THE IDENTIFICATION IN ADULT BONE MARROW OF PLURIPOTENT AND RESTRICTED STEM CELLS OF THE MYELOID AND LYMPHOID SYSTEMS*

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The bone marrow of the adult mouse contains stem cells that continuously replenish the myeloid and lymphoid systems (1-4). The best characterized of these stem cells is that which differentiates into myeloid cells, i.e., erythrocytes, granulocytes, and megakaryocytes. This cell can easily be measured by its ability to form macroscopic colonies in the spleens of irradiated mice (5). Using radiation-induced chromosome aberrations, Wu et al. (6) showed conclusively that each spleen colony was a clone and that the stem cells, hemoglobin-synthesizing cells, and granulocytes in individual colonies were derived from a single precursor. Numerous indirect studies have shown a close relationship between these spleen colony-forming units (CFU-S)[1] and the lymphoid system (7-12), but no one has demonstrated directly that CFU-S are the precursors of functional lymphocytes. Because of the existing uncertainty regarding the precise relationships between the differentiation of myeloid cells and of B and T lymphocytes, we have attempted to devise an experimental system in which to analyze the early events in the differentiation of these cells. The work described below had two objectives: (a) To obtain direct evidence for the existence of a class of pluripotent stem cells that can be the progenitors of functional B and T lymphocytes, as well as myeloid cells, and (b) to look for the existence in adult bone marrow of stem cells restricted to differentiation along one pathway. That is, we looked for stem cells that give rise only to myeloid cells or only to lymphoid cells.

Since the lymphoid system regenerates slowly and because functional lymphocytes circulate throughout the body, we used the technique of Wu et al. (6) to generate stem cells bearing unique, radiation-induced chromosome aberrations. By the use of these markers, it was possible to identify cells as members of the same clone regardless of their location in the recipient animal. The data below provide a direct confirmation of the hypothesis that a common stem cell gives rise to both myeloid and lymphoid cells including both B and T lymphocytes. In addition, we have obtained evidence for stem cells restricted in their capacity for differentiation.

Materials and Methods

Mice. Female mice of strain C57BL/6J aged 2-3 mo and female F_1 hybrids produced by crossing mice of strains WB/ReJ-W/+ and C57BL/6J-+/W^v (WBB6F$_1$) were obtained from The

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[1] *Abbreviations used in this paper:* CFU-S, spleen colony-forming units; FCS, fetal calf serum; LPS, lipopolysaccharide; MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin; S_m, stem cell restricted to myeloid differentiation; S_p, pluripotent stem cell; S_t, stem cell restricted to T-cell differentiation.

Jackson Laboratory, Bar Harbor, Maine. This cross yields mice of four different genotypes: +/+, W/+, +/W^v, and W/W^v. Mice of genotype W/W^v have a macrocytic anemia resulting from defective CFU-S (13); the other genotypes have a normal phenotype. The mice were housed two to a cage and given free access to food and water. All the mice were dusted twice with sulfur powder to eliminate ectoparasites (14).

Irradiation of Mice. Mice were irradiated in a ^{137}Cs irradiator at a dose rate of 87 rads/min (15).

Cell Preparations. Marrow cell suspensions were prepared as described previously (16). Large and small debris was removed by sedimentation through fetal calf serum (FCS) as described by Shortman et al. (17).

Culture Conditions. α-Minimal essential medium (18) supplemented with 10% heat-inactivated FCS (lot no. 455664; Flow Laboratories, Inc., Rockville, Md.) and 5×10^{-5} M 2-mercaptoethanol (lot no. 303312, J. T. Baker Chemical Co., Phillipsburg, N. J.) was used as the culture medium. 2-ml volumes of spleen cells (4×10^5 cells/ml) were cultured in trays (model FB16-24TC; Linbro Chemical Co., New Haven, Conn.) with 10 μl phytohemagglutinin (PHA) or with 50 μg lipopolysaccharide (LPS) for 3 days at 37°C. Preliminary experiments showed this time to be optimal for observing proliferating cells.

Reagents

PHA. Wellcome, reagent grade (lot K9453; The Wellcome Research Laboratories, Kent, England). The dry contents of one vial were reconstituted with 5 ml of sterile distilled water.

LPS. Difco LPS W, *Escherichia coli* 055:B5 (lot no. 503830; Difco Laboratories, Detroit, Mich.). Stock solutions were made by suspending LPS at 1 mg/ml in PBS and heating for 1 h in a boiling water bath.

Analytic Procedures

CELL SEPARATION. Velocity sedimentation was used to separate spleen suspensions according to cell size (19). Spleen cells, suspended in 20–30 ml and at a total cell concentration of 8×10^6 cells/ml were allowed to sediment at unit gravity for 4–5 h in a chamber 11.3 cm in diameter. Fractions of 15 ml were collected. Cells sedimenting between 2.5 and 4.0 mm/h were pooled to give a population of lymphocytes which were depleted of myeloid cells known to proliferate in culture (20).

