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STRIKING AUGMENTATION OF HEMATOPOIETIC CELL CHIMERISM IN NONCYTOABLATED ALLOGENEIC BONE MARROW RECIPIENTS BY FLT3 LIGAND AND TACROLIMUS[1]

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The influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and the recently identified hematopoietic stem-progenitor cell mobilizing factor flt3 ligand (FL) on donor leukocyte microchimerism in noncytodepleted recipients of allogeneic bone marrow (BM) was compared. B10 mice ($H2^b$) given 50×10^6 allogeneic (B10.BR [$H2^k$]) BM cells also received either GM-CSF (4 μg/day s.c.), FL (10 μg/day i.p.), or no cytokine, with or without concomitant tacrolimus (formerly FK506; 2 mg/kg) from day 0. Chimerism was quantitated in the spleen 7 days after transplantation by both polymerase chain reaction (donor DNA [major histocompatibility complex class II; I-E^k]) and immunohistochemical (donor [I-E^{k+}] cell) analyses. Whereas GM-CSF alone significantly augmented (fivefold) the level of donor DNA in recipients' spleens, FL alone caused a significant (60%) reduction. Donor DNA was increased 10-fold by tacrolimus alone, whereas coadministration of GM-CSF and tacrolimus resulted in a greater than additive effect (28-fold increase). A much more striking effect was observed with FL + tacrolimus (>125-fold increase in donor DNA compared with BM alone). These findings were reflected in the relative numbers of donor major histocompatibility complex class II^+ cells (many resembling dendritic cells) detected in spleens, although quantitative differences among the groups were less pronounced. Evaluation of cytotoxic T lymphocyte generation by BM recipients' spleen cells revealed that FL alone augmented antidonor immunity and that this was reversed by tacrolimus. Thus, although FL may potentiate antidonor reactivity in nonimmunosuppressed, allogeneic BM recipients, it exhibits potent chimerism-enhancing activity when coadministered with recipient immunosuppressive therapy. This property of FL may offer considerable potential for the augmentation of microchimerism, with therapeutic implications for organ allograft survival and tolerance induction.

In organ allograft recipients, the persistence of donor leukocytes within recipient tissues for indefinite periods has been construed as evidence of the transfer of hematopoietic stem and progenitor cells (*1, 2*). Moreover, multilineage donor leukocyte microchimerism has been postulated to be an essential condition for organ allograft acceptance (*1–3*). Accounts of the multilineage nature of microchimerism have emphasized the invariable prominence of donor-derived dendritic cells (DC*) (*1–5*). Both DC progenitors and other leukocytes of donor origin that engage the host immune system may have tolerogenic properties (*6*).

Current efforts to enhance "spontaneous microchimerism" in human organ transplantation focus on the administration of unmodified donor bone marrow (BM) by either single or multiple infusions (*7, 8*). This is undertaken without recipient cytoablation. Conventional immunosuppressive therapy is believed to facilitate donor cell survival and to promote the reciprocal donor-recipient leukocyte engagement that may provide the basis of allograft acceptance (*9*). A potential chimerism-enhancing strategy that is well-recognized in BM transplantation, but that has not been evaluated formally in organ allograft recipients, is the use of hematopoietic growth factors. Many of these can either enhance, inhibit, or bifunctionally affect the proliferation and differentiation of hematopoietic progenitor cells (*10–13*). They comprise granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and a variety of other cytokines that act at an earlier point in the hematopoietic cascade, such as interleukin (IL) 3, stem cell factor (c-*kit* ligand), and the recently cloned *flt3* ligand (FL) (*14*).

