Brief Definitive Report

PRIMARY IN VITRO CYTOTOXIC RESPONSE OF NZB SPLEEN CELLS TO *Qa-1*^{b}-ASSOCIATED ANTIGENIC DETERMINANTS*

By ROBERT R. RICH, DEBORAH A. SEDBERRY, DANIEL L. KASTNER, AND LILY CHU

From the Howard Hughes Medical Institute Laboratory, and the Departments of Microbiology and Immunology and Medicine, Baylor College of Medicine; and The Methodist and Veterans Administration Hospitals, Houston, Texas 77030

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*^{a}-associated determinants are specifically blocked by addition of anti-Qa-1 antiserum to effector-target cell mixtures (2). Moreover, although *Qa-1*^{b}-encoded antigens have not yet been serologically defined, CTL responses are readily obtained in the *Qa-1*^{a} anti-*Qa-1*^{b} 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 lymphocytes 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-1*^{b} 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-2*^{d}, *Qa-1*^{a}) were provided by Dr. D. P. Huston, National Institutes of Health, Bethesda, Md. BALB/c mice (*H-2*^{d}, *Qa-1*^{b}) were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, Tex. A-*Tla*^{b} mice (*H-2*^{a}, *Qa-1*^{b}) and B6-*Tla*^{a} mice (*H-2*^{b}, *Qa-1*^{a}) were gifts of Dr. E. A. Boyse and Dr. F. W. Shen, Memorial Sloan-

* Supported in part by U. S. Public Health Service research grant AI 13810. Computational assistance was provided by the CLINFO Core Facility, U. S. Public Health Service research grant RR 00350.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/79/12/1555/06 $1.00
Volume 150 December 1979 1555–1560

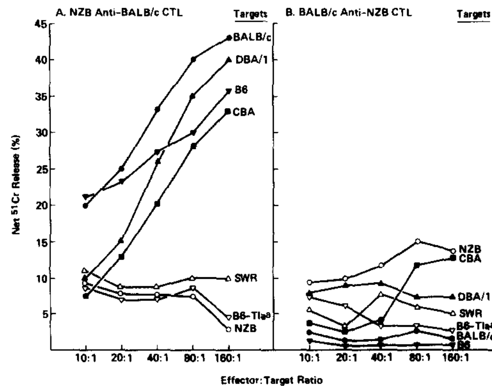

FIG. 1. (A) Primary cultures of NZB anti-BALB/c CTL were assayed for cytotoxicity against the ^{51}Cr-labeled concanavalin A blasts indicated. (B) Primary cultures of BALB/c anti-NZB CTL against the same target cell panel. *Qa-1*^{a} targets are designated with open symbols and *Qa-1*^{b} targets with closed symbols. Spontaneous release values ranged from 20 to 41% and errors did not exceed 7% of total releasable counts.

Kettering Cancer Center, New York. Mice of the following strains were obtained from The Jackson Laboratory, Bar Harbor, Maine, or were bred in our animal facilities from Jackson Laboratory breeding trios: A/J (*H-2*^{a}, *Qa-1*^{a}); B10.A (*H-2*^{a}, *Qa-1*^{a}); C57BL/6 (B6, *H-2*^{b}, *Qa-1*^{b}); B10.D2 (*H-2*^{d}, *Qa-1*^{b}); B10.BR (*H-2*^{k}, *Qa-1*^{a}); CBA/J (*H-2*^{k}, *Qa-1*^{b}); SJL (*H-2*^{s}, *Qa-1*^{a}); A.SW (*H-2*^{s}, *Qa-1*^{b}); SWR (*H-2*^{q}, *Qa-1*^{a}); and DBA/1 (*H-2*^{q}, *Qa-1*^{b}). All mice used as donors of responder and stimulator cells were sex-matched. Responder cells were obtained from mice 5–10 wk of age.

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, 6×10^6 responder cells were cocultured with 1×10^6 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 $Na_2[^{51}Cr]O_4$, as previously described (12). 3-h ^{51}Cr-release assays for cell-mediated lympholysis were performed at various effector to target cell ratios, adjusted for viable cells. Data are expressed as:

$$\text{percentage of net } ^{51}\text{Cr release} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{freeze-thaw} - \text{spontaneous release}} \times 100.$$

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 ^{51}Cr-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-2*^{q}, *H-2*^{b}, and *H-2*^{k}, 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*^{a} 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-*

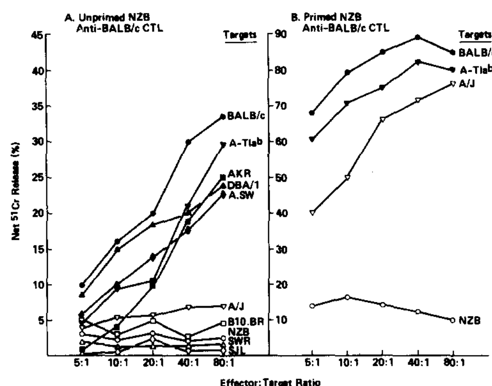

FIG. 2. (A) Primary cultures of NZB anti-BALB/c CTL were assayed for cytotoxicity against the ^{51}Cr-labeled concanavalin A blasts indicated. Spontaneous release values ranged from 27 to 45% and errors did not exceed 6% of total releasable counts. (B) CTL responses of NZB mice primed 3 wk previously by injection with 5×10^7 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-1*^{a} targets are designated with open symbols and *Qa-1*^{b} 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-*Tla*^{a} 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-1*-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-1*^{b}-associated cytotoxic 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 ^{51}Cr-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-1*^{b} target cells were lysed; *Qa-1*^{a} target cells were not); second, the congenic strain pair utilized in this experiment, A/J and A-*Tla*^{b}, 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-2*^{a} target cells are *H-2D*^{d}. In contrast with A-*Tla*^{b} targets, however, A/J targets were not lysed. Thus, the data provide no evidence for generation

