PRIMARY IN VITRO CYTOTOXIC RESPONSE OF NZB
SPLEEN CELLS TO Qa-1<br>ASSOCIATED ANTIGENIC
DETERMINANTS*

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We recently reported (1, 2) that cytotoxic T lymphocytes (CTL) with specificity for
Qa-1-associated determinants are generated when spleen cells from mice immunized
with H-2-identical, but Qa-1-disparate, lymphoid cells are restimulated in vitro with
Qa-1-disparate cells. Similar data have now been independently obtained from several
laboratories (3-6). The most notable feature of such cytotoxic reactions, in contrast
with CTL responses to other minor histocompatibility antigens (7), is that H-2
homology between effector and target cells in the cytotoxicity assay is not required
(1-6). CTL responses directed against Qa-1-associated determinants are specifically
blocked by addition of anti-Qa-1 antiserum to effector-target cell mixtures (2).
Moreover, although Qa-1-encoded antigens have not yet been serologically defined,
CTL responses are readily obtained in the Qa-1a anti-Qa-1b direction (2).
In no instance have we or others previously noted primary in vitro CTL responses
directed against antigenic determinants encoded within the Qa-Tla interval (2, 8, 9).
As a result of the Qa-1 antigenic disparity of the strains involved, however, we were
intrigued by the observation of Botzenhardt, et al. (10), recently confirmed by
Theofilopoulos, et al. (11), that CTL responses were obtained in primary cultures of
NZB splenocytes stimulated in vitro with H-2-identical BALB/c lymphoid cells. In
neither report was the antigenic target of these responses identified, nor was the
question of possible H-2 restrictions on these reactions clearly resolved. In this paper
we explore the antigenic specificity of such primary CTL responses of NZB lympho-
cytes stimulated with H-2-identical lymphoid cells. We provide evidence that the
major antigenic determinants recognized in these circumstances are those associated
with the Qa-1b genotype. These observations thus also constitute the initial report of
a primary in vitro immune response to antigens of the Qa-Tla system.

Materials and Methods

Mice. NZB/N mice (H-2d, Qa-1a) were provided by Dr. D. P. Huston, National Institutes
of Health, Bethesda, Md. BALB/c mice (H-2a, Qa-1b) were obtained from the Department of
Cell Biology, Baylor College of Medicine, Houston, Tex. A-Tla mice (H-2a, Qa-1b) and B6-
Tla mice (H-2b, Qa-1b) were gifts of Dr. E. A. Boyse and Dr. F. W. Shen, Memorial Sloan-

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In Vitro Generation of CTL. CTL were generated as previously described (1, 12). Except as noted, responder spleen cells were obtained from unimmunized mice. Briefly, 6 × 10⁶ responder cells were cocultured with 1 × 10⁶ irradiated H-2-identical stimulator cells for 6 d in 2 ml Eagle's minimal essential medium supplemented with fetal calf serum and 2-mercaptoethanol. Target cells were prepared from concanavalin A-stimulated blast cells and labeled with Na₂¹⁵⁸CrO₄, as previously described (12). 3-h ¹⁵⁸Cr-release assays for cell-mediated lympholysis were performed at various effector to target cell ratios, adjusted for viable cells. Data are expressed as:

\[
\text{percentage of net } ¹⁵⁸\text{Cr release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{freeze-thaw} - \text{spontaneous release}} \times 100.
\]