In the hemopoietic system, the expression of *flt3* (a mem-

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Abbreviations: BM, bone marrow; CTL, cytotoxic T lymphocyte; DC, dendritic cell; FL, flt3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

ber of the type III receptor tyrosine kinase family that also includes c-*kit* and *fms*) is believed to be restricted to primitive progenitor cells that respond to FL (*14–17*). FL potently synergizes with the colony-stimulating factors and c-*kit* ligand to enhance growth of hemopoietic progenitors (*14–19*). Notably, there is recent evidence that in contrast to the case with GM-CSF, G-CSF, and c-*kit* ligand, administration of FL to normal mice leads to dramatic increases in DC numbers, both in lymphoid and nonlymphoid tissues (*20*). In this study, we examined the influence of short-term (7-day) FL treatment on donor leukocyte microchimerism, both in normal (noncytoablated) and tacrolimus (formerly FK506)-immunosuppressed recipients of unmodified allogeneic BM. For comparative purposes, similar experiments were undertaken with GM-CSF. The results show that when administered with tacrolimus, FL dramatically augments hematopoietic cell chimerism within recipient lymphoid tissue. We also show that donor major histocompatibility complex (MHC) class II^+ cells with distinct DC characteristics are a prominent feature of the greatly enhanced donor cell population. These findings may have important implications for the therapeutic application of FL to augment chimerism and to promote donor-recipient leukocyte interactions that may predispose to improved organ allograft survival.

MATERIALS AND METHODS

Animals. B10.BR ($H2^k$, I-E^k), C57BL/10J (B10; $H2^b$, I-A^b), and C3H ($H2^k$; I-E^k) mice, 6–12 weeks old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in the central specific pathogen-free facility of the University of Pittsburgh Medical Center.

Cytokines and tacrolimus. Recombinant mouse GM-CSF produced in *Escherichia coli* was a gift from Dr. S.K. Narula, Schering-Plough Research Institute, Kenilworth, NJ. Chinese hamster ovary cell-derived human FL (*21*) was kindly provided by Dr. H.J. McKenna, Immunex Research and Development Corp., Seattle, WA. GM-CSF (4 μg/day s.c.) or FL (10 μg/day i.p.) was injected in 0.1 ml of low-endotoxin phosphate-buffered saline (PBS). Control mice received PBS alone. Tacrolimus (FK506) was obtained from the Fujisawa Pharmaceutical Co., Osaka, Japan, in powder form and injected intraperitoneally (2 mg/kg) in suspension in 0.5 ml of PBS on days 0, 2, 4, and 6 (experiment 1) or days 0–6 (experiment 2).

Treatment protocols. BM transplantation was performed using both the B10.BR→B10 and B10→C3H combinations. Recipient mice (four per group) were given intravenous injections of 50×10^6 freshly isolated, unmodified donor BM cells in RPMI 1640 (Life Technologies, Grand Island, NY) via the lateral tail vein. In addition, they received either GM-CSF (experiment 1), FL (experiment 2), or no cytokine, with or without tacrolimus for 7 consecutive days, starting at the time of BM transplant (day 0). On day 7, the animals were killed by cervical dislocation. Spleens were harvested for analysis.

DNA extraction. Freshly isolated, monodispersed spleen cell lysates were treated with pancreatic ribonuclease as described (*22*), and whole genomic DNA was extracted using a DNA extraction kit (Qiagen, Studio City, CA). The DNA was quantitated by ultraviolet spectroscopy, and was at least 40 kb long, indicating that it was derived from intact cells. The concentration of the genomic DNA was measured by spectrophotometry at 260 and 280 nm. This was verified using low-voltage gel electrophoresis and ethidium bromide fluorescence (*22*) with subsequent densitometric standardization.

