Mta, THE MATERNALLY TRANSMITTED ANTIGEN, IS DETERMINED JOINTLY BY THE CHROMOSOMAL *Hmt* AND THE EXTRACHROMOSOMAL *Mtf* GENES.

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Mta,[1] the maternally transmitted antigen of mice, is a transplantation antigen that can be detected by H-2-unrestricted CTL (1, 2). Mta has been found on all types of lymphoid cells and on fibroblasts. Three genes are required for the expression of Mta: *Mtf, Hmt,* and *B2m*. The maternally transmitted factor, *Mtf,* is passed from mother to offspring via the egg (3) and is likely to be a mitochondrial gene (4, 5). The *Hmt* gene has been mapped to the *Tla* region of the MHC on chromosome 17 (6). Since β_2-microglobulin (β2m) is required for expression of Mta (6, 7),[2] the product of the *Hmt* gene is most likely a class I MHC antigen located at the cell membrane.

Until recently, we had identified only a single form of Mta, associated with Mtf^{α} and Hmt^{a}. Mta(α,a) is found in >80 strains of laboratory mice. This antigen was absent in mice with Mtf^{β} or homozygous for Hmt^{b}. Wild mice from many different sources were also mostly Mta(α,a), but two possible variant forms were identified among WLA76 and *spretus* mice (8). Genetic and immunological analysis of WLA76 mice showed that their particular form of Mta is determined by a new allele of the cytoplasmic gene, Mtf^{γ}. This led us to realize that each allele of *Mtf* (α, β, γ, etc.) determines a unique form of Mta.[3, 4]

Analysis of the *spretus* Mta seemed particularly promising. *Mus spretus* is found in Southern France, Spain, Portugal, and North Africa (9). Although living in the same area as *Mus musculus domesticus,* they do not readily interbreed, even

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[1] *Abbreviations used in this paper:* β2m, β_2-microglobulin; Mta, maternally transmitted antigen.

[2] F.-W. Shen, K. Fischer Lindahl, Y. Saga, J.-S. Tung. Genetic constitution and properties of variant leukemia cell lines lacking expression of class I antigens. Manuscript submitted for publication.

[3] K. Fischer Lindahl, H. Winking, J. L. Guénet, F. Bonhomme, U. Gyllensten, E. M. Prager, and A. C. Wilson: Distinct epitopes of the maternally transmitted antigen, Mta, determined by three allelic forms of the cytoplasmic gene *Mtf.* Manuscript in preparation.

[4] K. Fischer Lindahl, H. Yonekawa, and K. Moriwaki. Mta types of Asian mice: new alleles of *Mtf* and *Hmt.* Manuscript in preparation.

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in the laboratory, and they are recognized as distinct species (9–11). A variant form of Mta could be due to a change in any of the genes known to be involved in Mta expression. The mitochondrial genome of *spretus* mice differs by at least 8% from the nucleotide sequence of *domesticus* mitochondrial DNA (12). The β2m of *spretus* mice, determined by the $B2m^{w1}$ allele, is considerably more acidic than the β2m of inbred mice (13, 14). Little is known about the MHC genes of *Mus spretus*.

Materials and Methods

Nomenclature. Alleles of *Mtf* are designated by Greek characters (α, β, γ, etc.) and alleles of *Hmt* by Roman characters (a, b, c, etc.). The Mta antigen of, say, an $Mtf^{\beta}\,Hmt^{a/a}$ cell will be designated Mta(β,a), or (β,a) for short. An $Mtf^{\alpha}\,Hmt^{a/c}$ cell has two Mta antigens, (α,a) and (α,c). The symbol α^{s} will be used to indicate an *Mtf* derived from *Mus spretus*, even though it was indistinguishable in our tests from the α of old inbred strains.

In all crosses, the female is listed first. Thus (B6 × SPE1) × B6 stands for (B6 ♀ × SPE1 ♂) ♀ × B6 ♂. Alleles of heterozygous loci are listed in the order maternal/paternal.

Mice. Standard inbred strains were purchased from the Institut für Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland; Gl. Bomholtgaard, Ry, Denmark; Olac, Ltd., Bicester, United Kingdom; and The Jackson Laboratory, Bar Harbor, ME. NMRI/Han and NMRI/Bom are not inbred strains, but they are uniformly Mtf^{β}, and NMRI/Bom is homozygous for $H\text{-}2^{q}$. B10.CAS2 mice (abbreviated B.C2 in some tables) were bred at the Basel Institute for Immunology from breeding pairs obtained from Dr. J. Klein, Max Planck Institut für Biologie, Tübingen. B6.AK1 breeding pairs were obtained from Dr. L. Flaherty, New York State Department of Health, Albany, NY.

