HAPLOTYPE-SPECIFIC SUPPRESSION OF CYTOTOXIC T CELL INDUCTION BY ANTIGEN INAPPROPRIATELY PRESENTED ON T CELLS*

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Minor histocompatibility (H) antigens are non-H-2 transplantation antigens, of which ~40 have been identified (1). A vigorous cytotoxic T lymphocyte (CTL) response to minor H antigens requires in vivo priming, and CTL so generated are H-2 restricted in their activity (2, 3). Priming for minor H antigens generally involves injection of ~10⁷ viable spleen cells. Induction of CTL by in vivo priming with lymphoid cells appears to follow several pathways, some of which are diagrammed schematically in Fig. 1. We have previously shown that some component of the antigen-bearing spleen cell population is competent to present minor H antigens to CTL precursors directly (4). Recipient cells also appear capable of presenting injected minor H antigens, as evidenced by the phenomenon of cross-priming, which refers to the finding that (A × B)F₁ mice injected with H-2ᴬ minor-H-different spleen cells (A') will generate not only H-2ᴬ-restricted CTL activity (5). In our view, the most experimentally sound explanation for cross-priming is that antigen-presenting cells in an (A × B)F₁ animal injected with A' spleen cells can reprocess and present these minor H antigens in conjunction with both H-2ᴬ and H-2ᴮ (4, 6, 7).

Consistent with that view, it seemed likely that a kinetic study of F₁ mice injected with A' cells would reveal early direct priming to minor H plus-H-2ᴬ followed later, after antigen reprocessing, by cross-priming to minor H-plus-H-2ᴮ. To our surprise, 3–6 d after antigen injection the minor H-specific, H-2ᴬ-restricted CTL response in such animals is severely depressed relative to the minor H-plus-H-2ᴮ and third-party alloreactive responses. Experiments described below were designed to investigate the nature of this haplotype-specific CTL hyporeactivity.

Materials and Methods

Mice. C57Bl/10Sn (B10, H-2ᵇ), B10.D2SnSn (H-2ᵈ), B10.BrSgSn (H-2ᵏ), and (AKR/J × DBA/2)F₁ [(AKD2)F₁, H-2⁰/H-2⁰] mice were purchased from The Jackson Laboratory, Bar

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Abbreviations used in this paper: A', H-2ᴬ cells bearing foreign minor histocompatibility antigens; B', H-2ᴮ cells bearing foreign minor histocompatibility antigens; C, complement; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; H, histocompatibility; MLC, mixed lymphocyte culture; TNP, trinitrophenyl.
Harbor, ME. The following strains of mice were bred in our animal facilities at the Stanford University Medical Center: A.TH (H-2K^dD^b), A.TL (H-2K^kD^k), BALB/c (C, H-2^d), BALB.B (C.B, H-2^b), BALB.K (C.K, H-2^k), and the various BALB and C57Blik F1 mice. [Parent -> F1] chimera were prepared by injecting T cell-depleted parental bone marrow into F1 animals that had previously received 1,000 rad from a ^137Cs source (4).

Antisera and Monoclonal Antibodies. The A.TH anti-A.TL antiserum (anti-I(k)) was prepared by multiple intraperitoneal injections with spleen cells and screened for specific cytotoxicity against lymphoid cells from congenic strains of mice. The monoclonal anti-Thy-1.2 reagent 13-4 was prepared as previously described (8). T24/31.7 (T24) is a rat-mouse hybridoma that secretes a monoclonal antibody that recognizes a mouse-specific determinant on Thy-1 (9). AD4(15) is a monoclonal antibody that recognizes Lyt-2.2 (10). Rabbit anti-mouse immunoglobulin was prepared by multiple injections with purified mouse IgG in complete Freund’s adjuvant. The (Fab')2 reagents used for plate separation were prepared by N. Landau, Massachusetts Institute of Technology, Cambridge, MA.

Mixed Lymphocyte Culture (MLC) and Cytotoxicity Assay. Spleen or lymph node cells from responder animals were cultured as previously described (11) with an equal number of irradiated (1,000 rad) stimulator cells of the specified type. Trinitrophenyl (TNP) modification was performed according to Shearer (12). After 5 d of MLC, serial threefold dilutions of responder cells were added to 4 × 10^4 ^51Cr-labeled target cells in a total vol of 1 ml. The percent specific lysis after 4 h of incubation was calculated in the following manner:

\[
\frac{\text{counts per minute released by responders} - \text{counts per minute released by medium alone}}{\text{counts per minute released by detergent} - \text{counts per minute released by medium alone}} \times 100.
\]

Target cells were spleen cells which had been cultured for 2 d with concanavalin A (Con A) as previously described (3).

