

Crossing HLA Barriers by “Megadose” Stem Cell Transplants

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Introduction

Bone marrow transplantation (BMT) offers a curative treatment of choice for many patients with leukemia or other hematological disorders.^{1–5} Despite the world registry network, which includes more than 8 million HLA-typed volunteers, the odds of finding a matched unrelated donor in the registries vary with the patient’s race and range from approximately 60–80% for Caucasians to under 10% for ethnic minorities. Moreover, months are often required to identify the donor from a potential panel, establishing eligibility and harvesting of the BM cells. One must remember that age restrictions are extremely stringent for patients utilizing an unrelated donor, as morbidity and mortality rise with age in this type of transplant. Furthermore, with the development of molecular analysis, close matching has itself become more accurate over the years in an attempt to reduce the risk of graft failure and graft-versus-host disease (GvHD), decreasing even more the chance of finding a suitable matched

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donor. For all these reasons allogeneic BMT is not available for many candidates. On the other hand, virtually all patients have a readily available haploidentical family member. Using full haplotype mismatched related donors offers several advantages:

- (1) Immediate donor availability for all transplant candidates;
- (2) Ability to select the donor of choice from several available relatives on the basis of age, infectious disease status and NK cell alloreactivity;
- (3) Controlled cell harvest and graft composition;
- (4) Immediate access to donor-derived cellular therapies if required after transplantation.

However, the use of haploidentical donors has presented a major challenge over the past three decades, due to life-threatening immunological problems, namely GvHD and graft rejection.⁶

Crossing the HLA Barrier in SCID Patients

Numerous murine and clinical studies during the past three decades have demonstrated that effective T cell depletion of BM preparations can completely prevent the development of both acute and chronic GvHD, in the absence of any posttransplant prophylaxis.⁷⁻¹⁵ The proof of principle was initially established in the clinical setting during the early 1980s when it was demonstrated that effective T cell depletion can completely prevent GvHD in SCID patients, even when haploidentical three-locus HLA-mismatched BM is used.^{11,12,14,15} The T cell depletion procedure used in these early studies comprised differential agglutination with soybean agglutinin followed by E-rosetting with sheep red blood cells.^{10,16,17} The procedure was time-consuming but it yielded a 3.5-log depletion of T cells, which was adequate for the prevention of GvHD in our first 3 patients reported in *Blood* in 1983.¹¹ By now more than 200 patients have been treated by this approach and, as can be seen from the long term results of O'Reilly¹⁸ (Sloan Kettering) and Buckley¹⁹ (Duke), the two major groups using it, long term survival is around 80% (Fig. 1).

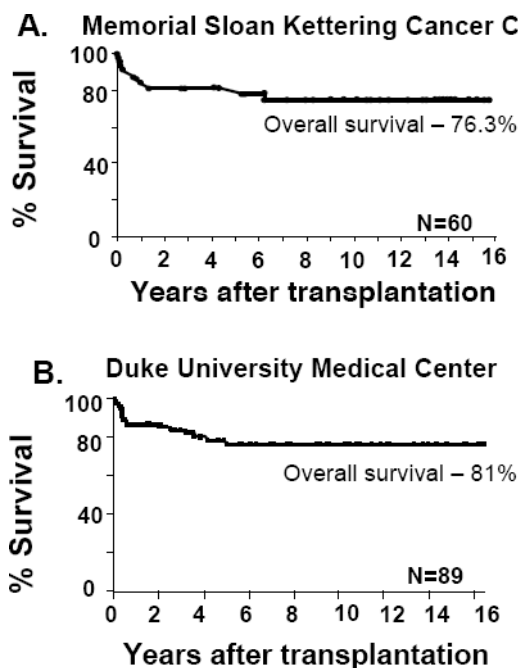


Fig. 1. Long term survival of SCID patients treated by transplantation of human T cell-depleted BM: results of O’Reilly *et al.* (A) (Sloan Kettering)¹⁸ and Buckley *et al.* (B) (Duke University Medical Center).¹⁹

The Experimental Basis for “Megadose” Transplants in Leukemia Patients

Following the encouraging results in SCID patients, it was reasonable to assume that in leukemia patients pretreated with supralethal radiochemotherapy, the remaining immunity at the day of transplant would be dramatically reduced, reaching levels similar to those found in SCID patients. This led us to believe that graft rejection should not represent a major problem. However, early results suggested that this was not the case and a high rate of graft rejection was documented.^{20–22}