SPE1 is an inbred strain of *Mus spretus*, initiated from wild mice caught near Granada, Spain (F. Bonhomme, personal communication). It was received from the Pasteur Institute, Paris, France at F_{17} and again at F_{21}, and then maintained in Basel by brother-sister mating. *Mus spretus* was established in Lübeck (Lub) by random breeding in a closed colony from wild mice caught near Porto Covo, Portugal (15). Mice carrying the H-2 complex of *spretus* /Lub and Mtf^{β} from NMRI/Han are designated N.SPL. Mice from closed colonies of Spanish (caught near Cadiz) and Moroccan *spretus* (caught near Azrou) were sent to us by Dr. M. Potter, National Institutes of Health, Bethesda, MD, who in turn had obtained these stocks from Dr. R. D. Sage, University of California, Berkeley, CA (16). H-2 complexes from *spretus*/Spain were backcrossed to C57BL/10 to give four lines, SPA1 (Mtf^{α} from B10.CAS2) and N.SP1 (Mtf^{β} from NMRI/Bom) with $H\text{-}2^{sp1}$, and SPA2 (Mtf^{α}) and N.SP2 (Mtf^{β}) with $H\text{-}2^{sp2}$.

Mice are being made congenic for mitochondrial DNA by repeated backcrossing, starting with a female of one strain, to males of another strain (8, 17). Thus, we have C57BL/6 (B6) mice with mitochondrial DNA from strain NMRI/Bom (B6.Mtf^{β}, previously called NMB [2]), WLA76 (B6.Mtf^{γ}), and Moroccan *spretus* (B6.mt^{spr}). The latter strain could be started only from a multiparous *spretus* female, since no viable offspring is obtained when virgin *spretus* mice are mated with laboratory males (11, 17). The mice used in the experiments were from the first up to the 13th generation of backcrossing. Similarly, we have NZB/Bom with mitochondrial DNA of strain A.CA (NZB.Mtf^{α}, previously called ANN [2]) and DBA/2 with mitochondrial DNA of strain WLA76 (D2.Mtf^{γ}). DNM4 mice come from the fifth generation of backcrossing to NMRI/Bom males, starting with a DBA/2 female (8).

Two stocks of wild mice were obtained from Dr. K. Moriwaki, National Institute for Genetics, Mishima, Japan; SUB-SHH is of the Mtf^{δ} type, and BAC1 has the Hmt^{d} allele.[4] C3H.R4 and B6.R9 mice have new recombinant MHC haplotypes derived from crossovers between H-2D (of C3H, and B6, respectively) and *Hmt* (from *M. m. castaneus*); the mice used in the present experiments were in the early stages of backcrossing.

Breeding and Sampling. All breeding was set up with one or two females to a male. To create H-2-congenic strains on the C57BL/10 background, inbred females were crossed

with *spretus* males, but for all subsequent generations, backcross females were mated to inbred males, as F_1 males were always, and in subsequent generations frequently, sterile (11).

At 5–7 wk of age, each mouse was sampled for typing (see below) by surgical removal of the spleen, a thymic lobe, or the cervical lymph nodes. Selected mice were mated when 7–12 wk old.

Mta Typing. All typing for Mta was done in the killer cell assay. Donors of responding cells were immunized with at least three intraperitoneal injections, 3 wk apart, of 15–20 $\times 10^6$ pooled spleen, lymph node, and thymus cells in phosphate-buffered HBSS. Bulk mixed lymphocyte cultures were incubated for 5 d. The killer cells were assayed in triplicate, at three concentrations (30, 10, and 3×10^4) of responder cells cultured, against 10^4 ^{51}Cr-labeled target cells for three and one-half hours. Cold-target competition was tested in duplicate in round-bottom microtiter plates with 60, 30, and 15 unlabeled competitors, and 30 initial responder cells per labeled target cell (8, 18).

Three strains were used as responders against Mta(α,a): B6.*Mtf*$^{\beta}$ was primed and boosted with B6, 129, or DDK cells (all *H-2*^{b}); NZB/Bom or NZB/B1NJ with NZB.*Mtf*$^{\alpha}$, NZB/Ibm, or B10.D2 cells (all *H-2*^{d}); and NMRI/Bom with NMRI/Lac, DNM4, or SWR (all *H-2*^{q}). In the tables, these killer cells are referred to simply by their H-2 types. In competition tests, the labeled Mta(α,a) target cells always differed from the responder at H-2.

The target cells and competitors were spleen (and occasionally lymph node) cells cultured for 2 d with Con A (2 μg/ml). The blast cells were purified by centrifugation over a density cushion of Ficoll-Isopaque or simply washed in later tests.