Lymphoid Cell Subpopulations. Cells to be treated with antiserum were resuspended at 10^7/ml in an appropriate dilution of antibody, incubated for 30 min at 4°C, washed, resuspended at 5 × 10^6/ml in a selected rabbit serum, and incubated for 45 min at 37°C. The dilutions of antibodies used were predetermined to kill plateau levels of the appropriate cells.

Purified T cells were obtained from spleen and lymph node cells nonadherent to Petri dishes coated with rabbit anti-mouse immunoglobulin, according to previously described techniques (13, 14). Selection on the basis of adherence to anti-Lyt-2.2- [AD4(15)] coated plates were performed in a similar manner. The efficacy of each separation was assessed by typing ^51Cr-labeled cells with anti-Thy-1, anti-Lyt-2, anti-Ia, and anti-H-2 sera plus complement (C). Nylon wool column enrichment of T cells was performed exactly as described previously (15).

**Fig. 1.** Minor-H-different (A') spleen cells are injected into an (A X B)F1 recipient. Although the anti-A' CTL precursors can recognize minor H antigens on both the injected cells and on host antigen-presenting cells, host CTL precursors specific for B' are activated by F1 host cells presenting the injected minor H antigens. Recognition of antigen directly on the A' cells may lead to temporary inactivation.
Results

Haplotype-specific Suppression of CTL Response to Minor H Antigens. To study the kinetics of direct- vs. cross-priming of minor H-specific CTL, \((C.K \times C)F_1\) \((H-2^k \times H-2^d)\) mice were injected intravenously with \(12 \times 10^6\) viable B10.Br \((H-2^k)\) spleen cells. We expected the in vivo expansion of CTL involved in the direct-primed response (anti-B10.Br) to appear early, activated by presentation of minor H antigens-plus-H-2 on the injected spleen cells themselves (Fig. 1; reference 4), whereas we expected the appearance of the cross-primed CTL response (anti-B10.D2) to follow, activated by minor H antigens processed by host F1 antigen-presenting cells (4, 7). To assay the results of such in vivo priming, responder spleens were removed 4–10 d after antigen injection and cultured for 5 d with irradiated \((B10.Br \times B10.D2)F_1\) stimulators that provide further in vitro stimulation of both the direct primed and the cross-primed CTL. Separate stimulation with B10.Br or B10.D2 cells does not activate both CTL populations, as cross-boosting of CTL to minor H antigens does not occur in vitro during a 5-d secondary MLC (3). Data representative of several such experiments are shown in Table I and demonstrate, surprisingly, that the cross-primed response is significantly greater than the direct-primed response early after priming. In most cases, the level of cross-primed CTL activity is invariant from days 5–10 postinjection, whereas the direct-primed response increases sharply during this time. Both the severity and the duration of the hypoactivity of the direct primed response are directly related to the dose of injected B10.Br cells, and this hypoactivity is not evident in animals injected with B10.Br cells that have been irradiated with 1,000 rad (Table I).

Full titration curves for the \(^{51}Cr\)-release assay from a single experiment are shown in Fig. 2. Fig. 2 A and B show the haplotype-specific hypoactivity of the direct-primed response; Fig. 2 C is a positive control to show that \((C.K \times C)F_1\) hosts develop similar levels of CTL activity to minor H antigens on B10.Br and B10.D2 Con A blasts if primed with \((B10.Br \times B10.D2)F_1\) spleen cells some weeks before in vitro boosting. Priming F1 hosts with minor-H-different F1 cells prevents the later induction of direct-primed hypoactivity, although it is not known whether this is a quantitative or a qualitative effect (data not shown).

The cross-primed response presumably results from the activation of CTL by F1 antigen-presenting cells, which should also activate the anti-B10.Br CTL, suggesting that injection of viable B10.Br spleen cells leads to a specific “depletion” or suppression of anti-B10.Br CTL. That the skewed ratio of the direct- and cross-primed responses results from a depression of the direct response rather than from a heightened cross-primed response is also demonstrated by priming with irradiated B10.Br cells (Table I). In this case, equally high levels of direct- and cross-primed responses appear early after priming.

