COMMITTED T LYMPHOCYTE STEM CELLS OF RATS

Characterization by Surface W3/13 Antigen and Radiosensitivity*

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T and B lymphocytes are descended from a common ancestor, a multipotent stem cell (1, 2). The question arises as to the stage at which these two lineages diverge. Do multipotent stem cells directly generate B and T cells according to their local microenvironment, or do they first give rise to descendant stem cells that are restricted in their potentiality? There would be prima facie evidence for such committed stem cells, able to yield only one or other type of lymphocyte, if procedures were found that differentially replenish the mature B and T compartments of repopulated irradiated hosts. Abramson et al. (2) induced chromosome markers by irradiation and followed the progeny of uniquely marked individual stem cells. They found signs of a committed T stem cell in bone marrow. However, their protocol did not permit the characterization or isolation of committed stem cells. The formal objection has been raised (3) that the induction of the marker may itself have caused the apparent commitment. In the studies reported here, we have capitalized on naturally occurring B and T cell markers in the rat. We set out to confirm the existence of committed T stem cells by studying their surface antigenic properties and their radiosensitivity in vivo.

In rats, a 95,000 mol wt, heavily glycosylated glycoprotein defined by the mouse monoclonal antibody W3/13 (4, 5) is found on some bone marrow cells, on thymocytes, mature T lymphocytes, and immunoglobulin-secreting cells, but not B lymphocytes. This peculiar distribution prompted us to study this antigen on stem cells assayed by their ability to form T and B lymphocyte chimeras (6). Marrow from donors simultaneously carrying T and B alloantigenic genetic markers was incubated with W3/13 and sorted into bright or dim or dull fractions before injection into syngeneic irradiated hosts along with a fixed dose of competing host-type marrow. We found differential T and B lymphocyte chimerism in the recipients of W3/13 dim marrow.

There are several reports (7–11) that some T cells are more radioresistant than B cells. We therefore studied whether B and T lymphoid stem cells could also be distinguished by their radiation sensitivities in vivo by competing the endogenous stem cells left immediately after irradiation with exogenous B and T genetically marked marrow. PVG rats were subjected to various doses of gamma irradiation and injected with a fixed dose of doubly marked marrow. We then looked for split chimerism in peripheral T and B lymphocytes.

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Materials and Methods

Rats and Irradiation. Male and female inbred PVG rats from our specific-pathogen-free colony were used at 7–12 wk of age as irradiated hosts. They were given 4.5–10 Gy (450–1,000 rad) ^{137}Cs gamma irradiation from dual opposed sources in a Gammacell 40 (Atomic Energy of Canada, Ottawa, Canada) at 0.95–1.0 Gy min^{-1} 6 h before cell transfer. The dose in experiments testing the function of W3/13-sorted cells was 9.5 Gy. Radiation doses were measured with a 0.6-ml thimble ionization chamber with a perspex build-up cap (Radiatron Ltd., Twickenham, England) calibrated against a National Physical Laboratory secondary standard.

Donor rats were 8–10-wk-old males from either the inbred PVG strain or the double-congenic PVG-(*Pta.A1*, *Pta.B*+ + *RI.1*^{a}), abbreviated PVG-(*Pta$_{DA}$* + *1a*). This carries peripheral T cell alloantigens and immunoglobulin kappa light chain alleles from the DA strain bred for, respectively, 9 and 18 backcrosses to the PVG background. The double-congenic was maintained under conventional non-specific-pathogen-free conditions. In some early experiments (see text), single-congenic rats were used carrying only one marker.

Bone Marrow. Femoral, tibial, and sometimes humeral marrow was flushed by conventional procedures (6). Approximately 20% of the marrow was recovered from these bones, as judged by label recovery from rats injected intravenously with ^{59}Fe 5 h before death. Typically, ~5 × 10^8 nucleated cells were recovered per donor. No deliberate attempt was made to recover endosteal marrow. The erythrocytes in suspensions destined for fluorescence-activated cell sorter (FACS)[1] analysis or sorting were lysed by 10-min exposure to Tris-buffered ammonium chloride at room temperature (6).

Fetal Liver. Pregnancy was timed from day 0 as the day when sperm were detected in the vagina. For each experiment, livers were microdissected from all the fetuses (~9 to 12) from a single dam, teased with watchmakers' forceps, and dissociated into cell suspension by gently drawing through 21- and 23-gauge needles. Cellularity and viability (routinely >85% by trypan blue exclusion) was assessed by hemocytometer.

