ON THE T CELL HYPERREACTIVITY OF NZB MICE AGAINST H-2-IDENTICAL CELLS
Evidence for Primary Response
Characteristics and an Increased Helper Potential*

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NZB mice spontaneously develop a systemic lupus erythematoses-like disease characterized by the occurrence of numerous autoantibodies and abnormalities in a number of immune functions and immunoregulatory circuits (1-3). In 1978 we described a unique capacity of NZB mice to develop cytotoxic T cells directed against H-2-identical other strains of mice in a primary in vitro system (4). In contrast to NZB mice, normal mice can react against H-2-identical targets only after in vivo preimmunization. These findings have been confirmed (5, 6).

Another striking observation described in the original report (4) was an apparent cross-reactivity of NZB cells sensitized against H-2-identical cells on various H-2-different targets. In a recent publication, Rich et al. (6) present evidence that the antigens recognized by NZB mice are $Qa-1^b$-coded determinants. $Qa-1^b$ is expressed in all H-2d strains typed for $Qa-1$ so far except NZB (which carry the $Qa-1^a$ allele) and provokes an H-2-unrestricted cytotoxic T cell response, which, in normal mice, requires in vivo presensitization (7, 8).

In experiments with different NZB hybrids ($Qa-1^b/Qa-1^a$), we will demonstrate that (a) the hyperreactivity of NZB mice is also apparent in F1 hybrids between NZB and normal mice despite of the fact that the targets are H-2 identical and possess the $Qa-1^b$ allele, and (b) this response is not restricted by the H-2 complex.

The fact that the hyperreactive cell-mediated lympholysis (CML)1 response against H-2-identical other strains of mice can only be observed in the autoimmune NZB mouse and its F1 hybrids, has provoked the assumption this unusual response might be directly related to the autoimmune status of these mice. In this context, we, as well as other investigators (5, 6), have put forward the following hypothesis: The NZB response observed in vitro might not be a primary, but, rather, a secondary, type of response that occurs as a consequence of some in vivo priming event against cross-reactive self-determinants. Experiments performed to clarify this question, however, did not confirm this hypothesis, but established the primary response character of the in vitro reaction of NZB mice against H-2-identical targets. Instead, some additional experiments would rather suggest that the response is connected with an unusually high helper potential in NZB mice as compared with normal mice.

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1 Abbreviations used in this paper: CML, cell-mediated lympholysis, Con A, concanavalin A, MEM, minimum essential medium
Materials and Methods

**Mice.** NZB mice (H-2^d and Qa-1^a) were purchased from The Jackson Laboratory, Bar Harbor, Maine; BALB/c (H-2^d and Qa-1^b); B10D2 (H-2^b and Qa-1^b), BALB/k (H-2^b and Qa-1^b), CBA (H-2^k and Qa-1^b), and A/J (H-2^a and Qa-1^a) were obtained from Olac, Shaw's Farm, Bicester, Oxon, England. The hybrid strains used were locally bred from parental animals obtained from the institutions mentioned above. Male and female mice were used at the age of 6–12 wk.

**Media.** Minimum essential medium (MEM) (Grand Island Biological Co., Grand Island, N.Y.) was used for cell washing. For cultures, RPMI-1640 (Grand Island Biological Co.) was supplemented with 2 mM L-glutamine, 5 × 10^{-5} M 2-mercaptoethanol, 10 mM Hepes buffer, 100 U/100 ml penicillin, 100 μg/100 ml streptomycin, and 5% human AB serum.

**Cell Suspensions.** Spleen cells were removed aseptically, minced in MEM, and washed three times. Viability was checked by trypan blue exclusion.

**Cell Culture.** A microculture system, originally described by Simpson et al (9), was used with slight modifications. Briefly, responder cells were adjusted to 5 × 10^6 cells/ml in culture medium, and 100 μl of the cell suspension was placed into flat-bottomed microculture dishes (C A Greiner und Söhne, Nürtingen, Federal Republic of Germany). Stimulator cells were adjusted to the same concentration and inactivated with 2,000 rad (RT 200, Philips Electronic Instruments, Inc., Mahwah, N. J.) or treated with mitomycin C (25 μg of mitomycin C/10^7 cells) (Serva Feinstenchemie GmbH & Co., Heidelberg, Federal Republic of Germany). Usually 12 wells of each responder cell suspension plus the respective stimulator cells were set up in parallel. Cultures were incubated for 5 d at 37°C in 5% CO₂ in normal atmosphere on a rocker platform at 5 cycle/min.