Quantitation of donor DNA by polymerase chain reaction. Oligonucleotides for the detection of donor MHC class II allele-specific DNA (I-E^k) in B10 recipients of B10.BR BM were custom-made at Oligos Etc. Inc., Wilsonville, OR. The sequences of the forward and reverse primers were 5′-ATTGAGGCCGACCACGTAGCT 3′ and 5′-ACCTTGGGGCTCAAATCTTCT 3′, respectively. The primers were designed to distinguish donor DNA from recipient DNA and yielded no visible polymerase chain reaction (PCR) product of 160 bp with recipient DNA template under ethidium bromide fluorescence, or a detectable product using an oligomer probe Southern blot. Using these allele-specific PCR primers and the target DNA from the BM transplant recipients, amplification was carried out using reagents from Perkin Elmer Inc., Norwalk, CT, under standard conditions. A series of reactions varying in cycling parameters alone were performed with ^{32}P-dCTP and 27 thermal cycles and found to be optimal over a range of 100 pg to 100 ng of donor DNA. The yield of radiolabeled PCR product from the unknown samples was measured by densitometric analysis with donor DNA as positive control DNA. PCR product yield of donor DNA template used as positive control in the experiments with the unknown samples was comparable to the standards. This served to calculate the amount of donor DNA in the unknown composite DNA samples. The semiquantitative assessment was further verified by a limiting dilution PCR assay (*23*). Briefly, varying dilutions of reference DNA were diluted serially to derive the dilution that yielded a qualitative yes-or-no reaction. Donor DNA in the unknown samples was quantitated by the degree of dilution necessary to replicate three to five positive events in 10 reactions. The previously mentioned semiquantitative analysis using ^{32}P-dCTP served as a guideline for the latter exhaustive analyses. All enzyme assays were performed three times to check for reproducibility. Results of the different donor template quantitative reactions were expressed as amounts of donor DNA per microgram of total composite DNA.

Immunohistochemistry. Cryostat sections of spleen from B10 recipients of B10.BR BM were equilibrated at room temperature, fixed in acetone, and stained for donor MHC class II using biotinylated mouse IgG2a anti-mouse I-$E^{k,d,p,r}$ (PharMingen, San Diego, CA) in an avidin-biotin-peroxidase staining procedure, as described previously (*24*). Controls included sections of normal donor or recipient strain tissue. The incidence of donor MHC class II^+ cells in sections was determined by two blinded observers. The results are expressed as the mean number of positive cells per 60 high-power fields.

Cell-mediated lymphocytotoxicity assay. Freshly isolated spleen cells obtained from C3H recipients of B10 BM 7 days after transplantation were used as effectors. They were restimulated in vitro for 5 days with gamma-irradiated (20 Gy) donor strain splenocytes. The EL4 ($H2^b$) mouse lymphoma cell line (TIB39; American Type Culture Collection [ATCC], Rockville, MD) was used as a source of specific target cells. The P815 ($H2^d$) mouse mastocytoma cell line (TIB64; ATCC) and the R1.1 ($H2^k$) lymphoma cell line (TIB42; ATCC) were used as third-party and syngeneic targets, respectively. The target cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (NEN, Boston, MA), washed, and plated at a concentration of 5×10^3 cells/well in 96-well round-bottomed culture plates (Corning, Corning, NY). Serial, twofold dilutions of effector cells were added to give effector to target ratios of 50:1, 25:1, and 12.5:1 in a total volume of 200 μl/well. The percentage of specific ^{51}Cr release was determined after the plates were incubated for 4 hr at 37°C in 5% CO_2 in air. The supernatant was recovered from each well using a supernatant collection system (Skatron Inc., Sterling, VA). Maximum ^{51}Cr release was determined by osmotic lysis of the cells. The percent specific cytotoxicity was calculated using the following formula: *% cytotoxicity* = 100 × [*experimental* (cpm) − *spontaneous* (cpm)/*maximum* (cpm) − *spontaneous* (cpm)]. The results are expressed as mean ± 1SD of percent specific ^{51}Cr release in triplicate cultures.

Statistics. The significance of differences between means was determined using the nonparametric Mann-Whitney *U* test.

RESULTS

Influence of GM-CSF alone or in combination with tacrolimus on levels of donor DNA (I-E^k) in allogeneic BM recipients.

