IN VIVO ADMINISTRATION OF HISTOINCOMPATIBLE LYMPHOCYTES LEADS TO RAPID FUNCTIONAL DELETION OF CYTOTOXIC T LYMPHOCYTE PRECURSORS

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Normally, exposure of an adult animal to novel antigen produces immunological priming. However, intravenous injection of allogeneic cells can result in specific inhibition (1-9). Thus, lymphoid cells from a mouse previously injected intravenously with viable allogeneic lymphocytes will mount a greatly reduced CTL response against cells identical to the injected cells, but not against unrelated cells, when cultured in an in vitro MLR (2, 5-8). The reduction in the ability of an injected host to mount a cytolytic response against the donor is very rapid, being detectable after <1 d, maximal at 2 d, and still detectable at 11 wk. By injecting fluorochrome-labeled donor cells we have been able to separate the resident donor cells from recipient cells before analysis of function in vitro. Using this technique, and the techniques of limiting dilution analysis of mixed responder cell populations, we have shown that the response reduction seen up to 8 d after injection of donor cells is due to functional deletion of CTL precursors (CTLp)[1] in vivo (6-8), rather than activation of suppressor cells of either host or donor origin (10-13) that mediate their effect in vitro, or redistribution of cells from lymph nodes to spleen within the recipient (14-16). We further show that donor cells can be recovered from a first recipient and used to induce response reduction in a second recipient. The observations are discussed with respect to the phenomenon of veto (17-20).

Materials and Methods

Mice. Male C57BL/6J (B6), DBA/2J (D2), (C57BL/6J × DBA/2J)F$_1$ (BDF$_1$), and B10.BR (BR) mice were obtained from The Jackson Laboratories (Bar Harbor, ME). Their H-2 haplotypes correspond to b,d, b/d, and k respectively. All mice arrived at our animal facility at 6 wk of age, and were subsequently used between 7 and 10 wk of age.

Preparation and Injection of the Donor Cells. Lymph node cells (LNC) were isolated from the superficial and deep cervical, inguinal, and mesenteric lymph nodes. Lymph nodes were placed in medium (αMEM with 10% FCS, 50 μM 2-ME, and 10 mM Hepes) at room temperature and teased apart by gently squeezing them through a wire mesh with a disposable syringe plunger. Released cells (in 6 ml) were layered over 3 ml of 6% BSA in PBS, centrifuged to separate lymphocytes from adipocytes, resuspended in 5 ml of medium, then underlayed with 5 ml Lympholyte-M (Cederlane, Laboratories, Hornby, Ontario), and centrifuged at room

[1] *Abbreviations used in this paper:* CTLp, cytotoxic T lymphocyte precursors; LNC, lymph node cells.

temperature for 20 min at 400 g to remove fine debris, erythrocytes, and dead cells. After washing with PBS containing 1% FCS (PBS 1% FCS), the LNC were adjusted to 25 × 10⁶ cells/ml and 0.4 ml of this suspension was injected into the lateral tail vein of a recipient mouse. At this point, the LNC viability was typically >96%.

Labeling Donor Cells with FITC.   Generally following Butcher and Weissman (21), cells prepared as above were resuspended to a concentration of 50 × 10⁶ cells/ml of 0.9% BSA in PBS and a FITC stock solution (600 µg/ml in PBS) was added to make a final concentration of 30 µg/ml. After a 20-min incubation at 37°C in a water bath placed in an incubator with a 10% CO_2/air atmosphere, the reaction was stopped by addition of 5 ml medium and centrifugation through 3 ml of 6% BSA in PBS, followed by washing with PBS 1% FCS.

Analysis and Separation of Donor Cells Retrieved from the Recipient.   LNC were isolated from recipients of an injection of 1–2 × 10⁷ FITC-labeled cells. The labeled cells were identified and could be sorted apart from the host cells using a Coulter Epics V flow cytometer. Dead cells were eliminated based on forward angle light scatter and analysis was typically based on 3 × 10⁴ to 1 × 10⁵ events. Flow cytometric analysis of labeled populations showed that 100% of the cells were initially brightly labeled.