Subsequently, using limit dilution analysis, we demonstrated the presence of residual alloreactive CTL-p in mice^{23,24} or primates²⁵ conditioned with radiochemotherapy, similar to that employed in the treatment

of leukemia patients. Likewise, the potential role of stem cell competition mediated by residual host hematopoietic stem cells surviving such lethal conditioning has been documented in mice.^{26,27} Thus, further ablation of these residual stem cells by selective myeloablative agents such as dimethylmyleran (DMM)²⁶ or after thiotepa²⁸ was found to markedly enhance short term engraftment, as well as long term donor type chimerism following transplantation of T cell-depleted BM allografts (Fig. 2). Furthermore, by using the latter agents it is possible to dissect stem cell competition from T cell-mediated rejection, so as to develop appropriate mouse models for the assessment of new modalities addressing these two barriers separately. In particular, based on these studies the conditioning protocol previously based on lethal TBI, in conjunction with antilympocyte agents such as ATG and cyclophosphamide, was supplemented with thiotepa. In addition, myeloablated mice exhibiting minimal stem cell competition were used successfully to construct an

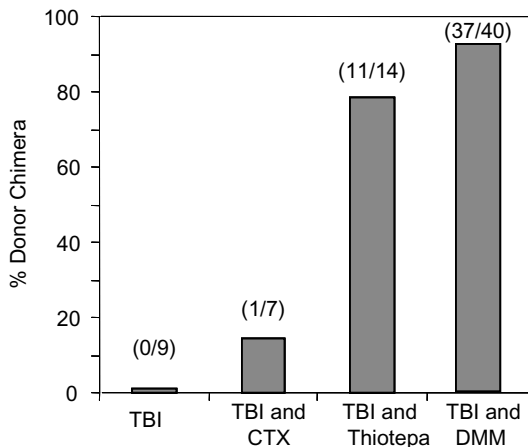


Fig. 2. Effect of conditioning with cyclophosphamide, thiotepa and DMM on the chimerism status of recipients. C3H/HeJ mice received 8 Gy TBI. Experimental groups were treated with cyclophosphamide (120 mg/kg body weight), thiotepa (10 mg/kg) and DMM 0.2 mg. T cell-depleted BM 3×10^6 from C57/BL donors was administered 1 or 2 days post-TBI. Chimerism status was evaluated 30 days posttransplant.^{26,28}

Table 1. Addition of PNA⁻ Thymocytes Prior to Bone Marrow Transplantation: Effect on Survival and Hematological Parameters 12 Days After Allogeneic Bone Marrow Transplantation²⁶

Addition of PNA ⁻ Thymocytes (×10 ⁶)	Transplanted Cells (×10 ⁶)	Survival*	Hematological Parameters [†]		
			Leukocytes (×10 ⁻³ /mL)	Hemoglobin g/dL	Platelets (×10 ⁻³ /mL)
5	3	0/8	–	–	–
5	20	0/8	–	–	–
1	3	0/8	–	–	–
1	20	0/8	–	–	–
0.3	3	3/8	1.5 ± 0.2	4.7 ± 1.2	78 ± 28
0.3	20	6/8	17.0 ± 13.9	12.0 ± 1.7	506 ± 159
–	3	7/8	4.5 ± 1.3	12.5 ± 2.1	211 ± 54
–	20	3/3	4.4 ± 0.5	14.9 ± 0.7	605 ± 30

Female C3H/HeJ mice were conditioned with 8 Gy TBI (day 0) and DMM (0.2 mg/mouse, day +1). Thymocytes from C3H/HeJ mice were fractionated by differential agglutination with PNA and the immunocompetent (PNA⁻) cells were injected IV (day +2). Transplantation of T cell-depleted bone marrow from C57BL/6 donors was performed on day +3.

* Chimerism analysis shows that all mice surviving were donor type chimeras 30 days posttransplant.

† Hematological parameters are presented as average values ±SD. All surviving mice were tested on day 12.

experimental model in which T cell-mediated rejection is reconstituted selectively by addition of graduated numbers of syngeneic mature thymocytes (PNA⁻).²⁶

As can be seen in Table 1, the reconstituted mice strongly reject BM allografts. A BM graft of 3×10^6 cells results in severe anemia, which is fatal even when the lowest number of 0.3×10^6 thymocytes are added. Graft rejection following this thymocyte dose can be overcome by a transplant of 20×10^6 BM cells, but it cannot be avoided by this large BM dose in recipients of 1.0×10^6 thymocytes.

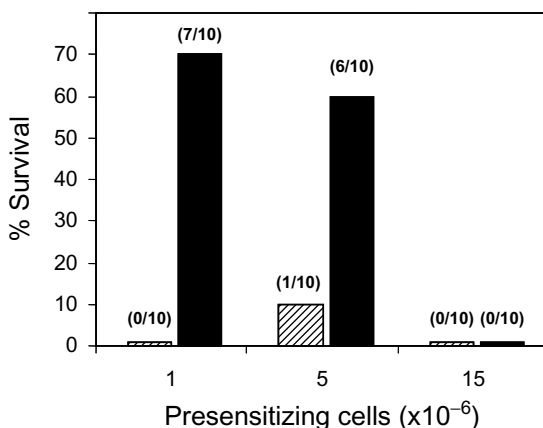


Fig. 3. Effect of the BM cell dose on survival 30 days after transplantation of T cell-depleted BM in presensitized mice. C3H/HeJ mice were presensitized with different numbers of irradiated (40 Gy) spleen cells from C57BL/6 mice 1 week prior to TBI (8 Gy). One day after TBI, the mice received either 3×10^6 (striped bars) or 20×10^6 (black bars) T-cell-depleted BM cells from C57BL/6 donors. Each group included 10 mice. Chimerism analysis showed that all mice surviving 30 days posttransplant were donor type chimeras.²⁶