Recombinant mouse GM-CSF has been shown to enhance hematopoietic reconstitution after syngeneic or allogeneic BM transplantation in lethally irradiated mice (*25, 26*). Alternatively, ex vivo preincubation of donor marrow cells with recombinant mouse GM-CSF significantly improves the seeding efficiency of hematopoietic stem cells and other progenitor cells after transplantation to irradiated recipients (*25, 27*). In view of these findings, we determined the influence of recombinant mouse GM-CSF (administered daily) on the level of microchimerism (donor DNA) within lymphoid tissue (spleen) of normal (noncytodepleted) and tacrolimus-treated B10 ($H2^b$) mice given 50×10^6 allogeneic (B10.BR [$H2^k$]) BM cells 7 days earlier. The results of quantitative PCR analysis for donor DNA (MHC class II; I-E^k) are shown in Table 1. Both GM-CSF and, to a greater extent, tacrolimus markedly increased the level of chimerism (5- and 10-fold, respectively) compared with that achieved with BM transplantation alone. When GM-CSF and tacrolimus were combined, however, a greater than additive effect was achieved, with almost 30 times the level of donor DNA detected compared with BM alone.

Influence of FL alone or in combination with tacrolimus on levels of donor DNA (I-E^k) in allogeneic BM recipients. In a separate experiment, using the same strain combination, FL was substituted for GM-CSF, and its influence on the level of donor DNA was determined. In contrast to the effect of GM-CSF, daily administration of FL significantly reduced (to less than 40%) the level of DNA encoding donor MHC class II within the spleen compared with mice given BM alone (Table 2). The level of donor DNA in mice given both FL and tacrolimus, however, was increased dramatically compared with that in either untreated or FL-treated animals (more than 120-fold and 300-fold, respectively). It was 10-fold greater than that observed in mice given tacrolimus alone. The level of chimerism in tacrolimus-immunosuppressed mice was fourfold greater in those also given FL compared with those also given GM-CSF. Since the total number of nucleated spleen cells in FL-treated mice was markedly increased compared with that in animals given BM alone (FL: 4.7-fold, FL+tacrolimus: 3.8-fold; Table 3), the increase in absolute numbers of donor cells in immunosuppressed, FL-treated animals was substantial.

Effects of GM-CSF and FL on donor MHC class II^+ cells in normal and immunosuppressed recipients. Cryostat sections of spleen from each mouse in each of the above experimental groups were stained using monoclonal antibody against donor MHC class II (I-E^{k+}). The mean number of chimeric leukocytes per unit area (60 high-power fields) was determined. The results (Table 4) showed a pattern similar to that obtained by molecular (PCR) analysis, although the quantitative difference in the numbers of donor cells among the groups was less than that observed by DNA quantitation. Nevertheless, the immunohistochemical data indicated a relative 35-fold increase in the number of donor class II^+ cells in mice treated with FL + tacrolimus compared with those given BM alone. Interestingly, the data confirmed that FL administration alone reduced the number of donor cells surviving within recipient lymphoid tissue, even when the fact that this cytokine significantly increased the total number of nucleated spleen cells was taken into account. The appearance of typical, donor MHC class II^+ cells is shown in Figure 1. Many of these "chimeric" cells displayed DC characteristics and were present both in T cell and other areas of the spleen.

Effects of FL and tacrolimus on antidonor cytotoxic T lymphocyte responses. To ascertain the possible functional (immunologic) significance of the changes in donor cell numbers induced by FL and tacrolimus, antidonor cytotoxic T lymphocyte (CTL) responses of spleen cells from allogeneic BM recipients were examined 7 days after transplantation. Using the B10 to C3H ($H2^b\rightarrow H2^k$) strain combination, it was observed that, compared with allogeneic BM alone, FL treatment significantly augmented the generation of antidonor CTL activity. The combination of FL and tacrolimus, however, resulted in reduced antidonor cytotoxicity compared with that seen with cells from either untreated mice or mice treated with tacrolimus alone (Fig. 2).

