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Immunology

Lymphohemopoietic reconstitution using wheat germ agglutinin-positive hemopoietic stem cell transplantation within but not across the major histocompatibility antigen barriers

(bone marrow transplantation/hemopoietic stem cell purification/immunological tolerance)

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ABSTRACT **Nonadherent (NA), low density (LD), wheat germ agglutinin-positive (WGA$^+$) murine hemopoietic stem cell-enriched preparations (HSCPs) were tested for the capability to reconstitute lymphohemopoietic elements in lethally irradiated mice. HSCPs from BALB/c mice reconstituted lethally irradiated, major histocompatibility complex (MHC)-matched DBA/2 mice to normal histology of the thymus and spleen and normal humoral and cellular immune functions. By contrast, lethally irradiated B6 mice could not be reconstituted after transplantation with NA, LD, WGA$^+$ cells from MHC-mismatched BALB/c mice. We previously observed frequent survival, stable chimerism, and normally vigorous functioning immune systems in B6 mice transplanted with T-cell-depleted bone marrow from both BALB/c and B6 donors. To extend these findings to a stem cell transplantation system, lethally irradiated B6 mice were transplanted with NA, LD, WGA$^+$ cells from both BALB/c and B6 mice. These mixed stem cell-enriched preparations did not reconstitute the lethally irradiated, MHC-mismatched mice. By contrast, such HSCPs from BALB/c plus DBA/2 into DBA/2 mice reconstituted the hematologic and lymphoid tissues and functional immune systems when the donor and the recipient pairs were matched at MHC and mismatched at multiminor histocompatibility barriers. These purified blood progenitors thus appear to lack certain cells/factors essential for engraftment and reconstituting recipients in a fully allogeneic environment.**

Bone marrow transplantation and stem cell transplantation are models for study of hemopoietic and lymphoid cell development and differentiation. Hemopoietic stem cells represent the most primitive cells that establish a fully self-renewing population and retain multilineage lymphopoietic and hemopoietic differentiation potentials. Like whole marrow transplantation, stem cell transplantation may be developed as treatment of numerous human diseases. Unlike bone marrow cells, stem cell-enriched populations do not contain mature lymphocytes capable of inducing graft-versus-host disease. Purified autologous stem cells might be used to treat malignant diseases that involve marrow without other forms of purging to remove tumor cells or mature T lymphocytes.

Efforts to purify hemopoietic stem cells have included separation based on cell density, size, and expression of surface antigens (1, 2). Low density (LD), wheat germ agglutinin-positive (WGA$^+$) cells appear to contain a true stem cell population since this cell fraction provides long-term protection from lethal total body irradiation (3, 4), is enriched for day-12 spleen colony-forming unit (CFU-s), and can develop into precursors for multiple cell lineages *in vitro* (5). LD, WGA$^+$ cells have already been used, respectively, to prevent or induce the development of autoimmune disease in autoimmune-prone or autoimmune-resistant mice (6). Autoimmune-prone New Zealand Black (NZB) mice do not develop autoimmune renal glomerular disease after lethal irradiation and transplantation of LD, WGA$^+$ cells from age- and sex-matched healthy, major histocompatibility complex (MHC)-matched DBA/2 donors. By contrast, autoimmune-free DBA/2 mice given a hemopoietic stem cell-enriched preparation (HSCP) from autoimmune-prone NZB mice developed autoimmune disease later in life. Thus far, the capability of LD, WGA$^+$ cells to develop into mature functioning lymphocytes and to completely repopulate the lymphoid and hemopoietic organs in lethally irradiated mice has not been tested.

In the present study, nonadherent (NA), LD, WGA$^+$ HSCPs were investigated for the capability to reconstitute, histologically and functionally, the immune system in lethally irradiated mice and for the ability to induce immunological tolerance within and across the MHC barriers. Our results provide evidence that NA, LD, WGA$^+$ hemopoietic cell populations contain genuine stem cells, which are capable of self-renewal; but MHC restriction limits the stem cell preparation from producing life-saving engraftment and reconstitution of hemopoietic and lymphoid systems in an allogeneic environment.

