Effects of Cord Blood Transfer on the Hematopoietic Recovery Following Sublethal Irradiation in MRL *lpr/lpr* Mice

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Abstract. **The potential of cord blood (CB) to serve as a rich source of stem cells and stem cell factors is receiving increasing attention. In addition, perhaps because of the early ontogeny of these cells or the lack of surface antigens, cord blood stem cells do not appear to require close identity with the recipient. In the present pilot study, we investigated the presence of a hematopoiesis enhancing effect (HEE) by assaying the ability of human cord blood cells to augment hematopoiesis across a species barrier. For these experiments, autoimmune-prone MRL-*lpr/lpr* mice were exposed to sublethal levels of irradiation and cord blood administration to study the role of factors present in human cord blood in augmenting the rate of lymphopoiesis. This strain was chosen because of the increased presence of peripheral T and B subpopulations, namely the B-1 and CD4/CD8 double negative T-cell subpopulations, which do not arise directly from bone marrow precursors, but rather accumulate with age. MRL-*lpr/lpr* mice were sublethally irradiated and reconstituted with syngeneic bone marrow (BM) cells or with human cord blood cells or peripheral blood mononuclear cells (PBMC), or were left unreconstituted. At 2 weeks post-treatment, lymphoid populations in the spleen and lymph nodes were studied as a measure of hematopoiesis. Factors present in cord blood were able to augment hematopoiesis over that which occurred endogenously. At 2 weeks postirradiation, recipients of BM cells displayed the fastest rate of peripheral lymphoid recovery, nonreconstituted mice showed the slowest lymphoid recovery, and recipients of cord blood recovered their lymphoid populations at an intermediate rate. Similarly, myelopoiesis was increased in irradiated SJL/J recipients of human cord blood. Thus, human cord blood cells appear to produce/induce factors that may act as an adjunct to increase stem-cell activity.**

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Human umbilical cord blood has been successful in the treatment of a variety of human malignant and nonmalignant disorders such as aplastic anemia, Fanconi's anemia, acute lymphoid and mueloid leukemias, and X-linked lymphoproliferative syndrome (1–4). For human disease, the lack of donors for bone marrow transplant and the serious complications associated with graft-versus-host disease (GvHD) are major obstacles. As such, significant attention has been directed at using human umbilical cord blood (CB) as a sourde of stem cells. Several reports have indicated that CB may only need to be matched for ABO blood group, and may not require close HLA matching (4–6), and that significant GvHD does not occur in patients receiving CB due to the immature and/or unresponsive nature of these cells (7–9). This point is further exemplified in xenotransplant studies by Ende *et al.* which show that CB can rescue mice from lethal irradiation (10–11). Since cord blood has also been found to contain stem cell factors that augment stem cell activation and differentiation (12, 13), the administration of human CB following irradia-

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tion may act on endogenous murine stem cells since human cytokines have been found to function in murine cells.

To study the effects of human cord blood stem cell factors *in vivo,* we employed a xenotransplant system involving sublethally irradiated MRL-*lpr/lpr* mice. These mice develop disease symptoms that closely parallel human systemic lupus erythematosus (SLE) and autoimmune lymphoproliferative syndrome (ALPS) (14–16). Autoimmune disease in these mice is characterized by the production of large amounts of autoantibodies including anti-DNA, anti-ribosomal proteins, anti-gp 70, anti-IgG (rheumatoid factor), and anti-RNA polymerase I (17–19). MRL-*lpr/lpr* mice also have increased percentages of B-1 cells that are long-lived B cells with self-renewal capabilities. MRL-*lpr/lpr* mice also develop a lymphoproliferation of a subset of T cells that are CD3+, but do not express CD4 or CD8 (double negative T cells, DNT) (20). These DNT cells can be seen in large numbers in the spleen, and especially the lymph nodes of these mice. At advanced ages (3–6 months of age), DNT cells may comprise greater than 90% of the total cells in the lymph node resulting in as much as 100 times normal size. Studies have shown that less than 5% of the DNT cells are actively cycling (21), suggesting that massive lymphadenopathy results from accumulation and faulty apoptosis due in part to the insertion of a retroviral transposon in the Fas gene for apoptosis rather than proliferation (22–26).