COLONY ASSAY. Bone marrow cells were assayed for hemopoietic function using the spleen colony assay of Till and McCulloch (5). Heavily irradiated (950 rads) mice of normal (+/+) or heterozygous (W/+, +/W^v) genotype were injected intravenously with 10^5 or 10^6 bone marrow cells, and on day 12 the spleen colonies were individually examined for chromosome markers.

CHROMOSOME ANALYSIS. 1.5 h before mice were analyzed for chromosome markers, they were injected intraperitoneally with Colcemid (Grand Island Biological Co., Grand Island, N. Y.) at a concentration of 4 μg/g body weight. Mitogen-containing cultures to be analyzed for markers received 0.06 μg/ml Colcemid 4.5 hours before harvesting. The procedure for preparing mitotic spreads was as described by Edwards et al. (10) with the modification that 0.075 M KCl was used in place of 1% sodium citrate as hypotonic solution. All metaphases were examined under oil immersion at a 1,180 magnification. Chromosome arm-length measurements were made on camera-lucida drawings of well-spread metaphases. Only well-spread metaphases where all the chromosomes were visible were scored. Spreads containing a marker but not a full chromosome complement were not scored. In some cases, the abnormality was 39 or 41 chromosomes; for these samples, only spreads containing exactly 39 or 41 chromosomes were scored as abnormal. We attempted to score 100 metaphases from each mitogen-containing culture. Since the majority of cells in a spleen colony are derived from a single precursor cell (6), the scoring of five metaphases was considered sufficient to characterize the karyotype of the cells in an individual colony.

EXPERIMENTAL DESIGN. Radiation-induced chromosome aberrations were used to trace the progeny of individual hemopoietic stem cells; no marked effect of such aberrations on the proliferation or differentiation of myeloid stem cells has been observed (21). We used a modification of the procedure developed by Wu et al. (6); Fig. 1 gives a diagrammatic outline of our experimental protocol. Normal WBB6F$_1$ mice of +/+ genotype were exposed to 700 rads from a ^{137}Cs irradiator. Shortly after irradiation, bone marrow suspensions were prepared, and 2×10^7 cells were injected into irradiated (200 or 350 rads) female WBB6F$_1$ mice of W/W^v genotype. This irradiated marrow cell concentration is near limiting dilution with respect to its content of CFU-S.
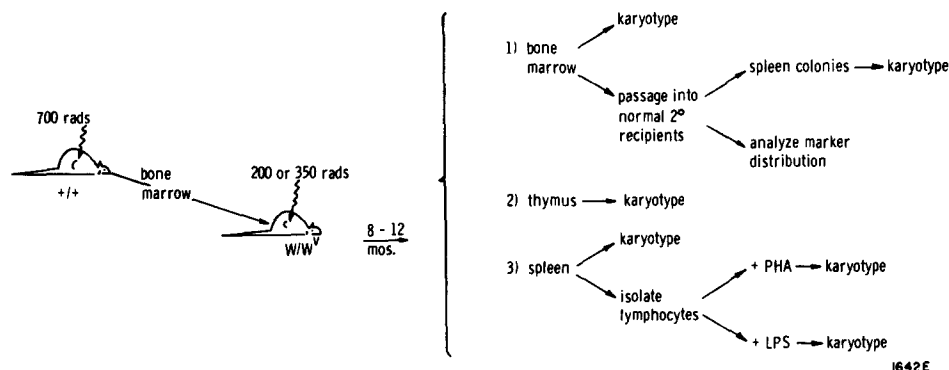
FIG. 1. Experimental protocol. (For details refer to text.)

Irradiated W/W^v mice were used because this host provides a suitable environment in which limited numbers of grafted stem cells have a selective advantage and can express their capacity for differentiation under conditions in which long-term survival of the host is possible (10, 12).

The grafted cells were allowed to grow and differentiate for 8-12 mo before the W/W^v recipients were analyzed. At the time of analysis, the bone marrow, spleen, and thymus were removed and cell suspensions made from each tissue of each mouse. Samples of each suspension were processed for karyotypic analysis. The lymphocytes were isolated from the spleen by velocity sedimentation and placed in culture with PHA or LPS mitogen to specifically stimulate T or B lymphocytes, respectively (22-24). After 3 days, the cells were harvested from the culture and analyzed for abnormal karyotypes. Preliminary experiments showed that the B- and T-cell contamination of PHA and LPS cultures, respectively, was less than 5%. Therefore, only marker frequencies greater than 5% were considered evidence for a marker chromosome in B cells in LPS-stimulated cultures or in T cells in PHA-stimulated cultures. Individual spleen colonies derived from bone marrow of the W/W^v donor were analyzed for abnormal chromosomes. If the same marker chromosome was observed in PHA and LPS blasts and in spleen colonies, we concluded that a chromosome aberration was induced in a stem cell that had the capacity to differentiate into T cells, B cells, and myeloid cells. The presence of an unusual karyotype only in LPS cultures would be evidence for a stem cell restricted in capacity to making only B lymphocytes.

