Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7414–7418, November 1985
Immunology

Enrichment of hematopoietic precursor cells and cloning of multipotential B-lymphocyte precursors

(B-lymphocyte differentiation/growth factors/cell surface antigens/hematopoietic differentiation)

JOHN P. MCKEARN*, JAMES MCCUBREY, AND BARBARA FAGG

Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005, Basel, Switzerland

Communicated by Niels K. Jerne, July 16, 1985

ABSTRACT **A simple one-step isolation technique significantly enriched mouse fetal liver cells that respond to interleukin 3 (IL-3), a multilineage hematopoietic growth factor. The fetal liver cell subpopulation isolated with monoclonal antibody AA4 contained 50- to 100-fold higher frequencies of multipotential (CFU-mix) or restricted (CFU-G/M, BFU-E) erythroid/myeloid precursors as well as precursors that differentiate to become mature B lymphocytes [CFU-mix = erythroid and myeloid colony-forming unit(s); CFU-G/M = CFU-granulocyte/macrophage; BFU-E = burst-forming unit-erythroid]. The B-lymphocyte precursors could be cloned in single-cell cultures when IL-3-containing supernatants were present. Growth of these clones was supported by purified IL-3 but not by purified IL-2. Stable growth has been maintained for >6 mo in the presence of IL-3. Such clones express on their cell surface low amounts of class I major histocompatibility complex antigens and high amounts of AA4, GF1, and leukocyte common glycoprotein 200 antigens. They lack detectable rearrangements of their Ig-encoding genes [joining region heavy and light (κ, λ) chain genes], even after subcloning, but maintain their capacity to differentiate to mature B lymphocytes committed to multiple Ig specificities.**

The mechanisms that enable multipotential stem cells to generate developmentally restricted progeny of each hematopoietic lineage remain relatively obscure. Despite their low frequencies among hematopoietic sites, multipotential and committed precursors of the myeloid and erythroid lineages can be quantitated by their ability to form colonies of differentiated cells (reviewed in ref. 1). Although similar assays exist for the study of mature lymphocytes and their committed precursors (2), virtually nothing is known about growth/differentiation requirements or frequencies of multipotential lymphoid precursor cells. Likewise, multipotential stem cells, which generate all lineages of blood-forming cells, remain largely uncharacterized. Simple selection techniques that enrich for hematopoietic precursor cells would greatly facilitate studies of their developmental program and its regulation.

One factor whose regulatory properties have been extensively characterized is interleukin 3 (IL-3) (1, 3–5). IL-3 serves as a growth/differentiation factor for multipotential erythroid/myeloid precursors [colony-forming unit (CFU)-mix] and the committed precursors of erythroid/myeloid lineages [burst-forming unit-erythroid (BFU-E) and CFU-granulocyte/macrophage (CFU-G/M)] with the exception of late-stage erythroid cells (CFU-E), which require erythropoietin (4), and late-stage megakaryocyte precursors (4, 6). Controversial evidence suggests that IL-3 may play a role also in lymphocyte development (7, 8). This issue will likely be clarified when functional analysis accompanies clonally restricted isolation procedures.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Animals. Fetal BALB/c mice, day 13 of gestation, were obtained from our breeding colony as were adult C.AL 20 mice. Adult BALB/c mice were obtained from The Jackson Laboratory.

Cell Lines, Culture Medium, and Growth Factors. FDC-P2 (9), WEHI-3B(D$^-$) (10), and CTL-L (11) cell lines were cultured in Iscove's modified Dulbecco's medium (IMDM) under an atmosphere of 5% CO_2 in air. WEHI-3 supernatant, which contains IL-3 and additional factors (1, 3–10), was stored at −70°C following centrifugation at 500 × *g* for 15 min and passage through a 0.22-μm filter. This supernatant, added at 10% (vol/vol) to IMDM containing 5% fetal calf serum, will be referred to as WEHI-3-conditioned medium (CM). Purified IL-3 and IL-2 (12, 13) were provided by J. Watson (University of Auckland).