Results are given as percent specific ^{51}Cr release = (experimental release − spontaneous release) × 100/(maximum release − counter background) (19). Spontaneous release was usually 25–30% of maximum release, and the specific release carries an error of 1–2% in terms of the maximum release. The tables show only the results obtained with 30 initial responders for direct killing and 60 competitors (unless otherwise indicated) for competition tests; results at other cell concentrations were consistent with those shown.

Long-term Lines. The two killer cell lines used were (NMRI/Bom × B6)F_1 anti-B6 and NZB/Bom anti-NZB/Ibm. Both were started from standard bulk mixed lymphocyte cultures that were fed fresh medium on day 7 and restimulated with irradiated (3,000 rad) antigen-bearing spleen cells (1:10 responder/stimulator ratio) on day 14 and day 21. The lines were then maintained by weekly stimulation with antigen in medium supplemented with T cell growth factors (20% supernatant from Con A–stimulated rat spleen cells [20]). They were used 5 d after the last restimulation at 10, 3, and 1, or 3, 1, and 0.3 killers per target cell. The results shown are from the highest concentration tested. The two lines showed the same reactivity pattern on target cells from inbred strains.

Mixed Lymphocyte Cultures. To measure proliferation, mixed lymphocyte cultures were set up in flat-bottom microtiter plates with 5×10^5 lymph node responders and 5×10^5 irradiated (1,750 rad) spleen cell stimulators per well, as described (2). The cultures were labeled overnight with 0.1 μCi [^{3}H]thymidine per well, and harvested on day 4.

Serological Typing. To characterize the *spretus* H-2 haplotypes, we measured the binding of a panel of [^{3}H]leucine-labeled mAb against H-2 and Ia antigens to Con A–stimulated spleen cells (usually $1–2.5 \times 10^6$ per tube) as described (21). To type animals for breeding, their lymph node cells were tested with one or two selected anti-H-2 and one or two anti-Ia antibodies, and thymocytes (5 and 10×10^6 per tube) were incubated with [^{3}H]leucine-labeled mAb against TL, the marker most closely linked to *Hmt*.

Qa-2 typing was done by a two-step complement-dependent cytotoxicity assay, using mAb D3.262, specific for Qa-2 (22), and nylon wool–purified T cells.

Typing for β2m. $1–2 \times 10^7$ Con A–stimulated lymph node or spleen cells were washed and incubated for 30 min at 37°C with 50 μCi [^{35}S]methionine in 200 μl methionine-free RPMI 1640 medium. The cells were lysed, and the β2m was immunoprecipitated and identified by SDS-PAGE or IEF (14).

Results

All *spretus* mice we have assayed for Mta had the same characteristic phenotype (Table I). Anti-Mta(α,a) killers from a 5-d mixed lymphocyte culture always lysed *spretus* targets, though sometimes not as well as standard Mta(α,a) targets. The same *spretus* targets were not lysed by our two killer cell lines specific for Mta(α,a), as shown by the appropriate reactions with positive and negative controls in Table I. Thus *spretus* cells have some crossreacting determinants that are covered by the more heterogeneous repertoire of the CTL from the fresh bulk cultures, but they lack the Mta epitope(s) recognized by the selected receptors of the long-term cultured lines. It is therefore not surprising that *spretus* cells cause no, or exceptionally weak, inhibition of Mta(α,a)-specific killing when used as competitors, even with fresh CTL (Table II).

To define the gene(s) responsible for the *spretus* Mta phenotype, we tested mice from a number of crosses (Table III). As it is conceivable that *spretus* mice have no Mta antigens at all, and that the observed killing was due to a crossreaction with H-2 or other class I antigens, it is important to note that the lysis was retained in (B10.CAS2 × *spretus*)F_1 hybrids, which are Mtf^{α}, but was lost in hybrids of NMRI mothers, which are Mtf^{β} (Tables III and IV).

The fact that (B10.CAS2 × *spretus*)F_1 hybrids display the *spretus* phenotype (Tables I and II) suggests that it is caused by the *Hmt* allele of *spretus*, to be defined as *c*. These hybrids have the common Mtf^{α} and the $B2m^{b}$ allele from C57BL/10 as well as $B2m^{w1}$ from *spretus*, and they have inherited the immunologically silent Hmt^{b} allele together with the *castaneus* H-2 complex (6). When the hybrids were backcrossed to B10.CAS2, the *spretus* phenotype segregated together with the E_{α} gene of the *spretus* H-2 complex, as would be expected of Hmt^{c}, and independently of the *spretus* $B2m^{w1}$ allele (Table III).