MATERIALS AND METHODS

Animals. Inbred, 6- to 8-week-old female mice of BALB/c (H-2d), C57BL/6 (H-2b), C3H (H-2k), and DBA/2 (H-2d) strains (The Jackson Laboratory) were maintained in sterilized cages on laminar air flow racks and were fed regular mouse chow and acidified water.

Cell Preparation. Bone marrow was flushed from the tibias and femurs with RPMI 1640 medium supplemented with 2% fetal bovine serum and 1% penicillin/streptomycin. Marrow cells from different donors were depleted of mature T lymphocytes by treatment with anti-Thy1.2 monoclonal antibody (mAb) (Becton Dickinson) plus low toxicity rabbit complement (Pel-Freeze Biologicals). Cell viability was determined by trypan blue dye exclusion.

Covalent Binding of Immunomagnetic Beads to WGA. Equal volumes of a uniform suspension of tosyl-activated magnetic beads (Dynal) were added to WGA (Sigma) protein solution [1 μg of protein per 50 ml of 0.5 M borate buffer (pH 9.5)] to achieve a protein/bead ratio of 75 μg/15 mg. The

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Abbreviations: CFU-s, spleen colony-forming unit; HSCP, hemopoietic stem cell-enriched preparation; LD, low density; MHC, major histocompatibility complex; mAb, monoclonal antibody; MPC, magnetic particle concentrator; NA, nonadherent; TCD, T-cell depleted; WGA, wheat germ agglutinin.

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suspension was incubated for 24 hr at 22°C using slow end-over-end rotation. The magnetic beads were collected by a magnetic particle concentrator [MPC; Dynal, Oslo], the supernatant was discarded, and the beads were washed three times for 10 min in 7–8 ml of borate buffer; then they were washed a fourth time for 30 min. A final wash was overnight at 4°C. The beads were collected with the MPC, the supernatant was discarded, and WGA-coated beads were resuspended in 0.1% bovine serum albumin (BSA) in phosphate-buffered saline at 4×10^8 beads per ml.

Separating WGA$^+$ Cells with WGA-Coated Magnetic Beads. NA marrow cells were separated by equilibrium density centrifugation on a discontinuous Percoll (Pharmacia) gradient as described (6). LD marrow cells (fraction 2) were washed twice, pelleted, and mixed with the magnetic beads at a bead/cell ratio of 3:1. The mixture was incubated on ice for 5 min and then resuspended in 8 ml of 0.1% BSA. The magnetic beads were harvested with the MPC, and the supernatant fluid containing WGA$^-$ cells was collected. The beads were resuspended in 8 ml of medium containing 20 mg of *N*-acetylglucosamine (Sigma) (300 mosmol). The mixture was incubated for 15 min at 4°C with occasional shaking (the high affinity of *N*-acetylglucosamine to WGA causes the dissociation of WGA$^+$ cells from the magnetic beads). The beads were separated from the medium by using the MPC, and the supernatant containing WGA$^+$ cells was collected. The process was repeated to collect cells with high affinity to WGA. The collected WGA$^-$ and WGA$^+$ cells were washed and adjusted to the desirable concentration before use.

CFU-s Assay. Recipients were irradiated with a ^{137}Cs γ-ray source 24 hr prior to transplantation with marrow, fraction 2 cells, WGA$^+$ cells, or WGA$^-$ cells. Day-12 CFU-s were scored according to Till and McCulloch (7).

Protocol for Bone Marrow and WGA$^+$ Cell Transplantation. Recipients were given 950 R (1 R = 0.258 mC/kg) of total body irradiation from the ^{137}Cs γ-ray source 24 hr before transplantation. On the day of transplantation, recipients were injected in a lateral tail vein with an appropriate number of T-cell-depleted (TCD) bone marrow cells or LD, WGA$^+$ cells. When transplanted with syngeneic or allogeneic LD, WGA$^+$ cells, recipients were injected with 5×10^5 cells. For transplantation of mixed populations, irradiated animals were given 5×10^5 syngeneic plus 15×10^5 allogeneic LD, WGA$^+$ cells.