Since it has been suggested that the regulation of stem cells involves a stochastic process (25, 26), it is possible that by chance a pluripotent stem cell capable of making B, T, and myeloid cells would only have produced T-cell progeny at the time it was analyzed in the W/W^v hosts. To determine whether the karyotypic patterns resulted from stochastic variations of pluripotent cells or were the result of the differentiation of stem cells of restricted potency, we also injected large numbers (2-7 × 10⁶) of bone marrow cells from the W/W^v recipient into heavily irradiated (950 rad) $WBB6F_1$ female recipients. If the pattern of marker expression in the W/W^v host represented stochastic fluctuations in the differentiation of pluripotent stem cells, different patterns would be expected in the secondary recipients. If, however, an unusual pattern in the W/W^v host resulted from the presence of a restricted stem cell, then the same pattern should be observed in the secondary recipient. Thus, to be classified as a restricted stem cell, a marker had not only to be observed in just one or two of the three pathways tested, but it also had to breed true; i.e., bone marrow from the W/W^v host had to generate the same marker distribution in a secondary recipient.

Results

Analysis of Recipients. A total of 28 primary W/W^v recipients were analyzed for the presence of abnormal markers according to the experimental protocol described above. A high frequency of marked cells was found in 10 mice. These results have been categorized into three patterns of hemopoietic regeneration and are described below. In one mouse, a chromosome marker was observed in bone marrow but was not found in either the lymphoid or myeloid cells exam-

TABLE I

Distribution of Chromosome Markers in T, B, and Myeloid Cells

Mouse*	Type of marker	No. marked cells/total metaphases‡					Spleen§ colonies
		BM	SPL	THY	PHA blasts	LPS blasts	
WV3 (8¹/₂)	39 Chromosomes	12/15 (80%)	NMS‖	NMS	9/10 (90%)	10/17 (59%)	7/7 (100%)
WV5 (8³/₄)	Minute 4:1	4/11 (36%)	NMS	NMS	61/100 (61%)	63/100 (63%)	12/14 (86%)
WV31¶ (3³/₄)	Minute	7/25 (28%)	3/31 (10%)	0/1 (0%)	9/100 (9%)	10/37 (27%)	10/13 (77%)
2°WV31-1** (3¹/₄)	Minute	13/25 (52%)	0/14 (9%)	NMS	39/100 (39%)	48/100 /48%)	10/10 (100%)
WV32 (3³/₄)	Minute 4:1	10/25 (40%)	NMS	NMS	13/100 (13%)	13/100 (13%)	7/12 (58%)

* The time interval in months between marrow injection and sacrifice is shown in parentheses.

‡ Parentheses contain the percentage of marker karyotypes.

§ Fraction of spleen colonies containing the chromosome marker when bone marrow from the sacrificed animal was assayed for CFU-S by transplantation.

‖ NMS, no scorable metaphase spreads were observed.

¶ Typical karyotypes are shown in Fig. 2.

** Secondary recipient of marrow from mouse WV31.

ined; this marker is not included in the following tables. This marker was the only one eliminated from the analysis. One mouse (WV5) contained two different markers, and data from this mouse appear in two different tables.

Evidence for a Pluripotent Stem Cell. The analysis of karyotypes in the five mice shown in Table I demonstrated markers in all three pathways tested. Thus, in the case of mouse WV5, a minute marker (of chromosome arm-length ratio of 4:1) was observed in 36% of the bone marrow metaphases and in 61 and 63% of the cells in PHA- and LPS-stimulated cultures, respectively. The marker was also present in 86% (12/14) of the colonies generated from the bone marrow.

A similar distribution of marked cells was observed in three other mice. For one of these mice (WV31) a test of marker distribution in a secondary recipient was made. Mouse WV31 had a minute chromosome marker in 28% of the bone marrow metaphases and in 10% of the spleen spreads scored. The one metaphase seen in the thymus preparation was not marked. The minute marker was present in 9% of the PHA-derived blasts and 27% of the LPS-derived blasts. As well, 77% of the colonies generated from bone marrow cells had the marker. The karyotypes observed in these samples are shown in Fig. 2. Passage of the marked bone marrow into a lethally irradiated (950 rads) secondary recipient, 2°WV31-1 (see Table I), yielded the following results. 52% of the bone marrow metaphases were marked and 39 and 48% of the PHA- and LPS-derived blasts, respectively, contained the marker. All 10 of the spleen colonies scored were also marked. Therefore, the distribution pattern of the marker in secondary recipients was the same as in primary recipients. These observations provide direct cytogenetic evidence for the existence of a pluripotent stem cell capable of differentiating into myeloid and lymphoid progeny, including both B and T lymphocytes.

