Brief Definitive Report

PRIMARY CELL-MEDIATED LYMPHOLYSIS RESPONSE TO A MATERNALLY TRANSMITTED ANTIGEN*

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Cytotoxic T lymphocytes (CTL) generated by priming and restimulating murine splenocytes with *H-2*-compatible allogeneic cells are generally restricted to recognizing minor histocompatibility antigens on target cells of the same *H-2* haplotype (1). Two important exceptions to the rule of *H-2* restriction are the recognition of class I antigens encoded in the *Qa-Tla* region of the 17th chromosome (2–8) and the recognition of a maternally transmitted antigen (Mta) in secondary cell-mediated lympholysis (CML) responses of NZB mice (9). In most protocols, significant *H-2*-unrestricted cytolysis is only observed in secondary responses (2, 3, 6), but the NZB strain is unique in that such CTL can be generated without prior in vivo priming (5, 10–13). We previously reported that the NZB primary CML response to $H\text{-}2^d$ stimulators is predominantly *H-2*-unrestricted and specific for the Qa-1.2 determinant. However, when NZB mice are first primed in vivo to *H-2*-compatible cells, *H-2*-unrestricted lysis of target cells that do not express Qa-1.2 is also observed (9, our unpublished observations). Genetic analysis by Fischer Lindahl et al. (9) demonstrated that the additional antigen recognized by the NZB secondary CTL is maternally transmitted, hence the designation Mta.

Although other investigators have confirmed our finding that the primary NZB CML response to $H\text{-}2^d$ stimulators is predominantly Qa-1.2 specific (5, 9, 13, 14), some also observed low levels of lysis on $Qa\text{-}1^a$, non-$H\text{-}2^d$ target cells (10, 11, 13, 14). An additional observation that has been particularly difficult to interpret, in view of our previous findings of Qa-1.2-specific responses, is that heterozygous $Qa\text{-}1^{a/b}$ F_1 CTL [e.g., (NZB × B10.D2)F_1[1] anti-BALB/c] lysed target cells in an *H-2*-unrestricted manner (13, 15). Because, for genetic reasons, it appeared quite unlikely that *Qa-1*-encoded determinants were recognized in such cultures, the nature of the target antigen for this *H-2*-unrestricted response was unclear. Our interest in *H-2*-unrestricted CML responses and in the genetics of NZB hyperresponsiveness led us to investigate possible differences in specificities of NZB and F_1 CTL recovered from a primary culture with *H-2*-compatible stimulators. We found that a major target antigen recognized by the *Qa-1*-heterozygous F_1 CTL was Mta, thus constituting the first demonstration that Mta can stimulate a primary CML response.

Materials and Methods

Mice. BALB/c mice were obtained from the Department of Cell Biology, Baylor College of Medicine, Houston, TX. B6-Tla^a mice were bred in our colony. All other mice were obtained

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[1] By convention, the maternal strain is listed first in designating F_1 hybrids.

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from The Jackson Laboratory, Bar Harbor, ME, or were bred in our colony from Jackson Laboratory breeding stock. All mice used as donors of responder and stimulator cells were sex matched. Responder cells were obtained from mice 6–14 wk of age.

In Vitro Generation of CTL. CTL were generated using a modification of previously described procedures (12). Preliminary experiments demonstrated that higher levels of cytolysis could be obtained by increasing the concentration of stimulator cells from our standard density of 5×10^5/ml to a density of $2–3 \times 10^6$/ml. Thus, unprimed responder cells and irradiated *H-2*-compatible stimulator cells were mixed in equal numbers at a final cell density of $4–6 \times 10^6$/ml, and cultured for 6 d at 37°C in a humidified atmosphere of 5% CO_2 in air. The culture medium was Eagle's minimum essential medium with Hanks' balanced salt solution, supplemented as described (12), with 5% fetal calf serum (Gibco Laboratories, Grand Island, NY), and 2×10^{-5} M 2-mercaptoethanol (Eastman Kodak Co., Rochester, NY).

