Induction of Peripheral Tolerance to Class I Major Histocompatibility Complex (MHC) Alloantigens in Adult Mice: Transfused Class I MHC-Incompatible Splenocytes Veto Clonal Responses of Antigen-Reactive Lyt-2+ T Cells

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Summary

The efficacy and the mode of action of pretransplant transfusion with class I major histocompatibility complex (MHC)-disparate splenocytes in establishing a state of peripheral tolerance in adult mice is analyzed. Adult mice injected intravenously with a critical number of $\sim 5 \times 10^7$ allogenic splenocytes accept skin grafts and develop chimerism in the peripheral lymphatic tissues, but not in thymus and bone marrow. In parallel, a split tolerance evolves: the frequency of class I MHC-reactive Lyt-2+ cytotoxic T lymphocyte precursor (CTL-p) and interleukin 2 (IL-2)-producing T cells falls off in the peripheral lymphoid tissue, but remains unaltered intrathymically. In particular, high affinity CTL-p become clonally undetectable. In vivo generation of tolerant cells is cyclosporin A resistant, but dependent on recipient L3T4+ T cells. Loss of Lyt-2+ CTL-p and IL-2-producing T cell precursors is not due to active suppression, but is caused by clonal anergy. Donor-derived chimeric cells positively selected 7 d after intravenous transfusion exhibit in vitro the hallmarks of veto cells, i.e., paralyze CTL-p reactive to donor-type class I MHC alloantigens. We conclude that the peripheral (split) tolerance induced in vivo by pretransplant transfusion operates because donor-type cells develop in vivo efficiently into "veto cells," which in turn induce a state of clonal anergy within antigen-reactive Lyt-2+ T lymphocytes.

A prime aim in transplantation immunology is to define gentle methods able to convert immune reactivity to transplantation antigens into a state of immune unresponsiveness. This conversion is successful in neonates: introduction of foreign antigens into a developing immune system prevents the system from responding further on to these antigens (1, 2). Recent evidence indicates that both in natural tolerance and in experimentally induced neonatal unresponsiveness maturing antigen-reactive thymocytes become either clonally deleted (3-8) or at least clonally silenced (9-11).

Induction of unresponsiveness in a mature peripheral T cell pool meets difficulties: receptor occupancy by antigens primarily induces sensitization rather than tolerization (12-14). Therefore, most strategies attempt first to reduce the pool of immunocompetent peripheral T lymphocytes, and thereafter to induce unresponsiveness by exposing the regenerating (neonatal) immune system to antigen. In the first step, rather invasive techniques are used, such as whole body (15, 16) or total lymphoid irradiation (17), systemic application of antiproliferative drugs (18, 19), or a combination of either of these methods.

Evaluation of pretransplant transfusion effects have indicated that intravenous confrontation with allogeneic cells may induce specific immunosuppression rather than sensitization (20-28). Independently, Miller, Bevan, and associates (29-36) pioneered the veto cell concept. Accordingly, unlinked to the specificity of their own antigen receptor, veto cells paralyze in vitro the response of T cells reacting to antigens displayed by the veto cells (29-36). Interestingly, upon intravenous transfusion of allogeneic lymphocytes (23-28) or even MHC-transfected recipient cells (37), recipient mice develop a state of specific unresponsiveness as if the transfused lymphocytes were veto cells.

Since intravenous cell transfusion represents a gentle method, and, in addition, bears the promise to induce unresponsiveness in peripheral mature T cells, we have analyzed the efficacy of this approach in vitro and in vivo. The main findings described here are that: (a) injected mice develop a long lasting chimerism that is paralleled by an unresponsiveness to skin

Abbreviations used in this paper: CsA, cyclosporin A; CTL-p, CTL precursor; f, frequency; IL-2 Th4-p, IL-2-producing T lymphocyte precursor; LD, limiting dilution; 95% CL, 95% confidence limit; p, probability of single hit kinetics.
allografts; (b) injected mice display a state of split tolerance (i.e., peripheral but not intrathymic CTL precursors (CTLp)\(^1\) bearing high affinity tolerogen-reactive TCRs are clonally silenced); (c) L3T4\(^+\) recipient T cells are necessary for tolerance induction; and (d) donor-derived lymphocytes isolated from chimeric recipients display in vitro effective veto functions.