The magnitude of fluorescence of cells, retrieved from lymph nodes or spleens of intravenously injected mice, though remaining bright and easily detectable, decreased rapidly during the first few hours, then remained relatively constant, decreasing very slowly over the following days (~5–10% per day). Examination of labeled cells by fluorescence microscopy showed a correlation between the rapid loss of fluorescence and an altered pattern of staining, which changed from bright rim fluorescence to a diffuse cytosolic fluorescence that included the nucleus of all cells (although initially fluorescence is absent in the nuclei). In vitro cell mixing experiments between labeled and unlabeled cell populations showed no evidence for transfer of label between neighboring cells, supporting earlier in vivo findings by others (22).

Generation of CTL and the Cytotoxicity Assay.   A standard MLR was used to activate and expand reactive CTL within the responder LNC population. 3 × 10⁴ responder B6 LNC were cultured for 5 d with 3 × 10⁵ BDF₁ irradiated (2,000 rad) spleen cells in 96-well V-bottomed plates (Linbro; Flow Laboratories, Mississauga, Ontario), six replicates per group, then tested for the ability to kill ⁵¹Cr-labeled BDF₁ or D2 spleen cell Con A blasts, or ⁵¹Cr-labeled P815 (H-2ᵈ) tumor targets, as previously described (23). The specific cytolytic activity (23) of the experimental group was expressed as a fraction of the control (mean ± 1 SD) within each experiment. The control response was determined using LNC responder cells isolated from identically treated mice that had received 10⁷ fully syngeneic LNC in place of donor BDF₁ cells. The response against third-party BR was tested in each experiment to verify the specificity of a reduced response.

Limiting Dilution Analysis.   The CTLp frequency within responder LNC populations was determined by limiting dilution analysis as previously described (23). Briefly, 100, 300, 600, 1,000, or 3,000 responder cells were distributed into 30, 24, 24, 18, or 12 wells, respectively, in 96-well V-bottomed plates. Additionally, each well received 3 × 10⁵ irradiated spleen cells, as stimulators, and an optimal concentration of growth and differentiation factors, typically 10% rat Con A supernatant containing 45 mM α-methyl mannoside. After 5 d of culture, 3,000 BDF₁, D2, or BR ⁵¹Cr-labeled spleen cell Con A blast targets were added to each well. The spontaneous release ranged from 11 to 15% of total releasable radioactivity. This method has been previously characterized as having the sensitivity to detect the killing of a clonal population of CTL that have expanded from a single CTLp (23). Each well was scored as either plus (having lytic activity) or minus (not having lytic activity), and a fit to single hit limiting dilution was tested. Subsequently, the relative frequency of CTLp in the experimental LNC population was calculated as a fraction of the frequency of CTLp in the control population.

Results

Intravenous Administration of F₁ Cells Can Reduce the Parental Host Response.   Fig. 1 demonstrates the response reduction induced by a single intravenous injection of 10⁷ BDF₁ LNC into B6 mice when LNC from these mice were subsequently tested for their ability to generate CTL in vitro in a B6 anti-BDF₁ MLR. Significant
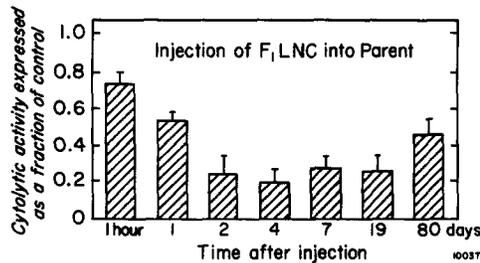
FIGURE 1. Cytolytic activity of B6 LNC against BDF$_1$ spleen cells in a 5-d MLR. The response by cells taken from B6 mice at various times after the injection of 10^7 BDF$_1$ LNC is shown as a fraction of the response by cells taken from control B6 mice simultaneously injected with 10^7 B6 LNC.

reduction of the response was detected by 24 h after the injection, approached a maximal level by 48 h, and persisted for at least 11 wk. The response of the injected mice against third-party cells, from BR or C3H, was not altered (e.g., see Fig. 2). Thus, the reduction of the response was specific for those T cells carrying receptors that were reactive with allogeneic determinants carried on the surface of the injected BDF$_1$ lymph node cells. Note that F$_1$ cells were used as donor cells to avoid the potential complications of a graft-vs.-host reaction.