Antibodies and Labeling. Monoclonal mouse IgG anti-rat thymocyte antibody W3/13 was a gift from Dr A. F. Williams, MRC Cellular Immunology Unit, Oxford. Its binding to bone marrow was revealed by indirect immunofluorescence using immunoadsorbent fluorescein$_3$-rabbit F(ab′)$_2$ anti-mouse Fab (12). To estimate the background labeling, monoclonal mouse anti-HLA supernate W6/32 (13) was used to replace W3/13. Twice-washed cells were incubated at 4°C for 1 h with intermittent resuspension at an antibody concentration of 30 μg ml^{-1} (saturating) and cells at 10^8 ml^{-1}. Cell recovery after labeling was >70% of starting numbers.

For analysis of chimerism, fluorescein-F(ab′)$_2$ rat anti-RI-1^a immunoadsorbent-purified antibody, congenic PVG anti-PVG-*Pta$_{DA}$* antiserum, fluorescein-rabbit F(ab′)$_2$ anti-rat IgG$_2$, rabbit F(ab′)$_2$ anti-rat F(ab′)$_2$, and fluorescein-horse F(ab′)$_2$ anti-rabbit IgG have been previously described (6). Congenic PVG-*Pta$_{DA}$* anti-PVG serum was a generous gift from Dr G. W. Butcher, (ARC Institute for Animal Physiology, Cambridge), and was used at a concentration of 1:10. Monoclonal rat IgG anti-RI-1^b anti-allotype, MRC OX 11, was derived from a fusion of NS1 mouse myeloma cells with spleen cells from a DA rat immunized for 15 mo with *Bordetella pertussis* coated with PVG serum (14). It was cloned and propagated in tissue culture by conventional procedures (15, 16). The supernate was diluted 1:10 for use. The binding of both of these alloantibodies was revealed by a second layer of fluorescent rabbit F(ab′)$_2$ anti-rat IgG. Details of labeling are in ref. 6. In brief, thrice-washed thoracic duct lymphocytes (TDL) from the first overnight collection after cannulation were split into three aliquots; 5 × 10^6 were labeled with anti-RI-1^a allotype, 5 × 10^6 with anti-rat F(ab′)$_2$ to determine the total B cell frequency, and 5 × 10^7 were depleted of surface immunoglobulin (sIg$^+$) cells by a rosetting procedure. The depleted cells were checked for B cell contamination by relabeling with anti-rat F(ab′)$_2$, and an aliquot of 5 × 10^6 was labeled with anti-Pta$_{DA}$ serum. To detect the host component in the radiation dose experiments, a further aliquot of whole TDL was labeled with MRC OX 11 antibody and an aliquot of the depleted TDL was labeled in two stages with anti-Pta$_{PVG}$ serum and fluorescent rabbit F(ab′)$_2$ anti-rat IgG$_2$.

Cell Sorting. A Becton-Dickinson FACS II (B-D FACS Systems, Mountain View, Calif.)

[1] *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; TDL, thoracic duct lymphocytes.

with an argon laser at 488 nm was used at the following settings: laser, 100 mW; photomultiplier, 680 V; fluorescence gain 8/1 for bone marrow, 16/1 for fetal liver. Low-angle light scatter thresholds were set to exclude erythrocytes, dead cells, and aggregates. 5,000 cells were sorted per second for 6–8 h. Cell recovery was 30–40% of the input, the loss being due to a combination of cells aborted through coincidence in one drop, cells falling outside the chosen fluorescence windows, adherence to the feed tube, disintegration on passage through the nozzle, and handling losses in the collecting tubes. Suspension of the input cells in isopycnic medium (70% Percoll [Pharmacia Fine Chemicals, Piscataway, N. J.] in Dulbecco's A plus B solution containing 10 mM NaN_3) helped to prevent aggregation in the sample and feed tubes. Purities, checked by re-analysis of sorted fractions, averaged 91% for bright cells, 81% for dim cells, and 93% for negative cells.

Results

W3/13 Labeling of Bone Marrow. A typical fluorescence intensity profile of W3/13-labeled cells among marrow nucleated cells (Fig. 1 a) reveals a bimodal distribution with a trough demarcating W3/13 bright cells and a more weakly labeled shoulder of a W3/13 dim population merging into but distinguishable from background labeling. The proportions of W3/13 bright and W3/13 dim cells stabilized at ~40 and 25%, respectively, at about 6 wk after birth. The dot plot (Fig. 1 b) of low-angle light scatter vs. fluorescence intensity showed the bright cells to be mostly large cells (granulocytes and blasts) and the dim cells to be mostly lymphoid. A differential count in smears of marrow sorted for these populations is shown in Table I, confirming this interpretation.

