

MATERNALLY TRANSMITTED TARGET ANTIGEN FOR UNRESTRICTED KILLING BY NZB T LYMPHOCYTES*

BY K. FISCHER LINDAHL, M. BOCCHIERI,‡ AND R. RIBLET‡

From the Basel Institute for Immunology, Basel, Switzerland; and the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

NZB mice spontaneously develop autoimmune diseases (1). This fact has prompted extensive studies of all aspects of their immune system. Among other phenomena, it was found that stimulation of normal NZB lymphocytes *in vitro* with *H-2*-identical BALB/c or DBA/2 lymphocytes readily induced cytotoxic T cell responses (2, 3). We (4) and others (5) have shown that this primary *in vitro* response is directed against antigens of the *Tla* region, where NZB mice differ from other *H-2^d* strains (6). In the course of that study, we observed that when primed NZB lymphocytes were restimulated with BALB/c cells *in vitro*, they became cytotoxic to target cells of all strains tested, with the exception of NZB. Here we analyze the specificity of this secondary response. Whereas the response itself is a normal T cell response to an antigen present in nearly all other strains but missing in NZB, the antigen is unusual.

Materials and Methods

Mice. Adult mice of both sexes were obtained from the sources listed in Table I. The NZB/Icr × C58/J (NX8) recombinant inbred strains were established and maintained at the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. by brother-sister mating for >20 generations (7).

F₁ hybrids between NZB/Icr and BALB/c or C58/J were bred at the Institute for Cancer Research; (SJL × NZB/Füll)F₁ and (A.CA × NZB/CrBom)F₁ hybrids and first generation backcross progeny to NZB/CrBom were bred at the Basel Institute for Immunology, Basel, Switzerland.

Tumor Cells. The following tumor target cell lines were kindly given to us by Dr. R. MacDonald, Ludwig Institute for Cancer Research, Lausanne, Switzerland: P815, a DBA/2 mastocytoma; EL4, a C57BL/6 lymphoma; AKR-A, a spontaneous AKR thymoma; and YAC-1, an A/Sn Moloney leukemia virus-induced lymphoma. They were all maintained *in vitro*.

Immunizations. Donors of responding cells were given a single (or occasionally two, at least 3 wk apart) intraperitoneal injection of 2×10^7 spleen cells, washed and suspended in serum-free medium.

Generation and Assay of CTL. Spleen cells from normal or immunized donors, taken at least 2 wk after priming, were stimulated by coculture with irradiated or mitomycin C-treated allogeneic spleen cells for 5 d (4). Their cytotoxic activity was assayed on ⁵¹Cr-labeled target cells stimulated 2 or 3 d before with lipopolysaccharide (LPS)¹ or concanavalin A (ConA), respectively (4, 8). The spontaneous release in the 3.5-h assay ranged from 12.3 to 44.3% of the maximum, with a median of 22.0% for 97 targets.

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‡ Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa.

¹ Abbreviations used in this paper: C', complement; ConA, concanavalin A; LPS, lipopolysaccharide; N, NZB/Icr; PHA, phytohemagglutinin; RI, recombinant inbred; 8, C58/J.

TABLE I
Mouse Strains Typed for Mta

Strain	H-2*	Qed-1*	Method‡	Source§
Negative				
NZB/BINJ	d	a	1	Jax
NZB/CrBom	d	a	1	Bom
NZB/Icr	d	a	1	Icr
NZB/Hz	d	a	1	Icr
NMRI	?	a	1	Bom
Positive				
B6.Tla ^a	b	a	1	Füll
BALB.B	b	b	1	B.B., Olac
C57BL/6	b	b	1	Füll
C57BL/10ScCr	b	b	1	Bom
C57L/J	b	b	2	Jax
C3H.SW	b	b	1	Icr
LP/J	b	b	1	Jax
BALB/c	d	b	1	Füll, Bom, Icr
DBA/2	d	b	1	Füll
NZB/Füll	d	a	1	Füll
NZB/BIPt	d	a	1	Pt
A.CA	f	a'	1	Füll
B10.M	f	a'	3	Füll
B10.BR	k	a	3	Olac, Jax
C58/J	k	a	1	Jax, Icr
AKR/J	k	b	1	Jax
BALB.K	k	b	2	C.B.
CBA/J	k	b	1	Füll, Bom
C3H/HeJ	k	b	1	Icr
C3H/Tif	k	b	1	Bom
B10.Y	pa	a	3	Jax
BUB/BNJ	q	a	1	Jax
SWR	q	a	1	B & K
RIIS/J	r	c	1	Jax
SJL	s	a	1	Füll, Bom, Icr
PL/J	u	a	1	Jax

* H-2 types from reference (6), Qed-1 types from reference (4) and recent, unpublished data (K. Fischer Lindahl).

‡ 1, cold target inhibition of Mta-specific lysis; 2, direct lysis by NZB/CrBom primed and restimulated with NZB/Füll; 3, direct lysis by NZB/CrBOM primed with BALB/c and restimulated with DBA/2.