Direct-primed CTL activity can be suppressed by injection of minor H different cells of both parental haplotypes in all strains of mice we have tested, including \((C.K \times C)F_1\) and \((AKD2)F_1\) mice injected with B10.Br and B10.D2, \((C \times C.B)F_1\) mice injected with B10 and B10.D2, and \((B10 \times B10.D2)F_1\) mice injected with C and C.B (data not shown).

Suppression of CTL activity is clearly haplotype specific \((H-2^k\) spleen cells deplete only H-2^k-restricted CTL) and is antigen specific (animals depleted for anti-B10.Br activity can still mount a good CTL response to TNP plus H-2^k, Table I). Thus, injection of F1 animals with viable, minor-H-different H-2 homozygous spleen cells...
leads to a severe, short-lived, haplotype and minor-H-antigen-specific suppression of CTL activity.

**Suppression of Minor-H-specific CTL by Spleen Cell Subpopulations.** By priming with spleen cells that had been separated by various means, we hoped to identify the cell types responsible for this haplotype-specific depletion of CTL activity. Spleen cells pretreated with anti-Ia serum plus C were able to suppress the induction of CTL more efficiently on a per cell basis than whole spleen cells as calculated from titration curves, a single point of which is shown in Table II. Depletion was also mediated by lymph node cells that were passed through nylon wool columns and then treated with anti-Ia plus C and were judged free of B cells and accessory cells by the loss of responsiveness to the mitogens lipopolysaccharide and Con A (data not shown).
Spleen cells were also pretreated with monoclonal anti-Thy-1 reagents (T24 and 13-4) or monoclonal anti-Lyt-2.2 (AD4(15)) plus C. In three separate experiments, Thy-1-negative spleen cells suppressed CTL responses from 30- to >50-fold less efficiently on a per cell basis than cells treated with C alone. High doses of cells pretreated with anti-Lyt-2 plus C were still able to deplete the appropriate CTL responses (Table II).

To determine the relative efficiency of suppression by Lyt-2-positive and Lyt-2-negative T cells, T cells selected as nonadherent to plastic dishes coated with rabbit anti-mouse immunoglobulin were separated into Lyt-2-positive and Lyt-2-negative cells on the basis of adherence to anti-Lyt-2.2-coated dishes. This latter separation is quite clean, as judged by antiserum typing of 51Cr-labeled separated cell populations (Table II). In two experiments, we assayed the ability of 3 × 10^6-75 × 10^6 separated spleen cells to suppress a CTL response specifically. As illustrated in Table II, Thy-1-positive cells are potent depleters, and both Lyt-2-positive and Lyt-2-negative T cells deplete, although Lyt-2 positive cells are 3-10-fold more efficient. As few as 3 × 10^6 Lyt-2-positive T cells mediate a 20-fold relative reduction in the direct-primed CTL response.

These data allow us to conclude that the cell type(s) responsible for suppression is radiosensitive, Ia-negative, nylon wool nonadherent, and Thy-1 positive, and is therefore a T cell. Suppression can be mediated both by T cells judged Lyt-2-positive by cytotoxicity and by adherence to anti-Lyt-2-coated plates, and those judged negative, although fewer Lyt-2-positive T cells are required to mediate a given level of depletion.

**Suppression by Tolerant Spleen Cells.** The realization that T cells are responsible for the suppression of the CTL response made it imperative to test whether this haplotype-specific depletion can be ascribed to a graft vs. host reaction on the part of the injected cells. It is important to point out that we can detect no CTL activity directed against host H-2 antigens at any time after injection of homozygous cells. Furthermore,
Table II

