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Genetics

Mta, a maternally inherited cell surface antigen of the mouse, is transmitted in the egg

(embryo transfer/cytotoxic T lymphocytes/bone marrow chimeras)

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ABSTRACT **Mta is a maternally transmitted cell surface antigen found in most mouse strains. It serves as a target for unrestricted T killer cells. All 49 embryos transferred between positive and negative mothers, even as early as 10–15 hr after fertilization, developed into mice with the Mta type of their original mothers. Therefore the genetic element that determines expression of Mta must be transmitted by the egg. Mta$^+$ and Mta$^-$ lymphocytes, coexisting for months in lethally irradiated F_1 mice reconstituted with a mixture of parental bone marrow cells, retained their Mta type. Thus Mta does not spread by infection.**

Most strains of mice carry cell surface antigen Mta, which can be detected by unrestricted cytotoxic T lymphocytes. Such killer cells can be raised in one of the few Mta$^-$ strains, NZB, NZO, or NMRI. Expression of the antigen is maternally transmitted: Mta$^+$ females bear only positive offspring and Mta$^-$ females bear only negative offspring, irrespective of the Mta type of the male (1). Because all foster-nursed mice had the Mta type of their original mothers, the Mta character is determined before birth and not transmitted via the milk (unpublished data). Here we examine whether the factor responsible for Mta expression is present already in the egg and whether it can spread by infection.

MATERIALS AND METHODS

Mice. Adult mice of both sexes were obtained from the Institut für Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland (Füll), from Gl. Bomholtgaard, Ry, Denmark (Bom), or bred at the Laboratory of Cell Differentiation, University of Geneva. All crosses were made in our animal facilities. The NMRI/Bom mice were all H-2^q, Mta$^-$, and albino, although they are not inbred and segregate for agouti. NZB/Füll were bred from NZB/BlPt and, like these, differ from other NZB substrains in being Mta$^+$ (1). CBA stands for CBA/H-T6J and B6 for C57BL/6J. The parental types of crosses are indicated as (♀ × ♂)F_1.

Embryo Transfers. Fertilized eggs were recovered about 10–15 hr after mating, by puncturing the ampullae of dissected oviducts in Whitten's medium (2) containing hyaluronidase at 0.2 mg/ml. After dissociation of the cumulus cells, the eggs were washed twice and stored in Whitten's medium under an atmosphere of 5% CO_2/5% O_2/90% N_2 (vol/vol). Two-cell embryos were recovered on day 2 of pregnancy (day of mating = day 1) by flushing the oviduct with Whitten's medium. Recipient females were anesthetized about 12–17 hr after mating by an intraperitoneal injection of 0.25 ml of tribromoethanol (25 mg/ml in Hanks' balanced salt solution) and their oviducts were exposed by a dorsal incision. Groups of about four one-cell or two-cell embryos were transferred into the ampulla of each oviduct by means of a fine, mouth-controlled glass capillary. Blastocysts were recovered on day 4 of pregnancy by flushing the uteri with Whitten's medium. Groups of about seven blastocysts were injected into each uterine horn of recipients on day three of pseudopregnancy. Females were caged individually after their operations. When the litters were very large, mice were later picked randomly for testing.

Bone Marrow Chimeras. Recipient F_1 mice (see Table 1) 9–14 months old were starved for 24 hr and then given 880 rads (1 rad = 0.01 gray) total body irradiation. Bone marrow was prepared from 2- to 10-month-old female donors by flushing the femurs and tibiae with Hanks' balanced salt solution plus 2% fetal calf serum. The cells were then suspended by pipetting and washed. T lymphocytes were eliminated by monoclonal anti-Thy-1.2 antibodies. The cells (25×10^6 per ml) were first incubated for 30 min on ice in a 1:1,000 dilution of F7D5 ascitic fluid (6) and then for 60 min at 37°C with an equal volume of selected rabbit complement diluted 1:10. Surviving cells were washed once, counted, mixed, and injected intravenously. The mice were kept in an isolator and given acidified water supplemented with antibiotics for the whole course of the experiment.