§ B.B., Dr. B. Blomberg, Basel Institute for Immunology; B & K, Bantin and Kingman Ltd., The Field Station, Grimston, Aldborough, Hull, England; Bom, Gl. Bomholtgaard Ltd., Ry, Denmark; C.B., Dr. C. Berek, Basel Institute for immunology, Basel, Switzerland; Füll, Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland; Icr, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa.; Jax, The Jackson Laboratory, Bar Harbor, Maine; Olac, Olac Ltd., Shaw's Farm, Blackthorn, Bicester, Oxon, England; Pt, Dr. M. Potter, National Cancer Institute, Bethesda, Md.

The percent specific ⁵¹Cr-release was calculated from the geometric means (8) of triplicate determinations as:

$$\frac{(\text{mean experimental release} - \text{mean spontaneous release})}{(\text{mean maximal release} - \text{mean spontaneous release})} \times 100.$$

The average SD of 2,800 sets of triplicates amounted to 8.2% of the respective means.

Cold target inhibition was carried out in round-bottomed microtiter plates; each well contained 1×10^4 ^{51}Cr -labeled target cells, 3×10^5 cold target cells, and effector cells generated from 3×10^5 responder cells (8). Maximal inhibition is to be expected with the specific target as cold target inhibitor; minimal inhibition should be observed with the responder itself as cold inhibitor. The degrees of lysis were always evaluated in terms of these two controls. With the number of combinations to be assayed, it was not feasible to test killers and inhibitors in several ratios. Thus, depending on the efficiency of the killers, revealed only by the assay, the reduction of lysis with a fixed number of effective inhibitors could therefore be complete or intermediate (cf. the two anti-H-2^d killers in Fig. 5) or occasionally barely significant (cf. the anti-H-2^k killers in Fig. 4). All experiments have been done at least twice, giving the same typing results.

T Cell Enrichment and Depletion. Spleen (and occasionally lymph node) cells were passed through nylon-wool columns (9) or columns of glass beads coated with mouse immunoglobulin (Ig) and rabbit anti-mouse Ig (10). The percentage of Ig-positive cells in the effluent was determined by indirect immunofluorescent staining (4). T cells were removed by treatment with a monoclonal anti-Thy-1.2 antibody, F7D5 (a generous gift from Dr. P. Lake, University College, London), and complement (C'). Spleen cells or cultured effector cells were incubated at $1 \times 10^7/\text{ml}$ of a 1:1,000 dilution of ascitic fluid for 30 min on ice, washed once, and then incubated for 45 min at 37°C with the same volume of a 1:10 dilution of rabbit C', selected for low natural cytotoxicity on BALB/c thymocytes. After two washings, responder and effector cells were assayed on the basis of the number of initial responder cells treated.

Results

Primary and Secondary NZB anti-BALB/c Responses. NZB mice differ from H-2-identical BALB/c and DBA/2 mice in the *Tla* region by the serologically defined TL and Qa-1 antigens (6) and by Qed-1, a target antigen for unrestricted T cell killing (4). Because Qed-1 stimulates primary cytotoxic responses *in vitro* in several strain combinations (4), it is likely to be involved in a primary NZB anti-BALB/c response. Table II confirms that normal NZB spleen cells from all four sublines tested responded in nearly all experiments to stimulation with BALB/c. Contrary to the claim of

TABLE II
Specificity of Primary *In Vitro* Responses of NZB Lymphocytes against H-2-Compatible BALB/c Stimulators

Responder	Stimulator	Experiment	Percent ^{51}Cr release from target cells*						
			BALB/c <i>d,b</i> ‡	BALB.B <i>b,b</i>	BALB.K <i>k,b</i>	C57BL/6 <i>b,b</i>	B6.Tla ^a <i>b,a</i>	C58/J <i>k,a</i>	NZB/BINJ <i>d,a</i>
NZB/BINJ	NZB/BINJ	1	7.9	2.9	—§	7.1	2.6	—	8.9
NZB/BINJ	NZB/BINJ	2	-0.5	0.6	—	0.8	1.4	-3.6	0.8
NZB/CrBom	NZB/CrBom	4	2.0	—	5.0	-4.0	—	4.1	2.4
NZB/BINJ	BALB/c	1	46.4	47.4	—	49.7	12.3	—	6.9
NZB/BINJ	BALB/c	2	24.8	29.6	—	15.0	1.2	5.6	2.6
NZB/BINJ	BALB/c	3	25.4	16.8	—	32.2	8.1	10.6	6.2
NZB/CrBom	BALB/c	3	28.7	22.4	—	22.7	5.4	6.4	8.4
NZB/CrBom	BALB/c	4	22.4	—	17.8	13.3	—	8.2	5.2
NZB/Füll	BALB/c	3	27.9	31.7	—	25.3	4.7	5.9	11.5
NZB/Füll	BALB/c	4	13.2	—	15.1	-2.4	—	-0.4	1.3
NZB/Icr	BALB/c	4	26.9	—	26.8	13.4	—	0.2	7.9
NZB/BINJ	C57BL/6	1	10.9	68.5	—	60.3	67.1	—	4.1
NZB/BINJ	C57BL/6	2	9.2	48.6	—	41.9	40.8	—	12.0
NZB/BINJ	C57BL/6	3	13.6	62.6	—	55.0	51.9	35.3	4.9

* LPS-stimulated spleen cells, 30 initial responder cells/target cell.

‡ H-2 and Qed-1 type.

§ —, not done.

