Brief Definitive Report

MATERNALLY TRANSMITTED ANTIGENS ARE CODOMINANTLY EXPRESSED BY MOUSE CELLS CONTAINING TWO KINDS OF MITOCHONDRIAL DNA

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A maternally transmitted factor, *Mtf*, determines the antigenic polymorphism of the major histocompatibility complex (MHC) class I–like maternally transmitted antigen (Mta) (1, 2). Most inbred strains of mice express Mta^{a} ($Mtf^{\alpha+}$), while NZB/BlNJ is Mta^{b+} ($Mtf^{\beta+}$). NZB mitochondrial DNA (mtDNA) is also unique (3, 4). Somatic cell hybridizations using $Mtf^{\alpha+}$ and NZB cells (5, 6) supported the idea that *Mtf* may be an mtDNA gene; cloned hybrids carried a single form of mtDNA and expressed the concordant form of Mta, while treatment of the donor with the mitochondrial-specific dye rhodamine 6G (R6G) inhibited the transmission of both mtDNA and Mta. *Mtf* may modify the expression of nuclear MHC class I–like genes (2).

To test whether *Mtf* uses positive or negative genetic mechanisms, we assayed *Mtf* dominance in hybrid cells carrying mtDNA from $Mtf^{\alpha+}$ cells and from NZB. Such cells were Mta^{a+}, Mta^{b+}, showing *Mtf* codominantly controls antigen polymorphisms via positive genetic mechanisms, and that this mechanism cannot be construed simply as induction of nuclear gene activity via defects in conventional metabolic mtDNA functions.

Materials and Methods

Generation of Heteroplasmic[1] *Cell Lines.* NZB-2 derived from an NZB/BlNJ ($H\text{-}2^{d}$, Mta^{b}) tumor. Primary cells were transformed with simian virus 40 (SV40), selected for pyruvate-independent resistance to 50 μg/ml chloramphenicol (CAP^{R}) (7, 8) and fused using 40% polyethylene glycol 1450 to the 2-heptyl-4-hydroxyquinoline-*N*-oxide resistant ($HQNO^{R}$) L cell derivative LA9HQNR11 (7) (mouse strain C3H, $H\text{-}2^{k}$, Mta^{a}). Hybrids were selected in pyruvate-free DMEM-based HAT medium with 25 μg/ml CAP and 5 μg/ml HQNO.

CTL Lines and Cytotoxicity Assays. CTL clones and ^{51}Cr-release assays were described (5, 6). CBA/J mice injected with 200 μg of poly(I-C) provided splenic natural killer (NK) cells. Lysis of adherent cells was also assayed by absorbance (A) (9); 15,000 cells were exposed to CTL for 18 h; survivors were stained with crystal violet and quantitated at

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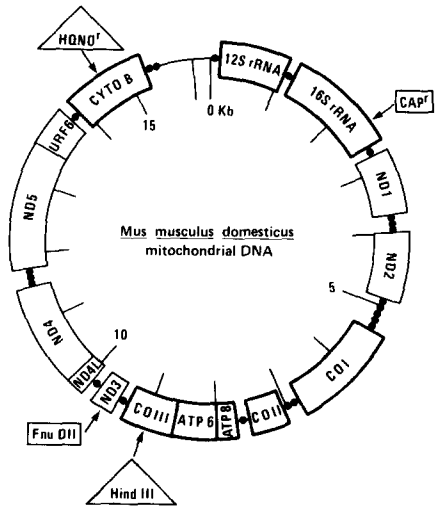
[1] The terms mono- and heteroplasmons refer to cells in which the specific cytoplasmic factors are derived from one or both parents. An *Mtf* heteroplasmon is $Mtf^{\alpha+}$, $Mtf^{\beta+}$.

a



b

GENETIC MARKERS USED TO CONSTRUCT HETEROPLASMIC HYBRID CELLS

	CELL LINE	
MARKER	L-HQNR11	NZB-2
Nuclear		
H-2	k	d
HAT	SENSITIVE	RESISTANT
Mitochondrial		
FnuD II 9595	-	+
Hind III 9136	+	-
HQNO	RESISTANT	SENSITIVE
CAP	SENSITIVE	RESISTANT
Cytoplasmic		
Mtf	α	β

FIGURE 1. Genetic markers used to construct heteroplasmons. (*a*) Map of mouse mtDNA genome. tRNAs (●) and open reading frames are indicated on the circle: *ND*, NADH dehydrogenase; *CO*, cytochrome oxidase; *ATP*, ATPase; and *CYTO B*, cytochrome *b* (12, 16). Markers in triangles, LA9HQNR11; in rectangles, NZB-2. (*b*) LA9HQNR11 and NZB-2 genetic markers.