Several reports have indicated that many autoimmune diseases stem from a disorder found at the stem cell level (27–30). As such, a form of treatment may include irradiation, followed by replacement with a source of normal stem cells. In mice, this has proven effective in various autoimmune disease models such as insulin-dependent diabetes mellitus (IDDM) in nonobese diabetic (NOD) mice, and SLE in NZB and BXSB mice (reviewed in Ref. 31). Our own studies in MRL-*lpr/lpr* mice show that in this strain, human CB can rescue only a small number of mice from lethal irradiation (30%); however, those that survive show a greatly increased life span, and a significant retardation of lymphoproliferative disease (11). Because we did not find any evidence of long-term human cell engraftment in these mice, we hypothesized that CB may provide factors that increase endogenous hematopoiesis. In the current study, we investigate the effects of sublethal levels of irradiation and transfer of either *lpr* bone marrow (BM), or human CB on the rate of lymphopoiesis as measured by the return of T and B cells in the periphery. By employing the MRL-*lpr/lpr* mice, we were able to take advantage of the presence of two unique subpopulations of lymphocytes that are long-lived and would not represent newly emerging bone marrow stem cell–derived lymphocytes. In MRL-*lpr/lpr* the B-1 cells and the DNT cells are the predominant radioresistant B- and T-cell populations, respectively. By employing this strain of mice, it is possible to differentiate the newly emerging lymphocytes from the populations remaining following irradiation. In this manner, the effects of cord blood factors on endogenous stem cell activity can be assessed.

Materials and Methods

Mice. Female weanling MRL-*lpr/lpr* and SJL/J mice were purchased from Jackson Labs (Bar Harbor, ME), and housed under standard conditions in the animal research facility at UMDNJ. Animals were 4 weeks of age at the time of irradiation.

Irradiation. Animals were irradiated with between 600–950 rad from the 8-MeV x-ray beam of a linear accelerator (Philips SL75-20, Elektra Oncology Systems, Inc., Shelton, CT). Mice were restrained in a lucite box during irradiation. Dose buildup and back scatter were provided by the placement of polystyrene blocks above and below the lucite box. Irradiated animals were left unreconstituted (rad ctr), or were transfused with either human CB, human PBMC or *lpr/lpr* bone marrow cells (*lpr* BM). Unmanipulated, age-matched female mice were used as controls (no rad ctr).

Bone marrow. MRL-*lpr/lpr* bone marrow cells were obtained from the femurs of donor mice at 4 weeks of age. Sterile PBS and a 25-gauge needle were used to flush the cells from the marrow cavity. The cells were washed, counted, and resuspended in sterile PBS for injection. Recipient mice received 10×10^6 lpr bone marrow cells intravenously immediately following irradiation.

Cord blood. Human umbilical CB was collected in sterile Iscove's media (Gibco, Grand Island, NY) supplemented with 50 U/ml preservative-free heparin. CB mononuclear cells (MNC) were isolated on a Ficoll gradient, washed three times with sterile PBS, counted, and resuspended in sterile PBS for injection. Multiple samples of cord blood MNC were pooled for injection. Mice receiving human cells were pretreated with 0.1 ml iv antiasialo GM1 serum (Waco Chemicals, Richmond, VA) to remove NK cells 24 hr prior to irradiation. CB recipients received 10–18 $\times 10^6$ CB-MNC intravenously immediately following irradiation.

PBMC. Human peripheral blood was collected and employed as described for the human cord blood.

Tissues. At various times post-transfer, mice were sacrificed, and lymph nodes (LN) were taken for RNA extraction. Inguinal, cervical, and axillary lymph nodes were removed and weighed. LN were macerated between frosted glass slides in sterile PBS to yield a single cell suspension. LN cells were counted and resuspended at levels appropriate for flow cytometry.

Flow Cytometry. 1×10^6 Lymph node or spleen cells were washed once in sorter buffer (PBS, 1% FCS, 0.01% NaN_3). The cells were stained with antibodies for 30 min at 4°C. For mouse T-cell subpopulations, the following combinations of antibodies were employed: anti-mouse CD4-FITC, anti-mouse CD8-BioPE, and anti-mouse CD3-Tri Color. For mouse B cells: anti-mouse IgM-FITC and anti-mouse CD5-BioPE. For human cells: anti-human CD19-FITC and anti-human CD5-PE. Isotype controls were

employed for each antibody, and all antibodies were obtained from Caltag (San Francisco, CA). After staining, the cells were washed once with sorter buffer, and appropriate tubes were stained with streptavidin-PE for 30 min at 4°C. The cells were washed once more and fixed in 1% paraformaldehyde.

Splenic Colony Forming Units (CFU). For CFU determination, mice received sublethal irradiation followed by either no treatment, or the transfer of human CB, human peripheral blood mononuclear cells PBMC (10×10^6), or lpr BM. The transfer of human PBMC was done to rule out the possibility of a nonspecific, xenogeneic effect of human cells on mouse stem cell activity. Stem cell activity was measured 9 days postirradiation. Spleens were removed, weighed, and fixed in Tellevesnicky's solution (20:1:1 70% ethanol, acetic acid, formalin v/v). Within 12 hr of fixation, colonies appeared as distinct, pale, raised bumps on the surface of the spleen and were indicative of sites of hematopoiesis. Colonies were counted under the 5× magnification of a dissecting microscope.