F_1 hybrids of *spretus* males with NZB/Bom or NMRI females fully express the

TABLE I

Lysis of Mus spretus Target Cells by Mta(β,a) anti-Mta(α,a) CTL

Exp.	Target cell	H-2	Mta	Percent specific ^{51}Cr release by			
				Fresh CTL		Long-term lines	
				q	b	q × b	d
896	*Spretus*/Lub	?	(α^s,c)	44	37	0	−1
	A.CA	*f*	(α,a)	53	56	37	34
	SUB-SHH	?	(δ,a)	5	7	−3	2
902	SPE1	*sp3*	(α^s,c)	24	30	0	−2
	NZB.Mtf^{α}	*d*	(α,a)	49	47	17	37
	B6.Mtf^{β}	*b*	(β,a)	14	1	0	0
951	*Spretus*/Spain	?	(α^s,c)	47	49	2	—
	Spretus/Morocco	?	(α^s,c)	50	51	0	—
	C3H/HeJ	*k*	(α,a)	57	49	29	—
	B10.CAS2	*w17*	(α,b)	8	3	0	—
	(B10.CAS2 × SPE1)	*w17/sp3*	(α,b/c)	48	46	−1	—

—, not done.

TABLE II

Failure of Spretus Cells to Inhibit Mta(α,a)-specific Lysis

Competitor	Mta	H-2	Percent specific ^{51}Cr release from labeled Mta(α,a) targets in Exp.:					
			888	896	902	906	951	954
None	—	—	49	57	28	52	48	49
Spretus/Lub	(α^s,c)	?	37	51	—	—	—	—
SPE1	(α^s,c)	*sp3*	—	—	21	39	—	—
Spretus/Spain	(α^s,c)	?	—	—	—	—	38	27
Spretus/Morocco	(α^s,c)	?	—	—	—	—	33	26
B6	(α,a)	*b*	0	9	—	—	8	—
A.CA	(α,a)	*f*	4	15	—	11	—	—
C3H/HeJ	(α,a)	*k*	—	—	−3	10	13	—
NZB.*Mtf*$^{\alpha}$	(α,a)	*d*	5	8	1	5	—	3
B6.*Mtf*$^{\beta}$	(β,a)	*b*	—	—	23	45	40	—
NZB/Bom	(β,a)	*d*	42	—	—	—	—	41
NMRI/Bom	(β,a)	*q*	45	—	—	—	41	43
(NMRI × WLA)F_1	(β,a)	*q*/?	—	55	—	—	—	—
B10.CAS2	(α,b)	*w17*	—	—	—	—	42	—
(B10.CAS2 × SPE1)F_1	(α,b/c)	*w17/sp3*	—	—	—	—	34	37

—, not done. Significant inhibition marked by box.

Mta(β,a) antigen, as evident by direct killing (Table IV) and competition (not shown), a result consistent with codominant expression of *Hmt* alleles. Hybrids backcrossed to B10.CAS2 males either expressed a normal Mta(β,a) antigen together with the H-2 of the NMRI/Han grandmother, or they were killed neither by anti-(β,a) nor by anti-(α,a) CTL. Among 18 *Mtf*$^{\beta}$ backcross mice tested, there was one recombinant between the E_α and the *Hmt* loci (unfortunately discovered too late to be tested for other markers on chromosome 17). Together with 12 *Mtf*$^{\alpha}$ backcross mice studied, this gives a recombination frequency of 1 in 30, consistent with previous mapping of *Hmt* 2.0 ± 1.2 cM distal to H-2D (6). Again, the *B2m*^{w1} allele had no effect on the Mta phenotype.

The maternally transmitted factor of *Mus spretus* was tested separately in mice from the fourth to the eighth generation of backcrossing the descendants of a Moroccan *spretus* female to C57BL/6 males. These mice have retained the *spretus* mitochondrial DNA (17), but they were non-agouti, suggesting loss of *B2m*^{w1}, which is linked to the A^w allele (14), and they were negative for serological markers associated with *spretus* H-2. The mice expressed an Mta(α,a) indistinguishable, within the limits of the assays, from that of standard C57BL/6 both in direct killing and in competition (Table V). Analysis of the very donors for these tests confirmed that they carried *spretus* mitochondrial DNA (M. Hirama and M. Phillips, personal communication). Thus, *spretus* mice seem to have the common *Mtf*$^{\alpha}$ allele carried by B6, despite the marked (8–17%) nucleotide sequence divergence from B6 mitochondrial DNA (12).