Assay for CTL Activity. Target cells were ^{51}Cr-labeled concanavalin A-stimulated spleen cells, prepared as previously described (12). Targets were plated at a density of 20,000 viable cells per well in 96-well round-bottomed tissue culture plates (Flow Laboratories, Rockville, MD). Cold targets were concanavalin A blasts prepared in the same manner, but not labeled. Effector cells and cold targets were adjusted for viability and added, in doubling dilutions, to the wells containing hot targets. The cells were incubated for 4–6 h at 37°C and harvested using the Titertek Supernatant Collection System (Flow Laboratories). Data are expressed as: percent net Cr release = ([experimental release − spontaneous release]/[maximum release − spontaneous release]) × 100, where the spontaneous release is the activity released from hot target cells in medium alone, and the maximum release is the activity released from hot target cells lysed with 0.5% sodium dodecyl sulfate, or by three cycles of freezing and thawing.

Results

We first compared the specificity of NZB and (NZB × B10.D2)F_1 primary CTL responding to *H-2*-compatible stimulators (Fig. 1). As previously reported (12), the NZB CML response to *H-2*^{d} stimulator cells was Qa-1.2 specific, as demonstrated by the differential lysis of targets from the *Qa-Tla* region congenic pair B6 (*H-2*^{b}, *Qa-1*^{b}) and B6-*Tla*^{a} (*H-2*^{b}, *Qa-1*^{a}) (Fig. 1 A). The greater degree of lysis of BALB/c targets than of B6 targets is consistent with *H-2*-restricted, minor histocompatibility antigen-specific cytolysis, as reported by others (10, 11, 13). We confirmed that (NZB × B10.D2)F_1 anti-BALB/c CTL can also be generated in a primary response, and the the cytolysis was *H-2*-unrestricted, with lysis of both *H-2*^{b} targets tested (Fig. 1 B). As expected, the *Qa-1*-heterozygous responders did not recognize Qa-1.2 on the target cells, as demonstrated by equivalent levels of lysis of B6 and B6-*Tla*^{a} targets. Similar to the NZB response, *H-2*-restricted, minor histocompatibility antigen-specific CTL may have been generated (compare lysis of BALB/c and B6 or B6-*Tla*^{a} targets, Fig. 1 B), but this was not observed in all experiments.

Because of the *H-2*-unrestricted nature of the F_1 primary CML response, and the report of secondary *H-2*-unrestricted lysis of cells bearing a maternally transmitted antigen (9), we asked whether *Qa-1*-heterozygous F_1 CTL could recognize Mta in a primary response. To study Mta-specific CTL, we assayed for lysis of targets from reciprocal F_1 females that share the same complement of chromosomes, but differ in their expression of Mta. Fischer Lindahl et al. (9) reported that Mta$^+$ mothers bear Mta$^+$ progeny, regardless of the male partner's phenotype. Similarly, Mta$^-$ mothers bear only Mta$^-$ progeny. All inbred strains typed by Fischer Lindahl et al., except NZB,[2] were Mta$^+$. Thus, (NZB × B10.D2)F_1 mice are Mta$^-$, but the reciprocal

[2] Outbred NMRI mice are also Mta$^-$. However, two sublines of the NZB strain, NZB/Füll and NZB/BlPt, typed positive for Mta. In all experiments shown, we used the Mta$^-$ subline NZB/BlNJ. Similar results have been obtained with the NZB/N subline, which was not typed by Fischer Lindahl et al. (9).

