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

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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-2d 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/F1)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 × 107 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.

* Supported by grants AI-13797, CA-09035, CA-06927, RR-05539, and by an appropriation from the Commonwealth of Pennsylvania.

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

J. Exp. Med. © The Rockefeller University Press • 0022-1007/80/12/1583/13 $1.00 1583
Volume 152 December 1980 1583-1595
Table 1

| Strain | H-2* | Qed-1* | Method † | Source § |
|--------|------|--------|----------|----------|
| Negative |      |        |          |          |
| NZB/Bl[NJ | 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 |      |        |          |          |
| B6.7Tha | 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 |
| RIHS/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 ¹¹¹Cr-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 $1 \times 10^4$ $^{51}$Cr-labeled target cells, $3 \times 10^6$ cold target cells, and effector cells generated from $3 \times 10^5$ 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$^o$). Spleen cells or cultured effector cells were incubated at $1 \times 10^7$ 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$^o$, 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

| Responder Stimulator | Experi- | Percent $^{51}$Cr release from target cells* |
|----------------------|--------|-------------------------------------------|
|                      |        | BALB/c | BALB.B | BALB.K | C57BL/6 | B6. T/a | NZB/BINJ |
| NZB/BINJ             |        | 1      | 7.9    | 2.9    | --      | 7.1     | 2.6     | 8.9  |
| NZB/BINJ             |        | 2      | --     | 0.6    | --      | 0.8     | 1.4     | 3.6  |
| NZB/Cr/Bom           |        | 4      | 2.0    | --     | 5.0     | --      | 4.0     | 2.4  |
| NZB/BINJ             |        | 1      | 46.4   | 47.4   | --      | 49.7    | 12.3    | 6.9  |
| NZB/BINJ             |        | 2      | 24.8   | 29.6   | --      | 15.0    | 1.2     | 5.6  |
| NZB/BINJ             |        | 3      | 26.0   | 16.8   | --      | 15.1    | 12.0    | 6.2  |
| NZB/Cr/Bom           |        | 2      | 28.8   | 22.4   | --      | 17.8    | 13.8    | 8.2  |
| NZB/BINJ             |        | 3      | 22.4   | 17.8   | --      | 15.3    | 4.7     | 11.5 |
| NZB/BINJ             |        | 3      | 27.8   | 31.7   | --      | 25.3    | 4.7     | 11.5 |
| NZB/BINJ             |        | 3      | 27.8   | 31.7   | --      | 25.3    | 4.7     | 11.5 |
| NZB/Full             |        | 2      | 13.2   | 15.1   | --      | 24.9    | 13.4    | 5.2  |
| NZB/ler              |        | 4      | 26.0   | --     | 26.8    | 13.4    | 5.2     | 7.9  |

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

§ H-2 and Qed-1 type.

$\ddagger$ not done.

**Table II**

Specificity of Primary In Vitro Responses of NZB Lymphocytes against H-2-Compatible BALB/c Stimulators
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^n) 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^b-restricted component in the response because BALB.B (H-2^b) does not inhibit the lysis of BALB/c (H-2^n), and because the lysis of (SJL × NZB)F1 is inhibited by the F1 cells (H-2^n × H-2^b) but not to the same extent by SJL (H-2^n). The B6. Tla^a target cells (H-2^b, Qed-1^a) define the

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**Fig. 1.** Lysis of LPS-stimulated spleen cells by NZB/BINJ cells stimulated in vitro with BALB/c (•) or NZB/BINJ (○); by NZB/C3H cells primed in vivo with BALB/c and restimulated with BALB/c (●) or NZB/BINJ (○); and by C57BL/6 stimulated with NZB/BINJ (□). H-2 and Qed-1 types of the target cells are indicated on each panel.
Target: BALS/c
Inhibitor: Heed-1
None
NZB/BINJ
d a
BALB/c
d b
BALB.B
b b
C57BL/6
b b
B6.T/a
b a
A.CA
f a'
C58/J
k a
SJL
s a
(SJLxNZB)F1
s/d a

| Inhibitor | H-2 | Qed-1 | % 51Cr-release |
|-----------|-----|-------|----------------|
| None      | -   | -     | 0 60 0 60 0 60 0 60 |
| NZB/BINJ  | d   | a     |                |
| BALB/c    | d   | b     |                |
| BALB.B    | b   | b     |                |
| C57BL/6   | b   | b     |                |
| B6.T/a    | b   | a     |                |
| A.CA      | f   | a'    |                |
| C58/J     | k   | a     |                |
| SJL       | s   | a     |                |
| (SJLxNZB)F1| s/d | a     |                |

**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. Tlaa, 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-1b-specific or H-2d-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 Biologische-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-1b, making direct Mta typing easier (Table III). However, some part of the response must be directed against minor histocompatibility antigens (presumably H-2d 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-1b-specific response (C57BL/6 vs. B6.Tlaa targets in Table III) and a response against minor histocompatibility antigens (lysis of NZB/BINJ targets), but no response against Mta (failure to lyse B6.Tlaa and C58/J).
### Table III