600 nm in a Bio-rad model 2550 EIA reader. Results were expressed as: percent specific lysis = 100 × [1 − (A experimental − A background)/(A no CTL − A background)].

Southern Analysis. mtDNA from sucrose-gradient purified mitochondria (10) was probed (11) with ^{32}P-labeled DNA from plasmid pMM4 (a gift of Don Robberson, M. D. Anderson Hospital, Houston, TX), containing the 3812 bp Pst I fragment of L cell mtDNA (12).

Results

We selected heteroplasmons from fusions of NZB-2 (CAP^R, HQNO^S, Mta^b, HAT^R) and LA9HQNR11 (CAP^S, HQNO^R, Mta^a, HAT^S) in HAT/CAP/HQNO medium (25 μg/ml CAP + 5 μg/ml HQNO) (Fig. 1). Initial selection required HAT. The independent clones F60 and F62 thrived with either or both mitochondrial poisons, carried both mtDNA types, and expressed both Mta forms (see below). The mtDNA and *Mtf* heteroplasmic condition was stable throughout cloning, subcloning, and maintenance for over 58 passages. The data presented refer to the F62 subclone Cl B2/G6.

We probed restricted F62 mtDNA with plasmid pMM4, which reacts with *FnuD* II fragments of 6434 and 1,638 bp (from L cell mtDNA [12]) and of 4,623, 1,638, and 1,811 bp (from NZB mtDNA) (Fig. 2*a*). Hind III (Fig. 2*b*) yielded bands of 15,407 and 888 bp (from NZB) and of 13,462 and 1,945 bp (L cell). Thus, as expected, F62 was heteroplasmic for mtDNA.

We phenotyped Mta with cloned CTL lines. In a ^{51}Cr-release assay, the Mta^a- and Mta^b-specific CTL lines 5F3 and 6G3, respectively, appropriately lysed Mta^a (BALB/c♀ × NZB♂)F$_1$ or Mta^b (NZB♀ × BALB/c♂)F$_1$ target lymphoblasts (Fig. 3*a*); both CTL lines lysed F62. In the crystal violet assay (Fig. 3*b*), CTL lines 5F3 and 6G3 appropriately lysed LA9HQNR11 (Mta^a) or NZB-2 (Mta^b) parental

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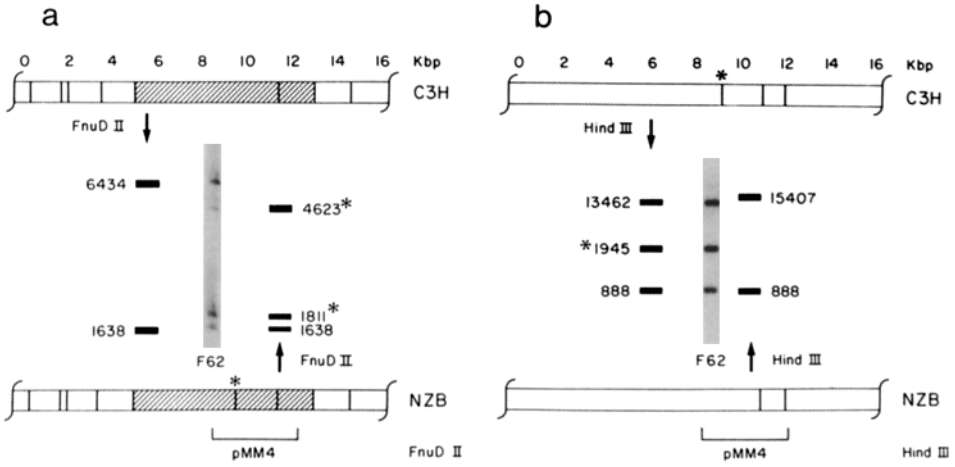

FIGURE 2. Biparental restriction patterns of mtDNA in heteroplasmons. (*a*) FnuD II pattern. *Above*, L cell mtDNA. *Below*, NZB mtDNA. Arrows, patterns expected for L cell (descending) and NZB (ascending) mtDNA. Hatched areas: sequences detected by pMM4. (*b*) Hind III pattern. In these gels, the 13,642 and 15,407 bands were not resolved.

cells, while both lysed F62 (as well as seven additional heteroplasmic clones and a dozen subclones).