If *spretus* mice have a new allele of *Hmt*, it should be possible to raise killers specific for the Mta determined by this allele, using the King Lear scheme of immunization. We bred F_1 hybrids from B6.*Mtf*$^{\beta}$ females and males of the inbred

TABLE III

Phenotype of Spretus Progeny Tested with CTL Specific for Mta(α,a) and Mta(β,a)

Cross (♀ × ♂)	Genotype				Number tested (*n*)	Mta phenotype
	Mtf	*Hmt**	H-2	*B2m*		
B10.CAS2 × *Spretus*/Spain	α	*b/c*	*w17/sp1* *w17/sp2*	*b/w1*	4	Spretus
B10.CAS2 × SPE1	α	*b/c*	*w17/sp3*	*b/w1*	6	Spretus
(B10.CAS2 × SPE1) × (B10.CAS2)	α	*c/b*	*sp3/w17*	*w1/b*	1	Spretus
	α	*c/b*	*sp3/w17*	*b/b*	1	Spretus
	α	*b/b*	*w17/w17*	*w1/b*	9	Blank
	α	*b/b*	*w17/w17*	*b/b*	1	Blank
NZB/Bom × SPE1	β	*a/c*	*d/sp3*	*a/w1*	3	NZB‡
NMRI/Bom × *Spretus*/Spain	β	*a/c*	*q/sp1* *q/sp2*	*a/w1*	3	NZB
NMRI/Han × *Spretus*/Lub	β	*a/c*	*?/sp4*	*a/w1*	3	NZB
(NMRI/Han × *Spretus*/Lub) × B10.CAS2	β	*c/b*	*sp4/w17*	*w1/b*	5	Blank
	β	*c/b*	*sp4/w17*	*a/b*	3	Blank
	β	*c§/b*	*?/w17*	*w1/b*	1	Blank
	β	*a/b*	*?/w17*	*w1/b*	4	NZB
	β	*a/b*	*?/w17*	*a/b*	5	NZB

* *Hmt* genotype inferred from E_α phenotype.

‡ Like cell from of NZB mice, these cells are killed (see Table IV) and completely inhibit lysis of labeled NZB target cells by anti-Mta(β,a) CTL.[2]

§ This mouse is presumed to be a recombinant between E_α and *Hmt*.

SPE1 strain. Such F_1 daughters were then immunized with cells from the father and other male SPE1 donors; the *H-2*^{b} haplotype allowed the F_1 to respond to the male antigen, H-Y, as a helper determinant (23). Immune F_1 cells were then restimulated in vitro with SPE1 female cells, which should differ from the Mta(β,a/c) F_1 only by the Mta(α,c) antigen, all other SPE1 histocompatibility antigens being codominantly expressed in the F_1. Table VI shows that CTL raised in this manner kill all (α,c) target cells independently of H-2; they fail to react with (α,b), (β,c), or (δ,a) target cells, but react weakly with (α,a), (γ,a), and (α,d) target cells. The lysis of SPE1 target cells can be efficiently inhibited only by (α,c) competitors, and it does not matter whether the *Mtf* of these is derived from *spretus* or laboratory mice (Table VII). There is an indication that *Mta*(α,c) is expressed less efficiently by *Hmt*$^{a/c}$ cells [which also express Mta(α,a)] than by *Hmt*$^{b/c}$ cells (which are not known to express a second form of Mta).

The F_1 anti-SPE1 killers are not restricted by an H-2K or -D molecule. They reacted equally well with all (α,c) targets, irrespective of whether their H-2 complex came from SPE1 or from *spretus*/Spain (mice with H-2 from *spretus*/Lub were not available for testing at the time of the experiments). Mice with the four *spretus* H-2 haplotypes that we are now backcrossing onto a C57BL/10 background (with either *Mtf*$^\alpha$ or *Mtf*$^\beta$) all differ serologically (Table VIII) and

TABLE IV

Effect of Spretus Hmt^c on Lysis by Anti-Mta(β,a) CTL

Target cells		H-2*	Mta*	*B2m*	Percent specific ^{51}Cr released by CTL‡ (α,a) Anti-(β,a)	(γ,a) Anti-(β,a)	(β,a) Anti-(α,a)
NMRI/Bom		*q*	(β,a)	*a*	46	55	4
DNM4		*q/(d?)*	(α,a)	*a*	6	8	44
B10.CAS2		*w17*	(α,b)	*b*	5	6§	5
(B10.CAS2 × *Spretus*/Spain)F$_1$		*w17/sp1/2*	(α,b/c)	*b/w1*	−8	−6	19
Spretus/Spain							
(NMRI/Bom × *Spretus*/Spain)F$_1$		*sp1/2*	(α^s,c)	*w1*	6	9	25
		q/sp1/2	(β,a/c)	*a/w1*	44	50	1
(NMRI/Han × *Spretus*/Lub) ×	1	*?/w17*	(β,a/b)	*a/b*	28	34	−3
B10.CAS2	2	*?/w17*	(β,a/b)	*w1/b*	32	39	−2
	3	*?/w17*	(β,a/b)	*w1/b*	32	34	−3
	4	*sp4/w17*	(β,c/b)	*a/b*	6	0	−2
	5	*sp4/w17*	(β,c/b)	*w1/b*	10	1	−2
	6	*sp4/w17*	(β,c/b)	*w1/b*	8	−1	−4