Depletion of CTL Response by Spleen Cell Subpopulations

| Injected B10.Br spleen cells* | Day of lysis of target cells | Ratio of activity: |
|------------------------------|-----------------------------|-------------------|
|                             |                             | B10.Br | B10.D2 |
| 1. 15 × 10^6 untreated     | -14                          | 50     | 42     | 3:1  |
| 75 × 10^6 C treated        | -6                           | 4      | 58     | <1:80|
| 12 × 10^6 anti-Ia + C treated§ | -6                      | 11     | 61     | <1:40|
| 75 × 10^6 anti-Thy-1 + C treated | -6                  | 30     | 40     | 1:2  |
| 75 × 10^6 anti-Lyt-2 + C treated | -6                  | 18     | 55     | 1:20 |
| 2. 15 × 10^6 unseparated   | -14                          | 61     | 64     | 1:1  |
| 30 × 10^6 unseparated      | -6                           | 8      | 70     | <1:80|
| 10 × 10^6 T cells§         | -6                           | 20     | 71     | 1:50 |
| 30 × 10^6 T cells          | -6                           | 5      | 63     | <1:80|
| 3.3 × 10^6 Lyt-2+ T cells  | -6                           | 25     | 72     | 1:20 |
| 10 × 10^6 Lyt-2+ T cells   | -6                           | 1      | 78     | <1:100|
| 10 × 10^6 Lyt-2+ T cells   | -6                           | 32     | 70     | 1:15 |
| 30 × 10^6 Lyt-2+ T cells   | -6                           | 26     | 75     | 1:23 |
| 60 × 10^6 Lyt-2+ T cells   | -6                           | 6      | 63     | <1:80|

* (C.K × C)F1 mice were injected intravenously with the indicated spleen cells. 6-14 d after antigen injection, responder spleen cells were cultured for 5 d with irradiated (B10.Br × B10.D2)F1 spleen cells and assayed for cytotoxic activity. No lysis of syngeneic (C.K × C)F1 targets was detected. Two separate experiments are shown.

§ As in Table I.

I "T cells" were nonadherent to plastic dishes coated with rabbit anti-mouse-immunoglobulin and were found to be 75% Thy-1+, 38% Lyt-2+ as assayed by antiserum + C treatment. Cells were further separated into Lyt-2+ and Lyt-2− fractions on dishes coated with AD4(15). Lyt-2+ cells were 100% Thy-1+, 96% Lyt-2+. Lyt-2− cells were 78% Thy-1−, 1% Lyt-2−.

preimmunization of the spleen cell donors with recipient spleen cells does not enhance their ability to suppress as judged from a comparison of the number of normal and immune cells able to mediate a given level of depletion (data not shown). These results argue against the dependence of depletion on an anti-H-2 or an anti-[host receptor idiotype] response by the injected cells. More definitively, suppression can be mediated both by spleen cells from [P→F1] chimeras and cells from animals fully tolerant to recipient H-2 and minor H antigens (Table III). For example, B10.D2 cells from a [B10.D2→(B10 × B10.D2)F1] chimera are tolerant of H-2b, but still deplete H-2b-restricted CTL activity from an F1 mouse as efficiently as do normal B10.D2 spleen cells. In addition, (C.K × B10.Br)F1 cells can suppress the anti-B10.Br activity from C.K mice to the same extent as can B10.Br cells. With homozygous mice as responders, the priming with H-2 syngeneic cells follows the same kinetics as the direct-primed response in F1 responders, i.e., much slower than the cross-primed response. However, in the case of homozygous recipients, the depleted minor H antigen response must be compared with an alloreactive control instead of with an unaffected minor H response as is possible in F1 mice (Table III).

We therefore conclude that no immunological recognition of the recipient animal by the injected cells is necessary for suppression of CTL activity. These results suggest
Table III

Depletion of CTL Response by Tolerant Spleen Cells

| Recipient* | Injected spleen cells | Day of injection | Percent specific lysis | Ratio of activity\f | B10.Br | B10.D2 |
|------------|-----------------------|------------------|-----------------------|---------------------|--------|--------|
| C.K        | 25 x 10^6 (C.K x B10.Br)F1 | -30              | 38                    | 36                  | 1:1    |        |
|            | 50 x 10^6 B10.Br       | -6               | 4                     | 70                  | <1:200 |        |
|            | 50 x 10^6 (C.K x B10.Br)F1 | -6               | 0                     | 70                  | <1:200 |        |
| C          | 25 x 10^6 (C x B10.D2)F1 | -37              | 75                    | 83                  | 1:2    |        |
|            | 30 x 10^6 B10.D2       | -6               | 73                    | 47                  | 12:1   |        |
|            | 30 x 10^6 (C x B10.D2)F1 | -6               | 72                    | 45                  | 10:1   |        |
| (C x C.B)F1 | 15 x 10^6 (B10 x B10.D2)F1 | -21              | 5                     | 75                  | 1:2    |        |
|            | 45 x 10^6 B10.D2       | -6               | 61                    | 23                  | 20:1   |        |
|            | 45 x 10^6 [B10.D2→F1] chimera | -6               | 66                    | 16                  | 50:1   |        |