mAbs. Spleen cells from control and transplanted B6 mice were analyzed for chimerism by flow cytometry using immunostaining with fluorescein isothiocyanate (FITC)-conjugated mAb specific for H-2b, H-2d, or H-2k MHC antigens (Pharmingen, San Diego). Thymus cells of control untreated BALB/c, DBA/2, or transplanted DBA/2 mice were used for immunostaining with anti-Ly1.1 or anti-Ly1.2 mAbs (Cedarlane Laboratories, Hornby, ON) or without antibody followed by incubation with FITC-conjugated F(ab')$_2$ fragments of goat anti-mouse IgG antibody (Cappel Laboratories). Thymocytes from these mice groups were also immunostained with FITC-conjugated anti-Thy1.2, anti-B220, anti-CD4, and anti-CD8 mAb, and phycoerythrin-conjugated anti-CD4 mAb (Pharmingen) to analyze reconstitution with lymphocyte subpopulations.

***In Vivo* Anti-Sheep Erythrocyte Plaque-Forming Cell Assay.** This was performed as described (8).

Mixed Lymphocyte Cultures. Spleen cells from recipients were cultured with irradiated syngeneic, allogeneic, or third party cells to assess simultaneously for tolerance and allore-activity as described (8).

Histological Analysis. Routine hematoxylin and eosin-stained histological sections were prepared from spleens and thymuses of the transplanted mice 100 days posttransplantation, as well as from age- and sex-matched control non-transplanted mice. Spleen sections were analyzed for general architecture and for the presence and extent of lymphoid follicles and hemopoiesis in the red and white pulps. The thymus was analyzed for lymphoid repopulation, with particular attention to the cellularity of the cortex and a distinct corticomedullary demarcation.

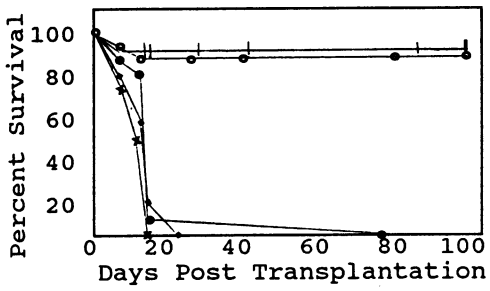

FIG. 1. Effects of transplantation of LD, WGA$^+$ cells on survival of B6 mice. ×, Irradiation control (n = 4); |, transplantation of syngeneic LD, WGA$^+$ cells (B6 → B6; n = 30); ●, allogeneic LD, WGA$^+$ cells (BALB/c → B6; n = 30); ◆, mixed syngeneic plus allogeneic LD, WGA$^+$ cells (BALB/c + B6 → B6; n = 30); ○, mixed syngeneic TCD bone marrow transplantation (B6) plus allogeneic LD, WGA$^+$ cells (BALB/c → B6; n = 10). Survival was monitored 100 days posttransplantation.

RESULTS

Survival of B6 Mice Transplanted with LD, WGA$^+$ Cells. B6 recipients were transplanted with LD, WGA$^+$ cells (Fig. 1) from syngeneic (B6 → B6), allogeneic (BALB/c → B6), or mixed syngeneic plus allogeneic donors. While transplantation of syngeneic LD, WGA$^+$ cells resulted in long-term survival, transplantation of allogeneic LD, WGA$^+$ cells across the MHC failed to protect the mice from lethal irradiation. When syngeneic (B6) TCD bone marrow cells (5×10^6 cells) were added to allogeneic (BALB/c) LD, WGA$^+$ cells (15×10^5 cells), long-term survival was regularly achieved. On the contrary, all mice transplanted with a mixture of syngeneic LD, WGA$^+$ cells plus fully allogeneic LD, WGA$^+$ cells died within 2 weeks posttransplantation. Control mice given allogeneic TCD bone marrow cells (data not shown) or a mixture of TCD syngeneic plus TCD allogeneic bone marrow cells achieved long-term survival and were free of graft-versus-host disease or evidence of immunodeficiency. LD, WGA$^+$ cells used in all experiments were purified by immunomagnetic bead sorting and were shown to be enriched >7-fold for day-12 CFU-s cells (data not shown).

