Brief Definitive Report

CROSS-PRIMING FOR A SECONDARY CYTOTOXIC RESPONSE TO MINOR H ANTIGENS WITH *H-2* CONGENIC CELLS WHICH DO NOT CROSS-REACT IN THE CYTOTOXIC ASSAY*

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Genes in the *K* and *D* regions of the mouse major histocompatibility-2 complex (MHC) determine the susceptibility of target cells to lysis by cytotoxic T cells which have been immunized to minor (non-*H-2* coded) antigens. T cells immunized against trinitrophenyl-modified (1) or virus-infected (2, 3, 4) syngeneic cells, or against the minor histocompatibility (H) differences of other cells (5, 6, 7), will lyse targets from the immunizing strain, but not targets from a congenic strain which carries all the same minor information as the immunizing strain but differs at *H-2*. F_1 (BALB/c × BALB.B) (F_1 (C × C.B) *H-2*$^{d/b}$) mice immunized in vivo and in vitro with C57BL/10 (B10, *H-2*^{b}) lymphoid cells generate cytotoxic cells which are capable of lysing B10 targets very efficiently, but which do not lyse B10.D2 (*H-2*^{d}) targets (6).

Such *H-2* restriction of cytolysis is almost absolute, there being less than 2% cross-reaction of F_1 (C × C.B) anti-B10 cytotoxic cells on B10.D2 targets. The results reported here show that the same *H-2* restriction does not apply during the in vivo priming phase of the induction. That is, B10 lymphoid cells do prime F_1 (C × C.B) mice for an excellent secondary in vitro cytotoxic response to B10 and B10.D2 cells (and vice versa). DBA/2 (*H-2*^{d}) and B10.D2 cross-react in the cytotoxic assay, and DBA/2 spleen or thymus cells prime F_1 (C × C.B) mice very well for a secondary response to DBA/2, B10.D2, and B10. When DBA/2 mastocytoma cells are used for priming, however, the *H-2* restriction holds true, i.e., P815 cells prime for a secondary response to DBA/2 and B10.D2, but not for a response to B10. Means to explain these results either in terms of the altered self or interaction antigen hypothesis (1, 3, 5) or in terms of the intimacy or dual recognition hypothesis (8) are discussed.

Materials and Methods

Mice. C57BL/10Sn (B10, *H-2*^{b}), B10.D2/nSn(*H-2*^{d}), and DBA/2 (*H-2*^{d}) female mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. F_1 (BALB/c × BALB.B) (F_1 (C × C.B), *H-2*$^{d/b}$) mice were bred at the Salk Institute. All mice used were female and 8-20-wk old at the start of the experiment.

Immunizations. In vivo priming injections were of viable allogeneic cells in Hanks' balanced salt solution given intraperitoneally. The source and number of cells injected is indicated in the text. In vitro immunizations were done in mixed lymphocyte cultures (MLC) using spleen as source of responder and stimulator cells. Cell concentration, medium, and culture conditions were exactly as described previously (6). It should be remembered that under these conditions F_1 (C ×

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C.B) spleen cells give no detectable primary cytotoxic response to B10, B10.D2, or DBA/2 cells. Cytotoxic cells immunized across broad non-*H-2*-coded genetic differences show extensive cross-reaction on allogeneic *H-2* identical cells (5, 6). Specificity for the immunizing minor *H* allele has been shown, however, using minor H congenic mice as responders and stimulators (9). Furthermore, priming has been shown to be specific also, e.g., B10.LP mice were derived from an initial cross between B10 and LP/J($H\text{-}2^b$) and differ from B10 only in a portion of chromosome 2 which carries H-3 and H-13; B10.LP mice injected once with B10 cells are primed for a secondary cytotoxic response to B10, but are not primed for a response to LP/J.