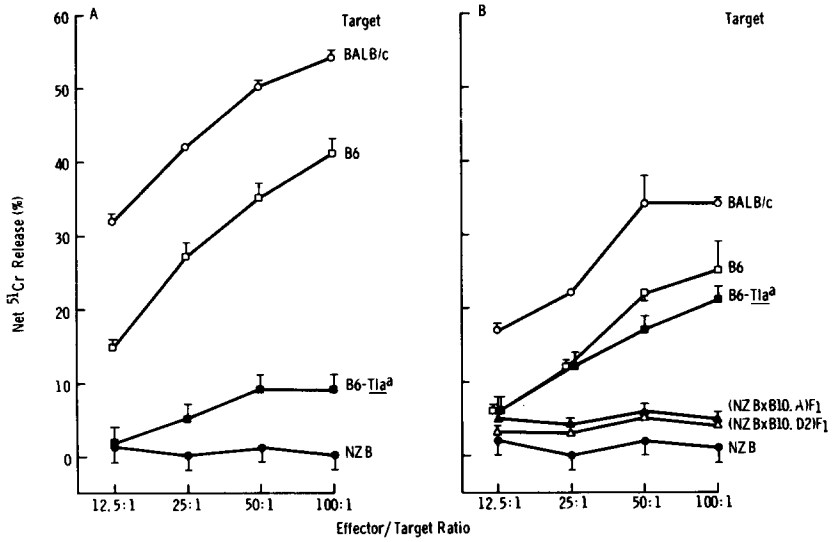

FIG. 1. NZB (A) or (NZB × B10.D2)F_1 (B) splenocytes were stimulated in vitro with irradiated BALB/c splenocytes and assayed for cytotoxicity against a panel of ^{51}Cr-labeled targets. Spontaneous release values were 5–11% of the maximum release for each target. The vertical bars represent 1 SE of the mean of triplicate determinations.

(B10.D2 × NZB)F_1 mice are Mta$^+$. The use of females as the donors of target cells rules out sex-linked transmission of the target antigen for the CTL. Fig. 2A depicts the results of such an experiment in which primary (NZB × B10.D2)F_1 anti-BALB/c CTL were assayed on a panel of hot targets. The Mta$^+$ targets, (B10.D2 × NZB)F_1 and (B10.BR × NZB)F_1, were lysed to the same extent as other Mta$^+$ targets used in this experiment, but the reciprocal Mta$^-$ F_1 targets, (NZB × B10.D2)F_1 and (NZB × B10.BR)F_1, were lysed at a much lower level. On the other hand, differential lysis of reciprocal F_1 targets was not observed when the CTL were generated from (B10.D2 × NZB)F_1 anti-BALB/c cultures, in which both responder and stimulator cells were Mta$^+$ (Fig. 2B). In addition, the Mta$^+$ F_1 CTL did not lyse the non-*H-2*^{d} targets (B6 and B6-*Tla*^{a}). The low levels of lysis on syngeneic and other *H-2*^{d} targets was observed in approximately one-half of our experiments with the F_1 responders. This may represent *H-2*-restricted lysis specific for serum components or for virus on the blasts used as targets.

Although the lysis of *H-2*^{b} targets (B6 and B6-*Tla*^{a}) in Fig. 2A demonstrated *H-2*-unrestricted lysis, it was important to demonstrate specifically that the lysis of the Mta$^+$ F_1 targets was not restricted by the *H-2*^{d}-encoded determinants shared with the effectors. Cold target inhibition experiments were performed to address this issue (Fig. 3). Lysis of the ^{51}Cr-labeled target, C57BL/10 (B10, *H-2*^{b}, Mta$^+$), was inhibited by cold targets from the Mta$^+$ (B10.D2 × NZB)F_1, but not by those from Mta$^-$ (NZB × B10.D2)F_1. Thus, the relevant antigen recognized by *H-2*-unrestricted CTL on the B10 targets is the same as that recognized on the Mta$^+$ F_1 targets.

Discussion

The nature of the target antigens for NZB anti-*H-2*^{d} primary CTL has been a subject of controversy. Although most investigators agree that Qa-1.2 is a major target antigen and that *H-2*-restricted minor histocompatibility antigen-specific lysis may