Long-Term Survival of DBA/2 Mice Transplanted with LD, WGA$^+$ Cells. In contrast to transplantation across the MHC barrier, LD, WGA$^+$ cells transplanted within the MHC but across multiminor histocompatibility barriers (BALB/c →

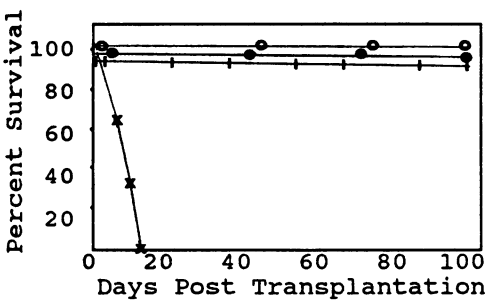

FIG. 2. Effects of transplantation of LD, WGA$^+$ cells on survival of transplanted DBA/2 mice. ×, Irradiation control (DBA/2; n = 3); ○, transplantation of syngeneic LD, WGA$^+$ cells (DBA/2 → DBA/2; n = 10); ●, allogeneic LD, WGA$^+$ cells (BALB/c → DBA/2; n = 15); |, mixed syngeneic plus allogeneic LD, WGA$^+$ cells (BALB/c + DBA/2 → DBA/2; n = 14).

Table 1. Phenotype of spleen cells in B6 mice transplanted with LD, WGA$^+$ cells

Group	Cell phenotype, %		
	H-2b	H-2d	H-2k
B6 (H-2b)*	94.7 ± 1.8	6.0 ± 1.7	7.6 ± 4.9
BALB/c (H-2d)*	2.2 ± 1.0	94.9 ± 0.3	1.9 ± 0.6
C3H (H-2k)*	5.4 ± 2.0	13.0 ± 2.9	83.3 ± 5.5
B6 → B6†	92.9 ± 5.5	11.9 ± 9.0	9.8 ± 5.3
BALB/c + B6 → B6‡	96.5 ± 0.3	1.9 ± 0.1	0.5 ± 0.1

Host spleen cells were analyzed 100 days posttransplantation; numbers represent percentage phenotype of three mice (mean ± SEM).
*Control nontransplanted mice.
†Transplantation of syngeneic LD, WGA$^+$ cells.
‡Transplantation of a mixture of syngeneic TCD bone marrow cells (B6) plus allogeneic LD, WGA$^+$ cells (BALB/c).

DBA/2) rescued the recipients from lethal irradiation (Fig. 2). DBA/2 mice transplanted either with syngeneic (DBA/2), allogeneic (BALB/c), or a mixture of both (BALB/c + DBA/2) LD, WGA$^+$ cells all showed survival for >100 days posttransplantation. In each transplanted group, the mice appeared healthy, gained weight, and showed no signs of graft-versus-host disease or gross immunodeficiency.

Flow Cytometric Analysis of the Chimeric State of B6 Mice and DBA/2 Mice Transplanted with LD, WGA$^+$ Cells. As shown in Table 1, B6 mice transplanted with a mixture of syngeneic TCD bone marrow cells plus allogeneic LD, WGA$^+$ cells across the MHC repopulated almost completely with host phenotype spleen cells (H-2b), indicating failure of engraftment of the fully allogeneic stem cell preparation. The chimerism of the transplanted DBA/2 mice is shown in Table 2. Thymocytes of DBA/2 mice transplanted with allogeneic LD, WGA$^+$ cells expressed mainly donor phenotype (Ly1.2) (although a considerable percentage of recipient phenotype existed), indicating that these thymocytes originated from the transplanted LD, WGA$^+$ cells. Transplantation of mixed syngeneic plus allogeneic LD, WGA$^+$ cells produced mixed chimerism that was more donor than host.

Primary Anti-Sheep Erythrocyte Plaque-Forming Cell Assay in B6 and DBA/2 Mice Transplanted with LD, WGA$^+$ Cells. B6 mice transplanted with syngeneic LD, WGA$^+$ cells (Fig. 3, bar C) produced a vigorous anti-sheep erythrocyte antibody response comparable to that achieved in normal nontransplanted mice. B6 mice transplanted with fully allogeneic LD, WGA$^+$ cells or a mixture of syngeneic plus allogeneic LD, WGA$^+$ cells did not survive. Spleen cells from DBA/2 mice reconstituted with LD, WGA$^+$ cells from syngeneic or allogeneic donors, or a mixture of syngeneic plus allogeneic cells from both BALB/c and DBA/2 donors exhibited vigorous primary antibody responses comparable to normal, nontransplanted DBA/2 mice (Fig. 3, bars E–H).