Cytotoxic Assay. Targets were spleen cells which had been cultured for 2 days with 10 μg/ml lipopolysaccharide (Salmonella typhosa, 0901, LPS, B-cell blasts) obtained from Difco Laboratories, Detroit, Mich. 4×10^4 [^{51}Cr]sodium chromate-labeled LPS blasts were titered against three or fourfold dilutions of the cytotoxic cells for 4 h as described previously (6). Percent specific release of ^{51}Cr was calculated as follows:

$$\frac{\text{experimental release cpm} - \text{spontaneous release cpm}}{\text{total cpm} - \text{spontaneous release cpm}} \times 100$$

Results

Spleen cells from F_1 (C × C.B) mice, which had been injected 5 wk earlier with 42×10^6 B10.D2 spleen cells, were cultured in vitro with irradiated spleen cells from F_1 (C × C.B), B10.D2, or B10 mice or an equal mixture of B10.D2 and B10 cells. After 5 days the cultured cells were assayed for lysis of B10.D2 and B10 LPS blasts. F_1 cells boosted with B10.D2 cells lysed B10.D2 targets (Fig. 1 a) but not B10 targets (Fig. 1 b). F_1 cells boosted with B10 cells lysed B10 targets (Fig. 1 b) but not B10.D2 targets (Fig. 1 a). There was almost no detectable cross-reaction of either population on the two targets in the cytotoxic assay. Unprimed F_1 cells were not cultured in MLC with irradiated B10 or B10.D2 cells in this experiment, but on three other occasions this did not give rise to measurable cytotoxic effects (see Table I). The activity of the B10.D2-boosted cells was about fourfold higher than the B10-boosted cells in this experiment (comparing lysis of the corresponding targets). Boosting with a mixture of B10 and B10.D2 cells gave activity against both targets (Fig. 1 a and b).

The results presented in Table I show that the kinetics of generation of cytotoxic cells in MLC was faster when the original priming strain was used for the boost than when *H-2* congenic cells were used. Spleen cells from normal F_1 (C × C.B) mice or from F_1 mice primed with B10 spleen cells were cultured with irradiated F_1, B10, or B10.D2 cells. The cytotoxicity against B10 and B10.D2 was assayed after 3 or 5 days of culture. On day 3 the B10-primed, B10-boosted cells lysed B10 targets significantly, while the B10.D2-boosted cells lysed neither target above the spontaneous release value. By day 5 both populations showed cytotoxic activity specific for the targets used for the boost. Activity against the original priming strain, B10, by the B10-boosted cells was about twofold higher than activity against B10.D2 by the cross-boosted cells. The B10.D2 boosted population lysed B10 targets weakly, 50-fold less effectively than they lysed B10.D2 targets. Normal F_1 cells cultured in vitro for 5 days with B10 or B10.D2 cells did not cause measurable lysis of either target.

The results so far have shown that cells from two strains which did not cross-react at the F_1 cytotoxic effector level did cross-react strongly during in vivo priming. There is a situation, however, in which the *H-2* complex restricts cytotoxicity and priming. Cytotoxic cells from BALB/c mice (5) and F_1 (C × C.B)

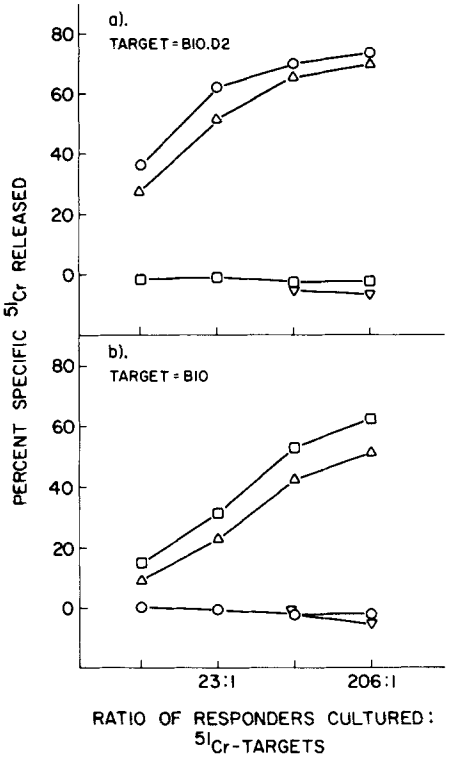

FIG. 1. Secondary in vitro cytotoxic response of B10.D2-primed F_1 (C × C.B) spleen cells to B10.D2 and B10 cells. Culture period was 5 days. Assayed against [^{51}Cr]LPS blasts from (a) B10.D2 and (b) B10. F_1 cells cultured with irradiated F_1 cells (▽ – ▽), B10.D2 cells (○ – ○), B10 cells (□ – □), or a 1:1 mixture of B10.D2 and B10 cells (△ – △). Spontaneous release of ^{51}Cr was 16.8 and 13.0%.