Results and Discussion

To evaluate the antigenic specificity of CTL generated in cultures of unprimed NZB responder splenocytes and H-2-identical BALB/c stimulator cells, cultures were harvested after 6 d of incubation and tested for CTL activity on a panel of ¹⁵⁸Cr-labeled target cells (Fig. 1 A). Two qualitatively distinguishable groups of target cells were identified, those that were lysed and those that were not. Moreover, there was no apparent H-2 restriction on this cytotoxic reaction, despite the fact that the response was directed against non-H-2 antigenic determinants. Thus, one of the two targets of each haplotype pair, H-2₃, H-2₄, and H-2₅, was lysed. Nevertheless, the antigenic target of this response is strongly suggested, because all targets sharing the Qa-1 b genotype with BALB/c were lysed, whereas Qa-1 a targets were not. The results with target cells from the congenic strain pair B6 and B6-Tla b are particularly informative. Because these mice are genetically identical both within H-2 and for background genes, and differ only by the insertion of an A strain chromosomal segment in the Qa-
Fig. 2. (A) Primary cultures of NZB anti-BALB/c CTL were assayed for cytotoxicity against the
51Cr-labeled concanavalin A blasts indicated. Spontaneous release values ranged from 27 to 45%
and errors did not exceed 6% of total releasable counts. (B) CTL responses of NZB mice primed 3
wk previously by injection with 5 × 10⁷ BALB/c spleen cells i.p. and then cocultured with
irradiated BALB/c cells for 6 d in vitro. Spontaneous release values ranged from 27 to 46% and
errors did not exceed 5% of total releasable counts. In both panels, Qa-1b targets are designated with
open symbols and Qa-1a targets with closed symbols.

Tla interval, this target cell pair maps the gene encoding this cytotoxic antigen to that
interval. Serologically identified determinants of the Qa-TL system other than Qa-1
seem unlikely to be involved in this response for several reasons: the strain distribution
of target cell lysis is compatible only with Qa-1 (13); NZB and BALB/c do not differ
for Qa-2 or Qa-3 (13); C57BL/6 and B6-Tlaa do not differ at Qa-2, Qa-3, Qat-4, or Qat-
5 (13, 14); and TL antigens have not been recognized on peripheral lymphoid cells
(15, 16). As previously noted, primary in vitro anti-Qa-1 reactions are not observed
with cells from most mouse strains. Fig. 1 B thus demonstrates that a significant
Qa-1a-associated cytotoxic reaction was not observed in the reverse direction (i.e., BALB/c
responder cells and NZB stimulator cells).

The foregoing experiment strongly suggests that a primary Qa-1b-associated cyto-
toxic reaction develops when NZB lymphocytes are stimulated in vitro with
BALB/c cells; it does not evaluate the possible concomitant generation of cytotoxic
responses against other cell surface determinants, such as minor histocompatibility
antigens or viral antigens, because the H-2 homology between effector and target cells
required for recognition of such responses was not provided (6, 12). Employing a
second panel of 51Cr-labeled target cells, the data in Fig. 2 A investigate this possibility.
Several points are readily apparent: first, utilizing several strains different from those
shown in Fig. 1, two groups of target cells were again distinguished. The cytotoxic
reaction was again independent of H-2 haplotypes, but correlated perfectly with the
Qa-1 type (Qa-1b target cells were lysed; Qa-1a target cells were not); second, the
congenic strain pair utilized in this experiment, A/J and A-Tlaa, which is identical
with A strain except for a B6 chromosomal segment in the Qa-Tla interval, again
provides evidence that the antigen recognized in this reaction is, in fact, associated
with Qa-1-encoded determinants. In addition, this strain pair also provides a degree
of H-2 genetic homology which should allow recognition of additional reactions
against minor histocompatibility antigens or viral antigens in NZB anti-BALB/c
cultures (6, 17), because H-2a target cells are H-2d. In contrast with A-Tlaa targets,
however, A/J targets were not lysed. Thus, the data provide no evidence for generation
of such H-2-restricted CTL responses. To provide assurance that possible minor histocompatibility locus-associated killing could be observed in this particular combination of stimulator and target cells, NZB mice were primed by injection of $5 \times 10^7$ BALB/c splenocytes i.p., and 3 wk later, spleen cells from primed animals were restimulated in vitro for 6 d with BALB/c stimulator cells (Fig. 2B). Because under these circumstances, both $A-Tla^b$ and $A/J$ target cells were lysed, we conclude that the background antigenic cross-reactivities between BALB/c and $A/J$, and the $H-2D^d$ homology between NZB and $A/J$ were sufficient to reveal minor locus-specific cytotoxic reactions of appropriately primed NZB splenocytes.