**Target Cells.** Concanavalin A (Con A) blasts or P815 (H-2^d) tumor cells (Qa-1^b positive; K. Fischer-Lindahl. Personal communication.) were used as targets for the cytotoxic reaction. Later (Results), we shall show that the lysis obtained with P815 tumor cells is comparable to that of Con A blasts with regard to specificity, but reaches higher values. Blasts were prepared by incubation of spleen cells at 4 × 10^6/ml in culture medium that contained 5 μg of Con A/ml for 48 h. Tumor cells were maintained in culture medium supplemented with 5% fetal calf serum instead of human serum.

For the cytotoxic assay, target cells were incubated with 100–200 μl sodium [51Cr]chromate (Amersham-Buchler, Frankfurt, Federal Republic of Germany) for 1 h at 37°C. Subsequently, they were washed three times (Con A blast cells were separated on a Ficoll-Isoaque [Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.] gradient after labeling to remove dead cells) and adjusted to 5 × 10^6 cells/ml. 100 μl of chromium-labeled target cells was added to the final assay.

**Cytotoxicity Assay.** Groups of 12 wells set up in parallel were pooled on day 5 of the culture. The cells were washed once and adjusted to 1.5–2.0 × 10^6 cells/ml. Serial dilutions of the effector cells were titrated against 5,000 [51Cr]-labeled targets and incubated for 3–4 h in a final vol of 200 μl. After incubation, the cells were spun down, and 100 μl of the supernate was transferred to another tube. Radioactivity of the corresponding tubes was measured in a gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.), and percent specific [51Cr] release was calculated according to the following formula:

\[
\text{Percent specific lysis} = \frac{\text{experimental counts per minute} - \text{background counts per minute}}{\text{maximal counts per minute} - \text{background counts per minute}} \times 100.
\]

Maximum release was determined by incubation of target cells in Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.).

**Preparation of Helper Cells.** Helper cells were prepared according to Pilarski (10), with the splenic lymphocytes of animals immunized 3 wk to several months before. T cells were purified over nylon-wool columns (11) and irradiated with 1,000 rad before adding them to the cell cultures.

**Preparation of Helper Factor.** Long-term mixed lymphocyte cultures of NZB cells against BALB/c stimulators were set up according to the method described by Ryser et al. (12). After
10 d of culture the cells were harvested and rechallenged with the originally used stimulator cells. 24 h after rechallenge, the cells were spun down, and the culture supernate was used as a source for helper activity.

Heat Treatment of the Stimulator Cells. Stimulator cells were incubated for 10 min at 45°C according to Röllinghoff and Wagner (13).

UV Treatment of the Stimulator Cells. UV-light treatment of stimulator cells was performed according to Lafferty et al. (14) by exposing 8 ml of cell suspension (15 × 10⁶/ml) in an open 80-mm Petri dish to a 40 W germicidal UV lamp at a distance of 14 cm for 10 min.

Results

Evidence for Stable Target Specificity of P815 Tumor Cells. In the experiments reported, a microculture system and P815 tumor target cells were employed to increase the sensitivity of the assay. Table I gives a comparison of the specific ⁵¹Cr release obtained at effector:target ratios of 30:1 and 5:1 for Con A blasts and the tumor cell line P815. The results indicate that P815 tumor target cells are comparable to Con A blasts with regard to specificity, but the values of specific lysis are much higher with P815 tumor cells.

Reactivity of F₁ Hybrids between NZB and Normal Strains of Mice. To test whether the cellular hyperreactivity demonstrated in NZB mice is also expressed in F₁ hybrids between NZB and normal strains of mice, the experiments shown in Table II were set up. The results indicate that in neither of the hybrids tested was there a cytotoxic response toward parental cells. However, there was reactivity toward unrelated H-2-identical cells, e.g., a response of NZB × B10 hybrids against BALB/c (Table II B), an anti-B10D2 response of NZB × BALB/c hybrids (Table II A), or an anti-BALB/c response of NZB × B10D2 hybrids (Table II C).