**Comparison of NZB Substrains**

| Stimulator | NZB Substrain | Percent \(^{11}Cr\) release* |
|------------|---------------|-------------------------------|
|            |               | CrBom | B6.Tla* | B6.Tla | C57Bl/6 | C58/J |
|            |               | In Vivo | In Vitro | In Vivo | In Vitro |
| 1 NZB/CrBom BALB/c 1×§ | BALB/c | 5.1 | 1.5 | 0.8 | 38.0 | 47.1 | 45.9 |
| 2 NZB/CrBom BALB/c 1×§ | BALB/c | 3.6 | 1.6 | 0.7 | 49.6 | 49.0 | 47.5 |
| 3 NZB/CrBom BALB/c 1×§ | BALB/c | 3.9 | 1.6 | 0.8 | 69.2 | 49.0 | 47.5 |
| 4 NZB/CrBom BALB/c 1×§ | BALB/c | 5.1 | 1.5 | 0.8 | 38.0 | 47.1 | 45.9 |
| 5 NZB/CrBom BALB/c 1×§ | BALB/c | 3.6 | 1.6 | 0.7 | 49.6 | 49.0 | 47.5 |
| 6 NZB/CrBom BALB/c 1×§ | BALB/c | 3.9 | 1.6 | 0.8 | 69.2 | 49.0 | 47.5 |

* LPS-stimulated spleen cells; 30 initial responder cells/target cell.
§ H-2 and Qed-I types.
\[\text{I}x, \text{once; 2x, twice.}\]
\[\text{I}x, \text{not done.}\]

### 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 \(^{11}Cr\) release from target cells* from |
|-----------|--------------------------|-------------------------|-----------------------------------------------|
|           |                          | BALB/c | B6.Tla* | C58/J |
|           |                          |       |         |       |
| NZB/CrBom-1*§ | Nylon wool purified§ | 66.2 | 40.3 | 74.2 | 61.0 |
| NZB normal (3 × 10^6) | Anti-Ig column passed | 64.8 | 33.6 | 70.9 | 52.2 |
| Anti-Thy-1,2 + C' (3 × 10^6) | C' alone | 64.8 | 33.6 | 70.9 | 52.2 |
| NZB normal (3 × 10^6) + NZB-1* (3 × 10^6) | Anti-Thy-1,2 + C' | 40.5 | 12.5 | 61.0 |
| NZB normal (3 × 10^6) + NZB-1* (1.8 × 10^6) | Anti-Thy-1,2 + C' | 40.5 | 12.5 | 61.0 |
| NZB/CrBom-1*§ | Anti-Thy-1,2 + C' | 63.8 | 43.3 | 80.2 | 61.0 |

* LPS-stimulated spleen cells; 30 initial responder cells/target cell.
§ T* 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.
\[\text{I}x, \text{not done.}\]

Immunization of NZB/Füll with NZB/Bom generates an H-2d-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/BiP. 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*, 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-2d) and C58/J (Mta+, H-2k). 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-2k.

The distribution of Mta among the RI lines showed no association with H-2 (among the positive mice, four were H-2k and four were H-2d; among the negatives, three were H-2k and two were H-2d) or with any other marker for which these mice have been typed (7). 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 (NZB/F1) × (NZB × (C57BL/6 × NZB)).
TABLE V
Presence of Mta on Various Tumor Cell Lines

| Inhibitor      | Anti-Mta* | Anti-H-2k* |
|----------------|-----------|------------|
|                | B6.Tla⁺  | C3H/Tif‡  | C58/J‡ | C3H/Tif‡ |
|                | Experiment 1 | Experiment 2 | Experiment 1 | Experiment 2 |
| None           | 39.0§     | 40.5      | 73.3    | 47.2     |
| NZB/CrBom      | 32.5      | 44.7      | 71.3    | 39.6     |
| NZB/Full       | 18.3      | 8.5       | 72.9    | 40.1     |
| BALB/c         | 9.2       | 9.5       | 72.7    | 39.1     |
| B6.Tla⁺        | 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/Full in experiment 2. Anti-H-2k: NZB/Full 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.

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
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

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**Table:**

| Inhibitor | H-2 | Anti-Mta | Anti-H-2<sup>2</sup> | Anti-H-2<sup>k</sup> |
|-----------|-----|----------|----------------------|---------------------|
| None      |     |          |                      |                     |
| NZB/ICr   | d   |          |                      |                     |
| C3H/HeJ   | k   |          |                      |                     |
| NZB/ICr   | d   |          |                      |                     |
| C3H/HeJ   | k   |          |                      |                     |
| SJL       | a   |          |                      |                     |
| NXB -     | k   |          |                      |                     |
| 4         | k   |          |                      |                     |
| 5         | d   |          |                      |                     |
| 6         | d   |          |                      |                     |
| 9         | k   |          |                      |                     |
| 13a k     | 13b k |        |                      |                     |
| 15        | d   |          |                      |                     |
| 16        | k   |          |                      |                     |
| 17        | k   |          |                      |                     |
| 18        | d   |          |                      |                     |
| 19        | d   |          |                      |                     |
| 20        | d   |          |                      |                     |