* Backcross mice were typed for E_α, and H-2 and *Hmt* types were inferred from the results. *Spretus*/Spain may have been *H-2^{sp1}* or *H-2^{sp2}*.

‡ α Anti-β, B6 anti-B6.*Mtf$^\beta$*; γ anti-β, B6.*Mtf$^\gamma$* anti-B6.*Mtf$^\beta$*; and β anti-α, B6.*Mtf$^\beta$* anti-B6.

§ Effector/target ratio of 10:1, all others 30:1.

TABLE V

Comparison of Mtf of Mus spretus with Mtf$^\alpha$ of B6 Mice

Competitor	*Mtf*	*Hmt*	H-2	Percent specific ^{51}Cr release from labeled Mta(α,a) target cells in Exp.: 998	1002	1004		1006		1137
				b*	b	b	d	d	b	d
None	—	—	—	31	45	46	60	55	38	35
B6	α	*a*	*b*	−3	0	2	20	16	5	3
C3H/HeJ or AKR	α	*a*	*k*	—	—	3	29	16	3	−4
B6.mt^{spr}	α^s	*a*	*b*	0	13	12	27	21	6	8
SPE1	α^s	*c*	*sp3*	10	—	25	54	—	24	26
B10.CAS2 × SPE1	α	*b/c*	*w17/sp3*	14	30	32	63	—	—	26
B6.*Mtf$^\beta$*	β	*a*	*b*	—	34	—	—	48	24	21
NMRI × *Spretus*	β	*a/c*	*q/sp*	16	—	36	60	46	27	21

—, not done. Significant inhibition marked by box.

* H-2 type of Mta(β,a) anti-Mta(α,a) CTL.

all stimulate each other in mixed lymphocyte cultures (Table IX). SPE1 is negative for Qa-2, and it expresses the *a* allele of *Qa-1* as measured with specific killers in a competition test (18, 24). All four *spretus* haplotypes bind the anti-TL mAb, 18/20, at a level comparable to BALB/c (21).

TABLE VI
Specificity of Killing by (B6.Mtf$^{\beta}$ × SPE1)F$_1$ Anti-SPE1 CTL

Target	*Mtf*	*Hmt*	H-2	Percent specific ^{51}Cr release in Exp.:				
				1102	1122	1129	1137	1138
SPE1	α^s	*c*	*sp3*	46	55	51	41	53
(B.C2 × SPE) × B.C2	α	*c/b*	*sp3/w17*	60	—	—	—	—
(B.C2 × SPA1)F$_1$	α	*b/c*	*w17/sp1*	—	—	—	45	23
(B.C2 × SPA2)F$_1$	α	*b/c*	*w17/sp2*	—	47	46	—	—
(N.SP2 × B.C2) × B.C2	β	*c/b*	*sp2/w17*	—	—	—	—	−2
B6.mt^{spr}	α^s	*a*	*b*	—	25	29	18	—
B6	α	*a*	*b*	46	—	—	21	—
CBA or C3H	α	*a*	*k*	—	26	12	14	—
B6.*Mtf*$^{\gamma}$	γ	*a*	*b*	—	18	—	—	—
D2.*Mtf*$^{\gamma}$	γ	*a*	*d*	43	—	—	—	—
B.C2 × (B.C2 × BAC1)	α	*b/d*	*w17/?*	—	—	20	—	—
B10.CAS2	α	*b*	*w17*	9	—	—	—	—
C3H.R4	α	*b*	*k*	—	—	5	0	—
B6.R9	α	*b*	*b*	—	18	—	1	—
B6.*Mtf*$^{\beta}$	β	*a*	*b*	3	—	—	0	—
NMRI/Bom	β	*a*	*q*	—	6	3	−1	—
(SUB-SHH × B.C2)F$_1$	δ	*a/b*	*?/w17*	—	—	−8	0	—

—, not done

Discussion

Mus spretus has a unique Mta phenotype that crossreacts with the standard Mta(α,a) antigen, and *spretus* mice do not carry the common *Hmt*^{a} allele. A gene closely linked to the H-2 complex and associated with at least four different *spretus* H-2 haplotypes is responsible for the *spretus* phenotype. We define it as a new allele, *c*, of *Hmt*.