The cytotoxicity obtained is, however, consistently lower than that in NZB parental mice (Table II D). Because these F₁ hybrids express both Qa-1 alleles—Qa-1a and Qa-1b—it is unlikely that the reactivity is directed against Qa-1b-coded determinants, unless a further heterogeneity of these determinants is assumed. In this context it was of interest to test whether the CML response of F₁ hybrids is restricted by genes of the H-2 complex. The respective data are given in Table III. NZB × B10D2 hybrids did not react against parental cells, but they developed cytotoxicity against BALB/c cells.

| Effector | Stimulator | Experiment | Percent specific lysis |
|----------|------------|------------|-----------------------|
|          |            |            | Con A blasts | P815 |
|          |            |            | 30:1 | 5:1 | 30:1 | 5:1 |
| NZB      | BALB/c     | 1          | 23.7 | 12.2 | 40.9 | 22.1 |
|          |            | 2          | 15.9 | 8.4  | 30.9 | 17.5 |
|          |            | 3          | 20.3 | 13.9 | 41.1 | 18.6 |
| BALB/c   | NZB        | 1          | -1.1 | -0.7 | -0.4 | -1.0 |
|          |            | 2          | 4.7  | 0.4  | 0.2  | -1.0 |
|          |            | 3          | 0    | -1.3 | 4.6  | -2.0 |
| NZB      | B10D2      | 1          | 28.9 | 4.8  | 45   | 24.6 |
|          |            | 2          | 33.9 | 18.7 | 32.7 | 17.1 |

The cytotoxic reactivity of various effector cells was tested against ⁵¹Cr-labeled P815 tumor cells and ⁵¹Cr-labeled Con A blasts identical to the respective stimulator cells at effector:target ratios of 30:1 and 5:1.
TABLE II
Reactivity of F1 Hybrids between NZB and Normal Mice

| Effector           | Stimulator | Percent specific lysis |
|--------------------|------------|-----------------------|
|                    |            | 30:1 | 5 1 |
| A NZB × BALB/c     | NZB        | 5    | 2    |
| NZB × BALB/c       | BALB/c     | 9.9  | 1.7 |
| NZB × BALB/c       | B10D2      | 21   | 3.6 |
| B NZB × B10        | NZB        | 3.7  | 0.3  |
| NZB × B10          | B10D2      | 9.9  | 0.1  |
| NZB × B10          | BALB/c     | 26.1 | 6.3  |
| C NZB × B10D2      | NZB        | -1   | 0    |
| NZB × B10D2        | B10D2      | 3.3  | 0.1  |
| NZB × B10D2        | BALB/c     | 22.3 | 9    |
| D NZB              | BALB/c     | 42.8 | 21   |

Spleen cells of F1 hybrids between NZB and different normal mice were cultured with NZB, BALB/c, and B10D2 stimulators (mitomycin C treated) and tested on 51Cr-labeled P815 tumor cells.

TABLE III
Lack of H-2 Restriction in the Response of NZB × B10D2 Hybrids against BALB/c

| Effector           | Stimulator | Target | H-2 | Qa-I | Percent specific lysis |
|--------------------|------------|--------|-----|------|-----------------------|
| NZB × B10D2        | NZB        | NZB    | K^dD^d | a | 3 5 |
| NZB × B10D2        | B10D2      | B10D2  | K^dD^d | b | 0 2 |
| NZB × B10D2        | BALB/c     | BALB/c | K^dD^d | b | 19.8 |
| NZB × B10D2        | BALB/c     | BALB/b | K^dD^b | b | 12 2 |
| NZB × B10D2        | BALB/c     | BALB/k | K^dD^k | b | 12 5 |
| NZB × B10D2        | BALB/c     | A/J    | K^dD^d | a | 3 9 |

Spleen cell cultures of NZB × B10D2 hybrids were cultured with parental or BALB/c stimulator cells (2,000-rad irradiated) and tested on various H-2-identical or H-2-different 51Cr-labeled Con A blasts. Values of specific lysis were obtained at an effector target ratio of 50:1.