DISCUSSION

Hematopoietic cell chimerism occurs in recipients of organ allografts and is persistent and long-lasting in those animals and humans that accept their grafts with or without the need for immunosuppressive therapy. Thus, we have shown previously that donor-derived progenitor cells (DCs) can be propagated from the BM of "spontaneously" tolerant organ (liver) allograft recipients, but not from those that reject (cardiac) transplants from the same donor strain (*28*). These findings implied that an organ (the liver) with a relatively large constituency of progenitor and stem cells was more likely to be accepted than one (the heart) with only a paucity of "passenger leukocytes." Related studies in noncytoablated, tacrolimus-immunosuppressed recipients of whole organ or BM transplants have shown that tissue chimerism is closely associated with tolerogenicity (*29*).

There is now considerable interest in the clinical use of donor BM to augment leukocyte microchimerism in conventionally immunosuppressed organ allograft recipients. An alternative/additional approach is the use (either alone or together with donor BM) of hematopoietic cytokines that

TABLE 1. Influence of GM-CSF and tacrolimus on levels of donor DNA (I-E^k) in spleens of allogeneic BM recipients[a] (experiment 1)

Treatment	Mean densitometry units ± SD (median)	Donor DNA/μg total DNA	% Donor DNA content	Fold increase in donor DNA[b]
None	309 ± 62 (282)	100 pg	0.01	—
Tacrolimus	2982 ± 185 (2998)[c]	1 ng	0.1	10.6
GM-CSF	1575 ± 111 (1576)[c]	0.5 ng	0.05	5.6
Tacrolimus + GM-CSF	8062 ± 558 (8083)[d]	3 ng	0.3	28.7

[a] Seven days after BM transplantation.
[b] Compared with BM alone (four mice/group). Strain combination: B10.BR (I-E^k) → B10 (I-A^b).
[c] $P < 0.025$ compared with BM alone.
[d] $P < 0.025$ compared with BM + GM-CSF alone using the Mann-Whitney U test.

TABLE 2. Influence of FL and tacrolimus on levels of donor DNA (I-E^k) in spleens of allogeneic BM recipients[a] (experiment 2)

Treatment	Mean densitometry units±1SD (median)	Donor DNA/μg total DNA	% Donor DNA content	Fold increase in donor DNA[b]
None	240±45 (227)	~100 pg	0.01	—
Tacrolimus	2,965±365 (2,882)[c]	~1.2 ng	0.12	12.7
FL	93±19 (89.5)[c]	~38 pg	0.004	0.4
Tacrolimus+FL	29,623±3,142 (28,272)[d]	~122 ng	12.2	124.5

[a] Seven days after BM transplantation (four mice/group).
[b] Compared with BM alone.
[c] $P<0.025$ compared with BM alone.
[d] $P<0.025$ compared with BM + FL alone using the Mann-Whitney U test.

TABLE 3. Influence of FL and tacrolimus on spleen weights and cellularity in allogeneic BM recipients (experiment 2[a])

Treatment	Spleen weight (mg/g body weight)	Total nucleated cells (×10^6)	Fold increase in cell number[b]
None	3.13±0.07	85.5±8.4	—
BM alone	3.71±0.15	102.3±12.5	1.2
BM+tacrolimus	2.68±0.08	68.5±8.0[c]	0.8
BM+FL	6.15±0.99	401.5±42.2[d]	4.7
BM+tacrolimus+FL	6.12±0.28	324.1±22.4[d]	3.8

[a] Results are expressed as mean ± 1SD obtained from groups of four mice 7 days after BM transplantation.
[b] Compared with normal mice.
[c] $P<0.05$ compared with normal mice.
[d] $P<0.01$ compared with normal mice.