Fig. 3 demonstrates that BALB/c cells were not unique in stimulating primary Qa-1$^b$-associated NZB CTL. When NZB spleen cells were cocultured with irradiated B10.D2 cells, good cytotoxic reactions were observed against both B10.D2 and $H-2b$, Qa-1$^b$ B10 target cells. In contrast, the Qa-1$^a$ target cells, B10.A and $A/J$, were lysed minimally, if at all. By providing targets congenic with the stimulator cell strain, Fig. 3 thus illustrates the marked difference in CTL responses against Qa-1-associated antigenic determinants and those with specificity for C57BL/10 minor locus antigens, as shown by the $H-2D^d$ homologous B10.A target cells. Finally, it should be noted that in neither this nor the preceding experiments were syngeneic NZB targets significantly lysed by BALB/c-stimulated NZB effector cells.

Mouse strains which do not develop the autoimmune diseases characteristic of NZB mice require presensitization in vivo for the induction of both H-2-restricted cytotoxic responses to minor histocompatibility antigens (7; D. L. Kastner. Unpublished observations.) and H-2-nonrestricted responses to Qa-1-associated antigens (1, 2). Nonetheless, after primary in vitro stimulation of NZB splenocytes with both Qa-1$^b$-associated determinants and the numerous minor locus determinants encoded by the BALB/c and C57BL/10 backgrounds, preferential stimulation of Qa-1$^b$-specific responses was demonstrable. Spleen cells from NZB mice thus behave in vitro as though they had been previously primed to Qa-1-encoded antigens, but not to other minor histocompatibility antigens. In this respect, the data suggest a similarity between Qa-1 antigens and the stronger antigens of the major histocompatibility complex. It is therefore also interesting that spleen cells from unprimed NZB mice display CTL
response kinetics which resemble secondary responses when stimulated in vitro with H-2-disparate cells (D. P. Huston, personal communication).

It is not yet determined whether Qa-1 hyperreactivity is a secondary effect of the autoimmune process in NZB mice or whether it may, in fact, be involved in the pathogenesis of the disease. It is provocative, however, that Qa-1\(^*\), Ly-1,2,3\(^*\) T cells appear to serve an important role in mediating feedback inhibitory effects for antibody responses (18), and that NZB mice exhibit substantial deficits in such cell populations, both in numbers and in functional activity (19). It is probable that the NZB anti-BALB/c CTL response does not reflect an autoimmune process per se, because it is directed against determinants specified by the Qa-1\(^k\) allele. In this context, however, it is interesting to note an unexplained difference in the observations of Theofilopoulos, et al. (11) from those of Botzenhardt, et al. (10) and ourselves; i.e., the former investigators did observe a substantial cytotoxic response of CTL harvested from NZB anti-BALB/c cultures for syngeneic NZB targets.

Theofilopoulos, et al. (11) also reported that similar cytotoxic responses were not generated in spleen cell cultures of MRL/1 or BXSB mice and H-2-identical stimulator cells, despite the fact that such mice also exhibit autoimmune syndromes (20). Nevertheless, it seems probable, based on the target cells utilized, that if MRL/1 and BXSB mice are capable of primary anti-Qa-1 CTL responses, this has not yet been tested with an appropriate target cell panel. Such studies, currently in progress, may provide further insight into the pathogenesis of those murine and human autoimmune diseases in which defective suppressor T-cell function has been demonstrable.

**Summary**

We have shown that cytotoxic lymphocytes generated in primary cultures of NZB spleen cells with H-2-identical BALB/c or B10.D2 stimulator cells exhibit specificity for Qa-1\(^k\)-associated antigenic determinants. This unidirectional cytotoxicity constitutes the initial demonstration of a primary in vitro response to antigens of the Qa-Tla system. Such responses do not require H-2 homology between effector and target cells in the assay system. In fact, when H-2\(^D\) homologous target cells were employed there was little, if any, evidence for development of primary H-2-restricted responses to minor locus histocompatibility antigens or viral antigens. In view of the recently defined role of Qa-1\(^*\), Ly-1,2,3\(^*\) cells as regulators of antibody responses, and of the deficiency of such cells in NZB mice, the observation of hyperreactivity for determinants of this system may be relevant to the development of autoimmunity in these animals.

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