Materials and Methods

**Mice.** Breeding pairs of C57Bl/6 (B6) (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)), B6.C.H2\(^{m1}\) (bmi1) (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)), B6.C.H2\(^{m12}\) (bmi2) (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.BR (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)) and B10.A (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)) mice were purchased from OLAC, Blackthorn, UK. CB17 (H2K\(^b\)I\(\alpha\)E\(\beta\)D\(\beta\)) mice were bred in the animal facility of the Ulm University. The mice were used at an age of 8–12 wk.

**Reagents.** Human IL-2 was a kind gift from Eurocetus, Amsterdam, The Netherlands (batch no. LP-370B). Con A was obtained from Pharmacia Fine Chemicals, Upplands Väsby, Sweden. Cyclosporine A (CsA) was generously provided by Dr. Borel, Sandoz AG, Basel, Switzerland. CsA (1 mg) was dissolved in 100 \(\mu\)l C\(\text{H}_2\)\(\text{O}\)F\(\text{H}\) and 20 \(\mu\)l Tween 80 (Sigma Chemical Co., Munich, FRG). A stock solution (1 mg/ml) was prepared by adding 880 \(\mu\)l PBS. Mice received 20 mg/kg body weight daily intraperitoneally. Selected rabbit low-tox complement was purchased from Cederlane, Hornby, Canada. Rat IgG was purchased from Sigma Chemical Co. PE-coupled streptavidin was from Becton Dickinson & Co., Heidelberg, FRG.

**mAbs.** FITC-conjugated anti-Lyt-2 antibodies and biotin-coupled anti-L3T4 and anti-Thy-1.2 antibodies were obtained from Becton Dickinson & Co., and used for cell sorter analyses. Hybridomas GK-1.5 (anti-L3T4, a kind gift from Dr. Fitch, University of Chicago), 53-6.72 (anti-Lyt-2; American Type Culture Collection, Rockville, MD), HO-13-4 (anti-Thy-1.2; American Type Culture Collection), and 34-4-20S (anti-H2D\(^\beta\); American Type Culture Collection) were grown as ascites. mAbs were purified from ascites fluid using standard methods. Anti-D\(^\alpha\) antibodies were coupled with FITC to a FITC/protein ratio of 2 using the method of Goding (39).

**Cell Staining and Cytofluorometry.** For analyses, 2–5 × 10\(^6\) cells were incubated with the respective mAb for 30 min at 4°C. Thereafter, the cells were washed twice with PBS, and, when necessary, a second incubation with PE-coupled streptavidin was performed. Subsequently, the cells were fixed with paraformaldehyde. 1–3 × 10\(^6\) cells were analyzed on a FacsScan (Coulter Immunology, Hialeah, FL). Cells were gated on forward angle light scatter, and the green (FITC) and red (PE) fluorescence was recorded.

**Complement Lysis.** Splenocytes (10\(^7\)/ml) were incubated for 30 min on ice with the respective antibody. After washing twice with PBS, the cells were incubated at 37°C with a 1:10 dilution of rabbit low-tox complement (Cederlane) for 30 min. This procedure was repeated twice. Cell sorter analyses revealed that the efficacy of complement lysis was >98%.

**Cell Preparation.** Thymi and spleens were removed aseptically and then teased. The cell suspension was freed from erythrocytes by a brief incubation in NH\(_4\)Cl. Spleen cells were always passed over a nylon wool column.