Target Cells from Chimeras. Suspensions of pooled lymph node and spleen cells were prepared from individual mice. Half of each suspension (25×10^6 cells per ml) was incubated 30 min on ice in a 1:100 dilution of ascites fluid containing antibodies against the H-2 antigens of one of the parental strains (see Table 1), followed by incubation for 45 min at 37°C with a 1:5 dilution of rabbit complement. The washed cells were then stimulated with concanavalin A (Con A) for 2 days and used as competitors in the cytotoxicity assay (7). A sample of Con A-stimulated blasts was tested with radiolabeled monoclonal anti-H-2 antibodies (different from those used above) for the presence of the eliminated type. The results were in all cases consistent with those obtained in the killer assay.

Killer Assay. Cytotoxic T cells were stimulated in mixed lymphocyte cultures as described (7), except that the cultures contained only 10% (vol/vol) fetal calf serum. H-2 specific killers were generated from nonimmune donors in the combinations indicated. Killers specific for Mta were obtained from Mta$^-$ donors immunized thrice, at least 3 weeks apart, with 2×10^7 Mta$^+$ cells (pooled spleen, lymph node, and thymus in serum-free medium). The Mta-specific killers were assayed on target cells of a different H-2 type to obviate H-2-restricted killing (1). Each experiment included specificity controls. The target cells were ^{51}Cr-labeled Con A-stimulated blasts of known H-2 and Mta type (7). Unlabeled Con A-stimulated blasts prepared in the same way from mice to be typed were then added in excess (8); if they shared the relevant antigen of the labeled target cells, they would compete for the killers and inhibit the release of ^{51}Cr.

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Abbreviations: B6, C57BL/6; CBA, CBA/H-T6J; Con A, concanavalin A.

Table 1. Characteristics of bone marrow chimeras

Irradiated host: Mice	No. injected	No. tested	Bone marrow donors	Cells $\times 10^{-6}$ per mouse	Anti-H-2 monoclonal antibodies	Ref.	% recovery of treated cells, median and range
(A.CA × NZB/Bom)F_1	5 ♀ + 5 ♂	10	A.CA (H-2^f, Mta$^+$)	8.7	H116-22 + H141-11	3	52 (10–103)
			+	+			
			NZB/Bom (H-2^d, Mta$^-$)	9.0	N2.11.6	4	55 (16–120)
(NZB/Bom × SJL)F_1	7 ♀ + 6 ♂	9	NZB/Bom (H-2^d, Mta$^-$)	7.7	15-5-5	5	68 (51–82)
			+	+			
			SJL (H-2^s, Mta$^+$)	12.0	H100-30/3 + 3-83	3, 5	38 (13–60)
(NMRI/Bom × B6)F_1	5 ♀ + 10 ♂	11	NMRI/Bom (H-2^q, Mta$^-$)	11.6	12-2-2	5	11 (5–20)
			+	+			
			B6 (H-2^b, Mta$^+$)	10.1	H141-29 + H141-31	3	53 (39–74)

RESULTS

Embryo Transfers. Because it cannot be changed by foster nursing, the Mta type of a mouse must be determined before birth. It could therefore either be transmitted in the egg or acquired from the mother during embryonic development. To distinguish between these alternatives we transferred embryos into Mta$^+$ BALB/c and Mta$^-$ NMRI females. The fully grown litters were then typed for Mta and for H-2, as an expression of their genotype.

In the first series the embryos were transferred into pseudopregnant recipients. The donor females were mated with noninbred ICR/Swiss males (Table 2). We have typed three ICR males and four females: all were Mta$^+$, two were *H-2^q* (most likely heterozygous), and none was *H-2^d* or *H-2^k*. The (NZB × ICR)F_1 embryos were obtained at the blastocyst stage. All 12 mice carried by NMRI females were Mta$^-$ as expected, because both NZB and NMRI are negative. The 13 mice transferred to Mta$^+$ BALB/c mothers were also all negative for Mta. (B6 × ICR)F_1 embryos were taken from their Mta$^+$ mothers at the two-cell stage, within 35 hr after fertilization. The eight mice carried by Mta$^-$ NMRI recipient females were positive for Mta and indistinguishable from three mice borne by Mta$^+$ BALB/c recipients. All mice had the expected H-2 type.