**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), NXB-17 anti-BALB/c (anti-H-2<sup>a</sup>), and NXB-20 anti-C3H/HeJ (anti-H-2<sup>k</sup>).
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/Full 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-1b. Responsiveness does not depend on the H-2d haplotype; cells from Mta- NX8-3 and NX8-4 (H-2b) 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. Mta+ NMRI mice are bred for their health and vigor, whereas the Mta+ NZB/BIPt
Fig. 6. Typing of first generation backcrosses of \((A.CA \times NZB/CrBom) F1\) 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 \((A \times N)\) No. 2 and 3 and \((A \times N) \times N\) No. 4 were considered positive for H-2\(^f\). The \((A \times N)F1\) was the father of the N x \((A \times N)\) backcross mice. All backcross mice were males.

**Table VI**

|Mta and H-2 Typing of First Generation Backcrosses of \((A.CA \times NZB/CrBom) F1\) to NZB/CrBom|
|---|---|---|---|---|---|
|Cross| A. Mta\(^+\)| Mta\(^-\)| Total| B. H-2\(^f+\)| H-2\(^f-\)| Total|
|---|---|---|---|---|---|---|
|N x \((A \times N)\)\(^*\)| 0| 13| 13| N x \((A \times N)\)| 6| 6| 12\(\|\)
|(A x N) \times N\(^\ddagger\)| 12| 0| 12| (A x N) \times N| 3| 8| 11\(\|\)
|Totals| 12| 13| 25| Totals| 9| 14| 23|

\(P = 1.9 \times 10^{-7}\)\(^\S\)

\(\|\) 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.

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-1b, H-2 restricting 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 MtaNZB/Icr and Mta C57/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.

We thank Dr. J. Bruce Smith for his interest and hospitality, Dr. Michael Potter for information and breeding pairs, Barbara Hausmann for skilful technical assistance, and Hans-Peter Meyer for careful handling of the Basel breeding program.

Received for publication 8 July 1980.

References

1. Howie, J. B., and B. J. Helyer. 1968. The immunology and pathology of NZB mice. Adv. Immunol. 9:215.

2. Botzenhardt, U., J. Klein, and M. Ziff. 1978. Primary in vitro cell-mediated lympholysis reaction of NZB mice against unmodified targets syngeneic at the major histocompatibility complex. J. Exp. Med. 147:1435.

3. Theofilopoulos, A. N., D. L. Shawler, D. H. Katz, and F. J. Dixon. 1979. Patterns of immune reactivity in autoimmune murine strains. I. Cell-mediated immune responses induced by H-2 identical and H-2 incompatible stimulator cells. j. Immunol. 122:2319.

4. Fischer Lindahl, K., and B. Hausmann. 1980. Qed-1—a target for unrestricted killing by T cells. Eur. J. Immunol. 10:289.

5. Rich, R. R., D. A. Sedberry, D. L. Kastner, and L. Chu. 1979. Primary in vitro cytotoxic response of NZB spleen cells to Qa-1-associated antigenic determinants. J. Exp. Med. 150:1555.

6. Klein, J., L. Flaherty, J. L. VandeBerg, and D. C. Shreffler. 1978. H-2 haplotypes, genes, regions, and antigens: first listing. Immunogenetics. 6:489.

7. Riblet, R., L. Claflin, D. M. Gibson, B. J. Mathieson, and M. Weigert. 1980. Antibody gene linkage studies in (NZB x C58) recombinant inbred lines. J. Immunol. 124:787.

8. Fischer Lindahl, K., and B. Hausmann. Expression of the I-E target antigen for T cell killing requires two genes. Immunogenetics, In press.

9. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645.

10. Wigzell, H. 1976. Specific affinity fractionation of lymphocytes using glass or plastic bead columns. Scand. J. Immunol. 5(Suppl. 5):23.

11. Altman, P. L., and D. D. Katz, editors. 1979. Inbred and genetically defined strains of laboratory animals. Part I. Mouse and rat. Federation of American Societies for Experimental Biology, Bethesda, Md. 16.

12. Schreier, M. H., and R. Tees. 1979. Clonal induction of helper T cells: Conversion of specific signals into nonspecific signals. Int. Arch. Allergy Appl. Immunol. 61:227.

13. Blair, P. B. 1968. The mammary tumor virus (MTV). Curr. Top. Microbiol. Immunol. 45:1.
14. Stutman, O., and F.-W. Shen. 1978. H-2 restriction and non-restriction of T-cell-mediated cytotoxicity against mammary tumour targets. *Nature (Lond.)* 276:181.

15. Klein, J. 1978. Genetics of cell-mediated lymphocytes in the mouse. *Springer Sem. Immunopathol.* 1:31.

16. Raveché, E. S., A. D. Steinberg, L. W. Klassen, and J. H. Tjio. 1978. Genetic studies in NZB mice. I. Spontaneous autoantibody production. *J. Exp. Med.* 147:1487.