**Limiting Dilution Cures.** Limiting dilution (LD) cultures were set up as detailed elsewhere (40). Briefly, replicates (\(n = 16\)) of graded numbers of responder cells were cultured together with 3 × 10\(^6\) irradiated (12 Gy) stimulator cells in 200 \(\mu\)l of medium. Culture medium (Click/RPMI; Biochrom, Berlin, FRG) was supplemented with 10% heat-inactivated FCS (Biochrom), 10 mM Hepes buffer, 2 mM glutamine, 5 × 10\(^{-3}\) M mercaptoethanol, 1 \(\mu\)g/ml indomethacin, and antibiotics. Note that LD cultures for the estimation of CTLp frequencies were supplemented with 20 U/ml rIL-2, while in LD cultures for the determination of IL-2 producer T cells, 10% WEHI-3 and 10% P388D1 supernatant (41) was added.

**Assay for IL-2 Activity and Cytotoxicity.** IL-2 activity in the supernatant of LD cultures was assayed after restimulation with 2 × 10\(^5\) irradiated (100 Gy) (41) stimulator cells using a colorimetric assay (41, 42). The \(^{3}\)HCr release assay was performed as described (40).

**Statistical Analyses.** In LD analyses, all cultures generating cytotoxicity or containing IL-2 exceeding the mean values plus three times the SD of cultures containing only stimulator cells were considered positive. Calculation of frequencies (f), 95% confidence limits of the frequencies (95% CL), and probabilities of single hit kinetics (p) were performed as described (40).

**Skin Grafting.** Mice were anesthetized with pentobarbital (0.6 mg/10 g mouse weight). Tail skin grafts (5 × 5 mm) were then transplanted onto the left chest wall according to the method of Brent et al. (43). Skin grafts were scored for viability three times a week.

## Results

**Transfusion with MHC Class I–Disparate Splenocytes Alters T Cell Reactivity.** Upon intravenous injection of spleen cells from bm1 mice into adult B6 recipients, the in vivo and in vitro reactivity of recipient mice changes dramatically. The majority of B6 recipients receiving 5 × 10\(^7\) allogeneic bm1 spleen cells tolerated bm1 skin grafts for >4 wk. As detailed in Fig. 1 A, the cell number injected was critical for the induction of unresponsiveness in vivo. In vitro, the frequency of anti-bm1-reactive CTLp in the recipient spleen dropped from 1:513 (control) to 1:10,500 within 8 d (Fig. 1B). This frequency reduction compares well with the loss of antigen-reactive CTLp observed in neonatally induced tolerance to class I MHC antigens (9–11). Reduction of anti-bm1-reactive CTLp occurred rapidly within 24 h, peaked at day 10–20, lasted for at least 4 wk (Fig. 1C), and was antigen specific, since the frequency of third party–reactive CTLp remained unaltered (data not shown). Fig. 1D further details that the selective reduction of anti-bm1-reactive T cells was not confined to CTLp, but also included anti-bm-1-reactive IL-2-producing T cells. Using cell sorter–purified Lyt-2\(^+\) T cells from recipient mice, we found that it is this subset that developed specific unresponsiveness (vide infra). Essentially similar results were obtained in the B10.A → B10.BR combination (H-2D\(^b\) difference).

**Not Thymic, but Peripheral CTLp Become Affected.** Next, the frequency of anti-bm1-reactive purified thymic Lyt-2\(^+\) L3T4\(^-\) T cells was compared with that of peripheral T cells 7 d after intravenous injection of 5 × 10\(^7\) bm1 spleen cells into B6 recipients. While in peripheral T cells, the frequency of anti-bm1-reactive CTLp was reduced to ~18% of the con-
control (Fig. 2 A), the number of thymic CTL-p remained unaffected (Fig. 2 B).

We also analyzed whether the few remaining anti-bm1-reactive CTL-p in the peripheral T cell pool of tolerant B6 mice could be scored as high affinity or low affinity CTL-p. To this, LD cultures were set up and clonally developing CTL colonies (probability for clonality >80%) were assayed for lytic activity towards bm1 targets in the presence or absence of blocking anti-Lyt-2 mAb (44). As detailed in Fig. 3 A, 7 of 30 (23%) colonies from splenic T cells of normal B6 mice were able to lyse bm1 targets in the presence of Lyt-2 mAb, and thus have to be scored as T cells bearing high affinity TCRs (44). On the other hand, no high affinity anti-bm1-reactive CTL-p were found in the spleen of tolerant B6 mice. (Fig. 3 B).