Theofilopoulos et al. (3), we found no evidence that this response was H-2 restricted: there was no significant difference between the lysis of BALB/c ($H-2^d$) and BALB.B ($H-2^b$) or BALB.K ($H-2^k$) target cells ($P > 0.05$; Wilcoxon's signed rank test). Nor did the response appear specific for BALB minor histocompatibility antigens, because C57BL/6 target cells were lysed equally well. B6. Tla^a target cells, on the other hand, which differ from C57BL/6 only in the Tla region where they share $Qed-1^a$ with NZB, and other $Qed-1^a$ target cells (see also Fig. 1) were not lysed, confirming that the response is specific for $Qed-1$ (4, 5).

When the NZB lymphocytes were primed in vivo with BALB/c and then restimulated in vitro, a strong cytotoxic response was seen on all target cells tested, irrespective of their $H-2$ or $Qed-1$ type (Fig. 1). The response was specific because NZB target cells were not killed. It required restimulation with BALB/c because primed NZB responder cells cultured with syngeneic irradiated cells displayed no cytotoxic activity.

With cold target inhibition of the killing of appropriately selected radiolabeled target cells, it is possible to distinguish the specificities involved in complex cytotoxic responses (4, 8). Fig. 2 shows that three major specificities can be identified in the secondary NZB anti-BALB/c cytotoxic response. The lysis of every target is inhibited by BALB/c, which carries all the stimulating antigens, but not by NZB/BINJ, which itself is not lysed. The lysis of BALB.B is inhibited by the three $Qed-1^b$ target cells, but not clearly by any $Qed-1^a$ target. There must be an $H-2^d$ -restricted component in the response because BALB.B ($H-2^b$) does not inhibit the lysis of BALB/c ($H-2^d$), and because the lysis of (SJL \times NZB) F_1 is inhibited by the F_1 cells ($H-2^s \times H-2^d$) but not to the same extent by SJL ($H-2^s$). The B6. Tla^a target cells ($H-2^b$, $Qed-1^a$) define the

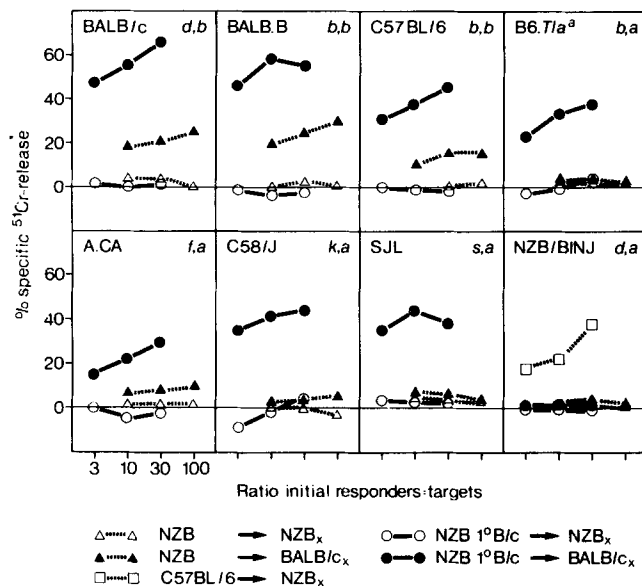


Fig. 1. Lysis of LPS-stimulated spleen cells by NZB/BINJ cells stimulated in vitro with BALB/c (\blacktriangle) or NZB/BINJ (\triangle); by NZB/CrBom cells primed in vivo with BALB/c and restimulated with BALB/c (\bullet) or NZB/BINJ (\circ); and by C57BL/6 stimulated with NZB/BINJ (\square). $H-2$ and $Qed-1$ types of the target cells are indicated on each panel.

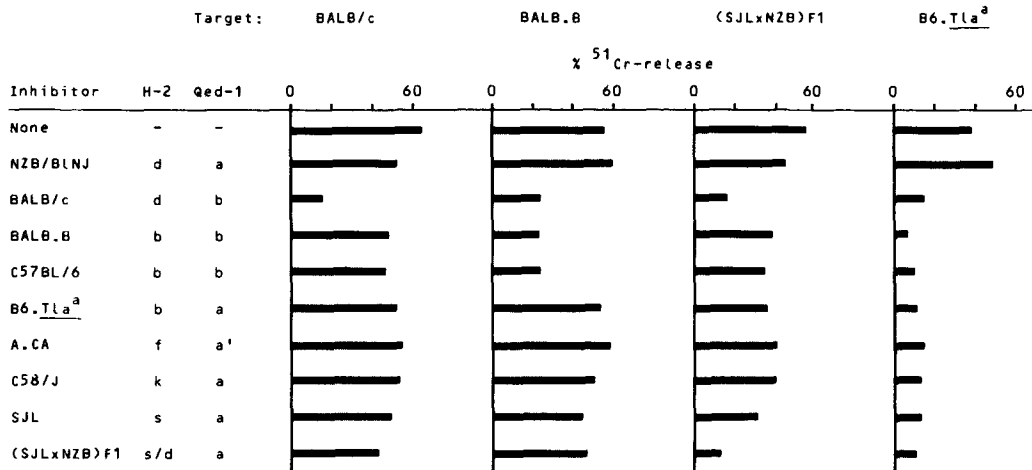


FIG. 2. Inhibition of lysis by NZB/CrBom effector cells primed and restimulated with BALB/c. Target cells were LPS-stimulated spleen cells.

last specificity. Every target cell that was killed in Fig. 1 inhibits the lysis of B6.Tla^a, showing that they all share the antigen detected here. We shall call this antigen Mta.