Effector cells generated against BALB/c, however, also lysed BALB/b (H-2^b) and BALB/k (H-2^k) targets, whereas they did not lyse A/J cells, which are compatible with BALB/c in the H-2D region, but possess the Qa-I^a phenotype.

Is the In Vitro Cytotoxic Response of NZB Mice against H-2-identical Targets a Primary or a Secondary Type of Response? In the following experiments we tested the hypothesis that the hyperreactivity of NZB mice might be the consequence of a naturally occurring autosoensitization against cross-reactive self-determinants in vivo. If this were the case, the response obtained in vitro should represent a secondary type of reaction. This assumption can easily be tested because it is known that primary cytotoxic precursors differ from secondary ones by their absolute requirement for metabolically active stimulator cells, whereas secondary cytotoxic precursors can be demonstrated after culture with UV-treated stimulator cells (13).

Accordingly, NZB cells, as well as BALB/c and B10D2 cells (derived from either preimmunized or untreated mice), were cultured with normal (2,000-rad irradiated), UV-treated (Table IV) or heat-treated stimulator cells (Table V).

Whereas effector cells of nonimmunized BALB/c or B10D2 mice cannot mount a
### Table IV

*Failure of NZB Cytotoxic T Cells to React in a Primary Culture against UV-treated Stimulator Cells*

| Effector | Stimulator | Presumunized | Percent specific lysis (normal stimulators) | Percent specific lysis (UV-treated stimulators) |
|----------|------------|--------------|---------------------------------------------|-----------------------------------------------|
| NZB      | BALB/c     | –            | 40.1%                                       | 40.1%                                         |
| NZB      | BALB/c     | +            | 81.8%                                       | 81.8%                                         |
| NZB      | B10D2      | –            | 48.6%                                       | 48.6%                                         |
| NZB      | B10D2      | +            | 94.7%                                       | 94.7%                                         |
| BALB/c   | NZB        | –            | 3.8%                                        | 3.8%                                          |
| BALB/c   | NZB        | +            | 88.2%                                       | 88.2%                                         |
| BALB/c   | B10D2      | –            | 1.4%                                        | 1.4%                                          |
| BALB/c   | B10D2      | +            | 88.2%                                       | 88.2%                                         |
| NZB      | CBA        | –            | 59.1%                                       | 59.1%                                         |
| NZB      | CBA        | +            | 87.7%                                       | 87.7%                                         |
| BALB/c   | CBA        | –            | 60.0%                                       | 60.0%                                         |
| BALB/c   | CBA        | +            | 82.2%                                       | 82.2%                                         |

In vitro spleen cell cultures of nonimmunized or in vivo pres immunized animals (15×10^6 cells 20 d before culture) were set up against normal (2,000 rad) or UV-treated stimulator cells and tested against ⁵¹Cr-labeled P815 target cells on day 5.

### Table V

*Failure of NZB Cytotoxic T Cells to React in a Primary Culture against Heat-treated Stimulator cells*

| Effector | Stimulator | Presumunized | Percent specific lysis (normal stimulators) | Percent specific lysis (45°C treated stimulators) |
|----------|------------|--------------|---------------------------------------------|-----------------------------------------------|
| NZB      | BALB/c     | –            | 55.7%                                       | 2.6%                                          |
| NZB      | BALB/c     | +            | 86.3%                                       | 86.1%                                         |
| BALB/c   | NZB        | –            | 13.8%                                       | ND                                            |
| BALB/c   | NZB        | +            | 94.1%                                       | 87.6%                                         |
| NZB      | CBA        | –            | 40.2%                                       | 2.9%                                          |
| NZB      | CBA        | +            | 69.8%                                       | 62.1%                                         |
| BALB/c   | CBA        | –            | 83.4%                                       | 28.2%                                         |
| BALB/c   | CBA        | +            | 78.9%                                       | 59.6%                                         |

In vitro spleen cell cultures of nonimmunized or in vivo pres immunized animals (15×10^6 cells 20 d before in vitro culture) were set up against normal (2,000 rad) or heat-treated (45°C for 10 min) stimulator cells. The values of specific lysis given in the table were obtained at an effector target ratio of 30:1 on ⁵¹Cr-labeled P815 targets. ND, not done.