**In Vivo Tolerance Induction of Lyt-2+ T Cells Is L3T4+ T Cell Dependent, but CsA Resistant.** In vivo injection of L3T4-, Lyt-2-, or even Thy-1.2-depleted splenic bm1 cells into B6 recipients was almost equally effective in causing a selective loss of anti-bm1-reactive CTL-p (Fig. 4). On the other hand, recipient L3T4+ T cells were necessary for in vivo induction of tolerance. Thus, B6 mice injected intraperitoneally with 2 mg of anti-L3T4 mAb to deplete their L3T4+ T cells (45-47) retained their ability to reject bm1 skin grafts...
Both Donor and Recipient T Cells Are Clonally Silenced, and Donor Lymphocytes Veto the Reactivity towards Their Own Class I MHC Antigens. Using an FITC-labeled H-2D^d mAb, we analyzed the state of chimerism in the B10.A → B10.BR mouse combination. While in bone marrow or thymus no donor cells were found (Table 3), significant numbers (3-9%) of D^d-positive B10.A donor cells were detected for up to 20 d in the spleen of recipient B10.BR mice (Table 3, Fig. 6). About 50–60% of these donor cells expressed the Thy-1.2 T cell marker (Table 3).

Upon depletion, by cell sorting, of donor cells from the spleen of tolerant mice, the recipient B10.BR T cells remained unresponsive towards donor-type stimulator cells (Table 4). Thus, unresponsiveness of recipient T cells is not due to active suppression by donor T cells, but represents a state of unresponsiveness due to either functional or clonal deletion. This type of result also extends to donor-derived B10.A lymphocytes from tolerized mice. As detailed in Table 5, B10.A cells positively selected from chimeric (tolerant) mice contained ~10-fold reduced frequencies of antirecipient (D^d)-reactive CTL-p.

Eventhough donor lymphocytes were found to be tolerant, i.e., clonally depleted for antirecipient (anti-D^d)-reactive CTL-p, the very same cells efficiently suppressed the primary activation of CTL-p with reactivity to donor class I MHC antigens; i.e., expressed veto cell function. For example, when B10.A (donor) cells that were positively selected from spleens of B10.BR (recipient) mice 7 d after intravenous injection of B10.A spleen cells were subsequently mixed at a ratio of 1:1 with normal B10.BR splenic responder cells, and thereafter cocultured at limiting dilution with 3 × 10^5 irradiated B10.A stimulator cells, a biphasic LD curve is obtained. At high cell input (>1,000 veto cells), the frequency of anti-B10.A-reactive CTL-p is effectively reduced (Fig. 7 A; Table 6, biphasic curve II), while at low cell numbers (<1,000 cells), the frequency remained unaltered (Fig. 7 A, biphasic curve I). Thus, as few as 1,000 veto cells are sufficient to block in LD cultures the primary activation of naïve B10.BR. CTL-p reactive to MHC antigens also expressed on veto cells. This suppression was antigen specific, since the reactivity to third-party antigens was not affected (Fig. 7 B; Table 6). Donor-derived B10.A cells from chimeric mice appear to be enriched for veto function, because B10.A cells from normal mice failed to suppress anti-B10.A responses in vitro (Table 6).