Monoclonal Antibodies (mAbs) and Quantitation of Cell Surface Determinants. Expression of cell surface determinants recognized by various mAbs was quantitated in a fluorescent antibody binding assay using rat anti-mouse mAbs as described (14, 15). mAbs M1/89.18 (16) and D468HLK (17), which recognize leukocyte common glycoprotein (LGP) 200 and rat major histocompatibility complex (MHC) antigens, respectively, were also used.

Cell-Separation Techniques. Subpopulations of fetal liver cells were obtained by "panning" on mAb-coated polystyrene dishes (14). Those cells removed after incubation and in the subsequent two rinses were pooled to produce the nonadherent (depleted) population. The adherent (selected) population was composed of cells that remained bound after 8–10 rinses and were recovered by repeatedly pipetting 5–10 ml of medium to gently resuspend cells.

Cellular-Proliferation Assays. Short-term proliferation was measured by resuspending 10^3 or 2.5×10^3 washed viable cells in 0.2-ml cultures. After 44 hr, triplicate cultures were pulsed with 0.5 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine (specific activity, 85 MBq) and harvested 4 hr later. Results are expressed as mean cpm (± SEM) of [^{3}H]thymidine uptake per culture.

Clonal Assays for Erythroid and Myeloid Precursor Cells. Fetal liver cells were grown in semisolid culture medium as

Abbreviations: IL-3, interleukin 3; mAb, monoclonal antibody; CFU-mix, colony forming unit-mix (erythroid and myeloid); CFU-G/M, CFU-granulocyte/macrophage; BFU-E, burst-forming unit-erythroid; CFU-E, CFU-erythroid; MHC, major histocompatibility complex; LGP, leukocyte common glycoprotein; IMDM, Iscove's modified Dulbecco's medium; CM, conditioned medium; SRBC, sheep erythrocytes; J, joining region; V, variable region.

*To whom reprint requests should be addressed at present address: E. I. duPont de Nemours and Co., Inc., Central Research and Development Department, Glenolden Laboratory, Glenolden, PA 19036.

Table 1. Enrichment of IL-3-responsive and WEHI-3-CM-responsive fetal liver cells

Cells in culture	[^{3}H]Thymidine uptake per culture			
			With IL-3	
	No factor added	With WEHI-3-CM	50 units/ml	100 units/ml
Unfractionated	315	600	765	255
	(2.50 ± 0.19)	(2.78 ± 0.14)	(2.88 ± 0.19)	(2.41 ± 0.34)
AA4-selected	470	7905	3635	4005
	(2.67 ± 0.13)	(3.39 ± 0.13)	(3.56 ± 0.19)	(3.60 ± 0.11)
AA4-depleted	230	375	450	345
	(2.36 ± 0.23)	(2.57 ± 0.15)	(2.65 ± 0.15)	(2.53 ± 0.36)
GF1-selected	325	310	NT	NT
Fc control (mAb D468HLK)	665	905	910	565
	(2.82 ± 0.01)	(2.96 ± 0.07)	(2.96 ± 0.02)	

Day 13 fetal liver cells (10^3) from BALB/c mice were cultured in IMDM containing 5% fetal calf serum, WEHI-3-CM, or purified IL-3. mAbs for AA4 and GF1 typically selected ≈0.7% of total cells, while mAb D468HLK, a negative control, routinely selected <0.1% of the total cells fractionated. Data with statistical analysis (mean ± SEM) were obtained from three or more separate experiments. Data without statistics were obtained in single experiments. NT = not tested.

detailed elsewhere (18). Cells were plated at two or more concentrations within the range of 10^3 to 5×10^4 per ml for mAb AA4-selected cells and 5×10^3 to 2×10^5 per ml for unfractionated and AA4-depleted populations. Mouse erythropoietin (0.2 unit/ml) and WEHI-3-CM were added in amounts sufficient to promote maximum colony formation. Small erythroid colonies (≥60 cells), derived from CFU-E precursors, were enumerated on day 2 of culture. After 7–10 days, the plates were examined for large colonies (≥50 cells) comprising (*i*) erythroid cells alone (BFU-E precursors) or mixed with any other differentiated cell types (CFU-mix precursors) and (*ii*) all other colonies (CFU-G/M precursors). The majority of these were comprised of neutrophils and macrophages.