Primary in vitro cytotoxic response against H-2-identical stimulator cells (whether these are normal, UV-, or heat-treated), nonimmunized NZB mice show a strong reactivity after culture with normal H-2-identical stimulator cells. No cytotoxic response, however, can be seen after culture of NZB cells derived from nonimmunized mice with UV- or heat-treated stimulator cells. After preimmunization in vivo, normal mice, as well as NZB mice, give a strong in vitro cytotoxic response against H-2-identical cells, which is not abolished by UV or heat treatment of the stimulator cells. In parallel, primary reactions of NZB and normal mice against allogeneic (H-2k) stimulator cells are abolished by UV or heat treatment of the stimulator cells, whereas...
secondary responses remain unimpaired. This experiment, then, suggests that the cytotoxic response of NZB mice toward H-2-identical targets obtained in vitro resembles a primary type of response.

Substitution of T Cell Help Enables Normal Mice to Mount a Primary In Vitro Response toward H-2-identical Cells. It has been established in the experiments described above (Tables IV and V) that the in vitro cytotoxic response of NZB mice against H-2-identical targets is not the consequence of an autoimmune-like presensitization in vivo, but represents a primary type of response. Thus, the question remains why, in contrast to normal mice, the autoimmune strain NZB develops a cytotoxic response that can easily be demonstrated after primary in vitro sensitization.

The data given below indicate that cytotoxic responses toward non-H-2 antigens require a level of T cell help that cannot be achieved by normal mice in a primary in vitro situation. In normal mice, the necessary amount of T cell help is available only after in vivo presensitization or, as our data demonstrate, after addition of helper cells or helper factors to the culture.

Table VI shows the response of BALB/c cells against NZB in the presence or absence of a helper factor derived from restimulated long-term cultures of NZB → BALB/c cells. The addition of this factor leads to a response that is comparable in magnitude to the primary response of NZB cells toward BALB/c. In contrast, no helper activity was generated in long-term cultures of BALB/c → NZB (data not shown). It was, however, possible to demonstrate helper cell activity of irradiated T cells obtained from BALB/c mice primed against NZB in vivo on primary cultures of NZB/c → NZB (Table VII D) or B10D2 → NZB (Table VII F). The irradiated cell fraction itself did not develop a cytotoxic response (Table VII G). The addition of T cell help to primary in vitro cultures of normal mouse cells is, thus, sufficient to induce a primary T cell cytotoxic response against H-2-identical targets.

These results indirectly suggest that an elevated helper T cell activity in NZB mice might facilitate their unusual primary cytotoxic response. Experiments for direct demonstration of hyperactive T cell help in NZB mice are currently in progress.

Discussion

In the experiments described in this paper, we further investigated the immunological basis underlying the unusual hyperreactive T cell response of NZB mice against H-2-identical targets.

Table VI

| Effector | Stimulator | Helper factor | Percent specific lysis |
|---------|------------|---------------|-----------------------|
| NZB     | BALB/c     | –             | 30.4 12.7             |
| BALB/c  | NZB        | –             | 18.1 9.6              |
| NZB     | BALB/c     | +             | 50.6 19.4             |
| BALB/c  | NZB        | +             | 26.9 9.6              |

Primary spleen cell cultures of NZB and BALB/c were set up against H-2-identical stimulator cells in the presence or absence of 100 μl of helper factor derived from NZB → BALB/c long-term cultures and tested on 51Cr-labeled P815 targets.
TABLE VII

| Effector       | Stimulator       | BALB/c helper cells added | Percent specific lysis |
|----------------|------------------|---------------------------|------------------------|
| A NZB          | BALB/c           | 30 5 10 1                 | 44 4 22 7              |
| B NZB          | B10D2            | 30 5 10 1                 | 42 4 10                |
| C BALB/c       | NZB              | 30 5 10 1                 | 88 8                   |
| D BALB/c       | NZB              | 30 5 10 1                 | 33 3 11 8              |
| E B10D2        | NZB              | 30 5 10 1                 | 13 0                   |
| F B10D2        | NZB              | 30 5 10 1                 | 15 8 2 5              |
| G BALB/c helper cells | NZB | 30 5 10 1                 | 0 0                   |