TABLE I

Kinetics of the Cytotoxic Response of Spleen Cells from B10-Primed F_1(C × C.B) Mice to B10 and B10.D2 Cells

Responding F_1(C × C.B) spleen cells*	Boosted with:	Aggressor: target ratio	Percent specific cytotoxicity after culture‡			
			Day 3		Day 5	
			^{51}Cr-B10	^{51}Cr-B10.D2	^{51}Cr-B10	^{51}Cr-B10.D2
B10 primed	B10	62:1	11.3	−8.2	59.4	−2.0
B10 primed	B10	21:1	8.1	−6.6	49.3	−0.7
B10 primed	B10.D2	62:1	−4.3	−0.5	7.4	54.8
B10 primed	B10.D2	21:1	−2.6	−1.5	3.6	38.1
Normal	B10	124:1	ND	ND	−0.1	−3.4
Normal	B10.D2	124:1	ND	ND	−2.1	−1.1

* Priming was with 42 × 10^6 B10 spleen cells.
‡ Spontaneous release from the [^{51}Cr]LPS blasts varied from 13.4 to 20.0%.

mice (Bevan, unpublished) immunized only with P815 (DBA/2) mastocytes cross-reacted on B10.D2 targets but not on B10 targets. Spleen cells from F_1 mice injected either with 50 × 10^6 B10 spleen cells or with 10^7 P815 cells were stimulated in MLC with B10, DBA/2, or B10.D2 spleen cells and assayed 5 days later for lysis of LPS blasts from the three strains. The results with B10 and

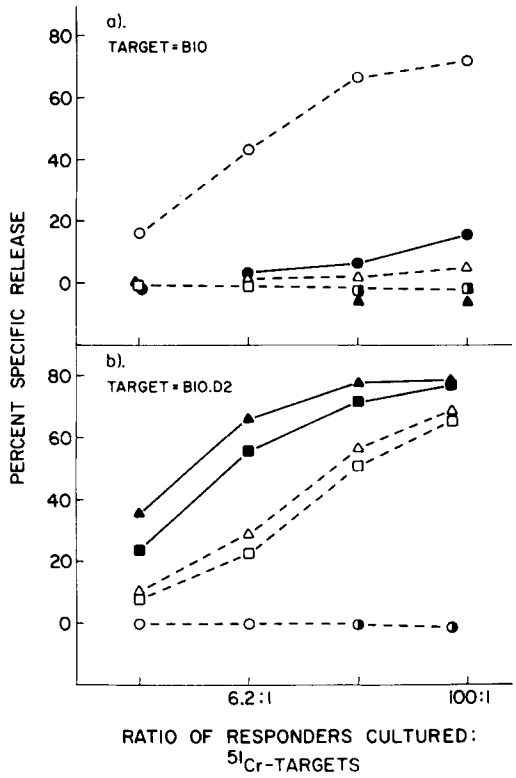

FIG. 2. Mastocytoma cells fail to cross-prime for a secondary cytotoxic response to *H-2* different cells. Spleen cells from F_1 (C × C.B) mice which had been primed either with B10 spleen cells (open symbols) or with DBA/2 mastocytes P815 (closed symbols) were cultured in MLC for 5 days with irradiated spleen cells from B10 (○ and ●), B10.D2 (□, ■), or DBA/2, (△, ▲). Assayed for lysis of LPS blasts from (a) B10 and (b) B10.D2 mice. Spontaneous release of ^{51}Cr was 16.0 and 12.1%. The assay on DBA/2 targets was almost identical to the assay on B10.D2 targets.

B10.D2 target cells are shown in Fig. 2. The F_1 mice injected with B10 spleen cells were primed for a secondary cyctotoxic response to B10 (Fig. 2 a) and for a response to B10.D2 (approximately threefold weaker) which could also be stimulated by DBA/2 spleen cells (Fig. 2 b). However, while the P815-primed F_1 cells gave an excellent cytotoxic response to B10.D2 or DBA/2 (Fig. 2 b) they responded very poorly to B10 cells. In a separate experiment P815-primed F_1 cells gave 39% lysis of B10.D2 targets at a ratio of 1.5:1 after boosting with B10.D2, but only 2.9% lysis of B10 targets at a ratio of 120:1 after boosting with B10 cells. In the experiment shown in Fig. 2, the pattern of lysis of DBA/2 targets was almost identical with that of B10.D2 targets (data not shown). In another experiment it was found that F_1 (C × C.B) mice primed 5 wk earlier with 10^7 spleen or thymus cells from DBA/2 were able to mount an excellent secondary cytotoxic response to DBA/2, B10.D2, and B10.