Cloning in Liquid Cultures. Graded numbers of 13-day fetal liver cells were grown in 0.2 ml of WEHI-3-CM. Wells containing viable colonies (minimum size, >50 cells) were scored after 14 days, and the frequency of colony-forming precursor cells was determined (19). In some experiments, colonies were expanded and recloned by single-cell micromanipulation. Cloning efficiencies ranged from 70% to 95%.

B-Cell Maturation Assay. Ig-secreting cells were measured in 1-ml cultures containing 10^4 cloned BALB/c fetal liver cells, 5×10^5 accessory cells from C.AL 20 bone marrow, 50 μg of agar extract mitogen (20), and 50 μg of lipopolysaccharide (*Salmonella typhosa* W 0901, Difco). C.AL 20 bone marrow accessory cells were prepared by removing B cells and their precursors (14). IgM-secreting cells from BALB/c and C.AL 20 were distinguished in an allotype-specific reverse plaque assay (14). IgM-secreting cells from both strains lysed sheep erythrocytes (SRBC) coupled with mAb 33-60 [rat anti-mouse IgM of all allotypes (21, 22)].

DNA Preparation and Southern Blot Analysis of Ig Genes. High molecular weight DNA from cell lines and adult BALB/c liver was prepared as described (23). DNA was digested to completion with *Eco*RI or *Bam*HI. Ten micrograms of restriction endonuclease-digested DNA was electrophoresed through 1% agarose gels, transferred to nitrocellulose paper (24), and hybridized with the following Ig-specific probes: joining region (J) heavy chain gene (J_H)-specific probe pJ_{11} (25), *c* κ light chain (c_κ) exon probe $p\kappa_1$ (26), or variable region (V) λ light chain gene (V_λ)-specific probe $V(J)\lambda_1$ (27).

RESULTS AND DISCUSSION

Enrichment of IL-3-Responsive Cells. Day 13 fetal liver cell subpopulations were selected by panning with various mAbs and tested for their ability to respond to IL-3. Results from short-term assays measuring cell proliferation indicated that only cell populations binding to mAb AA4 were enriched for IL-3 and WEHI-3-CM responsive cells (Table 1). This enrichment was not dependent on the Fc portion of rat IgG2b, the isotype of mAb AA4, since other IgG2b mAbs (GF1, 14.8, and D468HLK) failed to enrich responsive cells (Table 1 and unpublished results).

Clonal Frequencies of Hematopoietic Precursor Cells. The frequency of total erythroid/myeloid colonies found at 7 days in methylcellulose cultures was increased roughly 50-fold in the AA4-selected population (Table 2). None of the other mAbs (GF1, 14.8, or D468HLK) modified the precursor frequency relative to unfractionated fetal liver (unpublished results). The composition of colonies derived from AA4-selected cells indicated that this fraction was enriched in early precursor cells of all lineages (CFU-G/M, CFU-mix, and BFU-E). However, the frequency of late erythroid

Table 2. Clonal frequencies of erythroid/myeloid precursors among fetal liver cell subpopulations

Fetal liver cells analyzed	Number of colonies per 10^4 cells plated with and without addition of growth factors					
	Total colonies, Day 7 (CFU-G/M, BFU-E, CFU-mix)		Erythroid colonies			
			Day 2 (CFU-E)		Day 7 (CFU-mix, BFU-E)	
	No addition	With WEHI-3-CM/EP	No addition	With WEHI-3-CM/EP	No addition	With WEHI-3-CM/EP
Unfractionated	0 (3)	5 ± 1 (5)	16 ± 2 (4)	126 ± 25 (3)	0 (3)	1 ± 0 (3)
AA4-selected	0 (2)	270 ± 51 (4)	0 (3)	2 ± 2 (3)	0 (2)	73 ± 5 (3)
AA4-depleted	0 (2)	2 ± 1 (5)	2 (2)	194 (2)	0 (2)	1 (2)