Spleen cells of NZB and normal mice were cultured with H-2-identical stimulator cells. Helper cells added in D and F (2 x 10^6 cells/well) were nylon-wool-purified, 1,000-rad irradiated T cells from BALB/c mice primed with NZB cells 3 wk before. Percent specific lysis was determined on 51Cr-labeled P815 targets.

Because the capacity to mount an in vitro demonstrable response against H-2-identical other strains of mice without preceding immunization in vivo is only observed in the autoimmune NZB strain and not in any normal strain of mice, it was tempting to speculate the hyperreactive responsiveness of NZB mice might be a T cell autoimmune event. The demonstration of an autoimmune basis of this response would have been the first experimental evidence for a spontaneous development of autoimmune cytotoxic T cells.

However, from the beginning there was some doubt as to this hypothetical possibility caused, for example, by the failure of NZB cells to react against NZB stimulator cells. Furthermore, Rich et al. (6) and ourselves (4) have not been able to demonstrate cross-reactions of NZB effector cells sensitized against other H-2^d cells on NZB targets as was described by Theofilopoulos et al. (5). In spite of these concerns, we tested the possibility that the cytotoxic T cell response of NZB mice measured in vitro is a secondary type of response that results from an autosensitization against self-determinants cross-reactive to the antigenic determinants recognized during in vitro culture. The experiments performed, however, speak in favor of primary response characteristics of this reaction. The in vitro reaction of NZB against H-2-identical stimulators as well as the reaction against allogeneic cells set up in comparison, was totally abolished by UV or heat treatment of the stimulator cells, whereas secondary reactions of NZB and normal mice obtained after in vivo priming remained unimpaired regardless of whether they were directed against H-2 or non-H-2 differences. These results make it unlikely that the NZB hyperreactivity is the consequence of an in vivo autosensitization, although one cannot be sure that the priming procedure employed is comparable with a hypothetical autosensitization with respect to the antigenic requirements for restimulation.

A relatively simple alternative explanation for the high response potential of NZB mice in contrast to normal mice against H-2-identical cells might be a higher frequency of the respective cytotoxic precursor cells. A proportional lack of precursors against non-H-2 antigens in normal mice was, for instance, suggested by Bevan (15) to account for their inability to mount a measurable primary response against non-H-2 antigens in vitro.

For the estimation of relative precursor cell frequencies against H-2 differences,
Bevan et al. (16) described a system in which specific effector cells were induced antigen-independently by Con A. In these experiments they obtained lysis of H-2-different targets, but not of H-2-identical targets by unspecifically activated effector cells. By using this system, with modifications according to Bonavida (17), we tested the possibility of an expanded cytotoxic precursor cell pool against H-2-identical cells in NZB mice. NZB, as well as BALB/c, cells were polyclonally activated by Con A and subsequently tested in a 16-h $^{51}$Cr-release assay (in the absence of mitogen) against P815 and LS (H-2^k) target cells. Mitogen-activated NZB effector cells developed a cytotoxic reaction against P815 targets comparable to that against allogeneic H-2^k targets, whereas BALB/c effector cells did not lyse P815 targets but did lyse allogeneic H-2^k targets (B. Stockinger and U. Botzenhardt. Unpublished results.) according to the findings of Bevan et al. (16) mentioned above.

Because the NZB effector cells have not been characterized with regard to their T cell nature and specificity, we cannot be sure that the reactivity observed with polyclonally activated NZB cells actually reflects an increased pool of cytotoxic precursors against H-2-identical cells. Experiments with the more-sensitive limiting-dilution technique are currently in progress to clarify these questions.