Evidence for a Restricted Hemopoietic Stem Cell. A second pattern of regeneration, shown in Table II, was observed in five mice. In these mice, a chromosome marker was found only in myeloid cells. Mouse WV5, described as having a marked pluripotent stem cell, also contained bone marrow cells bearing a second characteristic marker: a long and minute chromosome of measured arm-length ratio of 9:1 (shown in Fig. 3a). This marker was observed in 45% of the marrow metaphases. Unlike the distribution of the first marker (Table I)
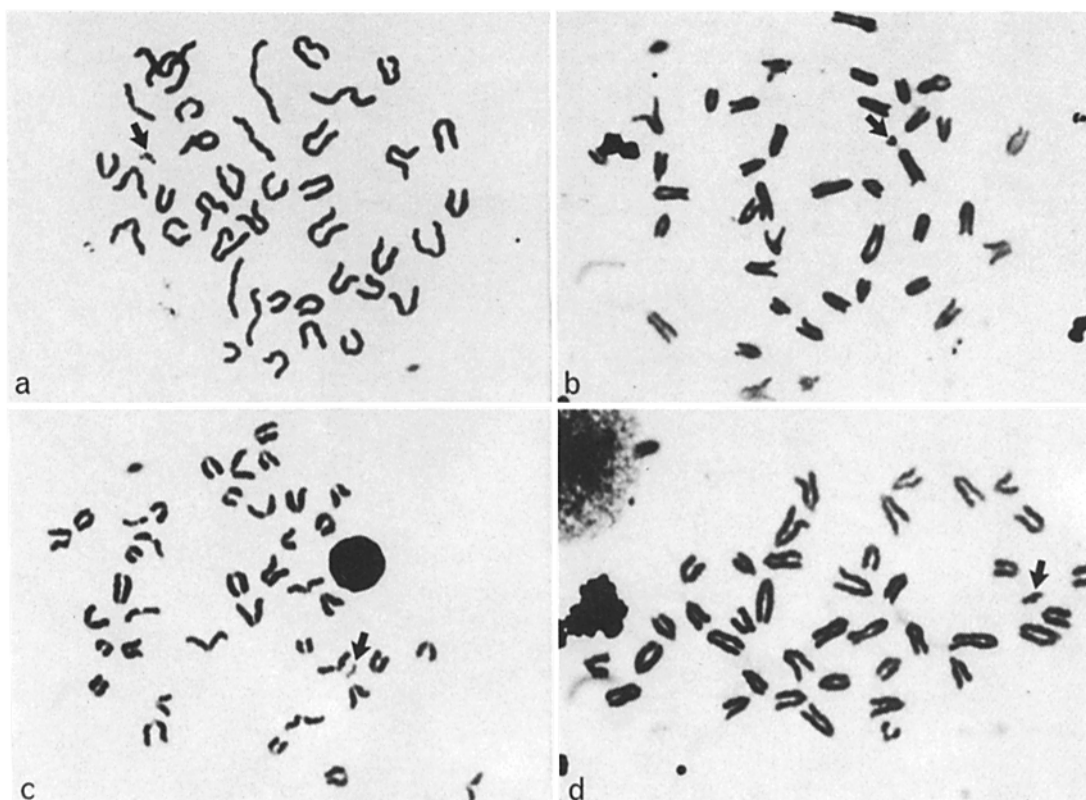
FIG. 2. Karyotypes of marked cells from W/W^v mouse WV31 (Table I). Metaphases shown were obtained from (a) marrow; (b) PHA cultures; (c) LPS cultures; and (d) spleen colonies. The arrows indicate the minute marker chromosome.

TABLE II

Distribution of Chromosome Markers in Myeloid Cells

Mouse*	Type of marker	No. marked cells/total metaphases‡					Spleen§ colonies
		BM	SPL	THY	PHA blasts	LPS blasts	
WV5‖ (8³/₄)	Long and minute 9:1	5/11 (45%)	NMS¶	NMS	0/100 (0%)	0/100 (0%)	2/14 (14%)
WV23‖ (10)	Metacentric	21/40 (53%)	5/9 (56%)	0/2 (0%)	0/100 (0%)	0/100 (0%)	9/12 (75%)
WV11‖ (10)	2 Metacentrics and minute	29/29** (100%)	5/5 (100%)	NMS	0/100 (0%)	0/100 (0%)	4/4 (100%)
2°WV11-1‡‡ (2¹/₂)	2 Metacentrics and minute	94/100 (94%)	7/9 (78%)	0/6 (0%)	0/100 (0%)	0/100 (0%)	8/9 (89%)
2°WV11-2‡‡ (2¹/₂)	2 Metacentrics and minute	23/25 (92%)	0/2 (0%)	0/2 (0%)	0/100 (0%)	0/100 (0%)	13/13 (100%)

* The time interval in months between marrow injection and sacrifice is shown in parentheses.