Strain Distribution of Mta. Table I lists the strains that have been typed for Mta, usually by cold target inhibition of lysis but occasionally, where Qed-1^b-specific or H-2^d-restricted lysis could be excluded, by direct lysis. The only Mta⁻ non-NZB strain found to date is the non-inbred NMRI strain. The 26 strains found positive for Mta included 9 different H-2 haplotypes and about 14 different non-H-2 backgrounds, of which at least 8 can be considered unrelated (11). The antigen was found in mice from six different sources, and was detected in experiments performed in Basel as well as in Philadelphia, making it unlikely that the antigen is the result of an infection in the holding facilities which spread to all incoming mice.

Mta was found even among NZB mice. Cold target inhibition tests showed that the NZB substrain maintained at the Institut für Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland (provisionally designated NZB/Füll) was Mta⁺. This line has been developed from breeding pairs provided in 1978 by Dr. M. Potter, National Cancer Institute, Bethesda, Md. Dr. Potter's line (NZB/BIpt) originated around 1968 from breeders obtained from Dr. L. W. Law, National Cancer Institute. Table III shows that although primed NZB/CrBom anti-BALB/c effector cells do not react with target cells from NZB mice obtained from The Jackson Laboratory, Bar Harbor, Maine or from the Institute for Cancer Research, they kill both NZB/Füll and NZB/BIpt target cells.

By immunizing NZB/CrBom mice with spleen cells from NZB/Füll mice, it is possible to generate Mta-specific CTL in the absence of a response against Qed-1^b, making direct Mta typing easier (Table III). However, some part of the response must be directed against minor histocompatibility antigens (presumably H-2^d restricted), because the lysis of NZB/Füll target cells is not inhibited by excess BALB/c targets that share Mta (data not shown). As expected, immunization of NZB/Füll mice with BALB/c cells induces a Qed-1^b-specific response (C57BL/6 vs. B6.Tla^a targets in Table III) and a response against minor histocompatibility antigens (lysis of NZB/BLNJ targets), but no response against Mta (failure to lyse B6.Tla^a and C58/J).

TABLE III
Comparison of NZB Substrains

Experiment	Responder	Stimulator		Percent ⁵¹ Cr release*								
		In vivo	In vitro	NZB substrain					BALB/c <i>d,b</i>	C57BL/6 <i>b,b</i>	B6.7la ^a <i>b,a</i>	C58/J <i>k,a</i>
				CrBom <i>d,a</i> †	BiNJ <i>d,a</i>	Icr <i>d,a</i>	Füll <i>d,a</i>	BIpt <i>d,a</i>				
1	NZB/CrBom	BALB/c 1×§	BALB/c	5.1	-0.6	8.3	58.0	—	72.9	55.6	47.1	45.9
2	NZB/CrBom	BALB/c 1×§	BALB/c	3.6	—	—	61.7	49.6	—	—	—	56.2
1	NZB/CrBom	NZB/Füll 2×	NZB/Füll	-3.9	-1.5	7.7	69.2	—	68.7	49.7	49.0	44.0
3	NZB/Füll	BALB/c 1×	BALB/c	—	51.9	—	—	—	84.1	68.2	6.3	10.3
1	NZB/Füll	NZB/CrBom 2×	NZB/CrBom	66.4	71.2	62.3	13.7	—	65.9	8.4	5.4	18.0
2	NZB/Füll	NZB/CrBom 2×	NZB/CrBom	60.6	—	—	4.6	5.4	—	—	—	—

* LPS-stimulated spleen cells; 30 initial responder cells/target cell.

† *H-2* and *Qad-1* types.

§ 1×, once; 2×, twice.

|| —, not done.

TABLE IV
Nature of the NZB Lymphocytes Responding In Vitro to Stimulation with BALB/c Spleen Cells

Responder	Treatment before culture	Treatment after culture	Percent ⁵¹ Cr-release from target cells* from			
			Experiment 1		Experiment 2	
			BALB/c	B6.7la ^a	BALB/c	C58/J
NZB/CrBom-1 ^o ‡	—	—	66.2	40.3	74.2	60.0
	Nylon wool purified§	None	64.8	33.6	70.9	52.2
	Anti-Ig column passed	None	—¶	—	72.8	49.2
	Anti-Thy-1.2 + C' (3 × 10 ⁶)	None	-3.8	-2.7	—	—
	C' alone (3 × 10 ⁶)	None	55.4	28.2	—	—
NZB normal (6 × 10 ⁶)	—	None	39.5	0.7	—	—
NZB normal (3 × 10 ⁶) + NZB-1 ^o (3 × 10 ⁶)	—	None	58.3	38.7	—	—
NZB normal (3 × 10 ⁶) + NZB-1 ^o (1.8 × 10 ⁶)	Anti-Thy-1.2 + C'	None	40.5	-12.5	—	—
NZB/CrBom-1 ^o	—	Anti-Thy-1.2 + C'	-2.4	-3.2	3.4	-2.5
NZB/CrBom-1 ^o	—	C' alone	63.8	43.3	80.2	61.0

* LPS-stimulated spleen cells; 30 initial responder cells/target cell.