Cytotoxic effector T cells develop from their precursors in cooperation with helper T cells (18). So, in addition to the probably expanded precursor cell pool in NZB mice, it seems feasible to assume an elevated helper T cell potential present in NZB mice that facilitates their cellular hyperreactivity. According to this interpretation, an in vitro response against H-2-identical cells requires a helper cell level that is not achieved in normal mice unless they have been primed in vivo. Our results are in accordance with this hypothesis, because it could be shown that a primary in vitro response of normal mice against H-2-identical targets can be induced if the cultures are supplemented with a helper factor derived from NZB → BALB/c restimulated long-term cultures (Table VI).

Long-term cultures of BALB/c → NZB did not yield helper activity; however, a helper effect was achieved by the addition of BALB/c helper cells from preimmunized mice to primary cultures of normal mouse cells (Table VII).

Based on these results, we draw the conclusion—although the experimental evidence is only indirect at the moment—that an unusual amount of T cell help is present in NZB mice that facilitates their hyperreactive immune response against H-2-identical cells. This interpretation is also supported by data from Cantor et al. (2), who demonstrated an increased number of Ly-1^+^2^-^3^- cells in NZB mice as compared with age- and sex-matched BALB/c mice.

The functional activity of cytotoxic T cells can not only be influenced by helper T cells, it is also under the control of suppressor T cells. Hints pointing to an expanded pool of T cell precursors and an increased amount of T cell help do not exclude the possibility that a defect in suppressor T cells might add to the hyperreactivity observed. Defects in number and function of suppressor T cells have been described in NZB mice in different experimental systems by several investigators (19–21). Concerning cytotoxic responses against H-2-identical target cells, as investigated in our experiments, the possible influence of suppressor cells has not been tested yet, either in NZB or in normal mice.

The target antigens recognized in the reaction of NZB mice against other H-2^d strains have been identified by Rich et al. (6) as Qa-1^b-coded determinants. These
findings easily explain the cross-reactions of NZB effector cells sensitized against H-2-identical cells on H-2-different targets as observed in the original paper by Botzenhardt et al. (4), because cytotoxic responses against Qa-coded determinants have been found not to be restricted by the H-2 complex (7, 8, 22).

Our experiments with Qa-1\(^a\)/Qa-1\(^b\) F\(_1\) hybrids between NZB and normal strains of mice either indicate the existence of an H-2-unrestricted response against further target antigens besides Qa-1\(^b\)-coded determinants, or one would have to assume antigenic diversity among different Qa-1\(^b\)-positive strains. This latter possibility was, however, negated by Kastner et al. (23), who failed to demonstrate any Qa-1\(^b\) diversity with a variety of different Qa-1\(^b\)-positive cells in cold target-inhibition experiments. It was notable that in our experiments all targets that were lysed by the F\(_1\) hybrid effector cells were of that Qa-1\(^b\) phenotype (although the F\(_1\) hybrids expressed the Qa-1\(^b\) phenotype themselves), whereas the Qa-1\(^a\) target was not lysed. In contrast to Kastner’s findings, this experiment suggests heterogeneity within the Qa-1\(^b\) system or within a system linked to it. A possible explanation for these divergent results might be the hyperreactive immunostatus of NZB mice, which might enable them to react against antigenic differences to which normal mice fail to respond.

The question how the T cell hyperreactivity of NZB mice is related to their autoimmune disease remains open. According to our results, it seems to reflect abnormalities on different levels of the immune response. In this interpretation, the T cell hyperreactivity of NZB mice might prove as a useful tool in further investigations of the pathomechanism of autoimmune disease.

Summary

Experimental evidence presented in this paper suggests that the T cell hyperreactivity of NZB mice against H-2-identical target cells is a true primary response and not the consequence of an in vivo T cell autoimmune priming event. Based on additional data, we believe an elevated potential of T cell help to be present in NZB mice, which facilitates the observed hyperreactivity. F\(_1\) hybrids of NZB and normal strains of mice inherited the capacity to hyperreact against H-2-identical cells in an H-2-unrestricted fashion. Because the hybrids tested possess both Qa-1 alleles—Qa-1\(^b\) and Qa-1\(^a\)—our experiments either indicate the existence of heterogeneity within the Qa-1\(^b\) system or of an H-2-unrestricted response against additional target antigens. The T cell hyperreactivity might prove to be a valuable tool in further investigations of the pathomechanism of autoimmune disease.