‡ Parentheses contain the percentage of marker karyotypes.

§ Fraction of spleen colonies containing the chromosome marker when bone marrow from the sacrificed animal was assayed for CFU-S by transplantation.

‖ Photographs of these karyotypes are shown in Fig. 3.

¶ NMS, no scorable metaphase spreads were observed.

** 14 more metaphase spreads looked normal but the number of chromosomes could not be clearly counted.

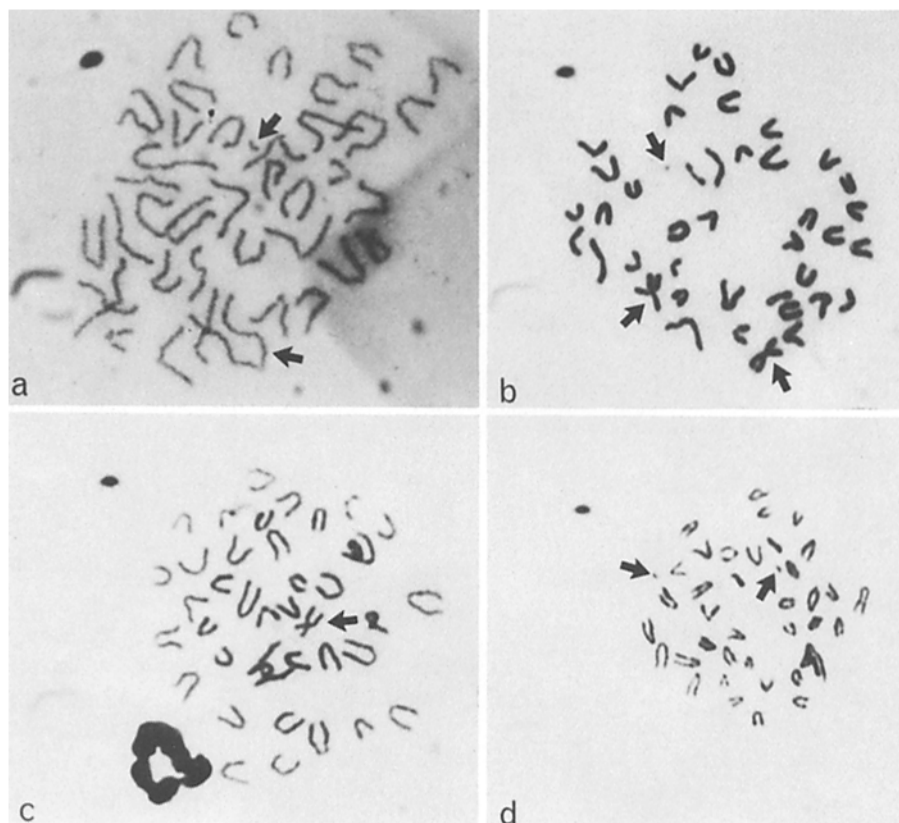‡‡ Secondary recipients of marrow from mouse WV11.

FIG. 3.   Karyotypes of marked cells from various W/Wᵛ mice. Arrows indicate marker chromosomes. (a) mouse WV5: long and minute (9:1) marker (Table II); (b) mouse WV11: 2 metacentrics and minute marker (Table II); (c) mouse WV23: metacentric marker (Table II); and (d) mouse WV16: 2 minute markers (Table III).

neither the PHA nor the LPS blasts exhibited the second chromosome marker (Table II). However, 2 of the 14 colonies examined were composed only of cells bearing this marker.

The analysis of mouse WV11 deserves special comment because of the unusual marker observed. An abnormal karyotype with two metacentric and one minute chromosome was observed in all of the bone marrow metaphases scored. This karyotype is shown in Fig. 3b. Cells bearing this marker had a total chromosome number of 41. None of the 100 metaphases scored in either the PHA or LPS cultures were marked. The unique marker was observed in all the cells of the four spleen colonies analyzed.