Discussion

The results described here will be discussed in the context of pretransplant transfusion effects (20–28), the veto cell

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**Figure 2.** Frequency analysis of splenic T and mature thymocytes in tolerant mice. Splenic T cells (A) and cell sorter-purified Lyt-2^-L3T4^- mature thymic T cells (B) from normal (+) and tolerized (*) (7 d after intravenous injection of bm1 spleen cells) B6 mice were stimulated under LD conditions with bm1 stimulator cells. After 7 d, the LD cultures were tested for lytic activity against bm1 blast target cells. The fraction of negative cultures is plotted against the number of responder cells seeded.
We show here that after intravenous injection of a critical number of allogeneic class I MHC-incompatible donor lymphocytes, recipient mice accept donor type skin grafts for $>30$ d. This state of unresponsiveness is paralleled by a state of chimerism in peripheral lymphatic tissues, but not in bone marrow or thymus. This split chimerism correlates with a state of split tolerance. Thus, the frequency of anti-donor-reactive CTL-p as well as IL-2-producing T cells falls off dramatically in the peripheral lymphatic organs, reaching, after 4–5 d, 5–10% of control values. Yet, in the thymus, the frequency of donor-reactive CTL-p remains unaltered. Both anti-donor-reactive recipient CTL-p as well as anti-recipient-reactive donor CTL-p are clonally silenced. Interestingly, it is the subset of CTL-p bearing high affinity TCRs that becomes functionally deleted. In vitro, donor-type lymphocytes, positively selected from tolerant chimeric mice, bear the hallmarks of veto cells, in that they...
B6 mice were injected intraperitoneally with 2 mg anti-L3T4 antibodies, 2 mg rat Ig, or PBS twice a week. As indicated, some mice were further injected intravenously with $5 \times 10^7$ bml spleen cells. The frequency of bml-reactive CTL-p of splenic T cells was determined 7 d later.

### Table 1. L3T4⁺ T Cell Depletion Prevents the Induction of Tolerance to Class I MHC Alloantigens

| Anti-L3T4 injection | Rat Ig injection | Anti-bml tolerization | CTL-p |
|---------------------|-----------------|-----------------------|-------|
|                     |                 |                       | 1/f   | 95% CL | p   |
| No                  | No              | No                    | 681   | 429–1,103 | 0.55 |
| No                  | No              | Yes                   | 12,884 | 7,780–37,464 | 0.83 |
| Yes                 | No              | No                    | 488   | 352–795 | 0.99 |
| Yes                 | No              | Yes                   | 1,641 | 1,182–2,681 | 0.98 |
| No                  | Yes             | No                    | 381   | 275–681 | 0.95 |
| No                  | Yes             | Yes                   | 9,045 | 5,561–24,224 | 0.40 |

### Table 2. Effect of CsA during the In Vivo Induction of Tolerance

| CsA | Percent of control frequency |
|-----|------------------------------|
|     | B10.A (tolerogen) | bm12 (third party) |
| -   | 16 | 87 |
| -   | 23 | 109 |
| +   | 7  | 85 |
| +   | 20 | 97 |

B10.BR mice were injected intravenously with $5 \times 10^7$ B10.A splenocytes. Some mice ($n = 2$) received daily 20 mg/kg body weight CsA intraperitoneally. After 7 d, the number of B10.A (tolerogen)- or bm12 (third-party)-reactive CTL-p was determined. The percentages of the frequencies of control animals ($n = 3$) are given.

### Table 3. Donor Cell Chimerism in Tolerant Mice

| Tissue                | Control mice | Intravenously injected mice |
|-----------------------|--------------|-----------------------------|
| Spleen                | 0.5          | 9.2 (Thy-1.2⁺, 65.3%)       |
| Thymus                | 0.4          | 0.4                         |
| Mesent.               |              |                             |
| lymph node            | 0.2          | 8.4 (Thy-1.2⁺, 46.9%)       |
| Bone marrow           | 0.4          | 0.6                         |

B10.BR mice were injected with B10.A spleen cells. After 7 d, the splenocytes, thymocytes, lymph node cells, and bone marrow cells were prepared and stained with a monoclonal FITC-conjugated anti-Dd antibody and biotin-coupled anti-Thy-1.2 antibodies. After further incubation with streptavidin-PE, the cells were analyzed for red and green fluorescence.