TABLE 4. Influence of GM-CSF and FL on donor cell (I-E^{k+}) chimerism in spleens of allogeneic BM recipients[a]

Treatment	I-E^{k+} cells/60 high-power fields	Mean±1SD (Median)	Fold increase in donor MHC class II$^+$ cells
Experiment 1			
None	18, 6, 21, 8	13.3±7.4 (13)	—
Tacrolimus	31, 75, 40, 26	43.0±22.1 (35.5)[b]	2.7
GM-CSF	18, 52, 28, 11	27.3±17.9 (23)	1.8
GM-CSF+tacrolimus	64, 64, 87, 43	64.5±18.0 (64)[c]	4.9
Experiment 2			
None	24, 19, 14, 20	19.3±4.1 (19.5)	—
Tacrolimus	98, 66, 200, 207	142.8±71.4 (149)[b]	7.6
FL	3, 0, 2, 3	2.0±1.4 (2.5)[b]	0.1
FL+tacrolimus	601, 1025, 374, 796	699±277.4 (698.5)[d,e]	35.8

[a] Seven days after BM transplantation (four mice/group).
[b] $P<0.025$ compared with BM alone.
[c] $P<0.05$ compared with BM + cytokine alone.
[d] $P<0.025$ compared with BM + cytokine alone.
[e] $P<0.025$ compared with BM + tacrolimus alone, using the Mann-Whitney U test.

mobilize stem and progenitor cells. Because these growth factors, e.g., G-CSF, GM-CSF, c-*kit* ligand (*30*), IL-3, IL-4, IL-8, other C-X-C chemokine family members (*31*, *32*), and FL, induce the proliferation of various leukocyte lineages in vitro, they are key candidate molecules for the manipulation both of donor and recipient BM-derived cells in vivo. Indeed, there is evidence that in mice, GM-CSF enhances the effect of donor BM cells in prolonging skin allograft survival (*33*).

In the present study, the influence of GM-CSF (a growth factor already approved for human use) and the recently cloned hematopoietic cytokine FL on short-term chimerism in noncytoablated recipients of unmodified allogeneic BM was compared. In these immunologically intact hosts, the two cytokines alone exhibited opposing effects. Whereas GM-CSF increased the level of donor DNA and donor MHC class II$^+$ cells in recipient lymphoid tissue, FL reduced both substantially. In contrast, both cytokines strikingly augmented the level of chimerism when *combined* with systemic, T cell-directed immunosuppression (tacrolimus). Remarkably, the effect of FL in immunosuppressed recipients was much more dramatic than that of GM-CSF, and was associated with a much higher incidence and absolute number of donor cells, many exhibiting DC characteristics. The quantitative differences between the systemic effects of GM-CSF and FL on donor cell chimerism in immunosuppressed mice are accentuated when account is taken of the pronounced increases in absolute numbers of BM-derived cells in lymphoid and nonlymphoid tissue of FL-treated animals.

Brasel et al. (*21*) have shown recently that in vivo administration of FL dramatically increases the numbers of hematopoietic progenitors in normal mouse BM, blood, and spleen, which results in increased myelopoiesis and B lymphopoiesis (*21*, *34*). Although FL by itself is only a weak mitogen for primitive lineage Sca-1$^+$ c-*kit*$^+$ mouse BM progenitors (with