* Mice were injected intravenously with the indicated spleen cells 6-37 d before killing. Chimeras were constructed as described in Materials and Methods. C.K responder cells were cultured with B10.Br (minor H different) or B10.D2 (alloimmune) stimulators. C responders were cultured with B10.D2 (minor H different) or B10.Br (alloimmune) stimulator spleen cells. (C × C.B)F1 responder cells were cultured with (B10 x B10.D2)F1 minor H-different stimulator cells. After 5 d of MLC, CTL activity was measured against the indicated ^51Cr-labeled Con A blasts. No lysis of syngeneic targets was detected for any responder cell population.

\f Data for an effector to target ratio of 50:1.

\§ Ratio of activity on first target to activity on second target. Calculated from a complete titration of responders against a constant number of target cells.

very strongly that the specificity of the reduction in CTL activity resides in the one-way recognition of the injected cells by the host CTL precursors.

Attempts to Recover Direct-Primed CTL Activity. It is possible that direct-primed CTL are present in the host during the first 6 d of priming, but that they have localized to extra splenic lymphoid organs because of some antigen-dependent changes in their patterns of recirculation (16). However, this does not seem to be the case, as responder cells taken from lymph nodes of normal as well as splenectomized mice show the same pattern of suppression and recovery of the direct-primed CTL response as do spleen cell responders (Table IV). It is also possible that CTL activated by direct presentation of antigen by the injected cells are lost during the spleen cell preparation because of a heightened sensitivity to mechanical stress, a phenomenon that has some precedent (17). However, the extent of suppression is comparable from cultures of diced splenic fragments and of cell suspensions from the same animal (Table IV). Furthermore, in vivo-induced depletion cannot easily be reversed in vitro—reboosting cultures of cells that are suppressed for the direct-primed response at day 5 of MLC does not alter their reactivity profile (Table IV). Thus, if suppression is a result of a temporary block to antigen activation on the part of direct-primed CTL, these CTL cannot be rescued from their inactive state by reboosting in vitro.

Depletion In Vitro. We have mixed spleen cells from animals primed to F1 cells with those from mice injected 6 d previously with homozygous spleen cells in an effort to demonstrate whether depletion involves an active suppression that can alter the activity of pre-primed CTL in vitro. As shown in Table V, we found no evidence for
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### Table IV

**Direct-Primed Activity Cannot be Recovered at the Time of Suppression**

| Experiment | Responding cells* | Day of injection | Ratio of activity‡ |
|------------|------------------|------------------|--------------------|
| 1          | Spleen           | −5               | 1:200              |
|            | Lymph node       | −5               | 1:130              |
|            | Spleen           | −6               | 1:50               |
|            | Lymph node       | −6               | 1:27               |
| 2          | LN normal        | −6               | 1:20               |
|            | LN splenectomi- | −6               | 1:27               |
| 3          | Spleen suspension| −6               | 1:30               |
|            | Diced spleen     | −6               | 1:50               |
| 4          | Spleen 2°        | −5               | 1:20               |
|            | Spleen 3°        | −5               | 1:15               |
|            | Spleen 2°        | −6               | 1:12               |
|            | Spleen 3°        | −6               | 1:10               |

* In experiment 1, spleen and lymph node (LN) cells were taken from the same (C × C.B)F1 animal injected intraperitoneally with 15 × 10⁶ B10 spleen cells. Responder cells were boosted with irradiated (B10 × B10.D2)F1 cells. In experiment 2, lymph nodes were taken from normal (C.K × C)F1 or from mice that had been splenectomized 5 wk previously. Both groups had been injected intravenously with 20 × 10⁶ B10.Br spleen cells and boosted in vitro with (B10.Br × B10.D2)F1 cells. In experiment 3, one-half of a spleen from a (C.K × C)F1 injected intravenously with 25 × 10⁶ B10.Br spleen cells was teased and the other one-half diced into small fragments with a scalpel. The cell suspension and the fragments were separately cultured with irradiated (B10.Br × B10.D2)F1 stimulators. In experiment 4, spleen cells from (C.K × C)F1 animals injected with 12 × 10⁶ B10.Br spleen cells were boosted for 5 d with irradiated (C × B10.Br)F1 cells. Some of the responders were assayed for CTL activity (2°) and some were reboosted on day 11 of culture and assayed on day 4 of reboosting (3°).