### Table 4. Frequency Analysis of Donor Cell-Depleted Host Lymphocytes

| Intravenous tolerization (day 0) | Donor cell depletion (day 7) | B10.A (tolerogen) | bm12 (third party) |
|---------------------------------|-----------------------------|-------------------|-------------------|
|                                 | 1/F                         | 95% CL            | p     |
| No                              | No                          | 2,979             | 2,085–4,982 | 0.91 |
| Yes                             | No                          | >74,435           | 1,327 | 965–2,098 | 0.99 |
| Yes                             | Yes                         | 36,381            | 765   | 505–1,582 | 0.15 |

B10.BR mice were injected with B10.A spleen cells. After 7 d, splenocytes were depleted by cell sorting of donor-derived cells, and, subsequently, the frequency of CTL-p was determined with reactivity to either the tolerogen (B10.A cells) or third-party cells (bm12).

specifically block primary activation of CTL-p reactive towards the veto cells' own class I MHC alloantigens. Since in vivo the donor (veto) cells do not affect the repertoire of maturing thymocytes, we conclude that transfusion of adult mice with class I MHC-incompatible lymphocytes leads to a state of peripheral (split) tolerance and thus allows chimerism to be maintained. Why do donor and recipient T cells not reject each other? Peripheral tolerance appears not to be due
Figure 6. Donor cell chimerism in tolerized mice. B10.BR mice were injected with $5 \times 10^7$ B10.A splenocytes. After different time points, spleen cells from tolerized mice were stained with an FITC-coupled anti-Dd antibody and analyzed with an Epics V cell sorter. Staining of control B10.BR spleen cells was <0.5%.

Table 5. Frequency Analysis of Donor-Derived Lymphocytes from Chimeric (Tolerant) Mice

| Responder cells | Stimulator cells | 1/F | 95% CL   | p   |
|-----------------|------------------|-----|----------|-----|
| B10.A (control) | B10.BR           | 762 | 545-1,265| 0.94|
| Donor-derived   | B10.BR           | 8,843 | 5,454-18,688 | 0.99|

B10.BR mice were tolerized by intravenous injection of B10.A spleen cells. After 7 d, donor-derived cells were positively selected by cell sorting, and subsequently, the frequency of antirecipient (B10.BR)-reactive CTL-p was enumerated and compared with that of B10.A cells of control mice.

to active suppression, but due to clonal paralysis of antigen-reactive Lyt-2$^+$ CTL-p and IL-2-producing Lyt-2$^+$ T cells. Since donor lymphocytes exhibit clonal anergy to recipient class I MHC antigens, yet express in vitro remarkably efficient veto functions, we believe that the reciprocal peripheral tolerance in the adult chimeric mice is caused, and maintained, by veto activity of donor and recipient cells.

Deletion of recipient L3T4$^+$ T cells before pretransplant transfusion abrogates the ability of the recipient to be tolerized (Fig. 5; Table 1). Although establishment of tolerance to class I MHC antigens by pretransplant transfusion meets difficulties if the transfused allogeneic lymphocytes in addition are class II MHC incompatible (26, 27, unpublished data), a problem that is overcome by systemic application of anti-L3T4 mAb (27), our data, in addition, imply that recipient L3T4$^+$ cells are essential for the in vivo development of veto.

Figure 7. Donor-derived cells specifically suppress antidonor responses. B10.BR mice were injected intravenously with B10.A spleen cells. After 7 d, the frequency of splenic T cells from control and tolerized mice against the tolerogen (B10.A) and third-party MHC antigens (B6) was tested. A fraction of tolerized splenic cells was further stained with anti Dd antibodies, and donor-derived cells were positively selected with a cell sorter. These cells were mixed 1:1 with splenic T cells from normal nontolerized B10.BR mice and, subsequently, a LD analysis was performed. (A) (*) Control mouse, $f = 1:1,018 (1:711-1,796)$, $p = 0.87$; (+) tolerized mouse $f = 1:7,750 (1:5,128-1:15,846)$, $p = 0.99$; (O) 1:1 mixture of donor-derived cells and control B10.BR cells. (- -) Linear part I of the biphasic LD analysis; frequency estimate, 1:976; (-----) linear part II of the biphasic LD analysis; frequency estimate, 1:3,138. (B) (*) Control mouse, $f = 1:1,110 (1:815-1,739)$, $p = 0.97$; (+) tolerized mouse $f = 1:1,302 (1:950-1:2,071)$, $p = 0.99$; (O) 1:1 mixture $f = 1:572 (1:416-1:913)$, $p = 0.96$. 