The distribution of the marker in the secondary recipients is also shown in Table II. In mouse 2°WV11-1, the double metacentric and minute marker was observed in 94% of the marrow metaphases and in 78% of the spleen metaphases. The six thymus spreads observed were normal. No marked metaphases were observed in the PHA- or LPS-stimulated cell cultures. All of the lymphoid cells analyzed were derived from karyotypically normal cells. However, 89% of the colonies were composed of cells bearing only the characteristic marker. A

TABLE III

Distribution of Chromosome Markers in T Lymphoid Cells

Mouse*	Type of marker	No. marked cells/total metaphases‡					Spleen§ colo-nies
		BM	SPL	THY	PHA blasts	LPS blasts	
WV4 (8½)	39 chromosomes	1/20 (5)	NMS‖	NMS	9/65 (14%)	0/65 (0%)	0/9 (0%)
WV16¶ (11½)	2 minutes	0/63 (0%)	NMS	NMS	42/100 (42%)	3/60 (5%)	0/10 (0%)
2°WV16-1** (2¼)	2 minutes	0/50 (0%)	0/1 (0%)	0/18 (0%)	9/100 (9%)	1/100 (1%)	0/4 (0%)
WV24 (10)	41 chromosomes	3/25 (12%)	2/7 (29%)	17/25 (68%)	2/100 (2%)	0/100 (0%)	0/12 (0%)

* The time interval in months between marrow injection and sacrifice is shown in parentheses.
‡ Parentheses contain the percentage of marker karyotypes.
§ Fraction of spleen colonies containing the chromosome marker when bone marrow from the sacrificed animal was assayed for CFU-S by transplantation.
‖ NMS, no scorable metaphase spreads were observed.
¶ Karyotype shown in Fig. 3d.
** Secondary recipient of marrow from WV16.

similarly restricted pattern of marker distribution was found in another secondary recipient (2°WV11-2) grafted with the same marrow. The distribution of markers in both secondary recipients was identical to the original donor. These data indicate the existence of a class of stem cells in the bone marrow which are restricted to differentiate and repopulate only the myeloid system.

Evidence for Restricted T-Cell Stem Cells. A third pattern of regeneration was observed in three mice, where chromosome markers were identified in T-cell cultures but not in B-cell cultures or spleen colonies. These results are shown in Table III. A double minute marker was identified in mouse WV16 (shown in Fig. 3d). The marker was not observed in bone marrow metaphases but was seen in 42% of the PHA-cultured blasts. Only 5% of the LPS-cultured blasts were marked. The spontaneous proliferation of marked T-cell contaminants in the LPS culture could account for this low frequency. None of the 10 colonies analyzed were derived from the marked stem cell. Passage of the marker into a secondary recipient, 2°WV16-1, resulted in a similar marker distribution pattern but with a lower frequency of markers in cells stimulated by PHA.

Another somewhat difficult to classify marker was observed in mouse WV24 (last line, Table III). Here, a chromosome number of 41 was observed. The marrow had 12% of the metaphases marked while 29% of the spleen metaphases were marked. A large proportion, 68%, of the thymus cells had the marker, but surprisingly only 2% of the PHA cultures contained the marker. None of the metaphases from the LPS cultures or in spleen colonies contained the marker.

Discussion

Several investigators have demonstrated a close relationship between the lymphoid and myeloid systems. However, all of the previous studies failed to show conclusively that the abnormal karyotype was in functional lymphocytes. Wu et al. (12), for example, the originators of the technique we modified for the present studies, identified cells with unique markers in spleen colonies, thymus, and lymph nodes, but they did not isolate dividing lymphocytes to confirm the presence of the marker in functional cells of the immune system. Trentin and Fahlberg (8) and Yung et al. (9) injected cells from individual spleen colonies into irradiated recipients and observed reconstitution of both the myeloid and

the lymphoid response. Both of these studies involved the T6 chromosome marker which is not suitable for studying clones. All stem cells from a donor will have the T6 marker so that it is impossible to determine how many stem cells an individual recipient has. Grafting single, T6-marked spleen colonies does not overcome this problem because single colonies are easily contaminated with other cells (6). Edwards et al. (10) showed that some antigen-binding cells could be derived from stem cells that also produced myeloid progeny, but they did not prove that the antigen-binding cells were B cells. Nowell et al. (11), using Wu's technique to induce chromosome markers in hemopoietic stem cells (6, 12), showed that T cells are probably derived from hemopoietic stem cells. The mixed lymphocyte reaction (MLR) was used to identify a population of immunocompetent T lymphocytes (27). They demonstrated that a small proportion of cells stimulated in MLR had the same marker also found in CFU-S. However, the frequency of uniquely marked cells in the MLR was very low (<7%) and could have been myeloid cells stimulated by factors released during the MLR (28). The data described in this paper show directly for the first time that adult bone marrow tissue contains a population of pluripotent stem cells that can give rise to both myeloid and lymphoid progeny, including both B and T lymphocytes.

Perhaps the most interesting finding from this work was the identification of a class of restricted stem cells. The most convincing data are for the existence of a stem cell restricted to myeloid differentiation. Three independently induced markers (WV5, WV11, and WV23) occurred only in the bone marrow and in spleen colonies derived from bone marrow. No cells with the abnormal karyotype were observed in PHA or LPS blasts. Less convincing is our data on stem cells restricted to differentiate into T lymphocytes. In two cases (WV4 and WV16), abnormal karyotypes were observed in high frequency only on PHA blasts. Insignificant numbers of marked cells were observed in LPS blasts, and there were no spleen colonies with the abnormal karyotype. Recently, Kadish and Basch have described an assay for prethymic stem cells in bone marrow (29); it is possible that our two examples are instances where chromosome translocations were induced in this class of stem cell.