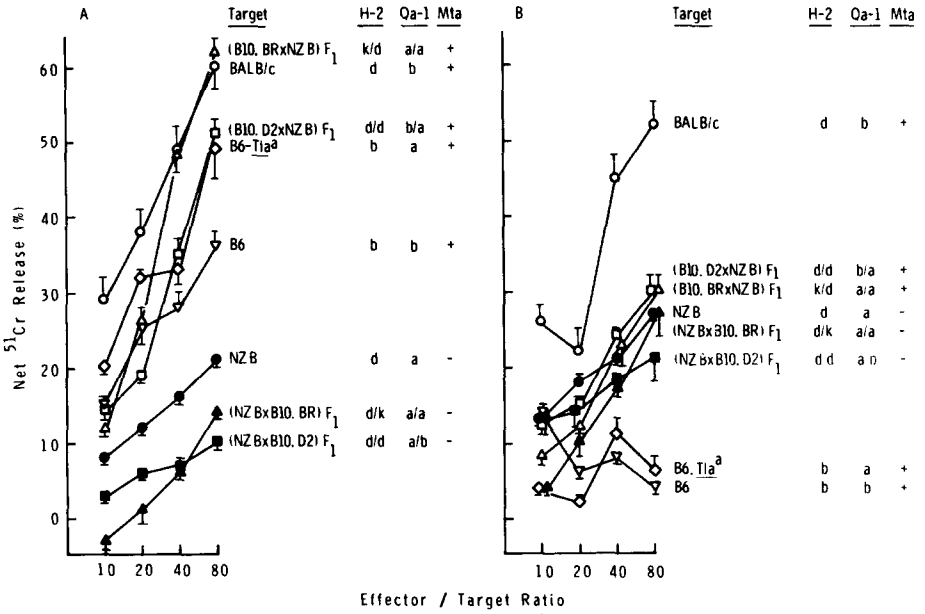

FIG. 2. (NZB × B10.D2)F_1 (A) or (B10.D2 × NZB)F_1 (B) splenocytes were stimulated in vitro with irradiated BALB/c splenocytes and assayed for cytotoxicity against a panel of ^{51}Cr-labeled target cells. Spontaneous release values were 19–27% of the maximum release for each target. The vertical bars represent 1 SE of the mean of triplicate determinations.

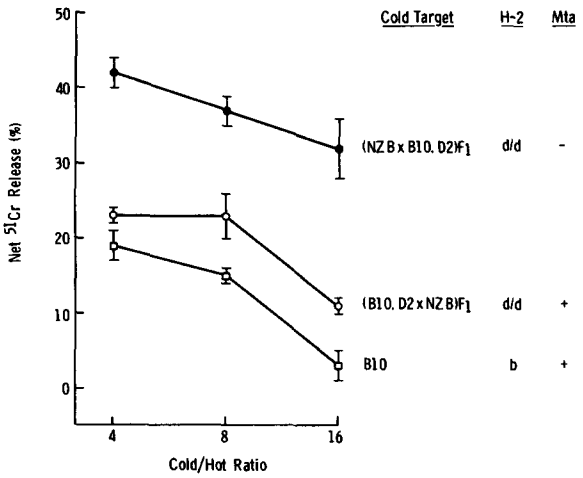

FIG. 3. (NZB × B10.D2)F_1 splenocytes were stimulated in vitro with irradiated BALB/c splenocytes, and assayed at an effector/target ratio of 64:1 against ^{51}Cr-labeled B10 targets and varying densities of unlabeled targets. In the absence of unlabeled inhibitors, net ^{51}Cr release was 52%. The spontaneous release was 28% of the maximum release. The vertical bars represent 1 SE of the mean of triplicate determinations.

occur, not all data can be accounted for by these findings. A particularly intriguing observation is that *H-2*-unrestricted, non-Qa-1.2-specific cytolysis has been detected in (NZB × B10.D2)F_1 responses (13, 15). The data presented in this report strongly suggest that Mta is a major target antigen for such responses. The differential lysis of