A similar ability to overcome rejection by cell dose escalation of T cell-depleted BM was observed in mice presensitized with irradiated donor type spleen cells (Fig. 3). Taken together, the quantitative relationship between residual host T cells and the number of T cell-depleted BM cells required to neutralize the resistance of these cells has been established and has led to several attempts to significantly increase the BM cell dose in humans. Initially, we hoped to achieve this goal by *ex vivo* expansion. However, with the advent of granulocyte-colony stimulating factor (G-CSF) mobilization in autologous transplants,^{29,30} in 1993 it became possible to test the concept of dose escalation in humans by supplementing BM with peripheral blood progenitor cells (PBPCs) collected after administration of G-CSF to the donor. This pilot study carried out between 1993 and 1995 demonstrated for the first time that in humans, as in mice, cell dose escalation can facilitate engraftment of T cell-depleted mismatched transplants.^{31,32}

How "Megadose" Transplants Overcome the Immune Barrier

Initial Evidence for Tolerance Induction by Human CD34⁺ Hematopoietic Progenitor Cells

Our results for the first series of leukemia patients receiving a larger inoculum of T cell-depleted transplants could be attributed to several types of accessory cells which are not removed by the lectin separation, as previously shown in murine models employing lethally irradiated recipients.^{27,33-41}

However, beginning in 1995, T cell depletion was substituted by positive selection of CD34 cells using magnetic beads. Further modification of the positive selection procedure in Perugia using Miltenyi magnetic beads and the clinical experience of 1999 are summarized by Aversa in this book. Importantly, this positive selection procedure in which all kinds of hypothetical facilitating cells are removed was not associated with any reduction in the engraftment rate, nor did it affect the speed of hematopoietic recovery. Thus, it seemed that cells within the highly CD34-enriched fraction might possess a marked capacity to overcome resistance to engraftment.

The intriguing question of how the CD34 cells overcome the barrier presented by host T cells was first addressed by Rachamim *et al.*⁴² who demonstrated that cells within the CD34 fraction are endowed with potent veto activity (Fig. 4).

Veto activity was defined in 1980 by Miller⁴³ as the capacity to specifically suppress CTL precursors (CTLp), directed against Ags recognized by the veto cells themselves, but not against third party Ags (Fig. 5). Thus, the recognizing T cell, with specificity directed against the veto cell, is killed upon binding to its veto target. This inherent specificity of veto cells, eliminating only host CTLp directed against the donor Ags, while sparing other CTLp, which can further persist and fight infectious pathogens, has suggested that veto cells could offer a specific and effective modality for the induction of transplantation tolerance.