Functional Studies on W3/13-sorted Bone Marrow Cells: B and T Lymphocyte Stem Cell Assay. Initial experiments used PVG-*1a* congenic rats as donors, carrying only the kappa chain marker, so only B and not T lymphocyte genesis was studied. PVG-*1a* bone marrow cells were competed in various doses against a fixed inoculum of 10^7 PVG marrow cells in irradiated PVG hosts. Four different fractions of PVG-*1a* bone marrow were used: unlabeled, labeled but unsorted, and the two sorted fractions, W3/13 bright and W3/13 dim-and-negative. Recipients were analyzed for chimerism of the B lymphocytes bearing the kappa allotype at various times after irradiation, usually 4–6 wk. We checked first the linearity of the stem cell assay to confirm previous observations (6). Competition between unlabeled PVG-*1a* and PVG marrow was equal. Ideally, 10^7 PVG-*1a* bone marrow cells should produce a chimerism of nearly 50%; in fact, four different animals were found to have 45, 46, 48, and 50% (Fig. 2). Labeling cells with antibody caused only a marginal reduction in stem cell activity (Fig. 2).

The data in Fig. 2 show that by sorting labeled bone marrow into W3/13 bright and W3/13 dim-and-negative fractions, B lymphocyte stem cell activity was contained entirely in the W3/13 dim-plus-negative fraction. The slight activity (3.6% chimerism) generated by the high dose of 10^7 W3/13 dim-plus-negative cells could be attributed to contamination with W3/13 bright cells. W3/13 bright cells, on the other hand, were able to generate more B cell progeny on a cell for cell basis than labeled but unsorted cells.

Similar experiments were then performed using PVG-(Pta_{DA} + *1a*) donor rats, which allow analysis of both T and B lymphocyte genesis simultaneously within one recipient. The use of these doubly congenic animals prevented artefacts due to variation in the thresholds used for sorting that otherwise might have occurred from one experiment to the next. Results from two experiments are summarized in Fig. 3.

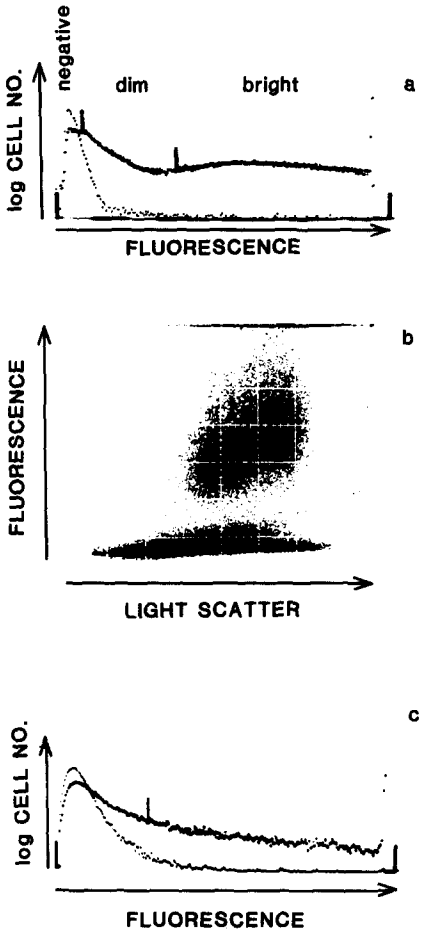

FIG. 1. (a) W3/13 labeling of young adult bone marrow. NH_4Cl-treated bone marrow cells for an 8-wk donor were labeled with either W3/13 (dark trace) or W6/32 (light trace), followed by fluorescein-rabbit anti-mouse Fab, and analyzed on the cell sorter. Fluorescence profile of 10^5 nucleated cells, with markers showing definition of negative, dim, and bright cells (35, 26, and 39% of all cells, respectively). Negative cells were defined as those with the staining intensity that 95% of W6/32 (control) stained cells showed. (b) Dot plot of low-angle light scatter, reflecting cell size, vs. fluorescence intensity of 10^5 labeled nucleated cells. Note the predominance of large cells (mainly granulocytes) among W3/13 bright cells, and of smaller cells (lymphocytes and normoblasts) in the dim and negative populations. (c) W3/13 fluorescence profile of 5×10^4 day-14 fetal liver cells (dark trace). Control labeling was with W6/32 (light trace). The fluorescence gain was adjusted to correspond to the bone marrow profile (top panel). The marker shows the threshold for sorting, equivalent to the division between dim and bright cells on the marrow curve. 27% of cells were brighter than this marker in this case; 0.7% in the control profile.

Both labeled and labeled but unsorted fractions of congenic marrow competed equally with PVG marrow. The lesser chimerism among T lymphocytes than B lymphocytes is the predicted pattern because T cells (defined as sIg^-) include ~25% Pta-negative cells (6): fully donor-type Pta-bearing cells would therefore register as only 75% chimeric among all T cells. The results from the sorted fractions show that, as before, B stem cell activity was confined to the W3/13 bright population, which was roughly twice as potent per cell as unseparated marrow, as would be anticipated from their