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B10.BR mice were injected with B10.A spleen cells. After 7 d, donor-derived cells were positively selected via cell sorting. Splenic T cells from B10.BR mice (line 1) were admixed with spleen cells from B10.A mice (line 2) or B10.A donor-derived cells (line 3) at a ratio of 1:1. These cell populations and splenocytes from tolerized mice (line 4) were subjected to a LD analysis. Mixing of donor-derived B10.A cells to normal B10.BR splenic responder cells resulted in a biphasic LD curve when the reactivity against B10.A stimulator cells was analyzed. Biphasic parts I and II give the frequency estimates of the linear parts of the respective segment of the LD analysis.

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| Responder cells                          | B10.A (tolerogen) | bm12 (third party) |
|-----------------------------------------|-------------------|--------------------|
|                                         | 1/F            | 95% CL        | p    | 1/F            | 95% CL        | p    |
| B10.BR                                  | 1,682          | 1,174–2,966    | 0.94 | 948            | 687–1,529      | 0.59 |
| B10.BR + B10.A (1:1)                    | 1,833          | 1,327–2,963    | 0.38 | 362            | 254–575        | 0.79 |
| B10.BR + donor-derived B10.A cells (1:1)|                 |                   |      |                |                  |      |
| biphasic part I                         | 1,619          |                   |      | 899            | 619–1,639      | 0.85 |
| biphasic part II                        | 6,953          |                   |      |                |                  |      |
| B10.BR, tolerized                       | 7,662          | 4,946–17,000    | 0.97 | 704            | 508–1,149      | 0.79 |

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Table 6. Donor (B10.A)-Derived Cells, but Not Normal B10.A Cells, Specifically Suppress Anti-B10.A Responses In Vitro

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Since there is evidence that in vitro veto cells do not clonally delete antigen-reactive T cells, but rather induce paralysis that can be overcome within time (unpublished data), we anticipate the continuous existence of clonally silenced CTL-p in adult mice transfused with class I MHC-incompatible splenic cells. The availability of TCR-transgenic mice (7, 8) will allow us to experimentally approach this question. There is already evidence that neonatal tolerization for reactivity to Mls antigens phenotypically abolishes anti-Mls reactivity in vitro and in vivo without clonally deleting the respective Vβ-bearing lymphocytes (52).

Even though there are numerous indications that pretransplant transfusion can induce specific immunosuppression rather than sensitization (20–28), the results presented here provide compelling evidence that pretransplant transfusion-induced peripheral tolerance to class I MHC antigens is associated with the in vivo activation and selection of remarkably efficient veto cells. Opposing thymic deletion of antigen-reactive T cells as a basis for negative selection of maturing T lymphocytes (4–8), veto cells operating in peripheral tolerance clonally appear to silence mature Lyt-2+ T cells. If so, then the in vivo induction and maintenance of tolerance to MHC class I antigens by veto cells might represent a paradigm for natural peripheral tolerance to self-antigens not present in the thymus. Accordingly, veto cells control the reactivity towards endogenously produced proteins and peptides, and might thus be further involved in immunoregulatory networks. On the other hand, pretransplant transfusion represents a gentle procedure to “open the window” of unresponsiveness in adult mice for the establishment of conditions for inducing a stable chimerism that then would imply an indefinite state of transplantation tolerance against foreign MHC alloantigens.
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