RTPCR Analysis of Cytokine Gene Expression. RNA was isolated from spleen and lymph nodes of control, irradiated, and irradiated recipients of either human cord blood or lpr bone marrow using RNAsol (Friendswood, TX). A portion of the spleen and lymph node (5×10^6 cells) was used as a source of RNA. In addition the donor, human cord blood was also analyzed, and a portion of the injected cord blood cells (5×10^6 cells) was employed as a source of RNA. The RNA was reversed transcribed and the cDNA amplified using kits obtained from Perkin Elmer (Branchburg, NJ). Primers specific for mouse IL-10 5′-CGGGAAGACAATAACTG and 3′-CATTTCCGATAAGGCTTGG (product size 180 base pairs), IFNγ. 5′-AACGCTACACACTGCATCTTGG and 3′-GACTTCAAAGAGTCTGAGG (product size 250 base pairs), and the housekeeping gene, HPRT were employed. For the human cord blood, RNA was analyzed for IL-10 5′-ATGCCCCAAGCTGAGAACCAAGACCCA and 3′-TCTCAAGGGGCTGGGTCAGCTATCCCA, IL-7 5′-CTGTTGCCAGTAGCATCATC, and 3′-TTTAACCTGGCCAGTGCAGT (product size 312 base pairs), and the human housekeeping gene β-actin 5′-ATGTTTGAGACCTTCAACAC and 3′-CACGTCACACTTCATGATGG (product size 495 base pairs). The PCR reaction consisted of one cycle of 94°C for 2 min, 35 cycles of 94°C for 1 min 57°C for 1.5 min, and 72°C for 2 min, followed by a final extension of 72°C for 5 min. PCR products were run on an agarose gel (4%) and visualized with ethidium bromide on a fluorimager (Molecular Dynamics, Sunnyvale, CA). Band densities were analyzed using Image Quant software. The results were expressed as a ratio of the band density for the cytokine band divided by the band density for the housekeeping gene.

Lymphocyte Responses. A single cell suspension was obtained from the lymph nodes of irradiated MRL-*lpr/lpr* recipients 2 weeks postirradiation. The irradiated mice were either untreated or given MRL-*lpr/lpr* bone marrow or human cord blood (see above). Lymph node cells were cultured with concanavalin A (Con A) at 1 μg/ml or phytohemagglutinin at 4 μg/ml for 72 hr. Cell proliferation was measured by incorporation of 1 μCi of tritiated thymidine. Cells were harvested onto glass fibers using an automated harvester (Cambridge Technology Inc, Cambridge, MA). Each experiment was performed in triplicate, and the background cpm (cells alone) was subtracted from the stimulated culture values.

Clonogenic Assays. Single-cell suspensions were prepared from the femurs and then used in clonogenic assays for CFU-GM. Cells were resuspended in complete culture medium, and then plated in duplicate in methylcellulose at 10^5/plate in a total volume of 1 ml. Cultures were supplemented with 4U of rMuGM-CSF. Colonies of greater than 20 cells were enumerated at Day 10 of culture.

Statistics. Group means and the standard deviation of the population were determined using Excel software. Significance of differences between groups was determined using an independent Student's *t* test and SigmaPlot 3.0 software.

Results

Hematopoiesis Following Irradiation. Hematopoiesis following irradiation was measured by flow cytometry of returning lymphoid populations in the periphery and by splenic colony-forming ability, both parameters used as indicators of stem-cell activity. The two populations of returning cells studied were B cells in the spleen, and T cells in the lymph nodes. B-cell lymphopoiesis was measured by the ratio of returning conventional (IgM+, CD5–) B-2 cells, and remaining B-1 (IgM+, CD5+) cells because B-1 cells have been shown to be radioresistant in these mice, and B-1 cells will not be reconstituted by bone marrow transplant (32). Following irradiation, B-2 cells increase in number, whereas B-1 cells will remain relatively stable in irradiated mice undergoing lymphopoiesis. Thus, changing percentages of conventional B cells to B-1 cells is indicative of stem-cell activity. Repopulation of the peiphery was used as a measure of stem-cell activity.

Figure 1 shows the percentage of B-1 cells out of the total B cells in the spleen 2 weeks following irradiation. With lethal irradiation (950 r) and no reconstitution, the B-1 cells comprise approximately 70% of the B cells in the spleen. These mice are not reconstituting their lymphoid system, and the predominant cell type remaining is the radioresistant B-1 cell. Mice that were lethally irradiated and given *lpr* BM are reconstituting their lymphoid system, and B-1 cells comprise a much lower percentage of total B cells (~15%) (Fig. 1). Even at sublethal doses of irradiation (650 r), unreconstituted animals showed the highest percentage of B-1 cells out of the total B cells as compared to mice receiving either CB or *lpr* BM following irradiation. Sublethally irradiated mice with the lowest percentage of B-1 cells are mice reconstituted with *lpr* BM (indicating the