‡ Ratio of activity on targets of the injected type to targets of the other haplotype. For experiment 1, it is the ratio of killing on B10 to B10.D2, and for experiments 2–4, it is the ratio of killing on B10.Br to B10.D2. In each experiment, a full titration of responders was performed; control animals primed at least 2 wk previously were always included. No significant killing on syngeneic targets was found in any experiment.

Suppression of CTL activity of the F1 primed cells. Thus, spleen cells from (C.K × C)F1 mice recently injected with B10.Br cells cocultured in a 4:1, 2:1, 1:1, or 1:2 ratio with spleen cells from long-term primed mice do not suppress the anti-B10.Br response of the latter population. Similar results are obtained with mixtures of cells from animals injected 6 d previously with homozygous cells (Table V). Therefore, haplo-type-specific depletion is not maintained during the period of MLC by a demonstrable suppressor mechanism. Experiments to test for suppression in vivo (by adoptive transfer, for example) are not possible because of the short time span of the depletion.

### Discussion

Our experiments show that the injection of (A') T cells into (A × B)F1 animals results in the temporary inactivation of the CTL specific for the injected cells. This loss of CTL activity could, in principle, be caused by either direct inactivation of
Table V
Lack of In Vitro Suppression by Depleted Populations

| Responding cells* | Percent specific lysis of target cells† | Ratio of activity§ |
|-------------------|----------------------------------------|-------------------|
|                   | B10.Br | B10.D2 |                   |
| A. day -6 B10.Br primed | 10 | 48 | 1:30 |
| B. day -6 B10.D2 primed | 42 | -2 | >100:1 |
| C. day -30 F1 primed | 62 | 70 | 1:1 |

Mix of A and C in the ratios
4:1 | 32 | 52 | 1:4 |
2:1 | 36 | 50 | 1:3 |
1:1 | 38 | 48 | 1:2 |
1:2 | 51 | 56 | 1:1 |

Mix of B and C in the ratios
4:1 | 44 | 18 | 9:1 |
2:1 | 44 | 32 | 5:1 |
1:1 | 54 | 50 | 3:1 |
1:2 | 56 | 52 | 2:1 |

Mix of A and B in the ratios
2:1 | 25 | 52 | 1:12 |
1:1 | 54 | 45 | 2:1 |
1:2 | 58 | 20 | 2:1 |

* (C.K × C)F1 mice were injected intravenously with: A, 35 × 10⁶ B10.Br spleen cells on day -6; B, 35 × 10⁶ B10.D2 spleen cells on day -6; or C, 15 × 10⁶ (B10.Br × B10.D2)F1 spleen cells on day -30. On day 0, responder spleen cells were cultured with irradiated (B10.Br × B10.D2)F1 spleen cells for 3 d. Responders were cells from A, B, and C alone, or mixtures, all totaling the same number of cells cultured. Cytotoxic activity was measured on 51Cr-labeled B10.Br, B10.D2, and (C.K × C)F1 Con A blasts. Spontaneous release was 17–23% with no detectable lysis of (C.K × C)F1 targets above background.

† Data for an effector to target ratio of 50:1.
§ Ratio of activity on B10.Br to B10.D2 targets, calculated from a full titration of responders against a constant number of targets.

CTL precursors or by inactivation of helper cells required for the induction of the CTL response. To explain our data, however, such helper cells would be required to recognize the A' cells themselves and would have to be able to distinguish F1 CTL specific for A' from those specific for H-2B cells bearing foreign minor H antigens (B'). We feel the existence of such cells is unlikely, and therefore favor the interpretation that A' T cells can inactivate anti-A' CTL and/or their precursors in vivo. Several hypotheses that can account for the antigen-induced haplotype-specific suppression of CTL induction we have reported are listed below.

