatibility complex (*H-2*) together form a "supergene complex"; and that the *T/t* system functions at the population level to maintain heterozygosity at *H-2* (5). This hypothesis depends upon and accounts for both the crossover suppression and the homozygous lethality of *t* mutants. Indirect evidence for a relationship between *H-2* and the *T/t* system has recently been obtained by using serological

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FIG. 1. Genetic map of the 17th mouse chromosome, linkage group IX. *T*, brachyury; *t*, various *t*-factors; *tf*, tufted; *H-2*, major histocompatibility complex; *TLα*, thymus leukemia antigen. Numbers represent map distances between relevant loci (13).

techniques to identify the *H-2* antigens of *t*-mutant mice (6). We have confirmed these results and extended them by using the mixed lymphocyte reaction (MLR) as a probe for *I*-region differences among mice carrying *t*-lethal mutations of the same and of different complementation groups. The *I* region maps between the *K* and *D* ends of the *H-2* complex, and is responsible both for the genetic control of immune responsiveness (7), and for antigens which elicit mixed lymphocyte reactions (8), graft-versus-host reactions (9), and skin graft rejection (10).

These experiments indicate that a given *t* factor is frequently associated with a unique *I* region; and that *t* factors which cannot be differentiated by complementation tend to share the same *I* region. This finding is taken to represent an association of individual *t* factors with certain *H-2* haplotypes. Possible mechanisms for such an association are discussed.

Materials and Methods

Mice. C3H/DiSn and C3H.SW mice were obtained from the breeding colonies of H. O. McDevitt. Balanced lethal *T/t^x* mice on the BT BRTF/Nev background were obtained from D. Bennett and K. Artzt, both from Cornell University Medical College, New York, and M. Lyon, MRC Radiobiology Unit, Harwell, England, and are here used, with two exceptions, in the 4th through the 7th back-cross generations to C3H/DiSn. The exceptions, *t⁶* and *t¹²*, were used as *F₁*'s with C3H/DiSn. For most of the experiments, *t*-mutant mice were progeny tested before use, in order to ascertain that the experimental mice actually carried the *t* factor in question. For the rest of the experiments, controls designed to detect noncarriers were run with the experimental cultures.

MLR Cultures. Unidirectional MLR cultures were set up in Falcon Microtest II plates (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) in 0.2 ml media/well containing 1×10^6 responder and 1×10^6 irradiated (3,000 rad from a ^{137}Cs source) stimulator cells.

Responder lymph node cell and stimulator spleen cell suspensions were prepared in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum, pelleted, and resuspended in a final medium of RPMI-1640 supplemented with 5% human serum, 0.002 mM glutamine, 5×10^{-2} M 2-mercaptoethanol, 100 U penicillin, and 100 μg streptomycin/ml. After 3 days incubation in a 37°C incubator in 95% air-5% CO_2 each well was pulsed with 1 μCi [^3H]thymidine (New England Nuclear, Boston, Mass.; 6.7 Ci/mol) for about 16 h. The cells were then harvested on glass wool fiber filters (Whatman GF/83) using a Mash II (Microbiological Associates Inc., Bethesda, Md.) cell harvester. The radioactivity was measured in a 2,5-diphenyloxazole, 1,4-bis(2-[5-phenyloxazolyl]) benzene and toluene scintillation mixture in a liquid scintillation counter. In each experiment, all assays were set up in triplicate.

Results

Mice carrying 10 different *t* mutations were selected to represent 5 of the 6 *t*-lethal complementation groups and the noncomplementing *t*-semiviable group (Table I). The data shown in Tables II and III form a pattern in which *t*-locus mutants of the same complementation group fail to stimulate in MLR and therefore appear to share the same *H-2* haplotype. Thus, *t¹²* and *t^{w32}*, and *t^{w1}*, *t^{w12}*, and *t^{w71}* comprise two groups, each of which is internally nonstimulatory.