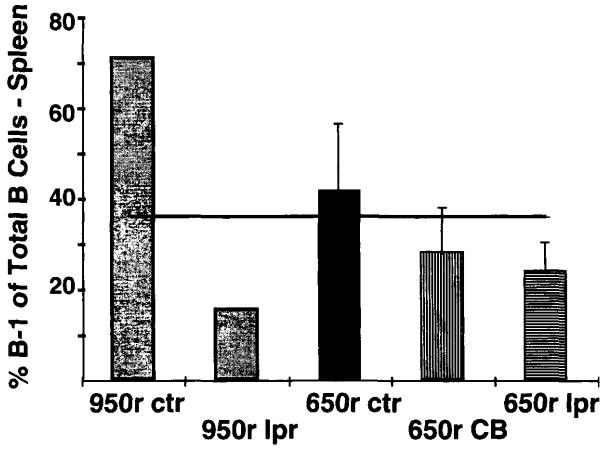

Figure 1. Percentages of B-1 cells in the spleen 2 weeks post-treatment. Each column represents the average values (plus standard error in sublethally irradiated mice), the horizontal line represents the mean value for untreated control mice. $n = 3$ for all groups. The experiment was repeated two additional times with similar results.

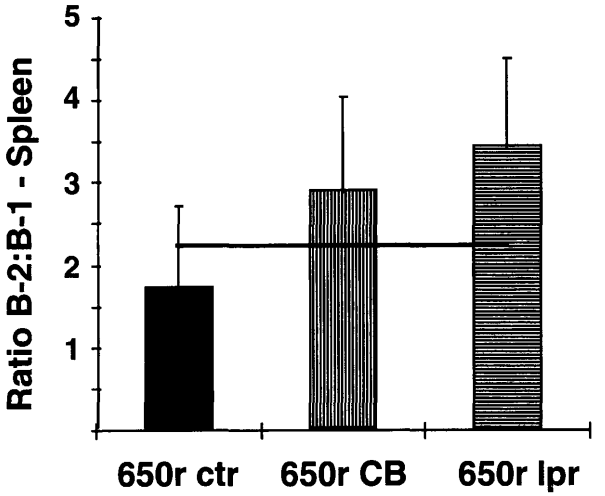

Figure 2. Ratio of B-2 (mainly contributed as a result of stem-cell activity) to B-1 (radio-resistant) cells in the spleen 2 weeks postsublethal irradiation. B2 cells were identified from flow cytometric analysis as IgM+, B220+, CD5-. B-1 cells were identified from flow cytometric analysis and were IgM+ and CD5+. The horizontal line represents the ratios measured in untreated, age-matched control mice.

highest stem-cell activity to regenerate normal B cells). In comparison to unreconstituted mice, *lpr* BM reconstituted mice have a lower percentage of B-1 cells due to increasing B-2 cell numbers from both endogenous repopulation, as well as differentiation of stem cells from the *lpr* BM graft. Mice receiving CB following sublethal irradiation have B-1 cell percentages between those of BM reconstituted and nonreconstituted mice. Absolute numbers of splenic B-1 cells followed a similar pattern. Age-matched MRL-*lpr/lpr* mice had a high absolute number of B-1 cells ($23.08 \pm 0.60 \times 10^6$). In the irradiated groups, the sublethal irradiated control mice had the highest absolute number of B-1 cells ($1.12 \pm 0.08 \times 10^6$) and sublethal irradiated recipients of *lpr* BM had the lowest absolute number of B-1 cells ($0.36 \pm 0.18 \times 10^6$). The CB recipients were intermediate ($0.59 \pm 0.31 \times 10^6$) (Table I). This suggests that, although B-cell lymphopoiesis (as measured by a decrease in B-1 cells) is not occurring as quickly as in BM recipients, CB recipients show amounts of lymphopoiesis above that which is endogenously occurring in the radiation control mice.

This same trend is seen in the ratios of B-1 cells and conventional B cells (Fig. 2). The lowest B-2 to B-1 ratios are seen in mice sublethally irradiated and not reconstituted. These ratios are lower than all animals reconstituted regardless of the level of irradiation, and are lower than age-matched control mice. In sublethally irradiated mice, the highest ratio of B-2 to B-1 cells is seen in *lpr* BM recipients. Sublethally irradiated CB recipients have B-2 to B-1 ratios between nonreconstituted irradiated mice and BM recipients. Again, this suggests that levels of hematopoiesis in sublethally irradiated CB recipients are greater than endogenous repopulation in nonreconstituted irradiated mice.