(a) A fraction of B10.Br T cells bears anti-idiotype receptors specific for (C.K × C)F1 CTL receptors that recognize H-2K-plus-B10 minor H antigens, and not H-2D-plus-B10 minor H antigens nor H-2K-plus-unrelated antigens. This population of B10.Br T cells would be capable of inactivating recipient CTL bearing the appropriate receptors. This T cell-mediated immune response is, in principle, similar to that described in rats by Bellgrau and Wilson (18). We feel it is unlikely that 2 × 10⁷
spleen cells would include enough of these hypothetical anti-idiotype-bearing T cells to suppress the CTL reactivity of an entire animal. Furthermore, it seems likely that priming a B10.Br mouse to immunocompetent C.K spleen cells would augment the number of B10.Br cells expressing such anti-(anti-H-2^k-plus-B10 minors) receptors. However, the ability of primed cells to suppress a CTL response is not enhanced compared with that of unprimed cells (data not shown). This type of anti-idiotype interpretation is, however, favored by Katz et al. (19) as an explanation for related observations.

(b) The injected spleen cells include CTL bearing receptors that recognize recipient alloantigens, but specifically inactivate only those CTL in close proximity, i.e., those CTL that themselves recognize the injected spleen cells. This model takes into account the T cell nature of the depleting cell and the haplotype-specific nature of the suppression. We find this hypothesis highly unlikely, because cells wholly tolerant to host antigens are quite competent at depleting a CTL response (Table III). It is therefore doubtful that immune recognition of the recipient cells by the injected spleen cells is required to elicit suppression. In contrast to our system, a graft vs. host reaction by parental (not minor H-different) spleen cells injected into F1 mice initiates the long-term generalized immunosuppression of the CTL response as documented by Shearer and Polisson (20, 21). In this system, coculturing of cells from injected mice with cells from normal F1 animals resulted in generalized suppression of CTL responsiveness (21, 22). Our observations clearly contrast with those of Shearer, leading us to reject graft vs. host reaction as an explanation for the haplotype- and antigen-specific suppression of CTL response we have studied.

(c) Another hypothesis would predict that suppression is mediated by minor-H-specific suppressor T cells of recipient origin. There is certainly a precedent for both in vivo (23) and in vitro generated suppression in minor H antigen responses (24-26). That tolerant spleen cells can suppress suggests that the induction of this suppression must be solely dependent on the host responding to the injected cells. The types of suppression mechanisms that can be envisaged are limited by the lack of detectable, ongoing suppression in vitro (21, 22) and the short lifespan of the phenomenon in vivo. Furthermore, this suppression must be haplotype specific and must be induced only by the injected cells themselves, not by F1 host cells presenting the foreign minor antigens.

(d) According to a fourth model, the injected spleen cell populations contain veto cells that can inactivate CTL that recognize some component of the veto cell surface. Here the recognition is one way: recipient CTL recognize the suppressor. It could be hypothesized that such veto cells are responsible for monitoring and eliminating autoreactive cells in normal mice. In in vitro experiments, Miller et al. (27-29) have presented evidence for the presence of such cells in normal thymus and bone marrow and in the nude mouse spleen. However, these veto cells demonstrable in vitro are specifically absent from unmanipulated normal spleens, which are clearly a good source for our suppressors. In addition, we have found that bone marrow cells are unable to deplete CTL activity in vivo, and high doses of thymocytes are required to reduce the appropriate CTL response (data not shown). The organ specificity and the absence of in vitro activity of the cells involved in the suppression we have observed make it unlikely that our depletion is mediated by the same sorts of cells described by Miller. However, it has been reported that T cells with "veto-like" activity can be
generated by in vitro culture of normal spleen cells and assayed by antigen-specific suppression of anti-minor H CTL induction during MLC. These spleen cell-derived veto cells could be suppressor cells specific for all types of antigens, but which suppress those minor-H-specific CTL or helper cells that recognize the veto cell itself. Again, the recognition is one way, but this modification of the veto cell model eliminates the necessity of inventing a novel spleen cell type. In our system, even Lyt-2-negative cells suppress, although less efficiently than Lyt-2-positive cells. This Lyt phenotype does not necessarily eliminate the donor cells as a potential source of suppressors. The basic concept of a T cell that inactivates all CTL precursors that recognize it adequately explains our findings, and perhaps the differences between our results and those previously reporting veto cell activity result from experimental design. In addition, we have found that long-term tolerance to subsequent syngeneic male skin grafts can be induced in neonatal females by injection of male T cells, but not B cells (I. L. Weissman, A. Greenspan, and L. Jerabek, manuscript in preparation). These results are consistent with a model of “one way” depletion or suppression of host cells recognizing antigenic donor T cells.