Tolerance and Alloreactivity of DBA/2 Mice. A mixed lymphocyte culture assay was used to test both tolerance and

Table 2. Phenotype of thymus cells of DBA/2 mice transplanted with LD, WGA$^+$ cells

Mouse strain	n	Cell phenotype, %		
		Ly1.1	Ly1.2	IgG
DBA/2 (Ly1.1)*	2	91.8 ± 4.7	9.8 ± 0.8	12.8 ± 6.2
BALB/c (Ly1.2)*	2	12.5 ± 2.4	75.5 ± 13.3	13.1 ± 6.4
DBA/2 → DBA/2	3	73.1 ± 14.7	10.0 ± 3.2	4.2 ± 1.5
BALB/c → DBA/2	3	16.3 ± 6.6	67.1 ± 9.7	6.0 ± 2.2
BALB/c + DBA/2 → DBA/2	2	28.3 ± 5.6	59.5 ± 4.4	2.7 ± 1.8

Thymus cells were analyzed 100 days posttransplantation; numbers represent percentage phenotype of n mice (mean ± SEM).
*Control nontransplanted mice.

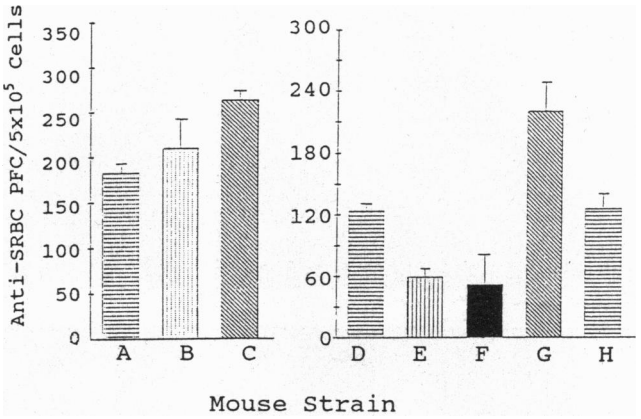

FIG. 3. Primary anti-sheep erythrocyte (SRBC) antibody response in mice transplanted with LD, WGA$^+$ cells. (*Left*) Bars A, BALB/c (control, nontransplanted); B, B6 (nontransplanted); C, reconstitution with syngeneic LD, WGA$^+$ cells (B6 → B6). (*Right*) Bars D, BALB/c (control nontransplanted); E, DBA/2 (nontransplanted); F, reconstitution with syngeneic (DBA/2 → DBA/2); G, allogeneic (BALB/c → DBA/2); H, mixed syngeneic plus allogeneic LD, WGA$^+$ cells (BALB/c + DBA/2 → DBA/2). Error bars represent SEM. PFC, plaque-forming cells.

alloreactivity of DBA/2 mice transplanted with LD, WGA$^+$ cells. As shown in Table 3, spleen cells from both DBA/2 mice reconstituted with MHC-matched allogeneic cells (BALB/c → DBA/2) and mixed chimeras (BALB/c + DBA/2 → DBA/2) were tolerant of both donor and recipient but were vigorously alloreactive to cells from a third party (B6).

Histological Analysis of Spleens and Thymuses of Mice Transplanted with LD, WGA$^+$ cells. Significant lymphoid follicular repopulation was observed in the spleens of DBA/2 mice reconstituted with syngeneic, allogeneic, or a mixture of syngeneic plus allogeneic LD, WGA$^+$ cells (Fig. 4). The architecture of the red and the white pulp of the spleen was preserved. Hemopoietic cells including megakaryocytes, myelocytes and macrophages, and lymphoid cells were present in distributions comparable to those of normal nontransplanted mice (Fig. 4*A*). Irradiated mice not reconstituted with stem cell preparations showed atrophic spleens and atrophic remnants of follicles and also exhibited accumulations of hemosiderin (Fig. 4*E*). In these irradiated mice, no evidence of erythropoiesis or lymphopoiesis was observed, and plasma cells were absent. Thymus sections from all transplanted DBA/2 mice showed normal thymic architecture with normal corticomedullary demarcation and normal ratios of cortex to medulla (Fig. 5). Irradiated DBA/2 mice not reconstituted with LD, WGA$^+$ cells showed dramatic atrophy of the