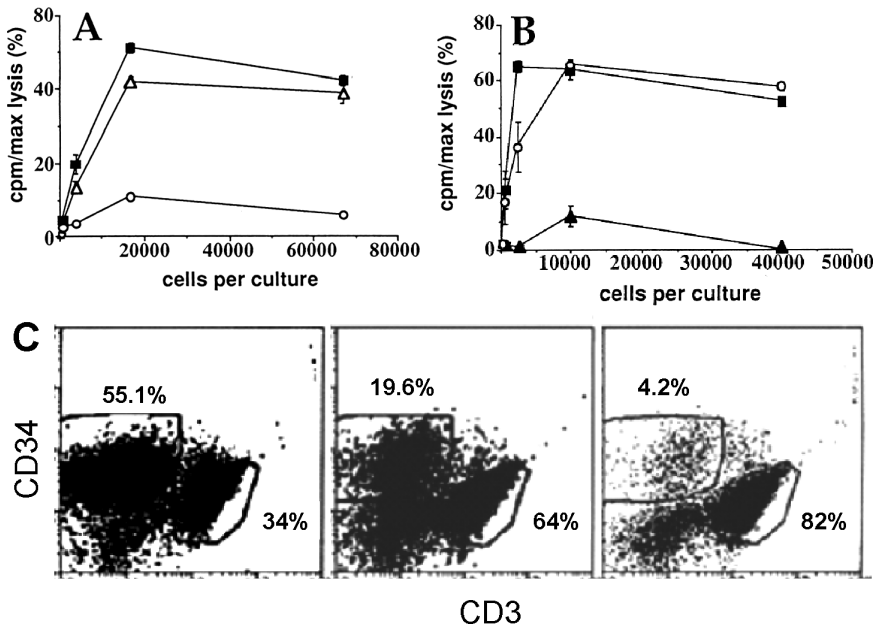


Fig. 4. The tolerizing effect of CD34⁺ cells is abrogated by irradiation and requires cell-to-cell contact. (A) Responder cells (1.2×10^6 /ml) were incubated with 1.2×10^6 /ml irradiated (30 Gy) stimulator cells (black square) for 5 days. Irradiated (30 Gy) (white triangle) or nonirradiated (circle) CD34⁺ cells from the same donor (1.2×10^6 /ml) were added to some of the cultures, as indicated. After 5 days, the cells were isolated and the CTL-p frequency was measured. (B) Responder and irradiated simulator cells were combined in the lower chamber of a Transwell culture system, which contains an upper and a lower chamber. Purified CD34⁺ cells were added to either the lower (black triangle) or the upper (circle) chamber, or were not added to the cultures (black square), as indicated. After 5 days of incubation, the cells were isolated and a limiting dilution assay for CTL-p was carried out. (C) Cellular composition after 5-day MLC. Equal numbers of responder T cells from donor C (84% purity) and irradiated allogeneic stimulator cells from either donor A (top panels) or donor B (bottom panels) were cocultured for 5 days, as described in *Materials and Methods*. Purified CD34⁺ cells (87% purity) from donor A were also added to the cultures, in different numbers, as follows: 5×10^6 (left column), 5×10^5 (middle column) and 5×10^4 (right column). After 5 days, the cells were analyzed by two-color flow cytometry, utilizing fluorescence-labeled monoclonal antibodies against CD34 and CD3. Percentages of CD34⁺ cells and of T cells are shown.⁴²

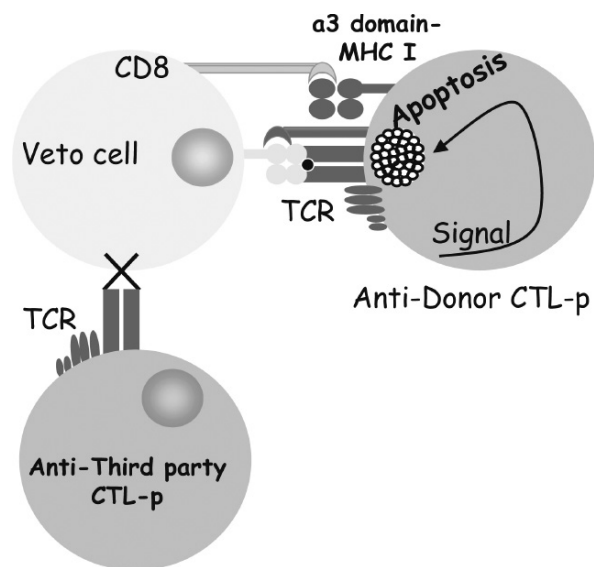


Fig. 5. The veto “concept.” A CTL-p specifically recognizes the veto cell by the binding of its T cell receptor (TCR) to the MHC class I molecule on the veto cell. Once the cells interact, instead of triggering stimulation and expansion of the CTL-p, the veto cell induces the transduction of a death signal (apoptosis) in the CTL-p. The veto activity is specific, as a CTL-p, which bears its TCR against the third party MHC molecule, does not recognize the veto cell, and thus survives. In CD8⁺ veto cells, the binding of the CD8 molecule to the $\alpha 3$ domain of the MHC class I molecule on the CTL-p, plays a role in the apoptosis signal.⁸³

Several important attributes of the CD34⁺ veto cells were shown by Gur *et al.*,⁴⁴ including the importance of the CD34⁺ HLA class I recognition for their activity and their specific inhibition of effector T cells expressing IL-2 and IFN- γ . The latter could provide a useful surrogate assay for the regulatory activity of CD34⁺ cells. This could be of importance considering the relatively low frequency of alloreactive CTL-p in the blood of normal individuals, which necessitates the use of indirect assays to monitor *in vitro* the fate of these cells upon interaction with a given immune regulatory population. Thus, it is possible to measure the inhibition of effector activity by functional assays, such as limit dilution analysis (LDA) for CTL-p (Fig. 6), or by a surrogate assay of effector cells expressing IL-2 and IFN- γ (Fig. 7).