T-cell lymphopoiesis was measured by the percentages of CD4+ T cells in the lymph node, which reflects normal lymphopoiesis, and their relationship to the accumulation of abnormal double negative T cells (DNT). In Figure 3, the flow cytometric two-color analysis of representative mice 2 weeks post-treatment is shown with the CD4+ T-cell percentages indicated in the lower right quadrant. The radioresistant T cells are predominantly the DNT cells. As was the case with recovering B cells, nonreconstituted mice displayed the lowest percentage of newly emerging T cells (CD4+ T cells) at 2 weeks post-treatment. Bone marrow recipients recovering from sublethal irradiation showed the greatest percentage of CD4+ T cells in the lymph node at 2

Table I. Splenic B-Cell Subpopulations 2 Weeks Post Irradiation

Treatment[a]	Number of Spleen Cells[b]	Abs Number of B[c]	Abs Number of B-1	Abs Number of B-2
650 r CTR	49.6 ± 9.3	3.0	1.1	1.9
650 r lpr	104.8 ± 20.9	1.5	0.4	1.1
650 r CB	199.3 ± 36.3	4.9	0.6	4.3
No rad CTR	457.0 ± 50.1	73.3	23.1	50.2

[a] Spleen Cells were obtained from sublethally irradiated MRL-*lpr/lpr* (650 r) given syngeneic bone marrow (*lpr*), human cord blood (CB), or no treatment (650 r CTR). Additional controls were age-matched nonirradiated mice (No rad CTR). Spleen cells were obtained 2 weeks following irradiation.

[b] Total number of spleen cells (excluding erythrocytes) ($\times 10^6$).

[c] Numbers are mean absolute numbers of cells ($\times 10^6$) with flow cytometric surface staining belonging to that subpopulation of B cells. B cells (total IgM^+), B-1 (IgM^+, $CD5^+$), B-2 (IgM^+, $CD5^-$).

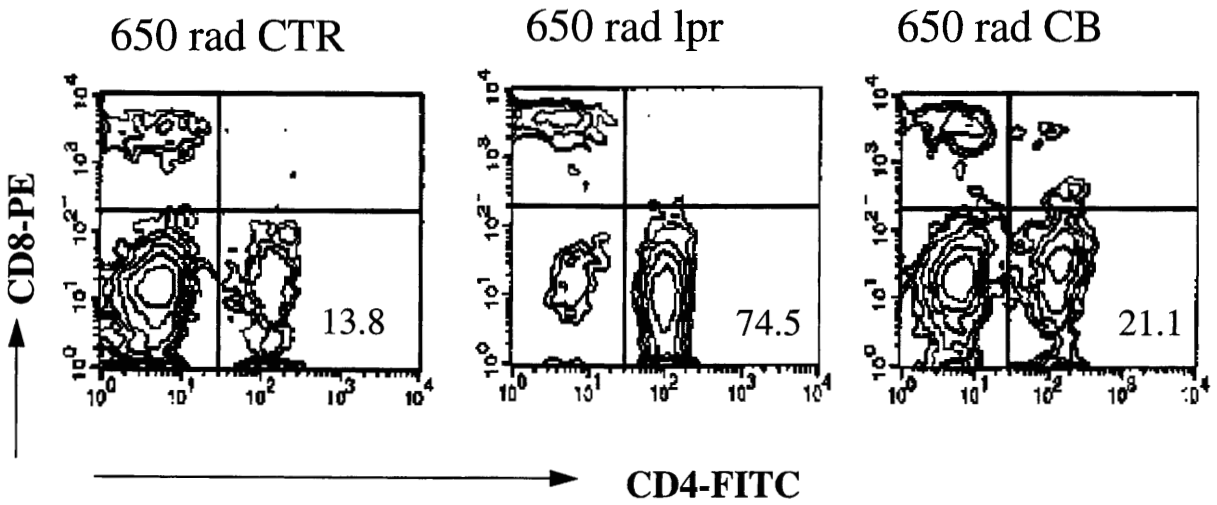

Figure 3. Two-color flow cytometric analysis of lymph node cells stained with CD4 FITC and CD8PE. The DNT cells are found in the lower left quadrant. The CD4+ T-cell percentage is indicated by the numbers next to the lower right quadrant that contains the single positive CD4 T cells. Lymph node cells were obtained 2 weeks following sublethal irradiation (650 r). Plots are identified by the treatment modality employed.

weeks post-treatment (Fig. 4). These percentages reflect normal T-cell hematopoiesis and are significantly increased in comparison to the percentage measured in unmanipulated control mice ($P < 0.05$) indicated by the horizontal line. Cord blood recipients show T-cell lymphopoiesis at a rate higher than nonreconstituted mice, which indicates increased hematopoiesis, most likely due to factors contributed by the human cord blood acting on murine stem cells. In the irradiated groups, the abnormal DNT cell percentages, which indicate the population of T cells remaining after irradiation, are highest in the group of animals sublethally irradiated and not treated (Fig. 5).