‡ 1^o, primed with BALB/c in vivo.

§ 1.2% Ig⁺ cells after passage over nylon wool, 9.6% after passage through anti-Ig column in experiment 2.

|| Equivalents based on number of cells initially treated.

¶ —, not done.

Immunization of NZB/Füll with NZB/Bom generates an H-2^d-restricted response against minor histocompatibility antigens (lysis of BALB/c, not BALB.B [data not shown]; the lysis of C58/J was not reproduced in four other experiments). The data in Table III suggest that NZB/Füll differs little, if at all, from NZB/BIpt. Cold target cells from both of these substrains have been shown to inhibit Mta-specific cytotoxicity, although on occasion less efficiently than cells from other strains (an example occurs in Table V).

Nature of the Responding Cell. The cell that responds to Mta is a T cell. It passes through nylon wool or rabbit anti-mouse Ig columns and is killed by monoclonal anti-Thy-1.2 antibodies and C', both before and after culture (Table IV). It requires priming, because normal NZB cells mixed with T-depleted primed NZB cells and stimulated with BALB/c could respond to Qed-1^b, but not to Mta.

Distribution of Mta on Lymphoid Cells. In all the above experiments, LPS-stimulated spleen cells were used as targets. As shown in Fig. 3, similar responses were obtained on phytohemagglutinin (PHA)- or ConA-stimulated lymphoblasts. Mta could also be demonstrated on fresh, normal spleen cells, T cells as well as B cells. The lower plateau levels of lysis are characteristic of normal spleen cells as targets, whatever the effector cell specificity (4). Mta was also detectable by direct lysis on a number of cloned C57BL/6 T helper cell lines (described in [12]).

Some frequently used tumor target cell lines have also been typed for Mta by cold target inhibition (Table V) and direct lysis (data not shown). All were strongly positive, even those with very low levels of H-2 antigens (K. Fischer Lindahl. Unpublished observations.).

Genetics of Mta. To determine whether Mta, like most other target antigens for unrestricted T cell killing, was encoded by genes linked to the H-2 complex, we typed the existing recombinant inbred (RI) strains derived from crosses of NZB/Icr (Mta^- , $H-2^d$) and C58/J (Mta^+ , $H-2^k$). The results show that five strains were Mta^- and eight strains Mta^+ (Fig. 4). The H-2 typing, done by cold target inhibition of killing in the same experiments as the Mta typing, agrees with previous results (7), except for NX8-9. The previously reported subline of this strain is extinct, and the surviving subline is $H-2^k$. The distribution of Mta among the RI lines showed no association with H-2 (among the positive mice, four were $H-2^k$ and four were $H-2^d$; among the negatives, three were $H-2^k$ and two were $H-2^d$) or with any other marker for which these mice have been typed (7).² On the other hand, the strain distribution pattern of Mta is perfectly correlated with the construction of the original matings from which the strains were derived. RI 3, 4, and 5 were derived from F₂ mice from the cross (N♀

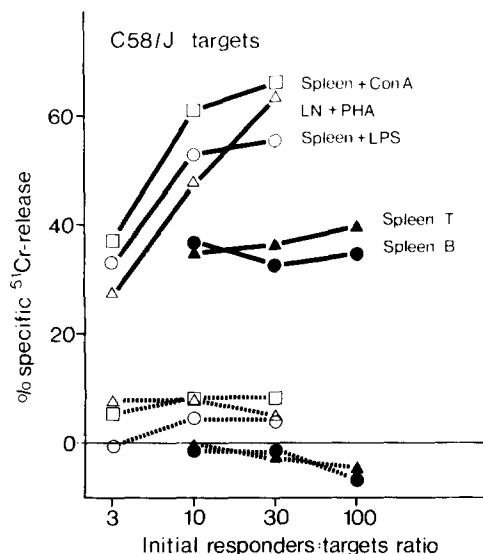


FIG. 3. Lysis of C58/J target cells by NZB/CrBom effector cells immunized twice and restimulated in vitro with NZB/Füll spleen cells (—) and by C3H/HeJ anti-B10.A(5R) (---); the latter effector cells gave 53% specific ⁵¹Cr-release from C57BL/6 LPS blasts at 30 initial responders/target cell.

² Bocchieri, M., R. J. Riblet, and J. B. Smith. Autologous mixed lymphocyte reactions in NZB mice: analysis in recombinant inbred lines shows a T cell defect unrelated to auto-antibody production. Manuscript submitted for publication.

TABLE V
Presence of Mta on Various Tumor Cell Lines

Inhibitor	Anti-Mta*		Anti-H-2 ^k *	
	B6.Tla ^a ‡	C3H/Tif‡	C58/J‡	C3H/Tif‡
	Experi- ment 1	Experi- ment 2	Experi- ment 1	Experi- ment 2
	%			
None	39.0§	46.5	73.3	47.2
NZB/CrBom	32.5	44.7	71.3	39.6
NZB/Füll	18.3	8.5	72.9	40.1
BALB/c	9.2	9.5	72.7	39.1
B6.Tla ^a	1.6	—	69.3	—
C3H/Tif	—	4.3	—	5.8
C58/J	8.2	—	31.4	—
P815	2.2	—	57.8	—
EL4	1.4	—	63.3	—
AKR-A	0.4	5.7	49.8	28.3
YAC-1	1.7	1.3	61.1	37.9

* Effector cells: Anti-Mta: NZB/CrBom primed and restimulated with BALB/c in experiment 1, with NZB/Füll in experiment 2. Anti-H-2^k: NZB/Füll anti-C58/J in experiment 1, BALB/c anti-C3H/Tif in experiment 2.