CTL usually recognize the outer two domains of class I antigens (25–28), and killers specific for allogenic H-2 antigens are not affected by allelic differences of β2m (29). Thus, it is not surprising that the *B2m*^{w1} allele of *spretus* mice had no effect on the Mta phenotype. The surprise was that the *Mtf* allele of *spretus* proved indistinguishable from the *Mtf*$^{\alpha}$ of old inbred strains.

Since *Mtf* is associated with mitochondrial DNA both in population studies[3] (4) and in somatic cell hybrids (5, 30), *Mtf* is presumed to be a mitochondrial gene. In view of the estimated 8–17% nucleotide sequence divergence between the mitochondrial DNA of *spretus* and laboratory mice (12), some antigenic difference was to be expected, but none was found. We have tried to raise killers directly in B6.mt^{spr} mice against standard B6, of which they should be tolerant apart from determinants encoded in mitochondrial DNA or other maternally inherited genes. A response could actually be obtained, but the specificity has remained variable and obscure.

The maternally transmitted factor, *Mtf*, contributes to the epitopes of Mta, and four different forms have been defined to date.[3, 4] If *Hmt* only determined the amount of Mta expressed (*a*, high; *b*, none; $c \leq d$, intermediate), then killers raised in Mta(β,a/c) mice against Mta(α,c/c) reacting with the homologous target should be completely inhibited by Mta(α,a/a) competitors, as these expressed

TABLE VII
Cold-target Inhibition of Lysis of Labeled SPE1 Targets by (B6.Mtf$^{\beta}$ × SPE1)F$_1$ Anti-SPE1 CTL

Competitor*	*Mtf*	*Hmt*	H-2	Percent specific ^{51}Cr release in Exp.‡			
				1122	1129	1137	1138
None	—	—	—	54	58	44	50
SPE1	α*	*c*	*sp3*	12	15	5	9
(B.C2 × SPA1)F$_1$	α	*b/c*	*w17/sp1*	—	—	4	6
(B.C2 × SPA2)F$_1$	α	*b/c*	*w17/sp2*	8	13	2	—
(B.C2 × SPE) × B.C2	α	*c/b*	*sp3/w17*	—	9	2	—
(B.C2 × SPA1) × B10.HTG	α	*c/a*	*sp1/g*	—	—	—	17
([B.C2 × SPA1] × B.HTG) × B10	α	*c/a*	*sp1/b*	—	31	—	—
N.SP1 × B10	β	*c/a*	*sp1/b*	—	62	39	46
N.SP2 × B.C2	β	*c/b*	*sp2/w17*	51	60	36	46
B6.mt^{spr}	α*	*a*	*b*	45	53	—	—
B6	α	*a*	*b*	46	54	37	—
NMRI/Lac	α	*a*	*q*	46	—	31	42
B6.*Mtf*$^{\beta}$	β	*a*	*b*	—	52	35	—
NMRI/Bom	β	*a*	*q*	50	—	39	—
B6.*Mtf*$^{\gamma}$	γ	*a*	*b*	51	—	36	—
B.C2 × (B.C2 × BAC1)	α	*b/d*	*w17/?*	—	56	—	—
B10 × (B.C2 × [B.C2 × BAC1])	α	*a/d*	*w17/?*	—	—	—	41
B10.CAS2	α	*b*	*w17*	—	—	37	—
B6.R9	α	*b*	*b*	48	54	36	—
(SUB-SHH × B.C2)F$_1$	δ	*a/b*	*?/w17*	—	49	39	—

* Competitor/target ratio was 30:1 in Exp. 1137, and 60:1 in all others.
‡ — not done. Significant inhibition marked by box.

more of the same antigen. Instead, such killers showed strong preference for Mta(α,c) targets. A ready interpretation of this result is that *Hmt* also contributes to the specificity of Mta, which is therefore determined jointly by *Mtf* and *Hmt*.

Without an appropriate recombinant, we cannot formally exclude the possibility that the killers specific for Mta(α,c) recognize an H-2K or -D molecule shared by all the *spretus* haplotypes. However, this seems unlikely for several reasons. The four haplotypes we have studied clearly differ by at least one H-2 and one Ia molecule, as shown by the strong mixed lymphocyte responses (Table IX), the serological differences (Table VIII), and the inability to crosscompete for anti-H-2 killer cells (data not shown). Furthermore, the *spretus* Mta, like Mta(α,a), is easily detected on thymocytes, which have very little H-2.