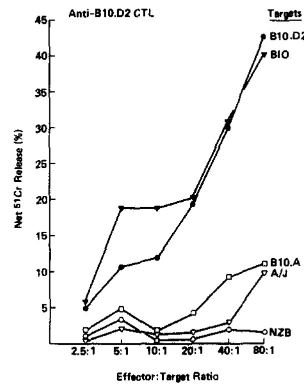

FIG. 3. Primary cultures of NZB anti-B10.D2 CTL were assayed for cytotoxicity against the ^{51}Cr-labeled concanavalin A blasts indicated. *Qa-1*^{a} targets are designated with open symbols and *Qa-1*^{b} targets with closed symbols. Spontaneous release values ranged from 32 to 44% and errors did not exceed 6% of total releasable counts.

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 × 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. 2 B). 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-2*^{b}, *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*^{b} 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*^{b}-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-2D*^{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.

We wish to thank Doctors D. P. Huston, E. A. Boyse and F. W. Shen for their gifts of mice; Dr. D. P. Huston for useful discussions and permission to include his unpublished observations; and Doctors S. S. Rich and T. Douglas for critically reviewing the manuscript. We are grateful to Mrs. Burnestine Givens and Miss Patricia Mullally for expert secretarial assistance.

Received for publication 4 September 1979.

References

1. Kastner, D. L., and R. R. Rich. 1979. *H-2*-nonrestricted cytotoxic responses to an antigen encoded telomeric to *H-2D*. *J. Immunol.* **122:**196.
2. Kastner, D. L., R. R. Rich, and F. W. Shen. 1979. *Qa-1*-associated antigens. I. Generation

of *H-2*-nonrestricted cytotoxic T lymphocytes specific for determinants of the *Qa-1* region. *J. Immunol.* **123:**1232.

3. Klein, J., and C. L. Chiang. 1978. A new locus (*H-2T*) at the *D* end of the *H-2* complex. *Immunogenetics.* **6:**235.
4. Lindahl, K. F. 1979. Unrestricted killer cells recognize an antigen controlled by a gene linked to *Tla*. *Immunogenetics.* **8:**71.
5. Forman, J. 1979. *H-2* unrestricted cytotoxic T cell activity against antigens controlled by genes in the *Qa/Tla* region. *J. Immunol.* In press.
6. Wernet, D., and J. Klein. 1979. Unrestricted cell-mediated lympholysis to antigens linked to the *Tla* locus in the mouse. *Immunogenetics.* **8:**361.
7. Bevan, M. J. 1975. The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. Exp. Med.* **142:**1349.
8. Widmer, M. B., D. J. Schendel, F. H. Bach, and E. A. Boyse. 1973. The H(Tla) histocompatibility locus: a study of in vitro lymphocyte reactivity. *Transplant. Proc.* **5:**1663.
9. 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.
10. 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.
11. 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.
12. Kastner, D. L., and R. R. Rich. 1978. Regulation of alloantigen-induced cytotoxic responses by concanavalin A-activated lymphoid cells: suppression by antigen elimination. *J. Immunol.* **121:**1732.
13. Klein, J., L. Flaherty, J. L. VandeBerg, and D. C. Shreffler. 1978. *H-2* haplotypes, genes, regions and antigens: first listing. *Immunogenetics.* **6:**489.
14. Hämmerling, G. J., U. Hämmerling, and L. Flaherty. 1979. Qat-4 and Qat-5, new murine T-cell antigens governed by the *Tla* region and identified by monoclonal antibodies. *J. Exp. Med.* **150:**108.
15. Old, L. J., E. A. Boyse, and E. Stockert. 1963. Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. *J. Natl. Cancer Inst.* **31:**977.
16. Stanton, T. H., and E. A. Boyse. 1976. A new serologically defined locus, *Qa-1*, in the *Tla*-region of the mouse. *Immunogenetics.* **3:**525.
17. Zinkernagel, R. M., and P. C. Doherty. 1975. *H-2* compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in *H-2K* or *H-2D*. *J. Exp. Med.* **141:**1427.
18. Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F. W. Shen, R. K. Gershon, and H. Cantor. 1978. Immunoregulatory circuits among T-cell sets. I. T-helper cells induce other T-cell sets to exert feedback inhibition. *J. Exp. Med.* **147:**1106.
19. Cantor, H., L. McVay-Boudreàu, 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.
20. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* **148:**1198.