TABLE I

Differential Count of W3/13-sorted Bone Marrow

	Percent of all cells	Purity	Percentage among all nucleated cells				
			"Lymph-oid"	Neutro-phils	Basophils	Eosino-phils	Blasts
Experiment 1							
Unsorted, labeled	100		48	33	1.5	3.1	14
W3/13 bright	38	93	8.4	72	3.8	4.3	12
W3/13 dim-plus-negative	62	97	82	3.4	0.3	0.8	13
Experiment 2							
Unsorted, labeled	100		58	23	4.0	3.1	11
W3/13 bright	33	96	13	66	1.6	9.6	9.1
W3/13 dim	9	80	75	5.6	3.5	2.3	13
W3/13 negative	50	94	86	5.1	1.6	3.5	6.1

PVG bone marrow from an 8-wk-old donor was treated with NH_4Cl to lyse mature erythrocytes and labeled with W3/13 and F1-rabbit anti-mouse Fab for sorting according to the thresholds shown in Fig. 1. Purities were determined by rerunning through the cell sorter. "Lymphoid" cells include NH_4Cl-resistant normoblasts. More than 300 cells were scored per Giemsa-stained smear.

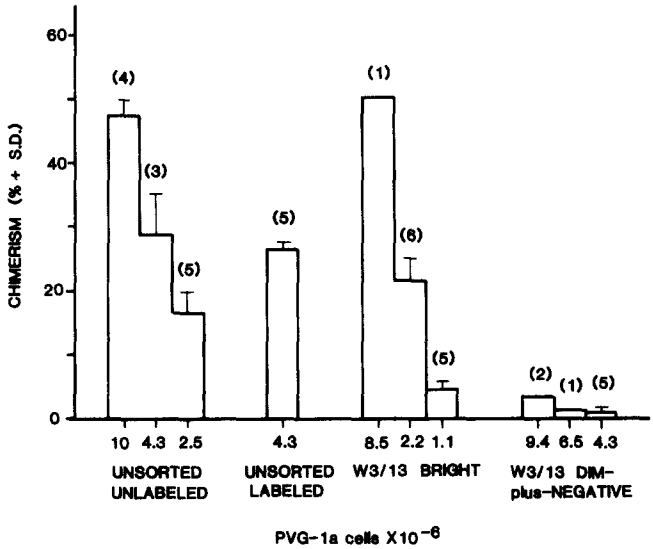

FIG. 2. B stem cell assay of bone marrow, either unsorted, or labeled but unsorted, or sorted into W3/13 bright and dim-plus-negative fractions. Two experiments with TDL analyzed 5 and 9 wk after reconstitution. Number of animals per group is shown in parentheses. All activity is in the W3/13 bright fraction.

enrichment. On the other hand, peripheral T lymphocytes were generated almost equally from W3/13 bright and W3/13 dim-plus-negative fractions of bone marrow. Recipients of W3/13 dim-plus-negative cells therefore unequally regenerated their T and B compartments. For instance, 6 wk after reconstitution, one chimera had 14% T lymphocytes of Pta_{DA} donor type, but only 4% B lymphocytes of Ig-1a type, the inverse of the normal pattern.

To check the stability of the chimerism, recipients were re-analyzed 24–48 wk after

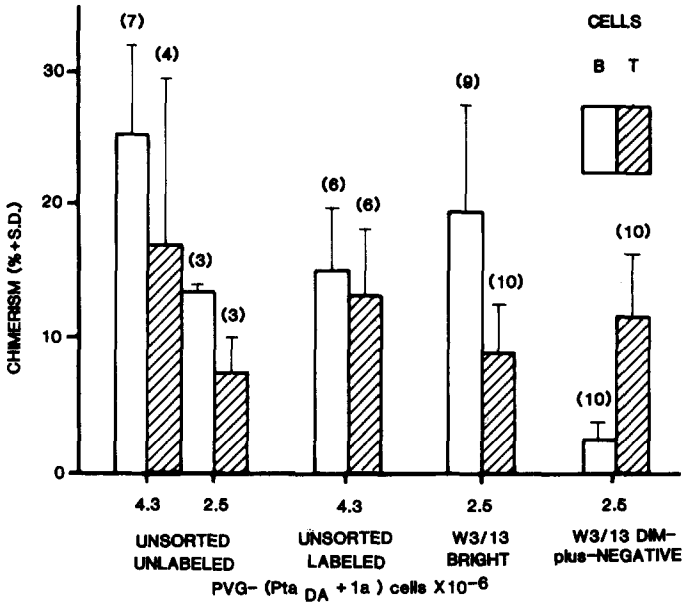

FIG. 3. B and T stem cell assay of bone marrow. Two experiments with TDL analyzed 5 and 9 wk after reconstitution. Number of animals per group is in parentheses. Note reversal of T and B chimerism in recipients of W3/13 dim marrow. See text for explanation of why T cell chimerism is normally less than B chimerism.