Since some CTL lines express NK-like activity (2), we tested our CTL for NK activity (Fig. 3*c*) and the heteroplasmons for NK sensitivity. NK cells lyse cell lines Y1 and F9 (Fig. 3*c* and data not shown) (13), which do not express Mta (2). The CTL lines 5F3 and 6G3 lysed neither F9 (not shown) nor Y1 NK targets, but did lyse F62 heteroplasmons. As expected, CTL line 5F3, but not 6G3, lysed the Mta^{a+} NK target line YAC-1 (not shown).

Discussion

The genetic issues we address required dual selection for mtDNA markers (see below). We note, however, that we were unable to generate $Mtf^{\alpha+}$, $Mtf^{\beta+}$ heteroplasmons in the absence of mtDNA selection. Previous fusions between BALB/c (Mta^{a}) myeloma and NZB spleen cells with no mtDNA selection (5, 6) yielded mtDNA monoplasmic hybrids only. Of 11 clones, one carried NZB mtDNA; the rest carried BALB/c mtDNA. Poisoning BALB/c donors with R6G increased the frequency of hybrids with NZB mtDNA but all were monoplasmic. Failure to recover heteroplasmons may reflect rapid segregation of mtDNA after fusion, or subtle incompatibility of NZB and BALB/c mtDNAs. Once formed, heteroplasmons containing both forms of mtDNA are very stable, resisting straightforward protocols designed to select mtDNA segregants.

mtDNA heteroplasmic hybrids of *Mtf*-disparate parents were Mta^{a+}/Mta^{b+}; they were also *Mtf* heteroplasmic. This implies our mtDNA monoplasmic hybrids, expressing a single form of Mta (5, 6), were *Mtf* monoplasmic, and that both transmission and R6G-sensitivity of *Mtf* and mtDNA are concordant.

Codominance of *Mtf* distinguishes between negative and positive genetic mechanisms: the former repress antigen expression while the latter activate expression of, or encode the antigen. Negative regulatory models predict alternative antigen

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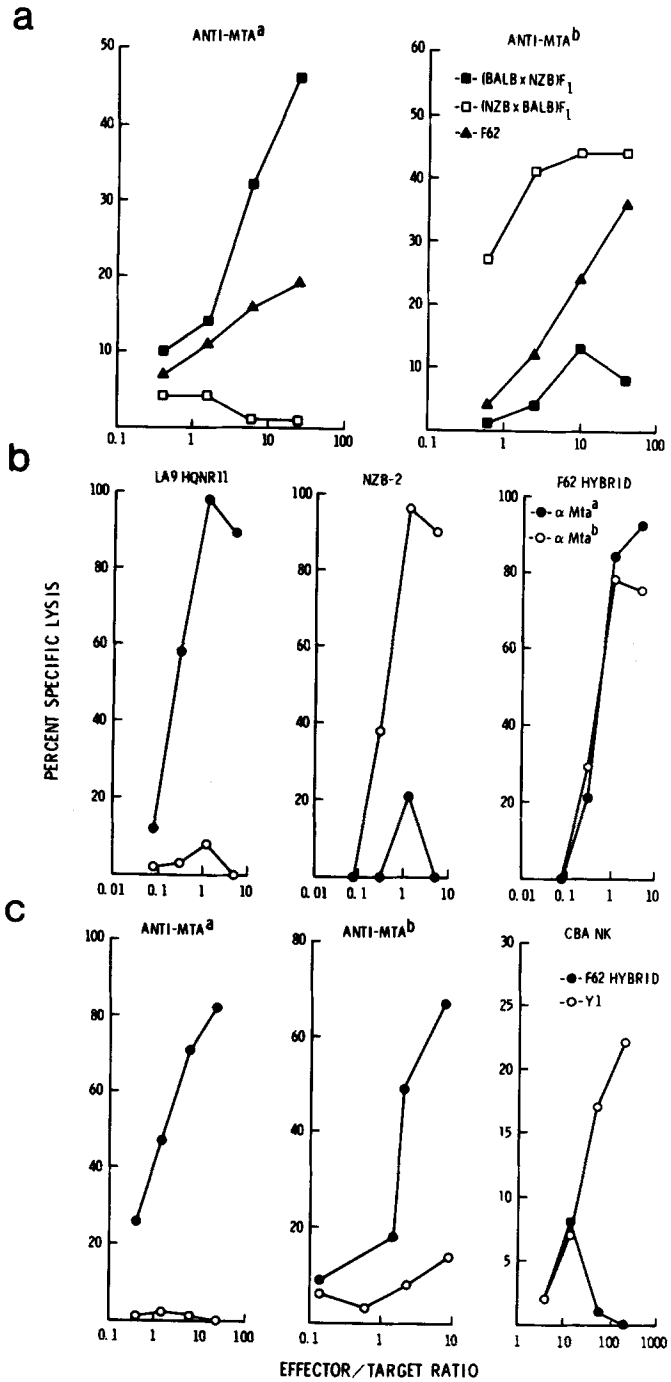