The requirement for β2m as well as the location of *Hmt* in the *Tla* region suggest that *Hmt* encodes a class I MHC antigen; this antigen then interacts with the product of the *Mtf* gene to create Mta. Thus, Mta would structurally resemble the target antigens created by interaction of viral or minor histocompatibility antigens and H-2. Even though this would be the first example in the mouse of a class I antigen other than H-2K, -D, or -L forming a self-plus-X determinant, such a case has been described in the rat by Livingstone and her colleagues (cited in 31). There, X is a minor histocompatibility antigen showing Mendelian

TABLE VIII

Preliminary Serological Characterization of Four Mus spretus H-2 Haplotypes Under Inbreeding

mAb	Reference	Immunizing specificity	*Spretus* line (H-2)			
			SPA1 (*sp1*)	SPA2 (*sp2*)	SPE1 (*sp3*)	SPL (*sp4*)
10-3.6	32	I-A^k	−*	+	+	+
H8.15.9	33	I-A^k	+	−	+	−
14-4-4	34	E^k_α	+	+	+	+
B8-24-3	35	K^b	NT	NT	−	−
H141-30	36	D^b	NT	NT	−	−
B??	—‡	?	NT	NT	−	−
31-3-4	37	K^d	−	−	−	−
34-2-12	37	D^d	−	−	−	−
34-4-21	37	D^d	−	−	−	+
34-7-23	37	D^d	+	+	+	NT
28-14-8	38	L^d	−	NT	−	−
H100-5/28	36	K^k	+	−	+	−
H100-27/55	36	K^k	±	−	+	−
H100-30/23	36	K^k	±	NT	−	NT
H116-22/7	36	K^k	+	NT	+	−
H142-45	36	K^k	−	−	−	±
3-83	34	K^k	±	−	−	±
12-2-2	34	K^k	±	±	+	±
15-1-5	34	K^k	+	−	+	+
15-3-1	34	K^k	−	−	−	−
15-5-5	34	K^k	−	−	−	−
16-1-2	34	K^k	±	−	±	−
D3.262	22	Qa-2	NT	NT	−	NT
18/20	36	Tla^a	+	+	+	+

* NT, not tested; −, negative, ±, weakly positive, +, positive.

‡ Hybridoma of unknown origin, received under a false name, which secretes IgM specific for H-2D^b and H-2D^q.

TABLE IX

Mixed Lymphocyte Culture Responses among Four Spretus Haplotypes

Responder cells	H-2	[^{3}H]Thymidine uptake (median cpm × 10^{-3}) with stimulator cells from:					
		B10.CAS2	SPE1	(B.C2 × SPA1)F_1	(B.C2 × SPA2)F_1	N.SPL × B.C2	B6.AK1
B10.CAS2	*w17*	2.8	17.3	15.7	16.6	14.5	14.6
(B10.CAS2 × SPE1)F_1	*w17/sp3*	2.3	2.4	14.6	12.4	9.0	10.5
(B10.CAS2 × SPA1)F_1	*w17/sp1*	1.7	21.6	2.0	26.4	22.5	9.0
(B10.CAS2 × SPA2)F_1	*w17/sp2*	1.9	16.7	18.0	1.5	17.8	14.9
N.SPL × B10.CAS2	*sp4/w17*	1.8	8.0	11.9	12.0	2.6	7.8
B6.AK1	*ak1*	8.8	16.0	19.9	11.0	11.9	2.5

Responses to H-2-compatible cells (negative controls) are underscored.

inheritance, and the restricting element has been mapped to the *RT1.C* region. The formation of such complexes between class I MHC antigens of the *Qa/Tla* regions and other cellular proteins may be a general phenomenon that has escaped detection for lack of polymorphism of the molecules involved.

Summary

Mus spretus from four stocks, originating in Spain, Portugal, and Morocco, were tested for the maternally transmitted antigen, Mta. All expressed a variant form not found in other species of mice. Analysis of appropriate crosses with inbred mice showed that the *spretus* form of Mta is determined by a new allele, *c*, of the *Hmt* gene. The Hmt^c allele has been isolated in coupling with four different H-2 haplotypes. It is possible to raise CTL specific for the *spretus* form of Mta. The maternally transmitted factor, Mtf^{as}, of *spretus* mice determines, in conjunction with the Hmt^a allele of C57BL/6, an Mta that is indistinguishable from the common form found in C57BL/6 and most other inbred mice. Our experiments show that the specificity of the cell surface antigen Mta is governed jointly by the cytoplasmic gene *Mtf* and the chromosomal gene *Hmt*. We propose that *Hmt* encodes a class I histocompatibility antigen that acts as a restricting element for the *Mtf* gene product, thus meeting the requirements of T killer cell recognition.

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