TABLE II

Chimerism Early and Late after Reconstitution in Recipients of W3/13-sorted Bone Marrow

Fraction and cell dose	Chimera	Chimerism			
		B cells		T cells	
		Early	Late	Early	Late
		%			
Unsorted, unlabeled	A	20.6	25.5	17.5	17.1
4.3×10^6	B	10.5	9.2	6.5	8.4
W3/13 bright	C	23.8	22.7	6.4	9.7
2.5×10^6	D	23.7	23.4	6.1	11.7
	E	24.8	23.6	10.3	20.5
	F	28.4	30.3	13.7	24.2
	G	26.6	24.8	13.1	22.2
W3/13 dim-plus-negative	H	3.8	3.2	14.2	5.4
2.5×10^6	I	4.3	2.7	14.1	7.9
	J	4.2	3.6	13.1	10.0

"Early" analysis was at 5–7 wk after reconstitution; "late" analysis was at 24–44 wk. Note stability of B cell chimerism, whereas T cell chimerism in W3/13 bright recipients increased and W3/13 dim-plus-negative decreased.

reconstitution (Table II). In the interval between the analyses, B cell chimerism remained unchanged both in the sorted and unsorted fractions. For example, in the W3/13 bright fraction, the drive to proliferate was sufficiently vigorously maintained

to continue competing equally with the whole PVG reference marrow inoculum. For T cells, however, although the chimerism generated by W3/13 bright cells almost doubled, that from W3/13 dim-plus-negative was halved though still detectable above background. The division between the T stem cell activities of bright and dim-plus-negative, initially equal, had shifted to about 3:1. These corresponding changes in T lymphocyte chimerism suggest that the more primitive T lymphopoietic stem cells are W3/13 bright.

In one further experiment, the thresholds for sorting were altered so as to separate W3/13 negative cells from W3/13 dim cells, discarding the bright fraction. Table III confirms the absence of B stem cell activity from dim or negative cells (Figs. 2 and 3) and shows that the T cell repopulating activity is in the dim not the negative population.

B and T Stem Cell Assays of Early Fetal Liver. The preceding studies with bone marrow suggested that it contained a mixture of different kinds of stem cell, both multipotential and committed. To characterize the surface phenotype of the most primitive stem cells, we studied cells from the developmentally earliest lymphopoietic organ. We first tried yolk sac cells. 10^6 viable yolk sac cells at day 10, dissociated with trypsin, yielded no detectable cell chimerism in the standard competition assay 7 wk after reconstitution. Turning then to fetal liver, the earliest practicable day for analysis of function was day 14, when in the B and T competition assays there was potent activity. Chimerism from 10^5 cells was readily measurable within 2 mo after reconstitution (Table IV). Activity per cell fluctuated from one experiment to the next (perhaps at this early time the exact stage of gestation is more crucial than we were able to control), but was usually more vigorous even than the day 17 to day 20 fetal liver previously examined (6). Table IV shows how the titration of unlabeled cells confirms the much greater potency of day 14 liver than marrow (17), by a factor of at least 20 on a per cell basis. The more cells, the greater was the chimerism, but unlike marrow (Fig. 2), the relationship was nonlinear and tended to saturate at high doses. However, at lower doses ($<10^6$), the assay could be used with confidence to monitor the enrichment or depletion of activity among sorted cells.

Day 14 fetal liver was labeled with W3/13 antibody. Fig. 1 c shows clear labeling above the background seen with a control incubation with an irrelevant monoclonal supernate, W6/32. There was a continuous gradation of intensity of labeling, and the thresholds for defining a cell as negative, dim, or bright were taken to correspond to

TABLE III

Stem Cell Activity of W3/13 Dim and W3/13 Negative Bone Marrow

Fraction	Purity	Percent of all cells	Cells × 10^{-6}	Percentage chimerism in	
				B lympho-cytes	T lympho-cytes
	%				
Unsorted and labeled	—	100	2.5	6.6	5.8
W3/13 negative	94	26	2.0	1.1, 0.5	0.6, 1.2
W3/13 dim	83	10	1.0	1.3, 0.8	12.8, 10.9

Chimeras were analyzed 8 wk after irradiation and reconstitution in the normal competition assay. In the dim fraction, the chimerisms show T stem cell activity to be approximately eightfold greater per injected cell than the unsorted cells. Note the absence of B stem cell activity from the sorted fractions.