FIGURE 3. Detection of Mta using antigen-specific cloned CTL. (*a*) Maternal restriction of Mta-specific CTL lines. Mta specificities of the CTL clones 5F3 (*left*) and 6G3 (*right*) were assayed on lymphoblasts derived from offspring of BALB/c♀ × NZB/BlNJ♂ (■) or NZB/B1NJ♀ × BALB/c♂ (□) matings and on F62 heteroplasmons (▲). (*b*) Specific lysis of heteroplasmic (*right*) and parental (*left* and *middle*) cell lines was assayed using CTL clones 5F3 (anti-Mta^a, ●) and 6G3 (anti-Mta^b, ○). (*c*) Y1 cells (○) but not F62 heteroplasmons (●) are targets for NK cells (*right*). The CTL lines 5F3 (*left*) and 6G3 (*middle*) did not lyse Y1 (○) but did lyse F62 (●).

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expression should be dominant, or codominant but unstable (14), leading to stable expression of one form. In contrast, active mechanisms predict stable codominant expression, as we observed.

Mtf codominance can also help exclude certain models of indirect regulation of nuclear genes by mtDNA defects. If *Mtf* defects in mtDNA functions induced nuclear gene compensation, converting one Mta antigen into the other, the nondefective form of *Mtf* would be dominant. Elimination of nuclear compensation by mtDNA complementation should restore the dominant Mta. Testing this idea required demonstration of genetic complementation between distinct mtDNAs, as has been shown for human mitochondria (15). Our mtDNA heteroplasmons thrived in the presence of both CAP and HQNO, a resistance demanding *trans*-complementation between the two mtDNA species, because $HQNO^R$ cytochrome *b* mRNA from LA9HQNR11 mtDNA must be translated using CAP^R rRNA from NZB-2 mtDNA. Because the two mtDNAs complemented their mutual defects, the putative demand for nuclear compensation should have been removed and a single form of Mta expressed, contrary to observation.

Demonstration of *Mtf* codominance implies *Mtf* itself shares transmission and R6G sensitivity properties with mtDNA. Codominance also rules out models based on repressors or nuclear compensation of mtDNA defects. Instead, *Mtf* must encode or activate the expression of Mta determinants. Most simply, *Mtf* may be an mtDNA locus encoding Mta determinants. Synthesis of *Mtf* heteroplasmons may permit experimental tests of this idea. Thus, if *Mtf* resides within mtDNA, then *Mtf* and mtDNA should cosegregate among mtDNA monoplasmic segregants. Ultimately, isolation of recombinant segregants would allow mapping of *Mtf* within mtDNA.

Summary

Mtf, a cytoplasmic, probably mitochondrial factor, controls Mta polymorphism. We tested for dominance between two forms of *Mtf* to determine whether Mta is controlled by positive or negative genetic mechanisms. We fused *Mtf*-disparate cells containing distinct mtDNA markers and selected for hybrids containing both. Such mtDNA heteroplasmons codominantly and stably express alternative Mta antigens. Stable codominance excludes negative genetic mechanisms as well as a model of induced nuclear compensation, and implies *Mtf* controls Mta expression through a positive genetic mechanism.

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