Analysis of the Fate of the Injected Cell Population. We sought a method to identify and, if desired, remove the injected F$_1$ cells before in vitro testing. It has been shown previously (22) that FITC-conjugated lymphoid cells appear to remain labeled and to recirculate normally when injected intravenously into mice. We found that FITC conjugation of BDF$_1$ cells did not alter their ability to specifically reduce the subsequent response of B6 recipients against D2 alloantigen (Fig. 2).

Fig. 3 shows two-dimensional profiles of forward angle light scatter versus fluorescence intensity for lymph node cells from B6 mice injected 2 d previously with 10^7
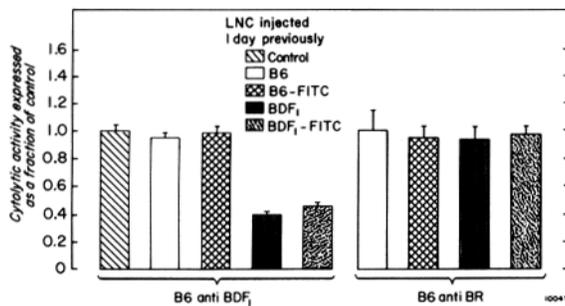

FIGURE 2. Prior conjugation of FITC to BDF$_1$ donor LNC does not alter the capacity to reduce the B6 anti BDF$_1$ response. B6 recipients were either not injected (i.e., unmanipulated control) or injected with 10^7 unlabeled LNC or 10^7 FITC-labeled LNC from the indicated donors. The relative response to a third-party alloantigen (anti B10.BR response) is shown in the right half of the figure.
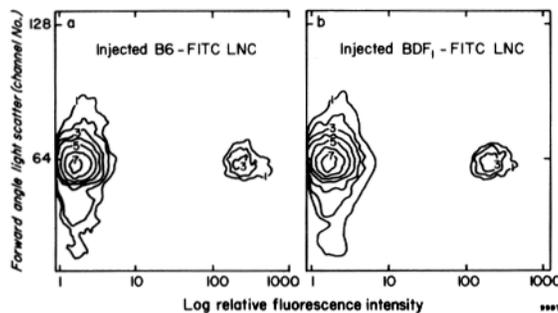

FIGURE 3. Two-dimensional profiles of forward angle light scatter versus relative fluorescence intensity of 10^5 LNC from B6 mice injected 2 d previously with either 10^7 B6-FITC (*a*), or BDF$_1$-FITC (*b*) LNC. The data are presented as contours, the number shown within a contour line corresponding to log$_2$ of the number of cells counted at that level. The B6-FITC cells comprised 1.8%, while the BDF$_1$-FITC comprised 1.7% of the total number of cells analyzed.

FITC-conjugated B6 or BDF1 lymph node cells. The injected cells can be readily distinguished from host cells. Note that there are no obvious differences in the distributions observed in mice injected with fluorescent BDF_1 or B6 (self) lymph node cells. As a function of time after injection, the fraction of FITC-labeled cells (either BDF_1-FITC or B6-FITC) approached the same steady-state value in both lymph node and spleen, varying from an average of 1.3–1.9% after 24 h in different experiments (Fig. 4), and remained constant for over 5 d. The results are numerically consistent with the proposition that the injected cells have homogeneously mixed with the host recirculating lymphocyte pool. This proposition is further supported by the observation that doubling the number of injected cells resulted in doubling the fraction appearing in the lymphoid tissues (data not shown).

Response Reduction in Lymph Node and Spleen. Previous results (2) indicated that intravenous injection of viable semiallogeneic F_1 lymphoid cells reduced the response of both lymph node and spleen cells against the injected alloantigen in a subsequent in vitro MLR. To make a more quantitative comparison, in this study we measured the response reduction in a defined population of host cells capable of reaching lymph nodes and spleen after intravenous injection: 10^7 FITC-labeled lymph node cells from B6 donors were injected into syngeneic B6 recipients, followed 24 h later by 10^7 unlabeled BDF_1 cells. Lymph node and spleen cells were prepared 5 d after the first injection and the B6-FITC cells were separated by FACS. When tested for their ability to respond in the MLR, the recovered B6-FITC cells from either lymph nodes or spleen showed equally reduced responses against BDF_1 stimulator cells but normal responses against BR (Fig. 5). These results imply that the response capability of all lymphocytes in the recirculating pool is equally reduced.