Table 3. Mixed lymphocyte culture response in DBA/2 recipients 100 days posttransplantation of LD, WGA$^+$ cells

Responder	Stimulator			
	DBA/2	BALB/c	B6	Self
DBA/2* (2)	1.0 ± 0.0	4.4 ± 3.0	5.4 ± 3.3	—
BALB/c* (2)	5.3 ± 2.6	1.0 ± 0.0	9.1 ± 1.1	—
B6* (2)	9.9 ± 2.2	9.9 ± 1.7	1.0 ± 0.0	—
DBA/2 → DBA/2 (3)	0.8 ± 0.1	1.8 ± 0.7	3.0 ± 1.8	1.0
BALB/c → DBA/2 (3)	1.1 ± 0.2	1.1 ± 0.5	7.0 ± 2.5	1.0
BALB/c + DBA/2 → DBA/2 (3)	0.8 ± 0.1	0.8 ± 0.3	3.1 ± 0.2	1.0

Number of mice is indicated in parentheses. Results are expressed as mean stimulation index (mean cpm of experimental culture/mean cpm of culture with autologous cells) ± SEM.
*Normal, nontransplanted mice.

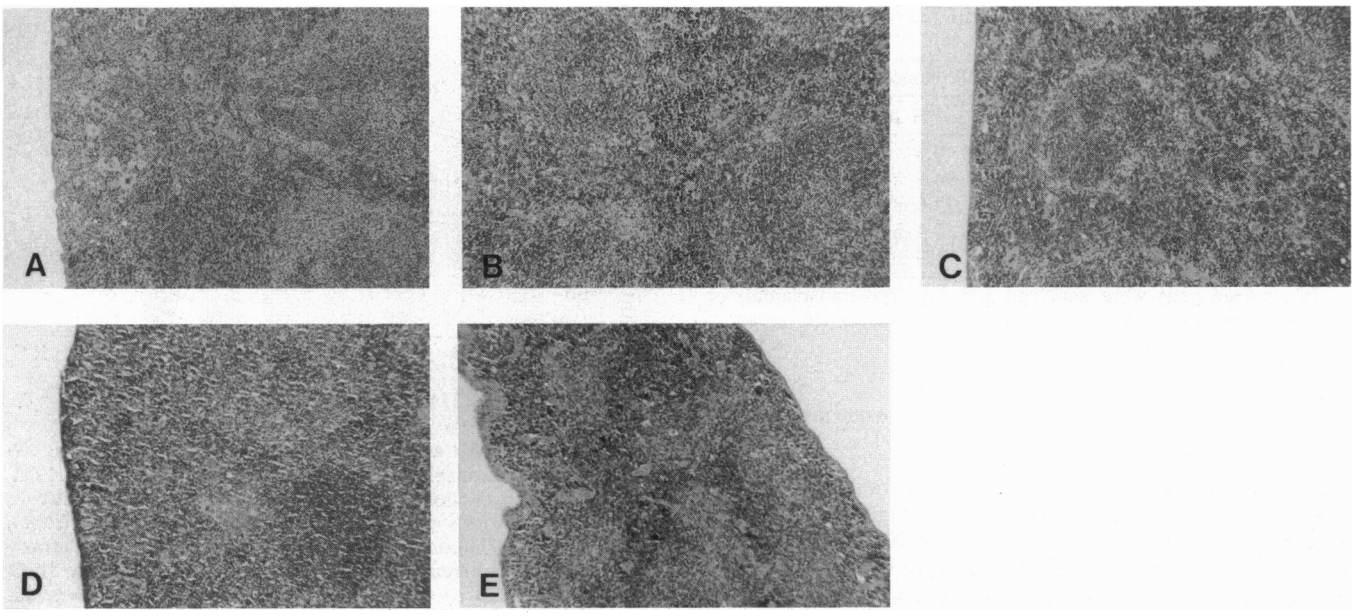

FIG. 4. Hematoxylin and eosin-stained spleen sections from DBA/2 mice transplanted with LD, WGA$^+$ cells. (*A*) Normal untreated DBA/2 mice. (*B*) Reconstitution with syngeneic LD, WGA$^+$ cells (DBA/2 → DBA/2). (*C*) Reconstitution with allogeneic LD, WGA$^+$ cells (BALB/c → DBA/2). (*D*) Reconstitution with a mixture of syngeneic plus allogeneic LD, WGA$^+$ cells (BALB/c + DBA/2 → DBA/2). (*E*) Irradiated nontransplanted DBA/2 mice 10 days postirradiation. (×70.)