TABLE IV
Stem Cell Activity in Day 14 Fetal Liver Fractions

Fraction	Purity	Percent of all cells	Cells × 10^{-5}	Percentage of chimerism in			
				B lymphocytes		T lymphocytes	
				Experiments 1 and 2	Experiment 5	Experiments 3 and 4	Experiment 5
	%						
Unlabeled	—	100	20			63.7, 63.7, 67.5	
			15	67.7, 69.3			
			10		61.0, 53.6		ND, 41.4
			5	70.5, 64.2, 66.7	36.5, 49.6	20.6, 16.5	27.1, 24.0
			2.5	30.1, 53.7			
			1		15.1, 12.3		10.2, 6.7
W3/13 bright plus dim	>86	36 to 34	10	49.5, 50.9			
			5	48.7, 53.0, 54.8		66.9, 64.9, 64.4, 67.3, 60.3	
			1	30.2			
W3/13 bright	89		1		59.6, 63.0		43.7, 47.9
W3/13 dim plus negative	95		10		3.3, 1.5		0.5, 1.3
			5		0.3		1.9
			1		1.1, 1.1		0.4, 0.4
W3/13 negative	>90		50			1.9, 2.1, 1.7	
			10–15	0.5, 0.3		2.1, 3.5, 3.2, 2.7	
			7.5	1.6, 0.4, 0.5			
			5	1.5, 2.1, 1.6		0.9, 1.4	

Day-14 fetal liver cells were labeled with W3/13 antibody and fluorescent rabbit anti-mouse Ig (experiments 1–5). Thresholds for sorting were set corresponding to those used for bone marrow. In experiments 1 and 2, donors were PVG-*1a* and chimeras were analyzed at 5 and 8 wk after reconstitution. In experiments 3 and 4, heterozygous (PVG × PVG-Pta_{DA}) donors were used and analysis was at 8 and 12 wk; allowance was made for the lower intensity of anti-Pta staining of heterozygotes during analysis. In experiment 5, donors were (PVG-(Pta_{DA} + *1a*) × PVG-*1a*)F_1 hybrids, heterozygous for Pta and homozygous for Ig-1a; analysis was at 10 wk.

the thresholds used for bone marrow. Labeled cells were sorted for assay of their stem cell activity, at first using separately B and T marked donors and later the double-congenic PVG-(Pta_{DA} + *1a*) when it became available. In Table IV, experiments 1, 2, and 5 (B cells) and 3, 4, and 5 (T cells) demonstrate that all stem cell activity was confined to W3/13 bright cells. There was no sign of activity in the dim or negative fractions, in particular none in the compartment from W3/13 dim cells, where it had been found in bone marrow. No split chimerism suggestive of committed stem cells was observed. The balance sheet of activity roughly shows the enrichment in the bright fractions that would be anticipated from 35% of cells being labeled.

Radiosensitivity of Endogenous Stem Cells. Three experiments were performed in which groups of PVG rats were exposed for various periods to the ^{137}Cs source and injected intravenously 6 h later with 10^7 PVG-(Pta_{DA} + *1a*) young adult bone marrow cells. Radiation doses ranged from 4.5 to 10 Gy (450–1,000 rad). Chimeras were analyzed at different times up to 9 mo after reconstitution, in some cases by cannulating the thoracic duct twice, before day 50 (early) and after day 100 (late). Even at early times the hourly output of lymphocytes from the thoracic duct was restored nearly to normal; the first night outputs were ~2×10^7/h at the lower radiation doses, falling to about 10^7/h at the highest. If this output can be taken as a guide to the total mobilizable pool (e.g., 60 times the initial hourly output [18]), then the total pool of lymphocytes in recipients of an intermediate dose, 850 rad, can be estimated at ~8×10^8, a very substantial expansion from the 10^7 inoculated. The proportion of B cells among all TDL was independent of radiation dose, ~60% at early times and 40–50%

(comparable with normal PVG rats) at later times. The general properties of these chimeras were thus very similar to the fully exogenous chimeras described earlier (6).

In addition to the usual sera detecting donor components, antibodies recognizing host Ig allotype (RI-1b) and host Pta (Pta_{PVG}) were used to determine directly cells descended from endogenous sources. Fig. 4 shows the percentage of host-type Ig-bearing cells among host plus donor allotype bearers (B chimerism) and correspondingly for Pta-bearing T cells, which were ~70% of surface Ig^- cells, irrespective of radiation dose. A crude but convenient index of radiosensitivity is the dose required to produce 50% chimerism, i.e., when the endogenous equivalent of 10^7 injected cells survive. For B lymphocytes, this was ~6.0–6.5 Gy, estimated within 50 d of reconsti-