Day 13 BALB/c fetal liver cells were fractionated by using mAb AA4 and methyl cellulose cultures prepared and evaluated as described. Values are the mean ± SEM of the number of separate experiments indicated in parentheses. EP, erythropoietin.

precursors (CFU-E) was dramatically reduced in the AA4-selected population. These data indicate that mAb AA4 recognized relatively immature erythroid/myeloid precursors. To confirm this, we examined erythroid precursors within selected and unfractionated populations in more detail. Table 2 shows the AA4-selected population was depleted of the relatively mature CFU-E but was enriched in BFU-E and CFU-mix. Inspection of the cultures at intermediate times confirmed that hemoglobinized colonies did not appear until at least days 4–5 of culture (unpublished data). These results indicate that mAb AA4 recognizes most types of immature erythroid/myeloid precursors (CFU-G/M, BFU-E, and CFU-mix).

Liquid cultures were initiated with cell numbers ranging from an average of 0.5 cells up to 256 cells per well for mAb AA4-selected fetal liver cells and 100 cells up to 3200 cells per well for unfractionated fetal liver cells. These cultures were examined after 14 days. AA4 selection again substantially increased the frequency of cells that proliferate in WEHI-3-CM (Fig. 1). Statistical analysis of colony formation in clonal liquid cultures revealed that cells selected by mAb AA4 were enriched for colony-forming precursors by approximately 100-fold, since 1 in 23 AA4-selected cells could proliferate to form a colony, whereas only 1 in 2470 unfractionated fetal liver cells formed a colony. Interestingly, whereas the data from unfractionated fetal liver cells exhibit "single-hit kinetics," data obtained with mAb AA4-selected cells generated a nonlinear curve with "multihit kinetics." Such multihit kinetics could result from limiting numbers of accessory cells required for colony formation or competition between different colony-forming cells. At least two types of colonies could be distinguished morphologically. One was composed of adherent "fibroblast-like" cells, while the second contained nonadherent "blast-like" cells.

Establishment of IL-3-Dependent Clones. We attempted to maintain long-term growth of both types of colonies in WEHI-3-CM. Nine nonadherent colonies and 10 adherent colonies were transferred to 0.5-ml cultures. None of the cells from adherent colonies survived more than 2 wk, whereas eight of nine nonadherent colonies did have long-term growth. Each of these colonies was recloned by micromanipulation, and their growth requirements were analyzed.

The results of proliferation assays (Fig. 2) clearly emphasize the ability of WEHI-3-CM or purified IL-3 to support growth (Fig. 2 *C–P*). Optimum cell growth was observed in the presence of 1.25–20% WEHI-3 supernatant or purified IL-3 at 12.5–100 units/ml. Purified IL-2 did not support growth even at 10 units/ml. We have maintained stable growth of these cells for >6 mo, with doubling times ranging from 9 to 13 hr when WEHI-3-CM is present. Attempts to grow these cells in factor-independent conditions have been unsuccessful.

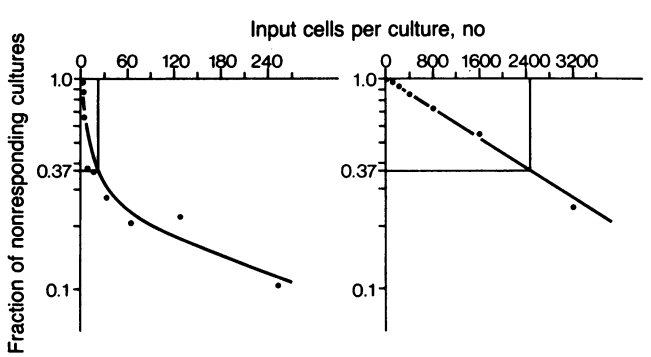

FIG. 1. Limiting-dilution analysis of fetal liver cells grown in WEHI-3-CM. (*Left*) AA4-selected fetal liver cells. (*Right*) Unfractionated fetal liver cells. Each data point represents 192–480 individual cultures.