thymus, which exhibited depletion of both epithelial cells and Hassall's corpuscles (data not shown).

B6 mice transplanted with fully allogeneic (BALB/c) LD, WGA$^+$ cells did not survive more than 15 days posttransplantation. Spleen sections from mice of this group taken at day 10 (data not shown) showed profound tissue atrophy and were similar to irradiated nontransplanted mice.

Lymphocyte Subpopulations of Thymus Cells in Transplanted DBA/2 Mice. To assess the repopulating efficiency of lymphocytes in thymuses of the transplanted DBA/2 mice, we stained thymocytes with markers for B-cell and T-cell subpopulations. As shown in Table 4, the distribution of Thy1.2$^+$ cells and their CD4$^+$ and CD8$^+$ lymphocyte subpopulations was similar in all tested groups to thymic cell distribution in normal, age- and sex-matched nontreated DBA/2 mice. The thymic repopulation with subtypes of lymphocytes and the immature CD4$^+$ CD8$^+$ population was impressive and did not differ whether the transplanted stem cell-enriched preparations were syngeneic, allogeneic within the MHC, or a mixture of both syngeneic plus allogeneic LD, WGA$^+$ cells.

DISCUSSION

In this report, we show that HSCPs of NA, LD, WGA$^+$ cells readily reconstituted lethally irradiated recipient mice when the source of the HSCP and the recipient mice differ at multiple minor histocompatibility determinants. However,

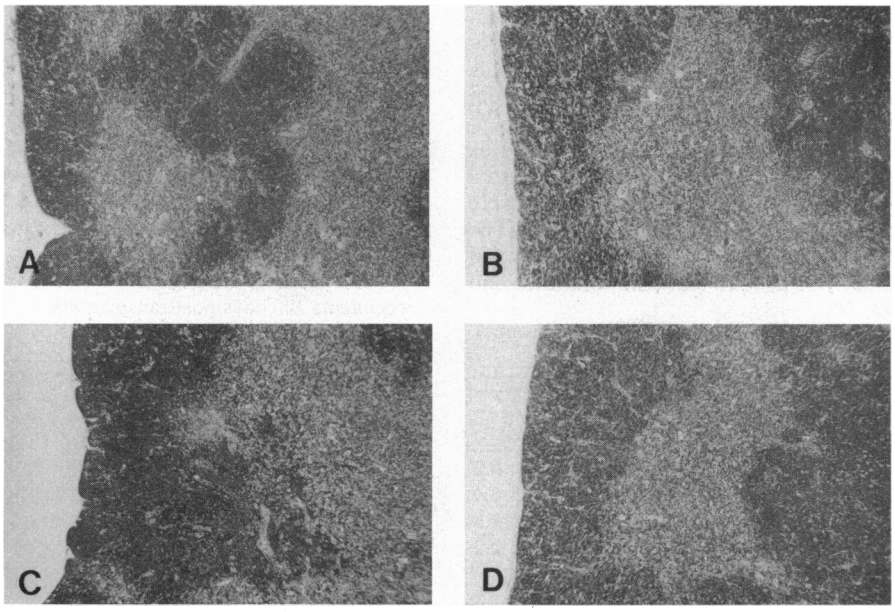

FIG. 5. Hematoxylin and eosin-stained thymus sections from DBA/2 mice transplanted with LD, WGA$^+$ cells. (*A*) Normal untreated DBA/2 mice. (*B*) Reconstitution with syngeneic LD, WGA$^+$ cells (DBA/2 → DBA/2). (*C*) Reconstitution with allogeneic LD, WGA$^+$ cells (BALB/c → DBA/2). (*D*) Reconstitution with mixed syngeneic plus allogeneic LD, WGA$^+$ cells (BALB/c + DBA/2 → DBA/2). (×70.)