These tests were performed between 7 and 32 weeks after the transfers. Several of the (NZB × ICR)F_1 females were pregnant by that time. They were allowed to bear their litters, and we typed a few of the offspring of mice that had been carried by Mta$^+$ BALB/c females. They were uniformly Mta$^-$, like their mothers.

These transfers showed that the Mta type of the offspring was determined before the embryo was implanted in the uterus, confirming and extending our earlier conclusion from foster-nursing experiments that contact after birth had no effect on Mta expression. In a second set of experiments we transferred early embryos, at the one-cell or two-cell stage, into already pregnant females to see whether embryos of different Mta types developing in the same uterus might affect each other. NMRI and BALB/c females were mated with either albino ICR or agouti CBA males in all four combinations, yielding mixed white and agouti litters (Table 3). Con A-stimulated blasts prepared from the spleens of the adult offspring were then typed for Mta and H-2 by unlabeled target competition.

Table 2. Mta types of mice transferred as embryos into pseudopregnant females

Transfer type	Donor ♀ (× mate)	Recipient ♀ (× mate)	Developmental stage of embryo	Litters, ♀ + ♂	Mta type, −/+
− → −	NZB/Bom	NMRI/Bom	Blastocyst	0 + 4	4/0
	(× ICR ♂)	(× ICR*)	Blastocyst	4 + 4	8/0
− → +	NZB/Bom	BALB/c	Blastocyst	3 + 5	8/0
	(× ICR ♂)	(× ICR*)	Blastocyst	2 + 3	5/0
+ → −	B6	NMRI/Bom	Two-cell	1 + 4	0/5
	(× ICR ♂)	(× ICR*)	Two-cell	1 + 2	0/3
+ → +	B6	BALB/c	Two-cell	2 + 1	0/3
	(× ICR ♂)	(× ICR*)			

* Vasectomized male.

Fig. 1 shows a test on all four kinds of mixed litters and their mothers. The experiment, otherwise typical, was chosen because it included the only mouse that appeared to have been affected by the embryo transfer (in the litter of ♀ no. 12, marked with a △). The mouse was an albino, expected to be an Mta$^-$ (NMRI × ICR)F_1, positive for H-2^q, and negative for both H-2^d and H-2^k, as were its white littermates. Yet cells from this mouse inhibited killing as if they expressed a double dose of H-2^q, a single dose of H-2^d, and possibly some H-2^k, as well as some Mta. It is conceivable that the mouse was a chimera with a low percentage of Mta$^+$ cells acquired from the carrying mother (which would account also for the H-2^d reaction) or from an agouti littermate (which would account for both the H-2^d and H-2^k). However, the records from the experiment show an abnormally low recovery of Con A-stimulated blasts from that mouse, suggesting a counting error that may have led to the cells being tested at more than twice the normal concentration. This would account for the observed inhibition by nonspecific effects.

The other eight females transferred as embryos had the H-2 type expected from their coat color. The Mta type matched their genotype in every case and differed from that of their differently colored littermates. The five females of the recipient litters all had the same Mta types as their mothers, and those remained unchanged after bearing and rearing a litter of another Mta type.

Many of the embryos in this series were transferred as fertilized eggs, yet they still expressed the Mta type of their genuine mother. We therefore conclude that the genetic factor that determines expression of Mta is transmitted in the egg and can become part of every cell of the adult mouse by cell division alone.

Irradiation Chimeras. To find out whether Mta could also spread by infection, we analyzed chimeric mice. F_1 hybrids between Mta$^+$ and Mta$^-$ strains that differed for *H-2* were irradiated and reconstituted with a mixture of parental bone marrow cells. After 2.5–8 months we separated the lymphocytes on the basis of their H-2 antigens and tested them for Mta expression. Because they carried the H-2 antigens of both parents, any surviving F_1 cells would have been eliminated.