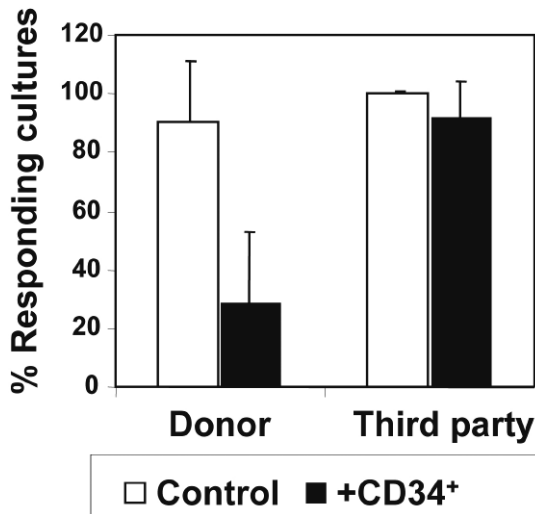


Fig. 6. The regulatory activity of CD34 cells: evidence for target specificity. The average CTL response (SD) in the presence (black bars) or absence (white bars) of CD34⁺ cells at a veto-to-responder cell ratio of 0.5. The veto effect was tested by a limiting dilution assay as follows: equal numbers ($1 \times 10^6/\text{mL}$) of responder cells and irradiated allogeneic stimulator cells from the donor of the CD34 cells and a third party donor were cocultured for 5 days. The responder cells were then cultured again for 7 days under limiting dilution, and the CTL activity was determined by ⁵¹Cr release assay. Data represent the average \pm standard deviation of 11 independent experiments using different donor and third party pairs. A significant difference ($P < 0.001$ on the *t*-test compared with control cultures without CD34 cells) between control cultures and those including CD34 cells was found upon stimulation against donor cells.⁴⁴

This veto activity of CD34⁺ progenitor cells may be mediated by cells other than the most primitive pluripotential hematopoietic stem cells and, therefore, while it is still very difficult to expand the latter cells *ex vivo*, it has been possible, recently, to expand the veto cells within the CD34⁺ cell fraction and increase their number by 20–80-fold simply by short term culture along the myeloid differentiation.⁴⁴

More recently, a major mechanism for tolerance induction exerted by several drugs or cell subpopulations involves anergy induction in the responder T cells. Thus, anergy can be induced by a costimulation

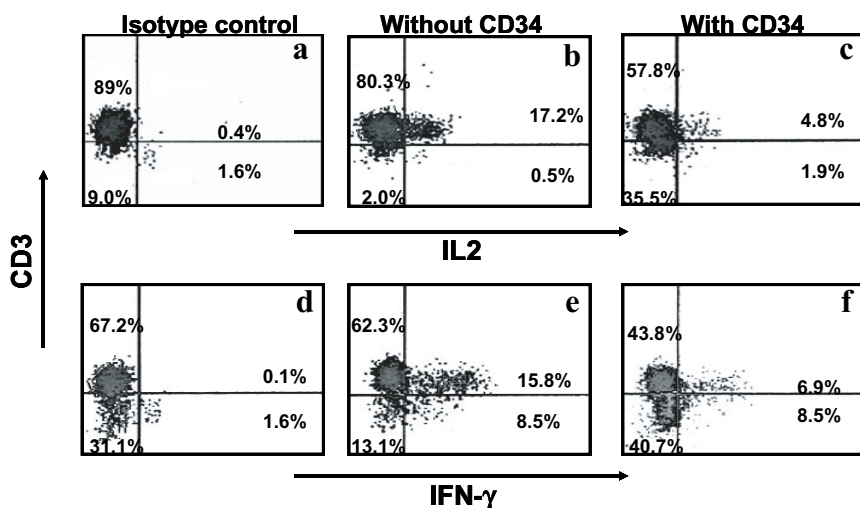


Fig. 7. Regulatory activity of CD34⁺ cells: effect on intracellular staining of IL-2 and IFN- γ in the effector T cells. Responder cells and irradiated allogeneic stimulator cells from the CD34⁺ cell donor were cocultured for 6 days in the absence or presence of CD34⁺ cells (0.5:1 CD34⁺ to responder cell). The cells were subjected to an additional 7-day limiting dilution culture. They were then incubated with phorbol myristate acetate, ionomycin and monensin. After that, they were fixed and stained to enable detection of the intracellular IL-2 (a–c) and IFN- γ (d–f). Lymphocytes were gated based on their FSC/SSC profile. The percentage of the gated double positive cells is indicated.⁴⁴

blockade with CTLA4-Ig,^{45,46} anti-CD40L,^{47,48} or anti-B7⁴⁹ antibodies by cytokines such as IL-10^{50,51} or by suppressor T cells such as CD4⁺CD25⁺ cells.^{52–56} In addition, stimulation with APCs of a DC2 subset^{57,58} or skewing the T cell response into a Th2 type by the appropriate cytokines, such as IL-4 or IL-10,⁵⁹ creates an unfavorable setting for the development of alloreactive CTLs. Our studies, using anti-CD28 mAb or the addition of exogenous IL-2 to bypass the requirement for costimulation via B7, as well as using anti-IL-10-blocking mAbs, have ruled out the possibility that CD34⁺ cells induce tolerance by such mechanisms (Table 2).