Mechanism of Response Reduction. The population of lymph node cells taken from an injected animal includes injected F_1 cells. It is possible that these cells, either directly or indirectly, induce suppression during the in vitro culture period. To address this question, BDF_1-FITC cells were injected into the parent at various time intervals before removing the lymph nodes, and before culture, labeled cells were removed by cell sorting. The results (Fig. 6) indicate that removal of the injected
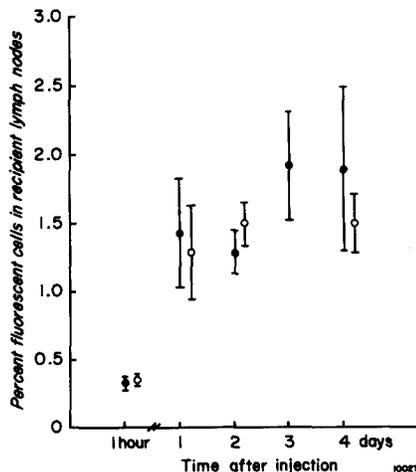

FIGURE 4.   The fraction of recipient LNC comprised of donor cells, as a function of time after injection. B6 recipients received either 10^7 B6-FITC LNC (*closed circles*), or 10^7 BDF_1 LNC (*open circles*). This analysis was performed by fluorescence flow cytometry. Each point represents results accumulated from two to five independent groups of experiments, and is expressed as a mean ± 1 SD.
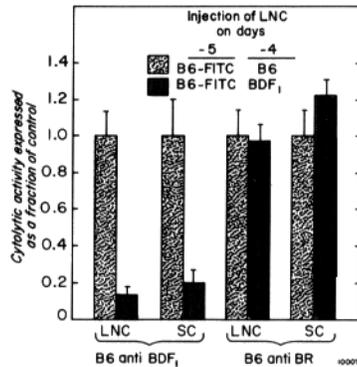
Figure 5.   The responses of lymphocytes recirculating through lymph node and spleen are reduced equally by exposure to semi-allogeneic cells. FITC-labeled B6 LNC were injected intravenously into syngeneic B6 recipients (day −5). One day later (day −4), the mice were further injected with either 10^7 BDF₁ or 10^7 B6 LNC. 4 d later (day 0), FITC-labeled cells were recovered by cell sorting from both lymph node and spleen of each group and tested for their ability to generate CTL as indicated. Reanalysis of 10^4 sorted cells indicated an enrichment to 92–96% fluorescence positive cells for LNC, and 76–78% for spleen cells.

BDF₁-FITC cells from the responder population did not alter the magnitude of the reduction detected in the MLR. Note that reanalysis of the sorted cells demonstrated that the fluorescent donor cells were typically reduced to 0.01% or less of the total population, indicating that no more than three fluorescent donor cells were added to each MLR (see Materials and Methods).

We have considered three possible explanations for the above results: (a) Suppressor cells of recipient origin became activated in vivo and mediated suppression in vitro; (b) suppressor cells of donor (F₁) origin became activated in vivo, in the process losing their fluorescent label, and mediated suppression in vitro; (c) recipient CTL precursors became inactivated in vivo before the cells were placed in the MLR. To distinguish among these possibilities, we used limiting dilution analysis to measure the frequency of CTLp reactive to BDF₁ cells in BDF₁-injected and control B6-injected mice. Fig. 7 shows that the reduction in the magnitude of the MLR response in F₁-injected mice at varying times after injection was reflected by an equivalent, proportional decrease in the CTLp frequency. This suggests that response reduction is entirely the result of functional deletion of CTLp in vivo and not due to the induction of suppressor cells of either recipient or donor origin in vitro.