Fig. 2 shows a test of (NMRI × B6) chimeras 8 months after

Table 3. Mta types of mixed litters from embryo transfers into pregnant females

Transfer type	Donor ♀ (× mate)	Recipient ♀ (× mate)	♀ no.	Developmental stage of embryo	Donor offspring: Tested, ♀ + ♂	Donor offspring: (Total)	Donor offspring: Mta type, −/+	Recipient offspring: Tested, ♀ + ♂	Recipient offspring: (Total)	Recipient offspring: Mta type, −/+
− → +	NMRI/Bom	BALB/c	1	Fertilized egg	2 + 0		2/0	2 + 1	(8)	0/3
	(× ICR ♂)	(× CBA ♂)	12	Fertilized egg*	3 + 0		2/(1)†	1 + 2	(10)	0/3
			18	Two-cell‡	1 + 2	(6)	3/0	1 + 0		0/1
− → +	NMRI/Bom	BALB/c	4	Fertilized egg	1 + 0		1/0	1 + 1	(3)	0/2
	(× CBA ♂)	(× ICR ♂)	14	Fertilized egg + two-cell	0 + 1		1/0	1 + 1	(5)	0/2
			21	Two-cell	2 + 3	(6)	5/0	1 + 2	(5)	0/3
+ → −	BALB/c	NMRI/Bom	3	Fertilized egg + two-cell	1 + 1		0/2	2 + 1	(11)	3/0
	(× ICR ♂)	(× CBA ♂)	17	Fertilized egg	1 + 2		0/3	1 + 2	(9)	3/0
+ → −	BALB/c	NMRI/Bom	7	Fertilized egg + two-cell	1 + 4		0/5	2 + 3	(11)	5/0
	(× CBA ♂)	(× ICR ♂)	8	Fertilized egg + two-cell	2 + 1		0/3	2 + 1	(6)	3/0

* Thirty fertilized eggs and 1 two-cell embryo.
† Exceptional mouse, illustrated in Fig. 1 and discussed in text.
‡ Twenty-two two-cell embryos and 1 four-cell.

reconstitution. The two populations of cells from each chimera were compared with graded mixtures of control cells for their ability to inhibit killing specific for Mta, H-2^b, and H-2^q. Assuming all-or-none expression, we could then estimate the percentage of cells that carried the antigen in question. Cells from each of the three chimeras selected for presentation could be separated into a subpopulation of H-2^b, Mta$^+$ (contaminated by a few H-2^q, Mta$^-$ cells) and a much larger subpopulation of H-2^q, Mta$^-$ cells. Even though we used an equal mixture of parental bone marrow cells, the majority of lymphocytes recovered from this type of chimera was of NMRI origin by 4 months (Table 1). Of 11 mice, 4 did not yield sufficient cells after treatment with anti-H-2^q plus complement to allow testing, and we failed to detect any H-2^b or Mta$^+$ cells in another 2 mice. In none of the 11 mice did we find Mta$^+$ cells that did not also express H-2^b, and vice versa (Table 4).

Two further sets of chimeras are included in Table 4. The Mta$^-$ bone marrow in these was from NZB/Bom, and the populations treated with anti-H-2^d plus complement were contaminated with H-2^d cells, because neither of our antibodies could lyse these cells efficiently. SJL lymphocytes attained a slight majority in the chimeras prepared in Mta$^-$ (NZB × SJL)F_1 hosts, and each of the nine mice had a sizeable H-2^s, Mta$^+$ subpopulation. By treatment with anti-H-2^s plus complement, we could also separate a cell population from each mouse with distinctly less or no Mta, the remaining H-2^s cells accounting for the low levels of Mta. The NZB cells seemed to dominate in the chimeras made in Mta$^+$ (A.CA × NZB)F_1 hosts, and in one of these we could not detect any H-2^f, Mta$^+$ cells. The Mta type of these chimeras, too, remained unchanged after 4 months of coexistence of Mta$^+$ and Mta$^-$ cells. It is likely that the slight deviations from the all-or-none expectation arose from the technical limitations of the assay.

DISCUSSION

Genetic analysis has shown that Mta is determined by maternal inheritance (1). To this we can now add that Mta expression is not affected by embryo transfer or any environmental influence. The stability of the Mta type, that is of an extrachromosomal feature, cannot be explained by some genetic incompatibility. By repeated backcrossing, any cytoplasmic factors in an Mta$^+$ strain can be combined with the chromosomal genes of an Mta$^-$ strain, such as NMRI or NZB. All mice from two successive backcrosses to the former and from nine successive backcrosses to the latter were Mta$^+$, showing that the genomes

Table 4. Correlation of H-2 and Mta types of separated cell populations from bone marrow chimeras