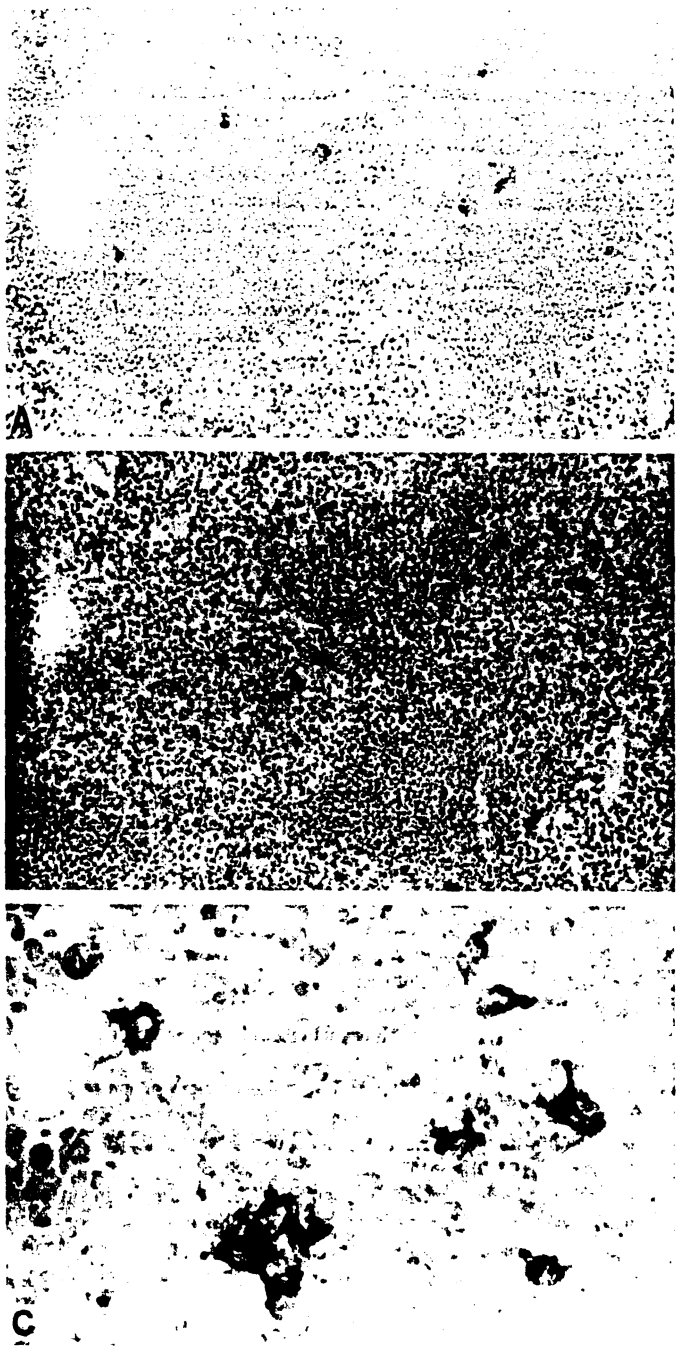

FIGURE 1. Identification of donor (B10.BR)-derived cells (donor MHC class II$^+$ [I-E^{k+}]) in spleens of allogeneic (B10) BM recipients 7 days after transplantation (on day 0) and treatment with FL and tacrolimus. Mice were given intravenous injections of 50×10^6 unmanipulated BM cells, and received either FL (10 μg/day), tacrolimus (2 mg/kg), or both agents daily from days 0 to 6. Donor MHC class II$^+$ (I-E^{k+}) cells were detected by immunohistochemical staining of cryostat sections using an avidin-biotin-peroxidase procedure (*24*), as described in *Materials and Methods*. Whereas an increase in donor class II$^+$ cells was observed in mice treated with (A) tacrolimus alone compared with controls, numbers were reduced substantially with FL alone (see Table 4). (B) The combination of FL and tacrolimus strikingly increased the incidence of donor cells, many of which (C) exhibited morphologic characteristics of DC. Cells were counterstained with hematoxylin (A, B: ×100; C: ×1200).