Table 4. Distribution of lymphocyte subpopulations of thymus cells of DBA/2 mice 100 days posttransplantation with LD, WGA^+ cells

Mouse strain	Phenotype of thymus lymphocytes, %				
	Thy1.2	B220	$CD4^+$ $CD8^-$	$CD4^-$ $CD8^+$	$CD4^+$ $CD8^+$
DBA/2*	97.0	2.2	13.0	3.0	78.0
BALB/c*	84.0	1.2	11.0	5.0	81.0
DBA/2 → DBA/2	97.0	2.8	9.0	2.0	82.0
	96.0	4.0	12.0	3.0	78.0
	97.0	4.0	7.6	2.1	88.4
BALB/c → DBA/2	98.0	7.2	9.0	4.0	81.0
	98.0	10.0	6.0	3.0	89.0
	93.4	7.5	8.1	3.3	86.2
BALB/c + DBA/2	87.6	5.9	10.0	2.1	84.0
→ DBA/2	90.0	1.1	9.0	3.0	84.0

Numbers represent percentage phenotype from one mouse.
*Control nontransplanted mice.

when allogeneic stem cell-enriched preparations were transplanted, either alone or together with syngeneic stem cell-enriched cells, to MHC-disparate mouse strains, LD, WGA^+ cells failed to reconstitute the hemopoietic and lymphoid systems or to restore the immunological functions of the lethally irradiated mice. The finding that syngeneic cells, within the mixture of syngeneic plus allogeneic LD, WGA^+ cells used, did not reconstitute lethally irradiated mice was somewhat surprising. This complex of cells used for transplantation must contain particular elements (cells/soluble factors) that have inhibited the syngeneic stem cells from reconstituting the recipients.

When allogeneic HSCPs alone were transplanted and the allogeneic cells differed from recipients only across multiminor barriers, full structural and functional immunologic, lymphoid, and hemopoietic reconstitution was seen. These findings must be considered in light of other investigations; Von Boehmer *et al.* (9) used parental marrow to successfully reconstitute lethally irradiated haploidentical F_1 recipients. Onoé *et al.* transplanted bone marrow differing from recipients at the entire allogeneic H2 barrier, but such animals showed both humoral (10) and cell-mediated (11) immunological deficits, especially during primary antibody responses. Ildstad *et al.* (12, 13) achieved immunologic and hematologic reconstitution of lethally irradiated recipients by transplanting TCD-donor plus TCD-recipient marrow cells across partial but not complete MHC barriers; the capacity to bridge complete barriers was not tested. Our own prior investigations (14) used the method of Ildstad *et al.* to achieve full immunologic, lymphoid, and hemopoietic reconstitution with T-cell-purged donor plus T-cell-purged recipient marrow across the entire MHC plus multiminor histocompatibility barriers. These findings taken together indicate that the complete bone marrow contained cells and/or factors that are essential for engraftment of stem cells in fully allogeneic recipients and are lacking in the LD, WGA^+ cell populations used here.

The cells or factors that account for these differences have not been defined. Ikehara and coworkers (15) presented evidence suggesting that radioresistant stromal cells should be considered as facilitators of the capacity to reconstitute hematologic and immunologic functions in bone marrow recipients. Kaufman *et al.*† have defined a non-T cell, which they call a facilitating cell, that can help in transplantation of T-cell-purged marrow.

Further experiments with stem cell preparations, which we have used here, or even more highly purified such as those reported by others (2, 16), are needed. Supplementation of hemopoietic stem cells with cells or factors derived from, or produced by, marrow cells must be analyzed for lymphohemopoietic capacity to achieve full reconstitution capacity when transplanted across the MHC barrier. Only when such cells have been defined will it be possible to develop further the use of stem cell preparations, when transplanted across the MHC barrier, to correct immunological, hematological, or lymphoid deficits—e.g., those produced by lethal total body irradiation.

†Kaufman, C. L., Wren, S. M., Hronakes, M. L., Simmons, R. & Ildstad, S. T., Symposium on Tolerance Induction, Breckenridge, CO, January 17–20, 1993, abstr. p. 9.

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