Cell treatment	H-2 tested	H-2$^+$ cells detectable	Mta$^+$ cells detectable: NT	<5%	>5%	5–20%	>20%
(NMRI × B6) chimeras, 4–8 months after reconstitution							
Anti-H-2^q + C	H-2^b	NT	4	0	0		
		<5%	0	2	0		
		>5%	0	0	5		
Anti-H-2^b + C	H-2^b	<5%	0	11	0		
		>5%	0	0	0		
(NZB × SJL) chimeras, 2.5–5 months after reconstitution							
Anti-H-2^d + C	H-2^s	<20%		0		0	0
		>20%		0		1	8
Anti-H-2^s + C	H-2^s	NT		1		0	0
		<5%		4		0	0
		5–20%		1		3	0
(A.CA × NZB) chimeras, 4–4.5 months after reconstitution							
Anti-H-2^d + C	H-2^f	<5%		1		0	0
		5–20%		0		2	2
		>20%		0		0	5
Anti-H-2^f + C	H-2^f	<5%		7		2	0
		5–20%		1		0	0
		>20%		0		0	0

The figures indicate the number of mice of a particular class. C, complement; NT, not testable.

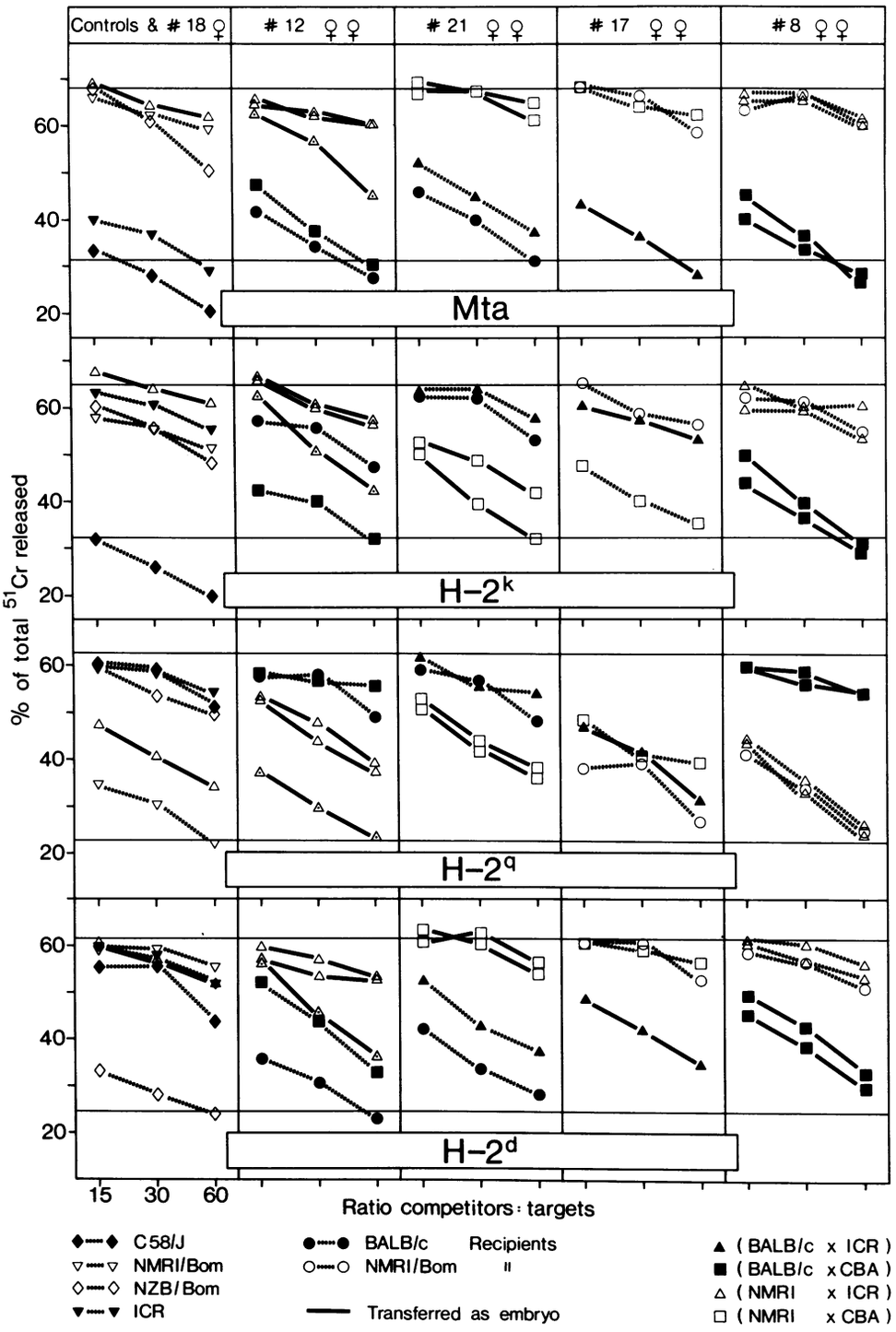

FIG. 1. Mta and H-2 typing of embryo-transferred mice (——), their normal littermates (·····), and recipient mothers (·····) by unlabeled target competition. Embryos were transferred as fertilized eggs or at the two-cell stage (see Table 3). Con A-stimulated blasts were prepared from the spleens of individual mice 10 weeks after the operation, and all foster mothers except no. 18 were tested together with the albino and agouti females from their litter. Mta-specific NZB/Bom anti-NZB/Füll killers were tested on ^{51}Cr-labeled C58/J target cells; similar results were obtained on B6-*Tla*^{a} targets. H-2^{k} was detected by B6 anti-C3H/HeJ assayed on C58/J targets, H-2^{q} by B6 anti-NMRI/Bom on NMRI/Bom, and H-2^{d} by B6 anti-NZB/Füll on NZB/Bom targets. All killer cells were tested at a ratio of 30 initial responders per target cell. The lower horizontal line in each panel indicates the spontaneous release in the absence of killers; the upper line indicates the killing in the absence of competitors.

of the Mta^{-} strains are compatible with expression of Mta. Likewise, all mice with the cytoplasmic genes of NMRI combined with the chromosomal genes of the Mta^{+} strain B6 by three generations of backcrossing were Mta^{-} (unpublished data).

We do not know whether both Mta types involve maternal transmission of a genetic message. Perhaps Mta^{+} mice receive the structural information for Mta, or perhaps Mta^{-} mice inherit a repressor. But it is now clear that the Mta type of an embryo, removed from its natural mother within 10–15 hr of fertilization, has already been fixed for life. The genetic element that determines whether or not Mta is expressed must therefore reside in the egg or, less likely, be acquired from the cumulus cells. The uterine environment or the milk of an adoptive mother could not change the Mta type thus laid down, and