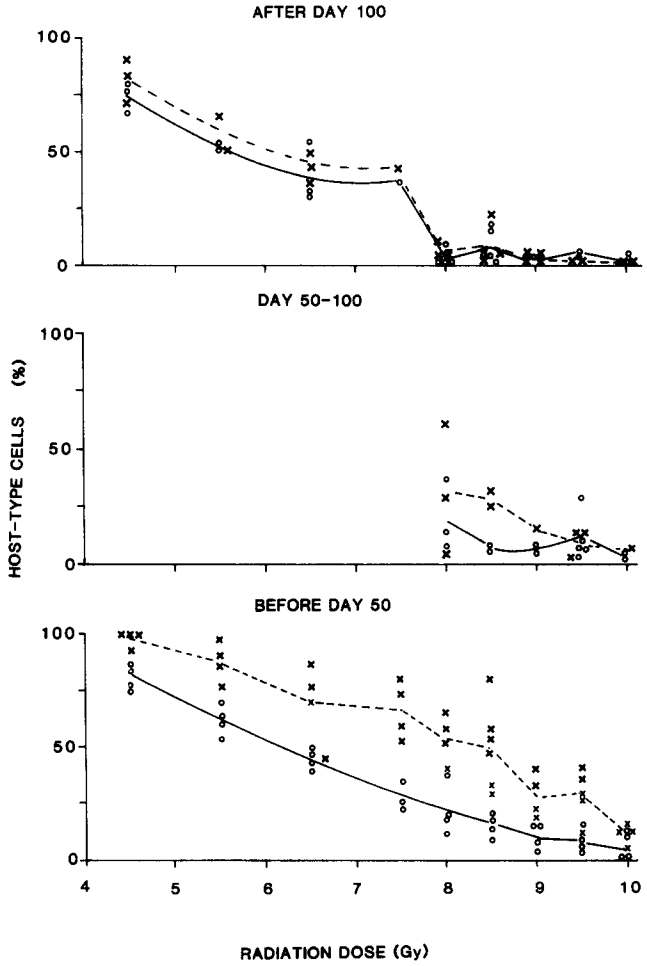

FIG. 4. Radiosensitivity of endogenous B and T stem cells. TDL from chimeras given various exposures to irradiation and reconstituted immediately with 10^7 PVG-(Pta_{DA} + *1a*) bone marrow were analyzed subsequently at different times, as shown (three experiments). Both host and donor cells were scored in these experiments, hence the chimerisms are recorded as values within each marked compartment, not just in the sIg^+ or sIg^- compartments as in Figs. 2 and 3. Individual points (B cells, O; T cells, ×) show frequencies of host-type cells. Note split chimerism in rats analyzed before day 50.

tution, and it hardly changed when examined much later, past 100 d. The late analyses included some chimeras analyzed at >8 mo, but still the B cell chimerism remained steady. However, for T cells, 8.5 Gy were needed to produce 50% chimerism upon early analysis (before day 50) dropping to the same dose as for B cells, 6 Gy, at later times. In other words, rats given 8.5 Gy when analyzed soon after reconstitution contained substantial numbers of host T cells in the virtual absence of host B lymphocytes, another example of split chimerism. Longer-term repopulation revealed that the stem cells yielding T lymphocytes were equally as radiosensitive as those producing B lymphocytes. For permanent chimerism they might thus be one and the same.

Discussion

The monoclonal mouse antibody, W3/13, originally raised against rat thymocytes and present also on granulocytes, was found to label brightly the cells that can permanently repopulate the T and B lymphoid compartments of irradiated hosts. Both fetal liver and young adult bone marrow gave comparable results except that with marrow a W3/13 dim population was found that generated mainly T cells and not B cells. This exception is further considered below. W3/13 antigen, a glycophorin-like molecule (5), is therefore a marker that extends back from the thymocyte and granulocyte lineages to a stem cell, just as Thy-1, a variable region-like molecule (19), extends back from the lymphoid lineage (20–22). The combination of these two markers narrows the surface antigenic specification of primitive stem cells in rats and suggests a method for their enrichment that may improve still further on the 100- to 300-fold purification achieved by Goldschneider et al. (23).

Two circumstances are reported here in which substantial T cell chimerism without B cell chimerism was observed in irradiated reconstituted rats. First, W3/13 dim bone marrow generated predominantly T cells and not B cells. Second, host lymphocytes recovering in rats given 8.5 Gy were mainly T for the first 50 d after irradiation. In both cases, the surplus T chimerism waned later on. We take this to suggest the existence of committed T lymphocyte stem cells. Ideally, a committed stem cell must be shown to have three properties: self-replication, production of mature lymphocytes, and preferential generation of one rather than another lineage, in this case a greater number of T rather than B cell progeny. The first point is not formally demonstrated here, but the very substantial and continued expansion in cell numbers during reconstitution would be hard to achieve without self-replication. The second point is met by confining our analysis to peripheral lymphocytes carrying the surface antigens, Ig and Pta, characteristic of mature cells. The third criterion depends on the interpretation of the percentage chimerisms. Both sets of experiments here were performed as competition experiments, with the test cells racing against a reference standard of 10^7 normal untreated cells. Provided the way in which these competing cells proliferate is unaffected by the test cells the percentage chimerisms reflect the numerical expansion of the test cells. Normal untreated test bone marrow competes in irradiated hosts equally with normal untreated reference marrow (6); furthermore, in the present radiation dose experiments there was no great systematic variation with dose in the numbers or T:B ratio of the chimeric lymphocytes. To this extent, the presumption of undisturbed proliferation of reference marrow is justified. However, there may remain some minor fraction of marrow exhibiting proliferative inequality

between test and reference inocula whose activity is amplified in the present experiments. Such disparity might result for example from the residual alloantigenic differences between the congenic lines, leading to artefactual imbalance in the relative production of T and B cells. However, these differences must be very small if they exist at all, because test skin allografts between the PVG and double-congenic strains are permanently accepted (our unpublished data). Unless the alloantigens used here as markers can be shown to be interfering, we interpret split chimerism as a manifestation of committed stem cells.