TABLE I
*t^x Chromosomes Used for MLR, Listed According to
 Complementation Group Assignments*

t^{12} ; t^{12} , t^{u32}
t^0 ; t^0 , t^6
t^{u5} ; t^{u5}
t^4 ; t^{u18}
t^{u1} ; t^{u1} , t^{u12} , t^{u71}
Semiviable: t^{u2}

TABLE II
Typical Data from a Single Panel of One MLR Experiment

Combination		cpm* $\pm$ SE	Stimulation index	<i>t</i> test <i>P</i> <
Responder	Stimulator			
t^0	t^6	14,036 $\pm$ 1,200	—	—
t^6	t^0	83,509 $\pm$ 2,355	5.9	0.001
t^6	t^{12}	87,862 $\pm$ 8,219	6.3	0.001
t^6	t^{u1}	9,958 $\pm$ 1,158	0.7	NS†
t^6	t^{u12}	13,521 $\pm$ 1,181	1.0	NS
t^6	t^{u71}	15,683 $\pm$ 333	1.1	NS
t^6	t^{u2}	30,273 $\pm$ 3,581	2.2	0.05
t^6	t^{u5}	91,731 $\pm$ 7,785	6.5	0.001
t^6	t^{u18}	50,998 $\pm$ 3,304	3.6	0.001
t^6	t^{u32}	54,548 $\pm$ 2,725	3.9	0.001
t^6	C3H.SW	195,780 $\pm$ 20,998	13.9	0.001
t^6	C3H/DiSn	7,562 $\pm$ 1,525	0.5	0.05

* Mean counts per minute based on triplicate assays.

† NS, not significant.

TABLE III
Mean Stimulation Indices from Four Experiments

Responder cell panel	Stimulator cell panel										CSW	C3H
	t^0	t^6	t^{12}	t^{u1}	t^{u12}	t^{u71}	t^{u2}	t^{u5}	t^{u18}	t^{u32}		
t^0	1.0	8.6	12.3	8.9	8.0	8.6	<u>0.7*</u>	12.3	7.4	6.7	19.1	0.8
t^6	5.9	1.0	6.3	<u>0.7</u>	<u>1.0</u>	<u>1.1</u>	2.2	6.5	3.6	3.9	13.9	0.5
t^{12}	12.9	16.5	1.0	14.0	11.5	18.9	6.6	13.0	6.0	<u>2.0</u>	27.3	1.4
t^{u1}	6.1	<u>1.2</u>	7.1	1.0	<u>1.2</u>	<u>1.3</u>	3.4	8.9	5.2	5.9	11.9	0.7
t^{u12}	4.3	<u>1.2</u>	3.9	<u>0.9</u>	1.0	<u>1.2</u>	2.6	5.0	3.8	3.4	9.0	0.8
t^{u71}	7.3	<u>0.8</u>	4.5	<u>1.0</u>	<u>1.2</u>	1.0	3.1	8.1	5.8	5.6	10.3	0.7
t^{u2}	<u>1.3</u>	10.4	9.2	7.3	8.6	9.2	1.0	14.2	7.6	9.0	15.3	1.0
t^{u5}	5.9	8.5	7.7	6.3	5.6	5.5	3.7	1.0	6.3	4.8	8.8	0.8
t^{u18}	4.4	2.9	2.5	4.2	3.4	4.0	2.2	6.2	1.0	3.6	11.9	0.7
t^{u32}	9.0	11.2	<u>1.3</u>	9.8	8.5	10.5	4.9	12.2	9.1	1.0	17.0	1.1
CSW†	10.1	11.3	8.7	10.3	9.9	10.4	6.8	10.8	13.0	7.6	1.0	8.5
C3H‡	9.5	12.9	9.5	9.0	7.7	9.3	5.1	7.6	5.7	4.5	15.8	1.0

* Underlined values indicate stimulation indices that do not reflect significant stimulation.

† C3H.SW.

‡ C3H/DiSn.

Regardless of the origin of the mice used, lymphocytes from mice which carry t mutations in the same complementation group do not stimulate each other in MLR. In contrast, lymphocytes from mice carrying t mutations from different complementation groups stimulate each other strongly in MLR.

It should be noted that in the negative control column of Table III, where lymphocytes from C3H/DiSn mice were used as stimulators, the stimulation indices vary from 0.5 to 1.4. In individual experiments, when the negative control was high or low, all other stimulation indices were correspondingly higher or lower. t^{12} lymphocytes generally gave high responses, and so in Table III, though an index of 2.0 is borderline, we feel that it reflects "no stimulation" in the case of t^{12} responding to t^{w32} . The reciprocal combination, in which t^{w32} responds to t^{12} , clearly shows no stimulation, since the stimulation index for the pair is 1.3.