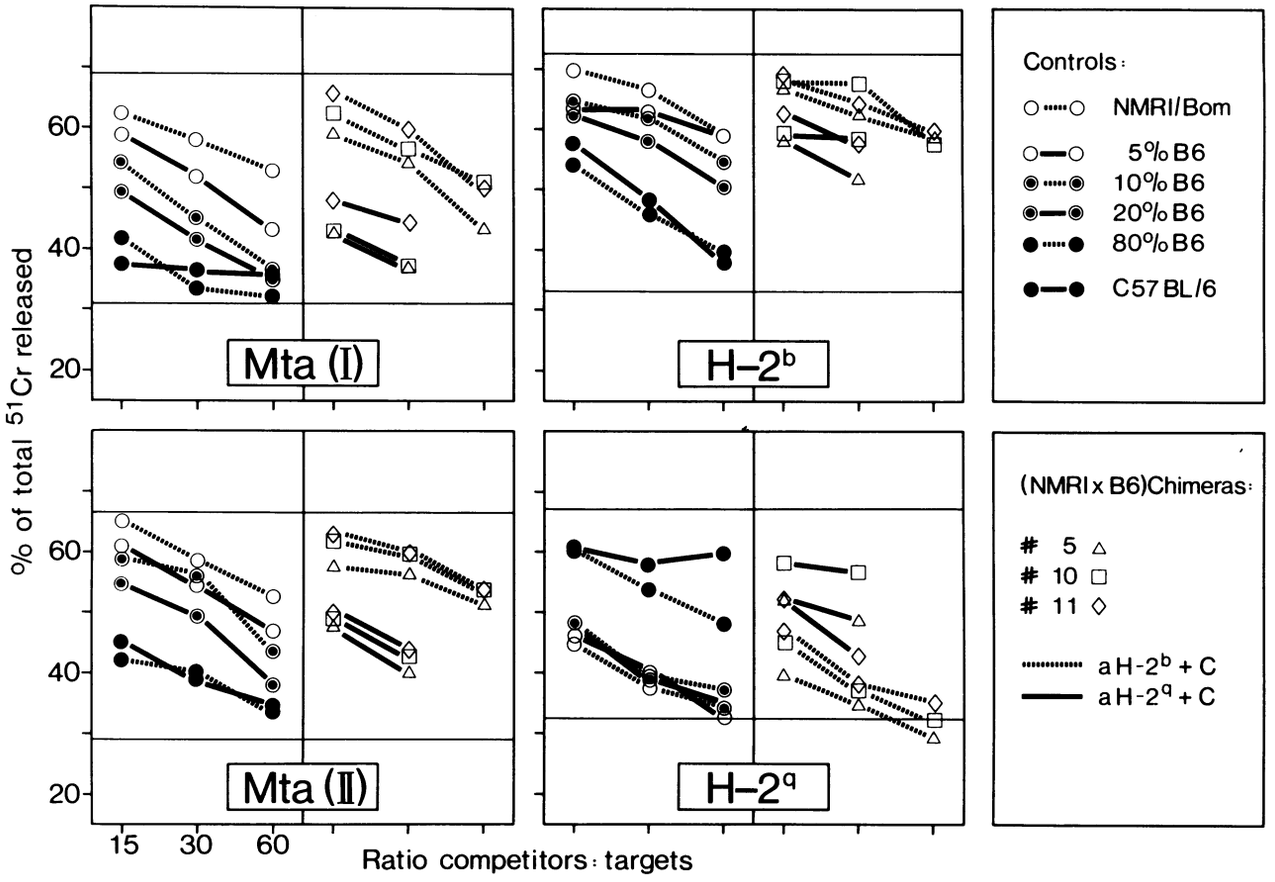

FIG. 2. Mta and H-2 typing by unlabeled target competition of cells obtained from bone marrow chimeras 8 months after reconstitution. Pooled spleen and lymph node cells from individual mice were treated with anti-H-2 antibodies (aH-2) plus complement (C) as indicated and cultured with Con A for 2 days before the assay. The left side of each panel shows the results with artificial mixtures of control Con A-stimulated blasts prepared on the day of assay. Mta-specific killers were (NMRI × B6)F_1 anti-B6 tested on B10.M targets (I) and NZB/Bom anti-NZB/Füll tested on B6 targets (II). H-2^b was detected by NMRI anti-B6 on B6 targets and H-2^q by B6 anti-NMRI on NMRI targets. The effector cells were all assayed at 30 initial responder cells per target cell. The lower horizontal line in each panel indicates the spontaneous release in the absence of killers; the upper line indicates the killing in the absence of competitors.

mixed company during fetal development was also without effect. Nor have we ever observed a spontaneous change of Mta type in our mouse colony, even among very old breeders of different types caged together.

Although the element that determines Mta expression does not spread from mouse to mouse, it might still have been infectious, given proper cell-to-cell contact. This did not seem to be the case in the 30 chimeras we examined. The unlabeled target competition assay has its limitations: it cannot distinguish between 80% and 100% positive cells, but it is sensitive to small fractions of positive cells. However, this sensitivity cannot be fully exploited because negative controls vary in their nonspecific inhibition. As a result, we would not consider the inhibition by a given cell population significant unless equivalent to 10–20% positive cells. Even so, the coexistence for months of Mta^+ and Mta^- cells of different genotypes could be demonstrated under conditions of vigorous growth and differentiation, irrespective of the host's Mta type. Maternal inheritance thus fully determines the Mta expression of individual cells. This is supported by the observation that some plasmacytoma lines of NZB origin passaged for 2 weeks to 2 months in Mta^+ mice remained Mta^- (unpublished data).

By all evidence, then, the genetic element responsible for Mta expression is transmitted in the egg from mother to offspring. Like some defective cytoplasmic virus or mitochondrial gene, it spreads by cell division, not by infection.

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