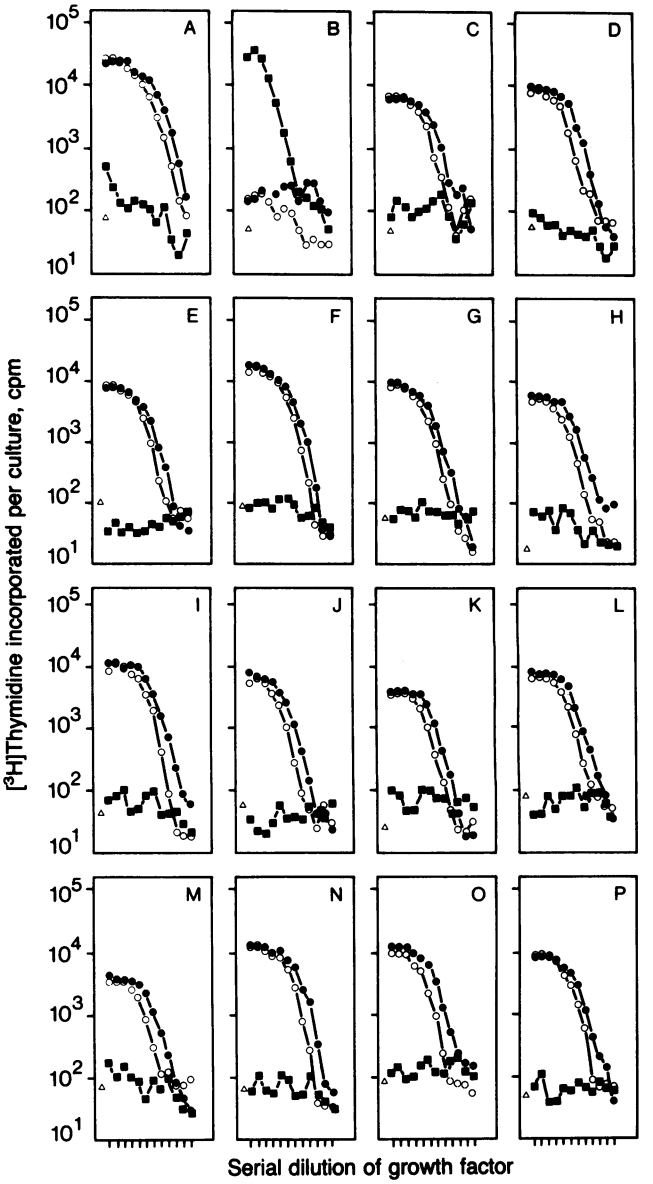

FIG. 2. Factor-supported growth of long-term fetal liver clones. Cells (2.5×10^3) derived from limiting-dilution cultures were incubated in the presence of WEHI-3 supernatants (●) (highest concentration, 20%), purified IL-3 (○) (highest concentration, 100 units/ml), purified IL-2 (■) (highest concentration, 10 units/ml); or IMDM with 5% fetal calf serum alone (△). Serial dilutions (1:1) of growth factor, plotted on the *x* axis in the order of decreasing concentration, were performed to quantitate their ability to support cellular proliferation (measured as in Table 1). Control cell lines included the IL-3-responsive clone FDC-P2 (9) and the IL-2-responsive clone CTL-L (11). The following cell lines were used: FDC-P2 (*A*), CTL-L (*B*), FL 1 parent cell line (*C*), FL1.2 subclone (*D*), FL1.6 subclone (*E*), FL2 parent cell line (*F*), FL2.1 subclone (*G*), FL2.3 subclone (*H*), FL3 parent cell line (*I*), FL3.1 subclone (*J*), FL3.3 subclone (*K*), FL4 parent cell line (*L*), FL4.7 subclone (*M*), FL5 parent cell line (*N*), FL5.5 subclone (*O*), and FL5.12 subclone (*P*).