In MRL-*lpr/lpr* mice, the disease is characterized by lymphadenopathy and the predominance of DNT cells in the lymph nodes. In contrast, irradiated mice do not demonstrate lymphadenopathy (Table II). In the irradiated groups, however, the sublethally irradiated mice that received cord blood or bone marrow cells have not only a decrease in the percentage of DNT but also a decrease in the absolute numbers of DNT cells. Functionally, the lymph node cells in these latter two groups are capable of responding to T-cell mitogens. In Table II the stimulation index in response to phytohemagglutinin was nearly double in the CB and *lpr* (SI 8.25 and 9.12)-treated groups when compared to the radiation control (SI 4.13). These results suggest that the cells found in the lymph nodes of both *lpr* BM and cord blood recipients are newly emerging thymus-derived functional T cells and not the radio-resistant DNT cells (which are highest in the radiation control) since these cells do not respond to mitogens. The lymph node cells of all groups were greater than 97% CD3+, and cytokine profiles of RNA extracted from the lymph nodes, determined by a semiquantitative RT PCR analysis, gave results suggesting that the recipients of

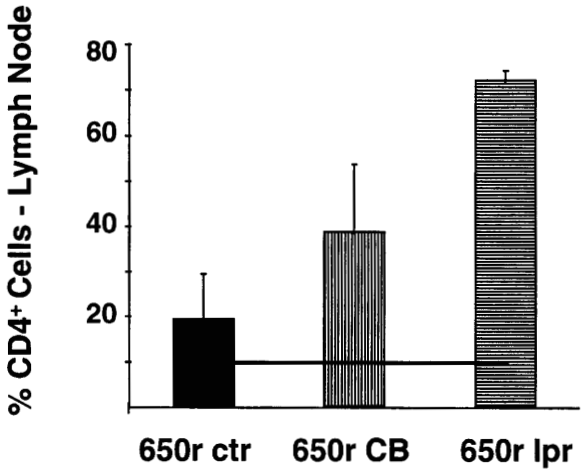

Figure 4. Percentages of CD4+ T cells in the lymph nodes at 2 weeks postirradiation. Columns represent means and standard error for each group, and the horizontal line represents the mean value for the untreated control group, $n = 3$ for all groups. The experiment was repeated two additional times with similar results.

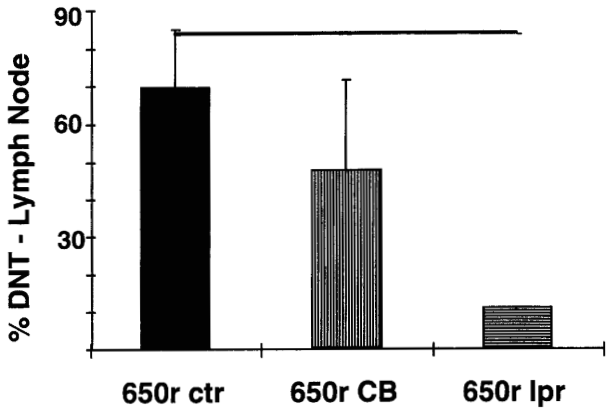

Figure 5. Percentages of DNT cells in the lymph nodes at 2 weeks postirradiation. Columns represent means and standard error for each group, and the horizontal line represents the mean value for the untreated control group. Data represent the means of three mice in a group. The experiment was repeated two additional times with similar results.

Table II. Effect of Cord Blood Transfer on Lymph Node Function

Source of LN	PHA (ΔCPM)	Con A (ΔCPM)	IL-10[a]	IFNγ[b]	Abs Number of DNT[b]	Abs Number of T ($CD4^+$ and/or $CD8^+$)	Abs Number of CD3
650 r CTR	1,674	10,222	51.3	268.8	7.6×10^6	3.5×10^6	11.1×10^6
650 r *lpr*	3,529	28,607	18.1	20.5	0.1×10^6	1.0×10^6	1.1×10^6
650 r CB	6,200	18,294	11.1	132.7	1.0×10^6	1.1×10^6	2.1×10^6
No rad CTR			82.1	96.0	166.0×10^6	30.0×10^6	196×10^6

[a] Numbers reported are normalized mean values (3 mice/group). Values represent the normalized values and are the ratio of the densitometric mean values for the observed band compared to the densitometric mean value for the housekeeping gene, HPRT.
[b] Numbers are mean absolute numbers of cells with flow cytometric surface staining belonging to that subpopulation of cells. Absolute numbers of lymph node cells are the total number of lymph node cells divided by the number of lymph nodes obtained.

human cord blood and *lpr* marrow have a decrease in IL-10 (T_H2 cytokine) when compared to the radiation control group. The amount if IFNγ produced may be due to both T_H1 cell production and DNT production, since DNT cells have been shown to produce IFNγ. In the experimental groups, the CB-treated mice had the lowest IFNγ and the lowest absolute number of DNT cells (Table II).