The differential radiosensitivity of T and B stem cells was an incidental finding from experiments whose first purpose was to ascertain the minimum dose at which the host component could be regarded as negligible in the chimerism assay. Fig. 4 shows this to be 9.5 Gy for chimeras assayed after day 50; earlier than this, 10.0 Gy should be used. The effect of residual host T cells in the chimerism assay would be to lower its sensitivity by reducing the percentage of cells of donor origin to below the expected value. The higher radiation doses needed for the eradication of host T stem cells than for B stem cells emphasize the need for careful monitoring of residual T cell activity when it is often presumed to be absent, for example in the preparation of B rats or of bone marrow chimeras for H-2-restriction experiments. Unwanted survival of host T cells could explain some of the discrepancies between findings on nude mice and B mice (24). The present results fit the observation that in semiallogeneic chimeras there is a detectable radioresistant host vs. graft component (10).

The experiments reported here do not establish whether the W3/13 dim committed stem cell sorted from marrow suspensions is the same as the more radioresistant component in the radiation dose experiments. At present, the only circumstantial evidence suggesting that they may be is that the surplus T cell chimerism was detected in analysis up to 2 mo after reconstitution, but in both cases, it diminished after that to approach the B cell chimerism, which stayed steady all the time. Thus the long-term proliferative capacity of the committed stem cell was weaker than the stem cell, presumably more primitive, that maintained the permanent chimerism. Further circumstantial evidence could be sought from a study of the subsets of T cells generated from committed stem cells that might distinguish them from progeny of more primitive stem cells. For more formal proof of identity it would be necessary to discover whether in vitro radiosensitivity corresponded to that in vivo and then to expose W3/13-sorted bone marrow to graded doses of radiation. The repopulation assay is not sufficiently sensitive to permit this kind of analysis.

In bone marrow there are a few recirculating T cells that can be removed by thoracic duct drainage (25). In addition, peripheral lymphocytes contain some T but not B repopulating activity when tested in chimeras (S. V. Hunt, unpublished data). This raises the question whether the W3/13 dim restricted T stem cell is pre- or post-thymic. At first sight, the latter seems unlikely, because peripheral T cells carry large amounts of W3/13 and so correspond to W3/13 bright, not dim, cells in marrow. Also, peripheral T cells lack the thymocyte and early erythroid marker W3/15 (4), and in unpublished experiments we have found no surplus T chimerism in recipients of W3/15-negative marrow. However, the conclusion that the committed T stem cell is pre-thymic would be fortified by examining other peripheral T cell antigens such as MRC OX 8, W3/25 (26), and Pta itself. The question would be properly resolved by comparing the T repopulating activity of the marrow of thymus-deprived rats

with that of the thymocyte-reconstituted B rats. Whether the committed T stem cell turns out to be pre- or post-thymic will not alter the conclusion that W3/13 is a prime example of a patchy or "jumping" antigen (27). It switches on, off, and on again during differentiation from the multipotent stem cell through both B and T lineages.

Summary

The existence of stem cells committed to the T lymphoid lineage was deduced from studying how rat T and B stem cells differ in their expression of membrane W3/13 antigen and in their susceptibility in vivo to gamma irradiation. Stem cell activity of rat bone marrow and fetal liver was measured in long-term radiation chimeras using B and T cell alloantigenic surface markers to identify the progeny of donor cells. Monoclonal mouse anti-rat thymocyte antibody W3/13 labeled ~40% of fetal liver cells and 60–70% of young rat bone marrow cells (40% brightly, 25% dimly). Bright, dim, and negative cells were separated on a fluorescence-activated cell sorter. All B and T lymphoid stem cells in fetal liver were W3/13 bright, as were B lymphoid stem cells in bone marrow. W3/13 dim bone marrow had over half the T cell repopulating activity of unseparated marrow but gave virtually no B cell repopulation. In further experiments, the radiosensitivity of endogenous B and T lymphoid stem cells was determined by exposing host rats to between 4.5 and 10 Gy of gamma irradiation before repopulation with genetically marked marrow. The results depended on whether chimerism was assayed before day 50 or after day 100. At early times, a radioresistant T stem cell was indicated, whose activity waned later. Thus committed T stem cells of rats carry moderate amounts of W3/13 antigen and are more radioresistant but less permanently chimeragenic than the stem cells that regenerate B lymphocytes.

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