Before accepting the existence of restricted stem cells it is necessary to rule out several artifacts which could account for the data shown in Tables II and III. First, it is necessary to rule out leukemia as a trivial explanation for the restriction. It is known that radiation can induce leukemia (30) and that some leukemic cells can form spleen colonies (31). In our experiments, however, several factors argue against the restricted stem cells having leukemic properties. There were no abnormal cells in the peripheral blood of animals whose myeloid system had been repopulated by cells with the unique karyotype. The differential and total blood cell counts were normal and all cells exhibited normal morphology. The long survival of both primary and secondary recipients suggests that the mice did not have a leukemia. Spleen colonies derived from bone marrow with an abnormal karyotype had normal morphological heterogeneity. The colonies did not contain cells having the homogeneous blast-like appearance of cells in colonies derived from known leukemic stem cells.

Second, it is necessary to rule out stochastic variation as a way of generating an apparently restricted stem cell population. Stochastic models for the differentiation of stem cells predict that by chance a single stem cell may give rise to

large numbers of progeny along one pathway (25, 26). A cursory examination of the progeny of such a stem cell could lead one to conclude mistakenly that the stem cell was restricted. In an attempt to rule out stochastic mechanisms as an explanation for our observations, bone marrow from the primary W/W^v recipients was injected into heavily irradiated, normal, syngeneic recipients and allowed to grow for 2-3 mo. If the restricted pattern in the primary W/W^v recipient resulted from a stochastic process, a different pattern of differentiation would be anticipated in the secondary recipient, since stochastic processes would be unlikely to give exactly the same distribution of differentiated progeny. However, in every case that we examined, the pattern of differentiation in the second recipient was identical to that in the first. These observations provide strong, albeit indirect, evidence against stochastic events influencing the patterns of differentiation described in this paper.

Third, one possible explanation for the observation of a chromosome marker restricted to the lymphoid system is that the aberration was generated in a pluripotent stem cell derived from the original W/W^v recipient. The genetic defect of W/W^v would result in this marker not being found in the myeloid series. This possibility is unlikely for two reasons. First, the radiation dose to the W/W^v recipients (200-350 rads) is unlikely to induce chromosome aberrations in a high frequency. Second, if the marker had been generated in a host pluripotent stem cell, the marker should have been observed in both B and T lymphocytes and not just in T lymphocytes. For these reasons, we favor the interpretation that adult bone marrow contains a class of stem cells programmed to differentiate only into T lymphocytes.

Fourth, it is necessary to rule out the possibility that the restriction, although real, is caused by the irradiation of the stem cell. That is, adult bone marrow may contain only one class of stem cell, a stem cell which is pluripotent and gives rise to both myeloid and lymphoid progeny. However, after irradiation to induce abnormal karyotypes, some of the stem cells may lose their differentiative potential and become restricted. On the basis of the experiments described above, we cannot rule out the possibility that restricted stem cells are generated by this mechanism. The only indirect evidence we have against such a mechanism is the observation that chromosome abnormalities are found in the pluripotent as well as the restricted classes of stem cells. Thus, the mere presence of abnormalities is not the basis for restricted differentiation.

On the basis of our data, we propose that lymphocytes, myeloid cells, and the restricted stem cells are related in the way shown in Fig. 4. In this model, we have assumed that the restricted stem cells are derived from the pluripotent stem cell (S_p). According to this model, B lymphocytes are derived directly from S_p. Certainly, many intermediate stages exist between S_p and mature B lymphocytes (e.g., reference 32). However, apparently none of the intermediate stages have sufficient self-renewal potential to have stem cell properties. The T-restricted stem cell (S_t) is shown in the model as a prethymic stem cell. We have no evidence for this assumption; it is based on the observations of Kadish and Basch, who found a prethymic stem cell in bone marrow (29).

It is perhaps surprising that we did not detect a lymphoid stem cell. For various reasons, one would have expected a lymphoid stem cell which had the capacity to give rise to either B or T lymphocytes. For example, in children with

Pluripotent   Restricted   Functional
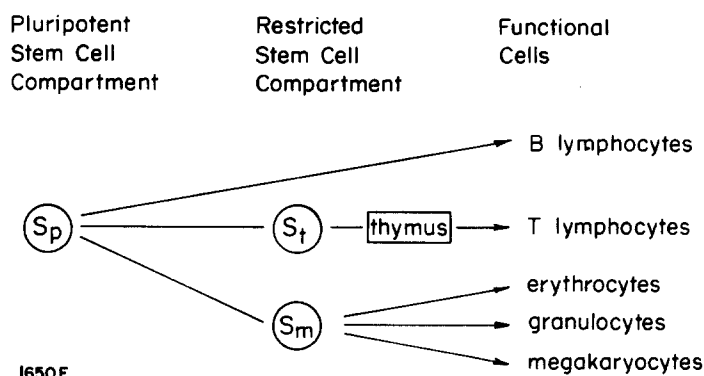Stem Cell   Stem Cell   Cells
Compartment   Compartment