These differing rates of hematopoiesis can be demonstrated in Day 9 splenic colony forming activity (Table III). Mice that receive BM have colony-forming units (CFU) too numerous to count, with pale, raised colonies covering most of the surface of the spleen. Human cord blood recipients show numbers of CFU less than that for BM recipients, but greater than numbers seen in human PBMC recipients. This activity of cord blood in augmenting endogenous stem-cell activity may be due to the hematopoiesis enhancing effect of cord blood and not due to xenogeneic effect because recipients of the same number of human PBMC show decreased numbers of CFU in comparison to CB recipients. Results of analysis of the cytokine mRNA present in human CB and PBMC was consistent with this since CB (and not PBMC) had elevated levels of IL-7 (data not shown). There was no evidence of donor human cell engraftment since mouse recipient spleen cells analyzed by flow cytometry as early as Day 9 post-irradiation and transfer of human cord blood showed no human B or T cells present (<1% above normal controls). Therefore the increase in splenic CFU is most likely endogenous murine stem-cell activity.

To determine if the hematopoiesis-enhancing effect of cord blood cells also occurred in strains of mice other than MRL-*lpr/lpr*, a similar series of experiments was performed in another mouse strain. SJL/J mice were given either 600 r or 800 r, and at each irradiation dose, one group was injected intravenously with 10^7 human cord blood cells within 2 hr of irradiation. A second group of irradiated mice was not reconstituted, and a third group was comprised of age-matched, unirradiated SJL/J mice. At Days 16 and 23 after irradiation, mice from each group were sacrificed, and the levels of CFU-GM in the BM were determined. As shown in Table IV, at both irradiation doses, mice that were injected with cord blood cells had higher numbers of CFU than nonreconstituted mice. This was particularly evident in mice that received the higher irradiation dose and were tested on Day 16 after irradiation. It is noteworthy that the total cell counts obtained from the BM were also higher in mice that received cord blood cells in comparison to the irradiated, nonreconstituted mice (data not shown). Therefore, there was an even greater difference between cord blood–injected and nonreconstituted mice with respect to the absolute number of CFU-GM in their BM. These results demonstrate that the hematopoiesis-enhancing effect of cord blood cells in this xenogeneic model system involved myelopoiesis, as well as lymphopoiesis, and is not an observation that is unique to the MRL-*lpr/lpr* strain.

Table III. Splenic Colony-Forming Ability—Day 9 Post-Treatment

Treatment	Avg. Number of CFU[a]
650 r *lpr*	TNTC[b]
650 r CB	34.5 ± 3.9
650 r PBMC[b]	26.3 ± 1.7[c]

[a] Splenic colony forming units in sublethally irradiated MRL-*lpr/lpr* (650 r) given syngeneic bone marrow (*lpr*), human cord blood (CB), or human peripheral blood mononuclear cells (PBMC). Splenic colony forming ability in the spleen was measured on Day 9 post-treatment. Values represent means and standard error for each group.
[b] TNTC = Colony number too numerous to count by Day 9 (>50).
[c] Significantly decreased ($P < 0.05$).

Discussion

Of late, much research has gone into the possibility of using human umbilical cord blood as a source of stem cells and factors that augment stem-cell activation and differentiation (12, 13). Human CB contains stem cells of the multipotent, erythroid, and granulocyte/macrophage lineages, which have shown, in culture, to have an increased capacity for clonogenic growth in comparison to the same cells found in bone marrow (33, 34). In the majority of the experiments described, the autoimmune prone MRL-*lpr/lpr* strain was employed. This strain was used because of the elevated percentages of specific subpopulations of T and B cells. The B-1 cells in the MRL-*lpr/lpr* mice do not arise from bone marrow stem cells in the adult, and the DNT cells are thymus-derived T cells that fail to undergo apoptosis and accumulate in the lymph node. Thus, T and B cells, which newly arise from stem cells, will not be predomi-

Table IV. CFU-GM Activity in BM of Sublethally Irradiated SJL/J[a] Mice

Group	CFU-GM/10^6 BM Cells	
	Day 16	Day 23
Unirradiated Controls	130	150
600 r – No Reconstitution	62	108
600 r + Cord Blood Cells	132	137
800 r – No Reconstitution	22	33
800 r + Cord Blood Cells	98	82

[a] SJL/J mice were irradiated at the indicated dose, and either injected iv with 10^7 human cord blood cells or not reconstituted. Age-matched, unirradiated mice were tested in parallel. At Days 16 and 23 after irradiation, BM cells were tested for CFU-GM activity (n = three mice per time point per group).

nantly B-1 cells or DNT cells. Because the MRL-*lpr/lpr* strain has abnormalities in the regulation of apoptosis, generalizations about the observed effects of CB on normal stem cells may not be possible. However, similar results employing human CB have been observed in other strains of mice studied. In both mouse strains employed, human CB increased the survival following lethal irradiation, increased CFU in the spleen and bone marrow, and increased the presence of functional T and B cells (35).