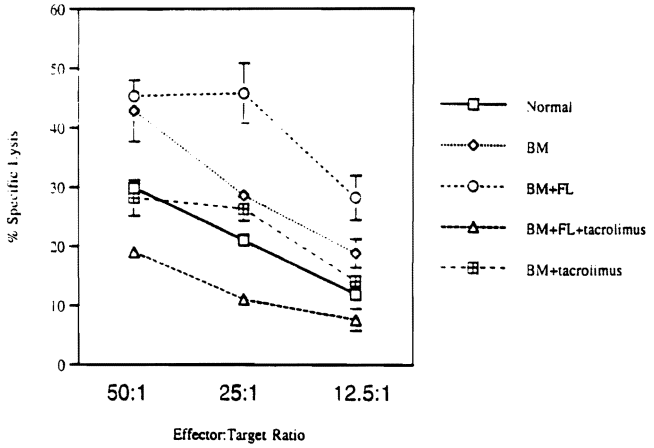

FIGURE 2. CTL responses of splenic T cells from normal C3H mice and from C3H recipients of allogeneic (B10) BM 7 days after transplantation. The mice (four per group) received either no treatment, FL (10 μg/day) alone, FL + tacrolimus (2 mg/kg/day), or tacrolimus alone. Results are expressed as mean percent specific target cell lysis in triplicate cultures ± 1SD.

combined myeloid and lymphoid potentials), it potently promotes in vitro survival of these cells, in part at least, by suppressing apoptosis (*35*, *36*). The FL receptor, flt3, is expressed on pluripotent, long-term reconstituting stem cells and early progenitors of the lymphoid myeloid lineages (*36*, *37*). This population is enriched over 100-fold in the spleens of FL-treated normal mice (*38*) and at least 20-fold in spleens and livers of normal rats given 50 μg of FL per day for 5 days (N. Murase, T. Sakamoto, and A.W. Thomson, unpublished observations). The latter findings are consistent with the very large increase in FL-responsive, donor-derived cells within the spleens of immunosuppressed allogeneic BM recipients observed in the present study. Significantly, FL has also been shown to mobilize stem-progenitor cells in nonhuman primates (*39*, *40*), highlighting the potential of FL for clinical use as a potent hematopoietic growth factor.

In contrast to GM-CSF, FL reduced the number of donor-derived cells in spleens of otherwise untreated BM recipients. This suggested that FL augmented the host response to donor alloantigens, and indeed, we observed augmentation of donor-reactive CTL precursors within the spleens of FL-treated mice. Reversal of this effect by concomitant tacrolimus-mediated immunosuppression is consistent with exhibition of the chimerism-promoting property of FL. The apparent ability of FL to augment immune reactivity is in keeping with recent observations of its capacity to dramatically increase functional DCs in normal mice (*20*), to exert anti-tumor activity (*41*), and, following donor pretreatment, to exacerbate experimental organ allograft rejection (R.J. Steptoe et al., manuscript in preparation).

Although DCs are only one of the hematopoietic lineages represented in the microchimerism after whole organ transplantation, and these cells have been implicated historically in the induction of antiallograft immunity, DCs also have tolerogenic properties (*6*). In particular, DCs at the "immature" precursor/progenitor stage may be capable of subverting T-cell responses in vitro and prolonging allograft survival (*42–44*). In addition, there is evidence that a major subpopulation of "lymphoid" DCs within normal secondary lymphoid

tissue can suppress responses of allogeneic $CD4^+$ and $CD8^+$ T cells by inducing apoptosis (*45*) or restricting IL-2 production (*46*), respectively.

The striking increase in allogeneic MHC class II^+ cells with DC characteristics in immunosuppressed mice treated with FL is consistent with the large increase in numbers of DCs in spleens and other tissues of FL-treated normal mice reported recently by Maraskovsky et al. (*20*). In contrast to the influence of FL, DCs in mouse lymphoid tissue are enhanced only to a moderate degree by GM-CSF (*20, 47*). These latter findings include observations on transgenic mice with excessive levels of GM-CSF (*47*). As numbers of both donor and host hematopoietic cells of multiple lineages are increased by FL administration, there appears to be considerable potential for this new recombinant growth factor in the investigation of the bidirectional immunologic paradigm of tolerance induction. In particular, it now appears important to ascertain the functional significance of the FL-induced augmentation of chimerism we have described in organ allograft recipients.

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