‡ Target cells: LPS-stimulated spleen cells.

§ Percent ⁵¹Cr release.

|| —, not done.

× 8♂)F₁♀ × (N♀ × 8♂) F₁♂, strains RI 6 and 9 from (N × 8) × (8 × N), strains RI 13 and 15 from (8 × N) × (8 × N), and strains RI 16–20 from (8 × N) × (N × 8); i.e., the maternal line of descent the Mta⁻ strains is derived from NZB, whereas the maternal line of descent of the Mta⁺ strains is derived from C58. These results forcefully suggest a maternal effect on the expression of Mta, and this was tested on reciprocal F₁ hybrids between Mta⁻ (NZB) and Mta⁺ (BALB/c and C58/J) strains (Fig. 5). Although the cells from F₁ hybrids of reciprocal crosses inhibited H-2^d-specific cytotoxicity equally well, only those derived from an Mta⁺ mother inhibited Mta-specific lysis. F₁ hybrids derived from a negative mother were negative. The female reciprocal F₁ hybrids between NZB and BALB/c are particularly informative, because these mice should be genotypically identical. We also included cold target cells from very old (>2 yr) NZB/Icr mice in these experiments. Plainly, Mta is not acquired with age (Fig. 5).

Backcrossing Mta⁺ (A.CA♀ × NZB♂)F₁ hybrids to NZB females produced Mta⁻ offspring, whereas backcrossing to NZB males yielded Mta⁺ offspring (Fig. 6). The results for all backcross animals are summarized in Table VI. The probability of getting the observed or a more extreme distribution of the H-2^d haplotype is 0.247 (Fisher's exact test); the probability of getting the observed distribution of Mta by chance alone is 1.9 × 10⁻⁷.

Discussion

Our experiments serve to define a new target antigen for unrestricted T cell killing. The antigen, termed Mta (for maternally transmitted antigen), is carried by most

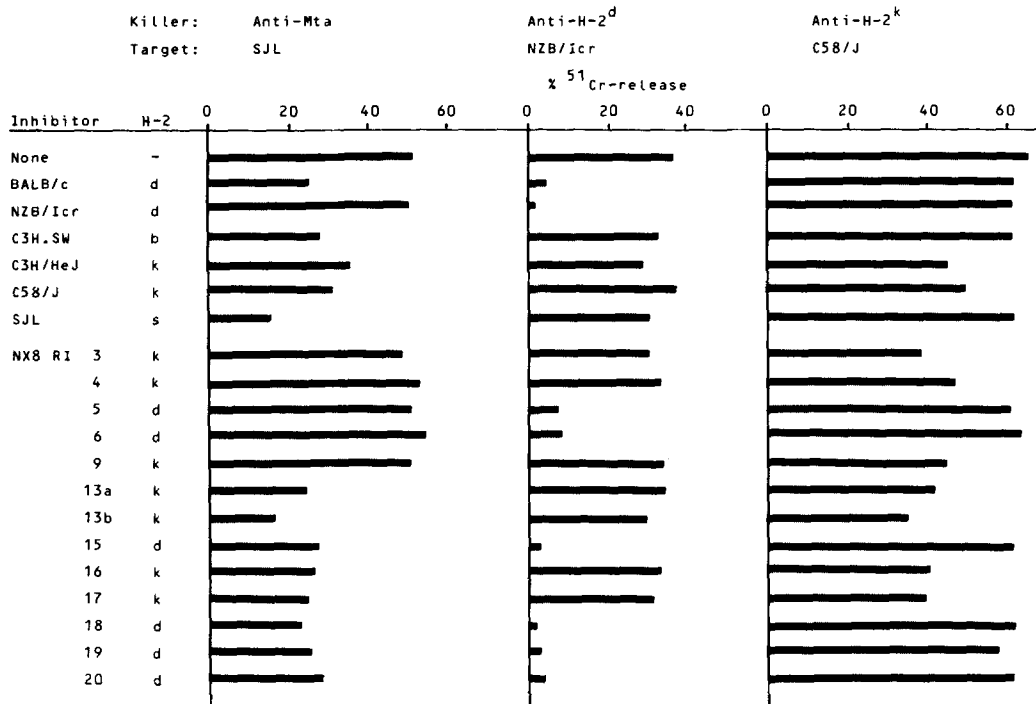


FIG. 4. Typing of NX8 recombinant inbred strains by cold target inhibition of lysis. All target cells were ConA-stimulated spleen cells. The effector cells were NZB/Icr primed and restimulated by BALB/c (anti-Mta), NX8-17 anti-BALB/c (anti-H-2^d), and NX8-20 anti-C3H/HeJ (anti-H-2^k).

mouse strains tested except for some NZB substrains and non-inbred NMRI mice. The antigen is displayed by all lymphoid target cells and a number of tumor cell lines. Mta did not segregate with *H-2* or any other marker; rather, the rule, to which we found no exception, is that Mta⁺ females bear positive offspring whatever the phenotype of the male may be, and Mta⁻ mothers bear negative offspring. The phenotypes of NX8 recombinant inbred strains (Fig. 4) and of backcross mice (Fig. 6 and Table VI) make sex-linkage of Mta unlikely, and the typing of reciprocal F₁ hybrids (Fig. 5) rules it out.