Furthermore, Gur *et al.*⁶⁰ showed that the only effective way to reverse the inhibitory activity of CD34⁺ cells was afforded by a caspase

Table 2. Effect of Different Regulatory Agents on the Inhibitory Activity of CD34⁺ Cells⁶⁰

Treatment*	MLR Cultures [†]	Number of Responding/ Nonresponding Cultures [‡]		Statistical Significance [§]		
		Responding	Nonresponding	P Value	P Range	Significance
Anti-HLA-1	A	16	0	ND	ND	ND
	B	9	7	0.0004	$P < 0.001$	S
	A [#]	16	0	ND	ND	ND
	D	15	1	0.3016	$P > 0.1$	NS
Anti-IL-10	A	16	0	ND	ND	ND
	B	7	9	5.7E-06	$P < 0.001$	S
	C	16	0	ND	ND	ND
	D	3	13	8.3E-17	$P < 0.001$	S
Anti-CD28	A	37	11	ND	ND	ND
	B	9	39	3.9E-25	$P < 0.001$	S
	C	30	2	ND	ND	ND
	D	5	43	1.4E-37	$P < 0.001$	S
Anti-CD2	A	6	10	ND	ND	ND
	B	2	14	0.0024	$0.01 > P > 0.001$	S
	C	10	6	ND	ND	ND
	D	1	15	1.4E-20	$P < 0.001$	S
IL-2	A	27	5	ND	ND	ND
	B	13	19	4.6E-07	$P < 0.001$	S
	C	28	4	ND	ND	ND
	D	10	22	6.6E-12	$P < 0.001$	S

(Continued)

Table 2 (Continued)

Treatment*	MLR Cultures [†]	Number of Responding/ Nonresponding Cultures [‡]		Statistical Significance [§]		
		Responding	Nonresponding	P Value	P Range	Significance
IL-12 [¶]	A	12	20	ND	ND	ND
	B	3	29	4.8E-08	$P < 0.001$	S
	C	31	0	ND	ND	ND
	D	7	25	3.8E-25	$P < 0.001$	S

ND — not done; S — significant; NS — not significant.

*The tested regulatory agents were added at the initiation of the MLR to the culture medium, at their optimal concentration, according to the manufacturer's recommendation and to preliminary titration experiments, except for anti-HLA-1. This agent was preincubated with the CD34⁺ cells and washed prior to their addition to the MLR culture.

[†]MLR cultures in which responder cells were stimulated against allogeneic PBMCs from the CD34⁺ cells' donor, in the absence (A and C) or presence (B and D) of CD34⁺ cells, were established.

[‡]The potential of different agents to reverse the inhibitory regulatory activity of CD34⁺ cells was evaluated by comparing the inhibition in the presence (C and D) and in the absence (A and B) of the specific agent. Briefly, a five-day MLR was established in which the responder cells were then recultured for seven more days under limiting dilution in microtiter plates. For each experiment, the number of positive and of negative cultures, tested at the highest effector cell concentration (40,000 cells per well), are shown. Wells were scored positive for CTL activity when Cr release exceeded the mean spontaneous release value by at least three standard deviations of the mean. The regulatory activity of CD34⁺ cells was evaluated by their capacity to inhibit alloreactive CTL-p clones in the MLR to which they were added at a ratio of 0.5:1 CD34⁺/responder cell. The addition of CD34⁺ cells to the MLR against third-party stimulators did not lead to a significant inhibition ($P > 0.1$). Thus, in a total of five experiments carried out in the absence of CD34⁺ cells, 3 of 96 anti-third-party MLR culture wells were scored negative while 6 of 96 were scored negative in the presence of CD34⁺ cells.

[§]The results were statistically analyzed by the χ^2 test.

[¶]The results represent a total of three experiments.

[¶]The results represent a total of two experiments.

[#]The role of anti-HLA-1 antibody was tested by incubating the antibody with CD34⁺ cells prior to their addition to the MLR. Thus, for the statistical analysis of results in culture D we used as a reference the results of culture A.

inhibitor such as BD-FMK, which induces resistance to apoptosis in effector T cells.⁶⁰ Collectively, these results strongly supported a deletion-based mechanism similar to that reported for veto CD8 T cells.^{61–64}

Two types of veto cells that have been widely characterized are the CD8⁺ CTL^{64–66} and CD8⁺ BM cells.^{67–69} In both instances it has been shown that FasL is likely involved in the killing of the effector cells by the veto cells. However, our failure to reverse CD34⁺ cell-mediated regulatory activity by anti-Fas antibody led us to investigate the role of other death ligands, such as TNF- α and TGF- β . In contrast to other studies showing that CD2⁺CD3⁻CD8⁺CD16⁺ veto cells in the monkey BM mediate their effect through TGF- β , our study indicates that the regulatory activity of CD34⁺ cells is likely mediated by TNF- α and not by TGF- β (Fig. 8).