As a further test for the presence of suppressor cells acting in vitro, we measured the frequency of CTLp in an equal mixture of cells from control and F₁-injected mice on the assumption that any suppressor cells present should have an effect on the CTLp of both populations. The measured CTLp frequency was equivalent to
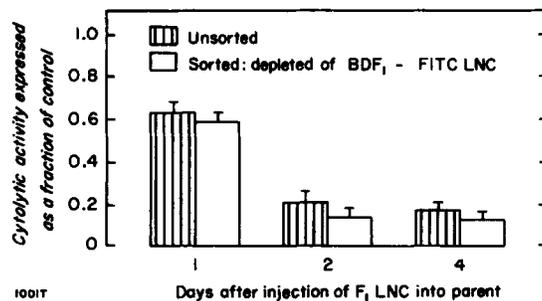

Figure 6.   Removal of the FITC-labeled resident BDF₁ LNC from the B6 responder cell population before the MLR does not reverse reduction of cytolytic activity.
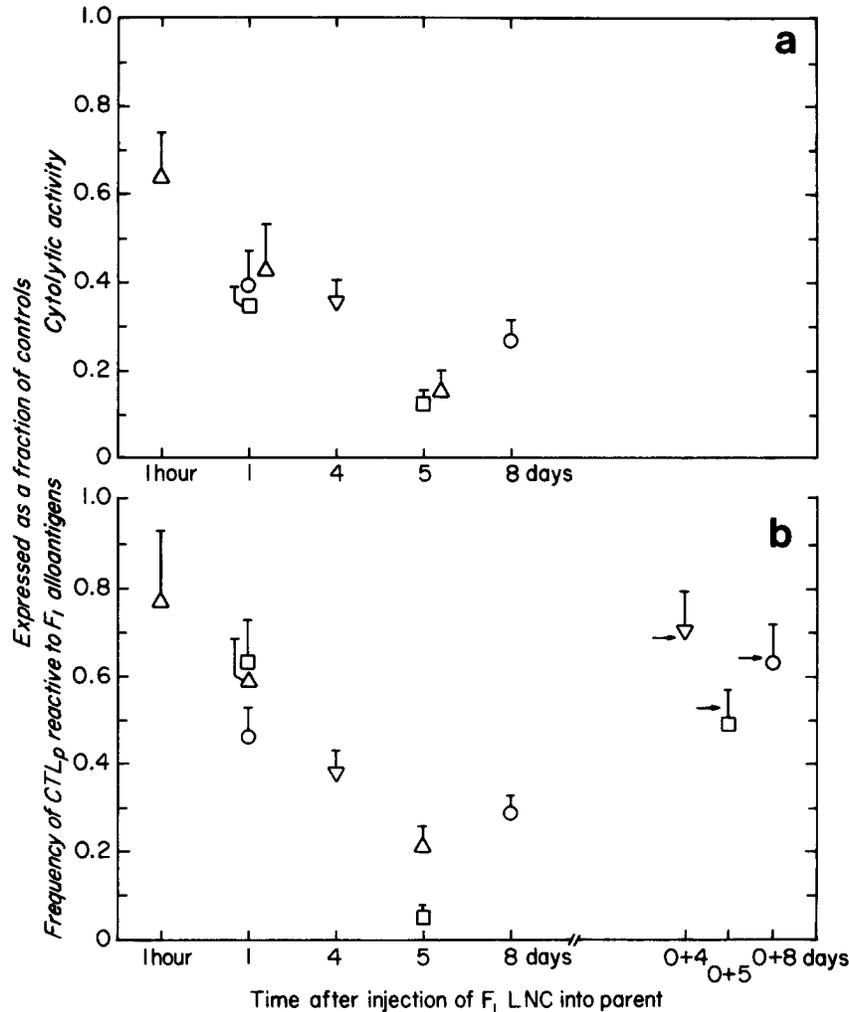
FIGURE 7. The relative degree of reduction in the MLR (a) is reflected by a similar decrease in the relative frequency of CTLp, as determined from limiting dilution analysis (b). Each symbol refers to a different experimental group of animals. Each point is derived from the analysis of the response of two to four mice. B6 mice were injected with 10^7 BDF$_1$ LNC, then killed after the indicated interval of time. Points labeled 0 + 4 day, 0 + 5 day, and 0 + 8 day indicate the relative frequency of CTLp measured in responder LNC taken from control strain A mice mixed in a 1:1 ratio with responder LNC taken from mice killed 4, 5, or 8 d after the injection of BDF$_1$ cells. The arrows indicate the predicted relative frequencies determined by calculating the mathematical mean of the individually measured frquencies. The range of CTLp frequency reactive to BDF$_1$ in the control populations was 1/330 to 1/445. The decrease in CTLp frequency was specific since the frequencies of CTLp reactive to an unrelated third party did not vary significantly between experimental and control populations (data not shown).

the predicted mean of the CTLp frequencies determined for each population measured separately (*right side*, Fig. 7 *b*), suggesting no suppressor cells were present.