Discussion

The finding that the same minor *H* alleles on *H-2* different congenic cells do not appear to cross-react in the cytotoxic assay (5–7) was not predicted from

previous work on the rejection of transplants in vivo. Thus Snell's immunization test for typing minor *H* alleles assumes cross-reaction in priming (10); the rejection of syngeneic male tissue by female mice can be tolerized or speeded up by exposure to male tissue of a different H-2 type (11); finally, F_1 specific antigens have not been detected in skin graft rejection (12), although the cytotoxic assay apparently detects them (5, 6). The work reported here makes the in vivo and in vitro phenomena more compatible by showing that although F_1 (C × C.B) cytotoxic effector cells detect no cross-reaction between B10 and B10.D2, there is a high degree of cross-reaction in priming for a secondary cytotoxic response.

The altered self or interaction antigen hypothesis states that the minor H antigens which F_1 cytotoxic cells recognize on B10 and B10.D2 are noncross-reactive because in B10 they are modified by $H\text{-}2^b$ genes and in B10.D2 by $H\text{-}2^d$ genes (5, 6). To preserve this idea intact while explaining the cross-priming with B10 and B10.D2, one can postulate the following: (*a*) Precursor cytotoxic T cells require helper T cells to respond. The determinants recognized by the F_1 helper cells are different from those recognized by cytotoxic cells, and they do cross-react strongly between B10 and B10.D2 lymphoid cells. (*b*) Expanding the pool of F_1 helper cells by priming with B10 is sufficient to give a secondary cytotoxic response to B10.D2. This is analogous to carrier priming resulting in an enhanced B-cell response to a hapten coupled to the carrier. This explanation predicts that in a B10-primed F_1 animal there is an expanded pool of cytotoxic cells reactive to B10, but not an expanded cytotoxic pool reactive with B10.D2.

The dual recognition or intimacy hypothesis states that the minor H antigens of B10 and B10.D2 are the same, but T-cell lysis requires a second interaction between killer and target which is controlled by *H-2*. This hypothesis might predict that an injection of B10 cells does expand the pool of F_1 cytotoxic cells reactive with B10.D2, but that they cannot express their lytic function on B10.D2 until they are confronted with B10.D2 cells for at least 2 days.

Using viable P815 tumor cells to prime F_1 mice, however, does demonstrate *H-2* restriction, i.e., whereas DBA/2 spleen or thymus cells cross-prime for a secondary response to B10, DBA/2 mastocytes do not (Fig. 2). T-cell-mediated cytotoxicity is restricted by the *K* and *D* regions of *H-2* (1, 2, 6, 7); helper T-cell function, on the other hand, is restricted by the *I* region of *H-2* (8). Helper T cells may recognize minor antigens in association with *I*-region products, and it is possible that I^b and I^d products cross-react. Spleen and thymus cells express *I*-region genes, but there is no evidence that mastocytes do. This could explain why mastocytes fail to cross-prime. Another possible explanation for the difference between normal cells and tumor cells during priming is simply that, unlike normal cells, the injected tumor cells grow rapidly for at least 7 days before succumbing to the host immune response. Such a great antigenic stimulus might bypass the normal induction pathways (e.g., host macrophages) which are followed when nongrowing cells are used to prime.

Summary

Cytotoxic effector T cells of F_1 (BALB/c × BALB.B) ($H\text{-}2^{d/b}$) mice immunized against the minor histocompatibility differences of C57BL/10 ($H\text{-}2^b$) can lyse

targets from C57BL/10, but cannot lyse B10.D2 ($H\text{-}2^d$) targets. Despite this lack of cross-reaction in the cytotoxic assay, C57BL/10 cells do prime F_1 (BALB/c × BALB.B) mice for a secondary cytotoxic response to B10.D2. C57BL/10-primed, B10.D2-boosted cytotoxic cells lyse B10.D2 targets but not C57BL/10 targets. DBA/2 ($H\text{-}2^d$) spleen cells or thymocytes prime F_1 mice for a secondary response to DBA/2, B10.D2, and C57BL/10 cells, but DBA/2 mastocytes, P815, do not prime for a response to C57BL/10. Whether *H-2* congenic lymphoid cells express minor histocompatibility determinants which cross-react at the cytotoxic T-cell level or the helper T-cell level is discussed.

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