Two alternatives remain. Perhaps all mice have the genetic information to produce Mta, and there is an epigenetic transmission from negative mothers of a principle that prevents expression of Mta for the lifetime of their offspring. It is not easy to envisage such a mechanism and, even though our experiments to date do not rule it out, we favor the alternative, a maternally transmitted genetic element coding for the antigen (Mta).

Such a maternally transmitted antigen might be passed on via the egg, through the placenta, or in the milk; it is likely to be a virus. Milk-transmitted mammary tumor virus is a well-established precedent (13); moreover, it has been reported to give rise to unrestricted T cell killing (14). Foster-nursing of hysterectomy-derived mice by NZB/Icr or BALB/c mothers gave no evidence that Mta is milk-transmitted (K. Fischer Lindahl, M. Bocchieri, and R. Riblet. Manuscript in preparation.), and

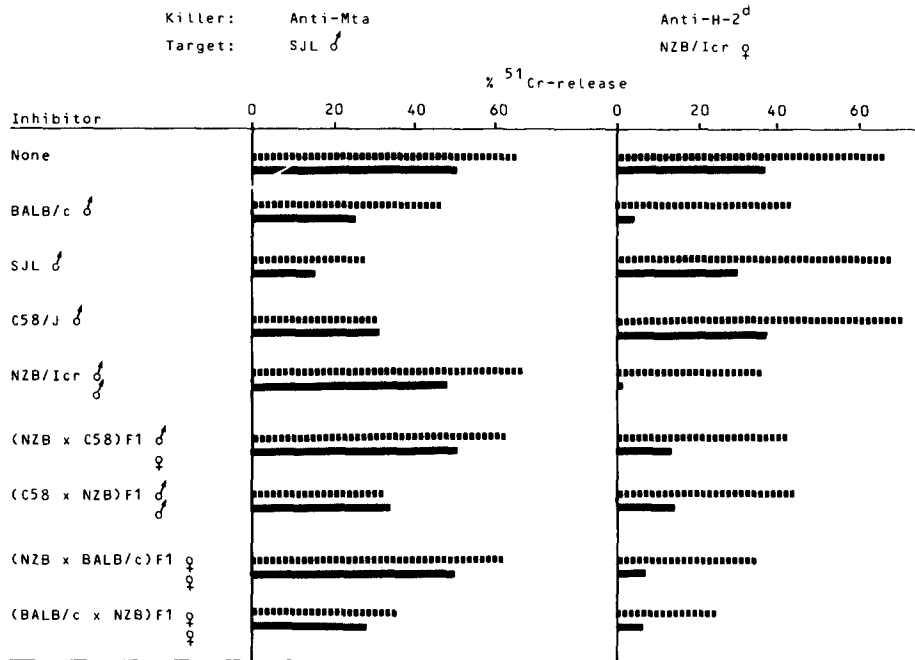


FIG. 5. Mta typing of reciprocal F₁ hybrids; results of two experiments using LPS- (---) and ConA- (—) stimulated target cells from one or two donors. Effector cells were NZB/Icr primed and restimulated with BALB/c (anti-Mta) and N × 8-16 (---) or N × 8-17 (—) anti-BALB/c (anti-H-2^d). The donors of the NZB/Icr cold target cells were >2 yr old.

embryo-transfer experiments are now required to distinguish between the other possibilities.

There is no evidence of horizontal transmission of Mta. Even very old NZB/Icr mice reared in an environment of positive strains stayed negative (Fig. 5); the Mta⁺ NZB/BIPt substrain and its derivative NZB/Füll differ from other NZB substrains by additional antigens, suggesting a genetic contamination of the line (Table III). If NZB mice were resistant to a ubiquitous virus because of a lack of receptors or interference by genomic viruses, one would expect backcrosses to NZB to yield some resistant progeny. However, in the first generation we have studied, 12 out of 12 (A.CA × NZB)F₁ × NZB mice were Mta⁺.

Mta is the first genetically analyzed target antigen for unrestricted T cell killing that is not evidently linked to the major histocompatibility complex (4, 8, 15). Its demonstration does not require prolonged assays (14), and it is detected under the same conditions as H-2-restricted responses. We saw no primary responses against Mta, but a single immunization in vivo induced strong responsiveness, which does not require a simultaneous response against Qed-1^b. Responsiveness does not depend on the H-2^d haplotype: cells from Mta⁻ NX8-3 and NX8-4 (H-2^k) mice primed and restimulated with C3H/HeJ cells responded to Mta (K. Fischer Lindahl, M. Bocchieri, and R. Riblet. Unpublished observations.).