reciprocal F_1 target cells by *H-2*-unrestricted CTL demonstrates specificity for an antigen that is indistinguishable from that described by Fischer Lindahl et al. (9) for NZB secondary CML responses. In that report, the investigators did not detect Mta-specific lysis in NZB primary CML responses. However, other investigators have reported *H-2*-unrestricted lysis of *Qa-1*^{a} target cells by primary NZB CTL (10, 11, 13, 14). We observed low levels of such lysis in approximately one-third of our NZB primary CML responses to *H-2*-compatible stimulators. In those experiments, we detected greater lysis on Mta$^+$ (B10.A × NZB)F_1 targets than on Mta$^-$ (NZB × B10.A)F_1 targets (our unpublished observations). Thus, Mta-specific CTL can also account for the low level of lysis detected on *Qa-1*^{a}, non-*H-2*^{d} target cells in the NZB primary CML response. In all paired comparisons of NZB and (NZB × B10.D2)F_1 responses, Mta-specific lysis by the Qa-1$^{a/b}$ F_1 CTL was always greater than that by the NZB CTL (compare lysis of the B6-*Tla*^{a} target in panels A and B, Fig. 1). Absence of foreign Qa-1 determinants in the stimulator population may therefore be a requirement for generating a strong primary Mta-specific response.

The finding that the *Qa-1* heterozygous (NZB × B10.D2)F_1 hybrids respond to Mta may clarify much of the controversy regarding the specificity of the NZB primary CML response. When NZB cells are stimulated with *H-2*-compatible stimulators, the major target antigen is Qa-1.2, although Mta-specific and *H-2*-restricted minor antigen-specific lysis may also occur. The same pattern holds for *Qa-1*^{a}-homozygous F_1 responders (e.g., [NZB × B10.BR)F_1; our unpublished observations]. However, if the F_1 responder is a *Qa-1* heterozygote, the specificity depends on whether the F_1 expresses Mta. *H-2*-restricted lysis occurs regardless of the Mta phenotype of the responder, and is the only reactivity seen for the Mta$^+$ responder. But for the Mta$^-$ F_1, *H-2*-unrestricted, Mta-specific lysis is the major component of the response.

That the NZB response is directed against a complex set of target antigens has important implications for studying the genetics of NZB hyperreactivity in the primary CML response. Because F_1 and backcross mice may not recognize as foreign the same antigenic determinants that the parental NZB mice recognize, one might mistake genes, such as *Qa-1*, that determine antigenic disparity for those that determine hyperreactivity. The finding that one of the target antigens does not segregate in Mendelian fashion makes such considerations even more important. Davidson et al. (13), studying the response of (NZB × B10.D2)F_1 mice and their backcrosses to the B10.D2 parent, concluded that two genes control the response to non-*Qa-1* antigens, and that one or both of these genes is semidominant, because the response of the F_1 mice was intermediate between that of the NZB and B10.D2 parents. An alternative explanation for the intermediate level of F_1 responsiveness is that the F_1 and NZB CTL respond to different stimulator cell antigens, because the F_1 mice were *Qa-1* heterozygotes. The inability of those particular F_1 mice to recognize the major target antigen for the parental NZB response, rather than gene dosage effects, may have caused the lower magnitude of the F_1 response. When the F_1 generation is *Qa-1*^{a}-homozygous [e.g., (NZB × B10.BR)F_1], the response is Qa-1.2-specific and the magnitude is equal to that of the NZB parent, suggesting that hyperresponsiveness to Qa-1.2 is a dominant effect (our unpublished observations). Thus, in studying the genetics of NZB hyperreactivity in the primary CML response, it is necessary to take into account not only the magnitude of the response, but also whether the F_1 and backcross mice are capable of recognizing Qa-1.2 or Mta as foreign determinants on the stimulator cells.

Summary

Mta-specific cytotoxic T lymphocytes (CTL) can be generated in primary cultures of (NZB × B10.D2)F_1 spleen cells with *H-2*-compatible BALB/c stimulator cells. The CTL lyse reciprocal Mta^+ (B10.D2 × NZB)F_1 as well as *H-2*-disparate targets, such as B10, B6, and B6-Tla^a; they do not lyse targets from NZB or any F_1 hybrid of an NZB mother. The lysis of ^{51}Cr-labeled B10 targets is completely inhibited by unlabeled targets from Mta^+ (B10.D2 × NZB)F_1, but not from the reciprocal Mta^- F_1, thus demonstrating *H-2*-unrestricted lysis of Mta.

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