One exception to the general pattern is that lymphocytes from mutant t^6 , a member of the same complementation group as t^0 , stimulate lymphocytes from animals carrying t^0 , but do not stimulate lymphocytes from animals carrying any member of the t^{w1} complementation group. Though this finding is consistent with the serological data of Hammerberg and Klein (6), we have set up genetic experiments to verify the complementation group assignment of our t^6 stock.

Another significant result is that lymphocytes from mice carrying t^{w2} , a semiviable mutation, do not react in either direction in MLR with lymphocytes from mice carrying t^0 , a lethal mutation. This suggests that MLR may be useful as a tool with which to assign t mutations of the viable and semiviable types to complementation groups, thereby aiding in the genetic analysis of the T/t system.

Discussion

In these experiments, the MLR was used as a probe for I -region differences among t -mutant-carrying mice. It is necessary, however, to consider the possibility that these results are not a function of t -factor-associated I -region genes; but are instead, a direct effect of t -region genes. That is, though strong MLR is governed by the I region (and the M locus, which can be dismissed here because of the genetic derivation of the mice), it is possible that the T/t region itself may determine an MLR response. Such T/t system determined MLR is unlikely for a number of reasons. First, both the stimulation indices and the patterns of reactivity with control cell populations were comparable to those observed for MLR across I -region differences. Second, no T/t -associated antigens have yet been demonstrated on lymphocytes (11, 12). Finally, these MLR results agree with serological findings obtained by Hammerberg and Klein (6) and confirmed in this laboratory (P. N. Goodfellow, personal communication). Definitive proof, however, must await appropriate blocking studies with antisera to both Ia and t -mutant-associated antigens.

The most striking observation made in these experiments is that each t factor tested is associated with a distinct $H-2$ haplotype, at least at the level of resolution afforded by the MLR. Furthermore, members of a given complementation group frequently share the same $H-2$ haplotype. In order to examine the implications of this finding, we must first consider a few facts regarding $H-2$ genetics and mouse population structure.

It is currently thought that wild mice live predominantly in small, closed breeding units known as demes (13). It is presumed that there is little interdemic genetic flow, even for populations which are geographically close; and so, given the fact that *H-2* is an extremely polymorphic system, it is not surprising that each new deme studied has been found to carry one or two new and unique *H-2* haplotypes. However, many demes also carry *t* factors; and we have shown here that when these *t* factors are not separable by complementation, they usually carry the same *H-2* allele. For example, the t^{w1} , t^{w12} , and t^{w71} factors, which were originally isolated from wild populations in New York, California, and Denmark, respectively, share a single *H-2* haplotype. This evidence suggests that all the *t* factors of each complementation group derived from the same ancestral chromosome. This in turn suggests that interdemic genetic flow is far more significant than it has been thought to be.

It is possible to account for the preservation of such an ancestral chromosome with either one of two mechanisms. First, and consistent with the evidence that many *t* factors suppress crossing over between *T* and the marker tufted, it could be that *t* mutations induce a physical change in this region of chromosome such that crossing over occurs very rarely or not at all. Alternatively, it could be that some gene-gene interaction exists between the products of the *T/t* and *H-2* systems, such that *t*-mutant chromosomes are not viable with any but one or a very few different *H-2* haplotypes. This mechanism is attractive insofar as it provides for the preservation of the ancestral chromosomal type without necessitating high levels of interdemic migration. That is, perhaps mutations from the wild type to *t*, which have never been observed in the laboratory, do occur, but are lethal unless they happen to occur on a chromosome with a compatible *H-2* haplotype.

If the latter mechanism is correct, it should be possible to detect double crossovers in the region between *T* and *H-2*. If the former mechanism is correct, *t* mutants which do not suppress crossing over to tufted, such as t^{w18} , should eventually pick up the *H-2^k* allele in our backcross. We are currently investigating these possibilities with genetic and immunologic methods.

Summary

Lymphocytes from *t^x*-mutant mice have a definite and consistent pattern of reactivity in MLR. Cells from *t*-mutant mice within a complementation group usually fail to stimulate each other, while cells from mutants in different complementation groups stimulate each other strongly. This indicates that *t^x*-mutant types are associated with certain *H-2* haplotypes, and that members of any given complementation group share the same *H-2* haplotype.

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