(e) The final hypothesis that we believe can explain our results suggests that the injected spleen cells eliminate the CTL response passively by temporarily inactivating, sequestering, or altering the transferability of the responding CTL precursors. For example, antigens injected intravenously tend to localize in the spleen and selectively recruit lymphocytes to this organ. Perhaps when spleen cells are injected and migrate to the recipient lymphoid organs, recipient cells interacting directly with these injected spleen cells are trapped, or rendered sensitive to mechanical manipulations, or become unresponsive to the proper activation signals. Thy-1-positive cells of any Lyt phenotype might be expected to interact with CTL efficiently, because they migrate to the T cell-rich regions of the host lymphoid tissues. In fact, recent in vivo experiments show that T cells are particularly efficient at presenting antigen to CTL precursors in draining lymph nodes. The “sequestration” theory predicts that minor-H antigen-bearing tolerant cells would be capable of depletion and predicts that suppression would be temporary. However, our experiments show that direct-primed activity could not be recovered in cultured splenic fragments, and both intraperitoneal and intravenous injection of minor H-different spleen cells result in depletion (data not shown). In fact, an intact recipient spleen is not required for depletion: direct-primed CTL activity is equally depleted in lymph node responders from splenectomized and from normal mice (Table IV). Thus, there is nothing peculiar about the splenic architecture that is a required component in depletion. If CTL precursors are trapped by virtue of recognizing the injected spleen cells, this sequestration can take place outside the spleen. These results contrast with those of Streilein and Niederkorn who find a strong splenic dependence of the loss of immune response to H-2-compatible tumors introduced in the anterior chamber of the eye of unprimed mice.

There are previous observations reported by Sprent and Miller of temporary unresponsiveness on the part of spleen, lymph node, and Peyer’s patch cells from animals recently injected with a high dose of heterologous erythrocytes. This antigen-
specific unresponsiveness was explained as a direct result of antigen activation, perhaps reflecting a transient high-zone tolerance. These authors were unable, as are we, to define and characterize the mechanisms involved.

Several elegant in vitro systems have been devised to investigate which cells are competent to present antigen to CTL and the events accompanying this presentation. The relative simplicity of these systems allows the investigator to manipulate important parameters, yet at the same time they cannot take into account the details of lymphoid organ architecture and antigen and cell trafficking that determine the types of cells that can interact in vivo and thereby influence the nature of the immune response. Our studies indicate that the in vivo presentation of minor H antigens by T cells can profoundly suppress the response of recipient CTL. Understanding this short-lived suppression, which may result from inactivation or sequestration of antigen-reactive cells early in an immune response, may influence considerations of the rejection of tumor cells and foreign tissue grafts.

Summary

To detect a strong cytotoxic T lymphocyte (CTL) response to minor histocompatibility (H) antigens in a 5-d mixed lymphocyte culture, it is necessary to use a responder that has been primed in vivo with antigen-bearing cells. It has previously been shown that minor-H-specific CTL can be primed in vivo both directly by foreign spleen cells and by presentation of foreign minor H antigens on host antigen-presenting cells. This latter route is evident in the phenomenon of cross-priming, in which H-2 heterozygous (A × B)F1 mice injected 2 wk previously with minor H-different H-2A (A') spleen cells generate both H-2A- and H-2B-restricted minor-H-specific CTL.

In a study of the kinetics of direct- vs. cross-priming to minors in F1 mice, we have found that minor H-different T cells actually suppress the induction of virgin CTL capable of recognizing them. CTL activity measured from F1 mice 3-6 d after injection with viable A' spleen cells is largely H-2B restricted. The H-2A-restricted response recovers such that roughly equal A- and B-restricted activity is detected in mice as early as 8-10 d postinjection. This temporary hyporeactivity does not result from generalized immunosuppression—it is specific for those CTL that recognize the foreign minor H antigen in the context of the H-2 antigens on the injected spleen cells. The injected spleen cells that mediate this suppression are radiosensitive T cells; Lyt-2+ T cells are highly efficient at suppressing the induction of CTL in vivo. No graft vs. host reaction by the injected T cells appears to be required, as suppression of direct primed CTL can be mediated by spleen cells that are wholly tolerant of both host H-2 and minor H antigens. Suppression cannot be demonstrated by in vitro mixing experiments. Several possible mechanisms for haplotype-specific suppression are discussed, including inactivation of responding CTL by veto cells and in vivo sequestration of responding CTL by the injected spleen cells.

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