Previous insights into the veto mechanism of CD8⁺ veto T cells have indicated that both CD8 and FasL on the veto cells might be required to

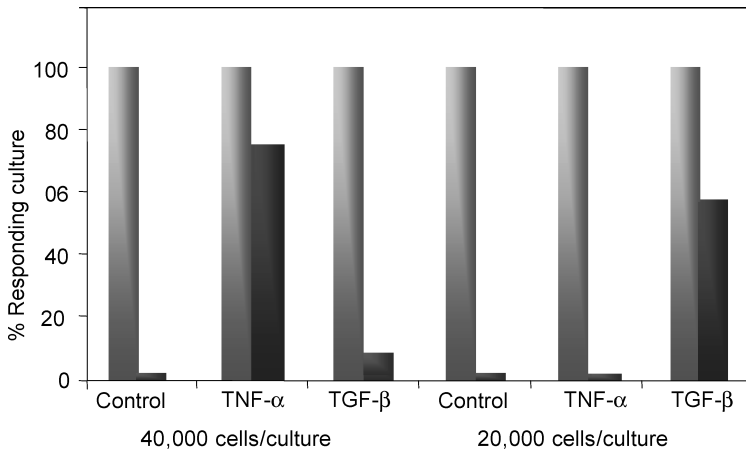


Fig. 8. CD34⁺-cell-mediated suppression of CTL responses is blocked by the addition of anti-TNF- α but not by the addition of anti-TGF- β . A 5-day MLR was established in which responder cells were stimulated against allogeneic cells from the CD34⁺ cell donor, in the presence (*gray*) or absence (*black*) of CD34 cells. Anti-TNF- α or TGF- β neutralizing mAbs were added at the beginning of the MLR at a concentration of 5 μ g/mL. The CTL activity was determined by the end of 7-day limiting dilution cultures. The data show the percentage of responding cultures at cell concentrations of 4×10^4 and 2×10^4 cells per well.⁶⁰

induce specific deletion of the effector cells.^{64,70} Such a mechanism involves initial recognition of the veto cell by the TCR of the effector cell, leading to expression of Fas upon activation and thereby allowing Fas–FasL apoptosis to take place, once inhibitory molecules such as FLICE-inhibitory protein (FLIP) or XIap are downregulated in the effector cell (Fig. 9). The extra affinity required to maintain the interaction between the effector cell and the veto cell might be provided through binding between CD8 on the veto cell and the class I $\alpha 3$ domain on the effector cell, but some form of signaling via this interaction might also occur.^{64,70,71}

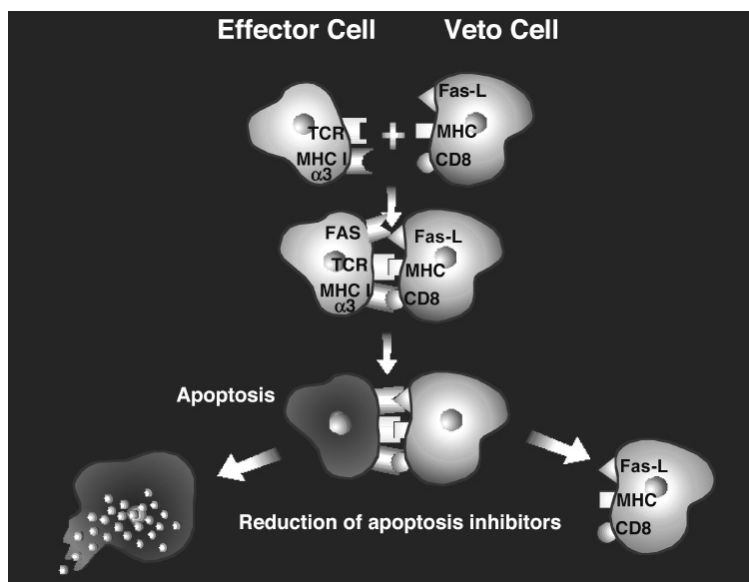


Fig. 9. Veto CTLs induce apoptosis in the effector T cells by the Fas–FasL mechanism. Upon engagement between the TCR of the effector cell and class I of the veto cell, the effector cell is activated and Fas is upregulated. However, the presence of FasL on the veto CTL is not sufficient to trigger apoptosis, as FLIP is also upregulated. The high affinity interaction between the CD8 on the veto cell and the $\alpha 3$ domain on the effector cell likely maintains the contact long enough (60–72 h) for FLIP and other inhibitory molecules to be downregulated, and for Fas–FasL killing to be completed.⁷¹

Considering that human CD34⁺ cells do not express CD8 molecules, our results indicate that the extra affinity afforded by CD8 on CD8⁺ veto cells could be provided by other adhesion molecules on the CD34⁺ cells. Preliminary results suggest that LFA1–ICAM1 might be involved in this context.

This veto activity of CD34⁺ progenitor cells may be mediated by cells other than the most primitive pluripotential hematopoietic stem cells and, therefore, while it is still very difficult to expand the latter cells *ex vivo*, it has been possible, recently, to expand the veto cells within the CD34⁺ cell fraction and increase their number by 20–80-fold simply by short term culture along the myeloid differentiation.⁴⁴ Furthermore, Gur *et al.* demonstrated that during *ex vivo* differentiation of myeloid cells from CD34⁺CD33⁻ hematopoietic stem cells, the veto activity is also exhibited by immature CD34⁺CD33⁺ as well as CD34⁻CD3433⁺ cells. The veto potential is apparently lost upon completion of maturation at the level of CD14⁺ monocytes or CD13⁺ neutrophils.