The present results show that sublethal irradiation and administration of CB can augment hematopoiesis beyond that which is seen in irradiated nonreconstituted mice. Hematopoiesis, as measured by the return of peripheral conventional B cells (a decrease in the percentage of B-1 cells and an increase in the B-2/B-1 cell ratio) and normal T cells (a decrease in the percentage of DNA and an increase in the ratio of $CD4/8^+$ T/DNT) occurs mostly in mice that were sublethally irradiated and injected with syngeneic bone marrow. This increased peripheral recovery is most likely due to both endogenous and exogenous hematopoiesis from the bone marrow graft as well as stem cell factors supplied by the bone marrow graft. Irradiated, nonreconstituted mice have only endogenous hematopoiesis, and thus, the ratio of their peripheral lymphoid populations is skewed toward the abnormal radio-resistant cells as the predominant lymphoid population in comparison to BM reconstituted or recipients of CB. These data suggest that CB recipients have a slight hematopoietic advantage over nonreconstitued mice due to augmentation by stem-cell factors from the CB. The absolute numbers of B-2 cells or $CD4/8^+$ T cells does not reflect this increased hematopoiesis (Tables I and II). Because in MRL-*lpr/lpr* mice splenomegaly and lymphadenopathy occur, the absolute numbers in control nonirradiated MRL-*lpr/lpr* mice of normal B and T cells is also increased when compared to normal strains. Thus ratios of subpopulations of B and T cells may give better insight into types of recovering cells rather than absolute numbers.

This observation is substantiated by splenic colony formation data. Following irradiation, stem cell activity can be measured by the appearance and number of pale, raised colonies on the surface of the spleen (36). As expected, mice reconstituted with BM showed Day 9 CFU far too numerous to count, with colonies covering almost the entire surface of the spleen, and discrete colonies being indiscernible. Recipients of CB showed augmented stem-cell activity as seen by a greater number of splenic CFU than sublethally irradiated mice receiving human PBMC. This augmentation of murine endogenous hematopoiesis mediated by human CB is seen only at early time points postirradiation and reflects an increase in murine CFU and human stem cell activity. Flow cytometric analysis of spleen cells at 9 days postirradiation and transfer of human CB cells failed to demonstrate the presence of human mature B or T cells in the peripheral organs of the recipient mice. The transient presence of human CB cells in irradiated mice (small amounts of human DNA can be detected in the bone marrow up to 13 days post-transfer, data not shown) suggests that the human CB cells either produce or induce factors that enhance murine hematopoiesis. We, as well as others, have shown that CB cells produce factors that augment hematopoiesis. The data show that in irradiated MRL-*lpr/lpr* mice, the administration of human CB may offer some hematopoietic advantage by augmenting stem-cell activity. In addition, the increase in endogenous mouse hematopoiesis and stem-cell activity by human CB may not be due solely to the xenogeneic effect. First, the human peripheral blood (also a xenotransplant) failed to result in increased hematopoiesis or CFU formation. Secondly, most xenogeneic responses that result in graft rejection are hyperacute due to "natural antibodies" in the recipient, and occur within minutes to a few hours (37), whereas augmented hematopoiesis seen in the mouse CB recipients was seen at 2 weeks following treatment. Thirdly, research has suggested that an immune response resulting in lymphocyte proliferation is weaker in a xenogeneic system as opposed to an allogeneic system, with reactivity decreasing with increasing discordance between species (37–39). Although CB may increase endogenous hematopoiesis, this does not appear to exacerbate the onset of lymphoproliferation or autoimmune disease in these mice. On the contrary, all irradiated mice, regardless of treatment following irradiation show reduced evidence of lymphoproliferative disease as well as autoimmunity in comparison to age-matched control mice (40). In addition, sublethal irradiation resulted in increased survival in all mice regardless of reconstitution when compared to nonirradiated MRL-*lpr/lpr* (data not shown).

In terms of clinical use, administration of cord blood may augment stem-cell activity (either transferred CB stem cells or endogenous stem cells). Factors that expand stem cells are enriched in cord blood (41). In summary, these experiments were designed to detect the newly emerging lymphoid cells derived from stem cell progenitors *in vivo* following sublethal irradiation. A decrease in the percentage of B-1 cells and an increase in the percentage of B-2 cells is an indication of early B lymphopoiesis following removal of mature B cells by radiation. Furthermore, taking advan-

tage of the lymphoproliferative disease in MRL-*lpr/lpr* mice, a decrease in the amount of abnormal double negative T cells was also an index of the level of T cell hematopoiesis. The increase in functional T cells as measured by mitogen responses in CB recipients relative to irradiated controls further indicates that an increase in T-cell hematopoiesis is occurring in the CB recipients. The lower the percentage of double negative T cells, the higher the contribution of newly emerging T cells to the total T-cell pool. By using these novel markers as indicators of hematopoiesis in MRL-*lpr/lpr* mice, we have shown that cord blood factors can cross xenogeneic barriers and augment endogenous stem cell activity in MRL-*lpr/lpr* mice.

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