F₁ Cells Recovered from a First Recipient Can Induce Response Reduction in a Second Recipient. We next asked whether cells from a first B6 recipient injected with FITC-

Table I

*Ability of Host or Recovered Donor Cells to Induce Response Reduction
When Reinjected into New Recipients*

		Experiment			
Group	Cells transferred	1	2	3	4
A	FITC-labeled (1.4-2.0 × 10⁶)	0.49 ± 0.11	0.30 ± 0.07	0.15 ± 0.04	0.32 ± 0.12
B*	Unlabeled (2.0 × 10⁷)	1.03 ± 0.12	1.14 ± 0.11	0.67 ± 0.11	1.18 ± 0.16
C	Unlabeled (1.6 × 10⁶)	ND	1.02 ± 0.06	ND	ND
D‡	Unsorted (2.0 × 10⁷)	0.94 ± 0.08	0.77 ± 0.15	ND	0.54 ± 0.10
E	Fresh BDF₁ (1.4-2.0 × 10⁶)	0.48 ± 0.07	0.20 ± 0.07	0.10 ± 0.04	0.10 ± 0.05
F	Control: Donors for A to D	0.14 ± 0.04	0.25 ± 0.06	0.06 ± 0.01	0.24 ± 0.08
A'	FITC-labeled (1.4-2.0 × 10⁶)	1.01 ± 0.09	0.76 ± 0.11	ND	ND
B'*	Unlabeled (2.0 × 10⁷)	0.93 ± 0.14	1.18 ± 0.16	ND	ND
C'	Unlabeled (1.6 × 10⁶)	0.92 ± 0.15	0.76 ± 0.14	ND	ND
D'	Unsorted (2.0 × 10⁷)	0.81 ± 0.12	0.82 ± 0.14	ND	ND
E'	Fresh BDF₁ (1.4-2.0 × 10⁶)	0.98 ± 0.08	0.85 ± 0.16	ND	ND

B6 mice were injected with 1.5 × 10⁷ BDF₁-FITC LNC. 6 d later, LNC were collected and injected in the numbers indicated into secondary B6 recipients either after sorting into labeled and unlabeled fractions or unsorted. LNC from these mice and various controls (see below) were collected 6 d later and cultured in anti-BDF₁ (groups A-F) and anti-B10.BR MLR cultures (groups A'-E'). Table entries are cytotoxic activity (mean of six replicates ± 1 SD) after 5 d of culture shown as a fraction of the untreated control. In groups A and A', animals were injected with 1.4 × 10⁶, 1.6 × 10⁶, 1.9 × 10⁶, or 2.0 × 10⁶ recovered labeled cells in Exps. 1-4, respectively. In control groups E and E', mice were injected with matching numbers of freshly collected BDF₁ LNC. Control group F is the response of LNC from a group of primary recipient mice tested in the MLR cultures at the same time as the secondary recipients, i.e., 12 d after injection of BDF₁-FITC LNC.
* In Exp. 3, 2.5 × 10⁷ cells were injected.
‡ Flow cytometric analysis of the injected cells showed that 4-6 × 10⁵ labeled cells were included in the injected cell population.

conjugated BDF₁ LNC could be used to induce response reduction in a second B6 recipient and, if so, which cells. B6 mice were injected with 1.5 × 10⁷ FITC-conjugated BDF₁ LNC. 6 d later a cell suspension was prepared from lymph nodes and sorted into fluorescent and nonfluorescent fractions, wich were then injected intravenously into other B6 mice. 6 d later LNC from these mice were tested for their ability to respond against BDF₁ and third-party stimulators. Mice injected with the fluorescent fraction (containing F₁ cells that retained their label) showed specific response reduction (Table I, group A); mice injected with the nonfluorescent fraction (containing host B6 cells and any injected F₁ cells that may have lost their label) showed a normal response (Table I, groups B and C). All groups showed a near normal response against third-party B10.BR stimulator cells (Table I, bottom section).