Preliminary results suggest that immature dendritic cells, previously shown to induce immune tolerance (L. Zangi *et al.*, unpublished results), exhibit marked veto activity on CD8 T cells while suppressing CD4 T cells through an MHC-independent mechanism mediated by the NO system.

Finally, NK cells which were shown to exhibit veto activity upon activation with IL-2 were also shown to appear rather early during the posttransplant period.^{72,73} Collectively, based on these observations, the following working hypothesis can be suggested. After transplantation of purified CD34 cells, the likelihood of activation of antidonor CTLp is proportional to the level of residual host T cells and is inversely correlated with the number of veto cells. Veto activity can be contributed initially by the CD34 cells infused and subsequently by the CD33 progeny of these cells which grow exponentially within the first few days posttransplant and also include CD11c⁺ immature dendritic cells. In addition, when using donors of HLA genotypes, which allow the generation of alloreactive NK cells, such cells can also be generated during the first few days after transplant and eradicate mature CTLs which were able to escape the veto cells

and to differentiate into antidonor CTLs. The establishment of the haploidentical graft is therefore greatly dependent not only on the ability of the initial inoculum of the CD34 cells to veto antidonor CTLp, but also on their ability to seed the BM and to generate as rapidly as possible the second or third derivatives which are required to complete the eradication of host antidonor T cells. Clearly, this working hypothesis is consistent with the role of agents that enhance homing of donor stem cells, as well lymphotoxic or myeloablative agents used in preparative regimens prior to transplantation.

A Major Remaining Challenge: Enhancing Immune Reconstitution

The reliable engraftment, in the absence of GvHD, attained by “Megadose” purified CD34 transplants has led to the use of mismatched haploidentical donor cells in thousands of patients with high-risk acute leukemia who urgently need a transplant and who do not have, or cannot find, a matched donor. Indeed, our observed transplant-related mortality (TRM) and event-free survival (EFS) are comparable to those described in patients at the same stage of disease who received transplants from matched unrelated donors.

A major remaining clinical problem is the slow immune recovery of the antimicrobial and antiviral responses. In fact, about 40% of the non-leukemic deaths in more than 250 patients transplanted in Perugia since 2002 were due mainly to bacterial or fungal infections. The incidence of infection-related deaths was linked to the delay in immune reconstitution and to the fact that most patients had a long history of disease, had been heavily pretreated and/or were in relapse at the time of transplant. Indeed, multivariate analyses showed that a history of infections and colonization at transplant were the most significant factors in infection-related deaths. Relatively high infection-related mortality rates and similar patterns of immune reconstitution are common to other T cell-depleted transplants, such as T cell-depleted matched unrelated transplants.⁷⁴

Several mechanisms are responsible for the posttransplant immune deficiency. Tissue damage by conditioning regimens prevents T cell homing to peripheral lymphoid tissues, where generation and maintenance of T cell memory take place. In adults, because thymic function is in decay, early immune recovery stems from expansion of the mature T cells in the graft and, months later, from *de novo* production of naïve T cells. In unmanipulated transplants, peripheral T cell expansion is antagonized by the immune suppressive therapy for GvHD prophylaxis. In T cell-depleted transplants, without any postgrafting immune suppression, homeostatic expansion of the T cells in the graft proceeds undisturbed. However, as the number of T cells has to be extremely low in order to prevent GvHD, immune recovery is inevitably slow.

Current research is focusing on two major approaches, namely investigating potential new agents that can boost thymic function in transplant recipients and adoptive transfer of host nonreactive T cells. The former includes KGF,^{75–78} IL7⁷⁹ and biochemical ablation of the male sex hormone. The latter can be further divided into *ex vivo*-expanded pathogen-specific T cells⁸⁰ or polyclonal expansion of host nonreactive T cells.^{71,81–83} Two promising methods currently in clinical trials for generating such cells are photodepletion of T cells found to respond in MLR of donor cells against the host⁸⁴ and generation of anti-third-party CTLs under IL2 starvation.^{71,81–83}

Another most promising approach based on the preclinical mouse studies of Negrin *et al.*^{85–90} is based on the capacity of Treg cells to neutralize the GvH reactivity associated with infusion of donor type T cells. Thus, promising preliminary clinical results have been obtained recently in more than 17 leukemia patients receiving “megadose” CD34 stem cell haploidentical transplants, in conjunction with infusion of 1×10^6 per kg body weight purified T cells and 2×10^6 per kg body weight Treg cells. In particular, these results indicate that the speed of recovery of peripheral donor CD4 and CD8 T cells is markedly enhanced following the infusion of donor T cells, reaching levels similar to those found in matched sibling transplants under a similar time frame. Most importantly, as expected from the mouse studies, GvHD is effectively prevented by the “umbrella” of the Treg cells.

If indeed a long term followup of this approach will confirm these promising results, the road will be open for wide use of haploidentical transplants in acute leukemia patients.

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