FIG. 4. Model for the relationship between stem cells of the myeloid and lymphoid systems.

severe combined immune deficiency disease, the myeloid system is normal, but there is an absence of both B and T lymphocytes (for review see reference 33). Since these children can be cured by a graft of normal bone marrow cells, it has been proposed that they have a defect in their lymphoid stem cells (33). However, it is important to emphasize that negative results in studies of the type reported here may not be conclusive. Thus, lymphoid stem cells (or even B-restricted stem cells) may exist in much lower frequencies than the other stem cells so that they are both more rarely marked and more easily diluted out. Alternatively, they may have a greater radiation sensitivity than other stem cell classes and thus be selectively depleted by the doses of irradiation used to induce chromosome translocations.

Another problem which our data fail to resolve is the question of the precise relationship between CFU-S and the various stem cells defined by the marker distributions. Presumably the myeloid stem cell (S_m) forms spleen colonies. We have no information on the colony-forming potential of S_p. S_p should form colonies if S_m are among its progeny. In this case, one would predict heterogeneity among spleen colonies derived from bone marrow. Colonies derived from S_p may contain B- and T-lymphocyte precursors, while S_m-derived colonies would have only myeloid cells and their precursors. However, testing this prediction will require more sensitive assays for lymphocyte precursors than are presently available.

Finally, one implication of the above model is that regulatory signals probably influence the direction of differentiation of pluripotent stem cells. If B cells are derived from S_p, then in mice such as the primary and secondary recipients of WV11, the B cells and T cells were derived from unmarked S_p while the majority of the myeloid cells developed from a marked S_m. Since these mice contained both unmarked S_p and marked S_m, it is surprising that so few unmarked myeloid cells were observed. One might have expected a restricted clone to be diluted out by new unmarked S_m derived from unmarked S_p. One interpretation of the long-term maintenance in the mice of a clone of restricted stem cells is that the pathway of differentiation of S_p is regulated according to the demand of the animal. If all of the requirement for myeloid cells is being met by restricted S_m, the pluripotent S_p stem cells would produce only lymphoid

progeny. While regulation at this level of differentiation is difficult to reconcile with stochastic models for stem cell differentiation (34), our data do not rule out such models. However, the chromosome data do place restrictions on the processes where stochastic events may be involved in regulation.

Summary

The precise relationship between the stem cells for the lymphoid system and those for the blood-forming system is unclear. While it is generally assumed that the hemopoietic stem cell, the spleen colony-forming unit (CFU-S), is also the stem cell for the lymphoid system, there is little evidence for this hypothesis. To investigate the stem cells in these two systems, we irradiated bone marrow cells to induce unique chromosome aberrations in the stem cell population and injected them at limiting dilution into stem cell-deficient recipients. Several months (between 3 and 11) were allowed for the injected cells to repopulate the hemopoietic system. At that time, the bone marrow, spleen, and thymus were examined for a high frequency of cells having the same unique chromosome aberration. The presence of such markers shows that the marker was induced in a cell with extensive proliferative capacity, i.e., a stem cell. In addition, the splenic lymphocytes were stimulated with phytohemagglutinin (PHA) or lipopolysaccharide (LPS) to search for unique chromosomes in dividing T and B cells, respectively. Finally, bone marrow cells were injected into secondary irradiated recipients to determine if the marker occurred in CFU-S and to determine whether or not the same tissue distributions of marked cells could be propogated by bone marrow cells in a second recipient. After examination of 28 primary recipients, it was possible to identify three unique patterns of stem cell regeneration. In one set of mice, a unique chromosome marker was observed in CFU-S and in PHA- and LPS-stimulated cultures. These mice provide direct evidence for a pluripotent stem cell in bone marrow. In addition, two restricted stem cells were identified by this analysis. In three recipients, abnormal karyotypes were found only in myeloid cells and not in B and T lymphocytes. These mice presumably received a marked stem cell restricted to differentiate only into myeloid progeny. In three other recipients, chromosome aberrations were found only in PHA-stimulated cells; CFU-S and cells from LPS cultures did not have cells with the unique chromosome. This pattern suggests that bone marrow contains cells comitted to differentiation only into T lymphocytes. For each of the three types of stem cells, secondary recipients had the same cellular distribution of marked cells as the primary recipients. This observation provides further evidence that unique markers can be induced in both pluripotent and restricted stem cells.

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