Discussion

Our experiments lead to the conclusion that lymphoid cells taken from a parental (B6) mouse injected 1-8 d previously with BDF₁ lymphoid cells, and subsequently tested in an in vitro MLR, have been functionally deleted of CTLp capable of recognizing the injected cells before being put in the MLR (Fig. 7). This functional deletion appears to apply to the whole recirculating lymphocyte pool (Fig. 5). It cannot be reversed by addition of an IL-2-containing supernatant, as equivalent reduction

was seen in the limiting dilution assay measurements, where such a supernatant was added (Fig. 7 b), and in bulk culture measurements or cytolytic activity, where supernatant was not added (Fig. 7 a).

Several lines of evidence suggest that functional deletion is not the result of induction in vivo of either host or donor antigen–specific (11) or antiidiotypic suppressor cells (10) that act during the in vitro culture period. Thus, if there were suppressor cells induced that acted in vitro, a mixture of lymph node cells prepared from parental controls and F_1-injected animals should produce a response less than the sum of the separate responses. However, the frequency of CTLp activated in such a mixture was equal to the sum of that observed in the separate populations (Fig. 7 b). Further evidence against a role for suppressor cells was obtained in cell sorting experiments. Thus, when response reduction was induced by injection of FITC-labeled F_1 cells, the same response reduction was observed in vitro whether or not labeled F_1 cells were removed before in vitro culture (Fig. 6). Here, suppressor cells, if present, must either be F_1 cells that have lost their label or host cells. However, if instead of being put into MLR culture, the labeled and unlabeled fractions were transferred into new recipients, only the labeled cells could induce response reduction in the new recipient (Table I). Thus, the labeled F_1 cells retained the ability to induce response reduction, perhaps, as discussed below, by producing a functional deletion of host CTL precursors that recognize them.

It is important to note that these data do not rule out the possibility that suppressor cells, of either host or donor origin, are induced at later times (i.e., later than day 8) and we are currently investigating these possibilities.

Sano et al. (24) have shown that intravenously administered allogeneic spleen cells, previously treated with neuraminidase, can lead to reduction in the anti-allo-delayed-type hypersensitivity (DTH) response, although untreated spleen cells have little effect. They argue that the neuraminidase-treated allogeneic donor cells concentrate in the liver, subsequently attracting circulating reactive T cells involved in the DTH response. Through an unknown mechanism, these events lead to long-term removal of effector precursor cells from the circulation. In contrast, we found that injection of neuraminidase-treated F_1 LNC was less effective at inducing CTL response reduction than an injection of untreated control F_1 LNC (data not shown), and we conclude that CTL precursor cells are not affected by the mechanism studied by Sano et al. Note that DTH reactions primarily involve class II MHC, whereas CTL reactions primarily involve class I MHC.

A veto cell is a cell that can inactivate CTLp that recognize it (17–20), and can be thought of as a functionally deleting APC. The observations that we describe here could be explained by this mechanism. Thus, donor F_1 cells would contain veto cells that, when recognized by host CTLp reactive against the foreign alloantigen, would lead to inactivation of these CTLp. Rammensee and colleagues have shown tht intravenous injection of allogeneic, T cell–containing lymphoid cells leads to an apparent functional deletion of both the host and the donor CTLp capable of recognizing each other (6, 8). Our results generally confirm their findings and provide additional evidence to show that the mechanism responsible for response reduction is active in vivo, and is not due to induction of suppressor cells acting in vitro.

The veto phenomenon was first characterized by in vitro studies of the MLR, where it was shown that certain cells (CTL, but also other cells) have the ability