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References

1. Talal, N 1976. Disordered immunological regulation and autoimmunity Immunol. Rev 31: 240.

2. Cantor, H, L. McVay-Boudreau, J Hugenberger, K Naidorf, F W Shen, and R K. Gershon 1978. Immunoregulatory circuits among T-cell sets. II. Physiologic role of feedback inhibition in vivo. absence in NZB mice J. Exp Med 147: 1116.
3. Creighton, W. D., D. H. Katz, and F. J. Dixon. 1979. Antigen-specific immunocompetency, B cell function and regulatory helper and suppressor T cell activities in spontaneously autoimmune mice. *J. Immunol.* 123:2627.

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

5. Theofilopoulos, A., 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.

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

7. Kastner, D. L., R. R. Rich, and F. Shen. 1979 Qa-1-associated antigens. I. Generation of H-2 nonrestricted cytoxic T lymphocytes specific for determinants of the Qa-1 region. *J. Immunol.* 123:1232.

8. Fischer-Lindahl, K. 1979. Unrestricted killer cells recognize an antigen controlled by a gene linked to Tla. *Immunogenetics* 8:71.

9. Simpson, E., R. Gordon, M. Taylor, J. Mertin, and P. Chandler. 1975. Micromethods for induction and assay of mouse mixed lymphocytes reactions and cytotoxicity. *Eur. J. Immunol.* 5:451.

10. Pilarski, L. M. 1977. A requirement for antigen-specific helper T cells in the generation of cytoxic T cells from thymocyte precursors. *J. Exp. Med.* 145:709.

11. 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.

12. Ryser, J.-E., J.-C. Cerottini, and K. T. Brunner. 1978. Generation of cytoxic T lymphocytes in vitro. IX. Induction of secondary CTL responses in primary long term MLC by supernatants from secondary MLC. *J. Immunol.* 120:370.

13. Röllinghoff, M., and H. Wagner. 1975. Secondary cytotoxic allograft response in vitro. I. Antigenic requirements. *Eur. J. Immunol.* 5:875.

14. Lafferty, K. J., J. S. Misko, and M. A. Cooley. 1974. Allogeneic stimulation modulates the in vitro response of T cells to transplantation antigen. *Nature (Lond.)* 249:275.

15. Bevan, M. J. 1976 Cytotoxic T cell response to histocompatibility antigens: the role of H-2. *Cold Spring Harbor Symp. Quant. Biol.* 41:519.

16. Bevan, M. J., R. E. Langman, and M. Cohn. 1976. H-2 antigen-specific cytoxic T cells induced by concanavalin A: estimation of their relative frequency. *Eur. J. Immunol.* 6:150.

17. Bonavida, B. 1977. Concanavalin A-mediated activation of antigen-primed lymphocytes into secondary cytoxic lymphocytes. *J. Exp. Med.* 145:293.

18. Cantor, H., and E. A. Boyse. 1975 Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly* cells in the generation of killer activity. *J. Exp. Med.* 141:1390.

19. Gershon, R. K., D. D. Eardley, K. Naidorf, and H. Cantor. 1978. Association of defective feedback suppressor T cell activity with autoimmunity in NZB mice. *Arthritis Rheum.* 21:180.

20. Krakauer, R. S., T. A. Waldmann, and W. Strober. 1976. Loss of suppressor T cells in adult NZB/NZW mice. *J. Exp. Med.* 144:662.

21. Steinberg, A. D. 1974. Pathogenesis of autoimmunity in New Zealand mice. V. Loss of thymic suppressor function. *Arthritis Rheum.* 17:11.

22. Forman, J., and L. Flaherty. 1978. Identification of a new CML target antigen controlled by a gene associated with the Qa-2 locus. *Immunogenetics.* 6:227.

23. Kastner, D. L., R. R. Rich, and L. Chu. 1979. Qa-1-associated antigens. II. Evidence for functional differentiation from H-2<sup>α</sup> and H-2<sup>β</sup> antigens. *J. Immunol.* 123:1239.