Mta shows no relationship to autoimmunity. The strain distribution pattern of any of several autoimmune traits among the NX8 RI strains does not correlate with Mta.² Mta⁻ NMRI mice are bred for their health and vigor, whereas the Mta⁺ NZB/BIPt

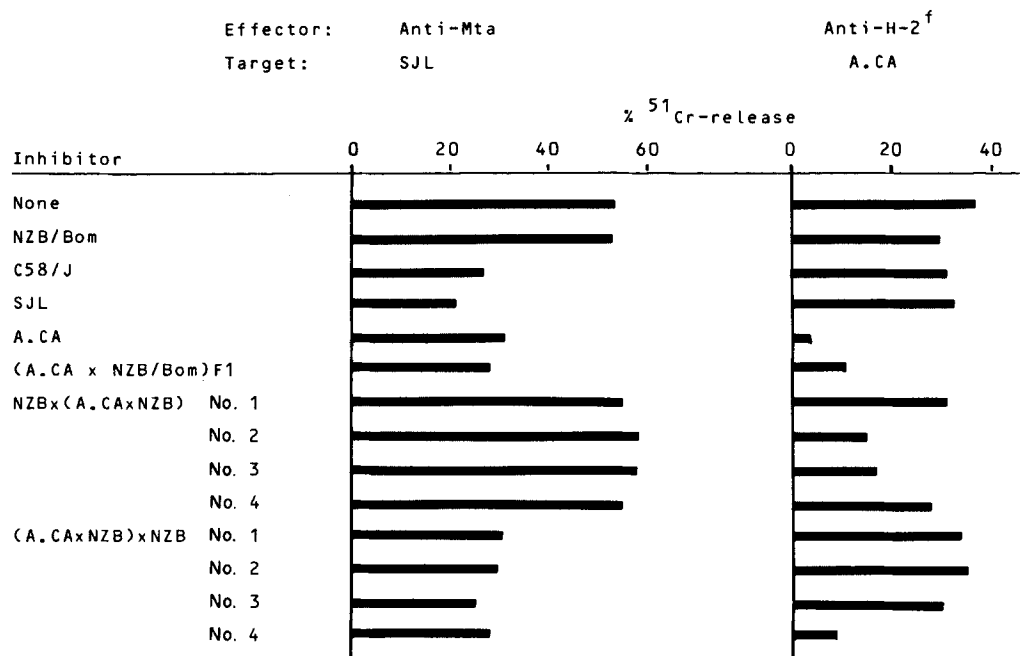


FIG. 6. Typing of first generation backcrosses of (A.CA x NZB/CrBom)F₁ to NZB/CrBom. Target cells were LPS-stimulated spleen cells and effector cells were NZB/CrBom primed and restimulated with BALB/c (anti-Mta) and BALB/c anti-A.CA (anti-H-2^f). N x (A x N) No. 2 and 3 and (A x N) x N No. 4 were considered positive for H-2^f. The (A x N)F₁ was the father of the N x (A x N) backcross mice. All backcross mice were males.

TABLE VI

Mta and *H-2* Typing of First Generation Backcrosses of (A.CA x NZB/CrBom)F₁ to NZB/CrBom

Cross	A.			B.			
	Mta ⁺	Mta ⁻	Total	Cross	H-2 ^{f+}	H-2 ^{f-}	Total
N x (A x N)*	0	13	13	N x (A x N)	6	6	12
(A x N) x N‡	12	0	12	(A x N) x N	3	8	11
Totals	12	13	25	Totals	9	14	23
	<i>P</i> = 1.9 x 10 ⁻⁷ §			<i>P</i> = 0.247§			

A: Inhibition of lysis of SJL and C58/J target cells by NZB/CrBom primed and restimulated with BALB/c. B: Inhibition of lysis of A.CA by BALB/c anti-A.CA effector cells.

* 3 ♀ + 10 ♂.

‡ 8 ♀ + 4 ♂.

§ Fisher's exact test.

|| The results did not permit unequivocal typing of one animal in either group. The *P* values for the four possible allocations ranged from 0.144 to 0.404.

strain is prone to various lymphoproliferative disorders (M. Potter. Personal communication.). Finally, a study of reciprocal F₁ hybrids between NZB and DBA/2 showed no difference in their ability to produce autoantibodies (16).

Summary

A new target antigen for unrestricted killing was defined by NZB T lymphocytes which were immunized and restimulated with H-2-identical BALB/c spleen cells.

These effector cells killed nearly all target cells tested, irrespective of their *H-2* type, but did not kill NZB target cells. The response was shown to have three major components: unrestricted killing specific for Qed-1^b, H-2^d-restricted killing specific for minor histocompatibility antigens, and unrestricted killing specific for a new antigen, Mta. Mta is present on normal and mitogen-stimulated T and B lymphocytes and on several tumor lines. It was found on cells from 26 mouse strains tested, including two substrains of NZB, representing 9 different *H-2* types and 14 different non-*H-2* backgrounds. Analysis of the NX8 recombinant inbred lines (derived from Mta⁻ NZB/Icr and Mta⁺ C58/J parents) suggested that Mta is maternally transmitted. This was confirmed by typing of reciprocal F₁ hybrids and backcrosses between positive and negative strains: Mta⁺ females bear Mta⁺ offspring and Mta⁻ females Mta⁻ offspring, irrespective of the phenotype of the males.

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