to produce functional deletion of CTLp capable of recognizing determinants on their surface. It has been previously argued that a T cell that receives a specific signal (i.e., antigen in association with MHC) but then fails to receive all the necessary secondary signals will result in functional anergization of the T cell, rather than activation (25). Consistent with this model, Jenkins et al. have recently shown that peptidic antigen presented by APCs previously treated with the crosslinking fixative ECDI (26, 27), or by purified Ia incorporated into planar lipid membranes (28), fails to activate class II–restricted, antigen-specific helper T cell clones. Instead, this encounter can induce long-term unresponsiveness of these T cells to antigen presented subsequently by normal, untreated APC. The authors have argued that the T helper cell clones become unresponsive when a co-stimulatory factor fails to be delivered concomitantly with the antigen-specific, MHC-restricted signal (as opposed to active delivery of suppressor factors). Support for this has come from the observation that addition of irradiated allogeneic B cells or macrophages to T cell clones cultured with ECDI-treated APCs and antigen can lead to their activation rather than anergization (27), putatively resulting from delivery of a co-stimulatory factor. These findings contrast with the in vitro veto phenomenon affecting CTLp, since it has been observed that CTLp are most sensitive to veto 1–3 d after exposure to antigen (29–31), and that mature CTL, (including CTL clones) are insensitive to inactivation by veto. Furthermore, the culture conditions used to detect activity of veto cells involve adding them to an MLR that includes irradiated allogeneic spleen cells as stimulators. To this point, no analogous co-stimulatory signals have been found that can overcome the activity of veto cells, or restore the response of inactivated CTLp. The mechanism of functional deletion in the studies we described here and in previous in vitro studies (17–20) remains unclear and may not be the same in the two cases.

Although the precise molecular events may be distinct, both class I–restricted CTLp and class II–restricted helper T cells are apparently susceptible to functionally deleting antigen presentation. Analogously, B lymphocytes may be made unresponsive subsequent to interaction with specific antigen under special conditions (32, 33). Delineation of the mechanisms involved in functionally deleting antigen presentation may be essential to understanding the regulation of specific immunity.

It would appear that a veto-like phenomenon could readily explain the mechanism of negative selection preventing the generation of autoreactive, pathogenic T cells during ontogeny in the thymus (34–38). In addition, the evidence presented in this paper raises the possibility that these mechanisms may persist among mature populations in the periphery of the adult animal. Since current evidence suggests that processed self antigen may be presented on the cell surface in association with MHC (39, 40), a peripheral veto mechanism increases the potential range of self antigens that may be presented to persisting autoreactive T cells and hence does not restrict the mechanism to privileged antigens presented solely in the thymic environment. It would then be intriguing to investigate the range of antigens that could be presented in a suppressive manner by such a system, the role of veto-like mechanisms in the downregulation of an immune response, and how such down regulating systems avoid interfering with effective immunity to infectious agents. Conversely, it would also be important to study if this system could be experimentally manipulated in order to selectively control or delete undesireable specificities from the T cell repertoire. This could be potentially useful for facilitating tissue allografting

or for inactivating pathogenic T cells responsible for certain autoimmune diseases. Clinical precedent for this approach already exists, such as the observation that patients previously transfused with blood are more likely to accept a subsequent kidney transplant (41). Animal studies indicate that this effect is optimized when the blood transfusion and subsequent graft are matched at MHC (3). We and others (7, 20) suggest that this might result from a veto mechanism similar to what we have discussed here.

Summary

It is well established that a single intravenous injection of F_1 lymphocytes can rapidly and specifically reduce the ability of a parental recipient to generate CTL against donor alloantigens in a subsequent MLR. By fluorescently labeling the injected cells, we have been able to identify, and if desired, remove them in cell suspensions prepared from recipient spleen and lymph node. The injected cells, whether F_1 or syngeneic, appeared to form part of the normal recirculating pool. Removal of injected F_1 cells from responder lymph node or spleen cell suspensions had no effect on the response reduction observed in the 5-d in vitro MLR (typically 80% reduction for responder cells taken 2 d after injection of F_1 cells). When the frequency of CTL precursors (CTLp) was measured by limiting dilution, it was reduced to the same degree as the MLR response, implying that response reduction is due to a reduction in the number of activatable CTL in the responder cell suspension. An equal mixture of responder cells from treated (i.e., F_1 injected) and control mice gave a measured CTLp frequency equivalent to the average of the separate frequencies, implying the absence of suppressor cells active in vitro. Labeled F_1 cells recovered from a first recipient could be used to induce response reduction in a second recipient. The results are discussed in terms of APCs that functionally delete rather than stimulate CTLp that recognize them (i.e., a "veto mechanism"). These experiments appear to rule out a role for in vivo–induced suppressor cells up to 8 d after injection of semiallogeneic cells but do not address the question of whether they are induced at later times.

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