Cell Surface Phenotype. A comprehensive survey of cell surface determinant expression by the fetal liver clones provided some insight into their identity. Expression of low amounts of class I MHC antigens and high densities of AA4, LGP 200, and GF1 (Fig. 3 *B*, *I*, *J*, and *L*, respectively) antigens were routinely observed among all of the factor-dependent clones, whereas κ light chain, IgM, J11d, class II MHC, Thy-1, Lyt-1, Lyt-2, and B220 antigens were not

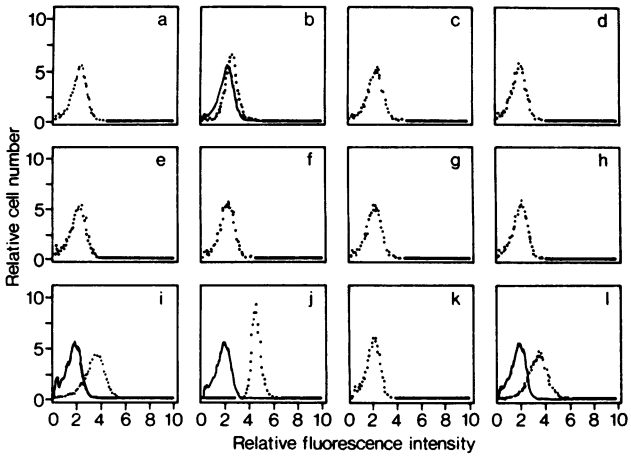

FIG. 3. Quantitation of cell surface determinants. Solid lines, controls presented when test samples exceeded background fluorescence levels; dashed lines, quantitation of cell surface expression of the following molecules: J11d antigen (*A*), class I MHC (*B*), class II MHC (*C*), Thy-1 (*D*), Lyt-1 (*E*), Lyt-2 (*F*), κ light chain (*G*), IgM (*H*), AA4 (*I*), LGP 200 (*J*), B220 (*K*), and GF1 (*L*). The histograms were generated with cell line FL5. Nearly identical results were obtained with all of the fetal liver cell lines.

detected (Fig. 3 *A*, *C–H*, and *K*, respectively). This phenotype clearly distinguishes these IL-3-dependent cell lines from the IL-3-dependent T-cell and myeloid lines described by others (7–9). More importantly, the clones expressed several markers (AA4 and GF1 antigens and LGP 200) normally found on B-cell precursors (14, 15).

Ig Gene Organization. Since the clones shared some properties of B-cell precursors, their Ig gene structure was analyzed. Southern analysis used probes that detect rearrangement of heavy chain genes [J_H-specific probe pJ_{11} (25)], κ light chain genes [c_κ-specific probe $p\kappa_1$ (26)], and λ light chain genes V_λ-specific probe $V(J)\lambda_1$ (27)]. DNA from the eight parent cell lines and 32 of their subclones were examined, and Fig. 4 contains results obtained with five parent cell lines (FL1-5) and nine subclones. The genes encoding the J_H segments (Fig. 4 *A* and *B*), κ light chain (Fig. 4*C*), and λ light chain (Fig. 4*D*) were present in germ-line configuration in every cell line tested, even when *Eco*RI and *Bam*HI digests were analyzed with each Ig probe (Fig. 4 and unpublished results). Thus, none of the factor-dependent fetal liver cell lines exhibit gene rearrangements found in committed B lymphocytes, even though they do express several cell surface determinants found on B-cell precursors.

Differentiation of Fetal Liver Clones. By coculturing BALB/c fetal liver clones (*H-2^d Igh-C^a*) with accessory cells from bone marrow of Ig congenic C.AL 20 mice (*H-2^d Igh-C^o*), we were able to induce significant numbers of *Igh-C^a* IgM-secreting cells detected in an allotype-specific reverse plaque assay (Table 3). Control cultures that contained syngeneic allotype disparate (C.AL 20) accessory cells alone or unfractionated bone marrow cells from C.AL 20 did not produce *Igh-C^a* IgM-secreting cells, thus confirming the specificity of this assay. None of the uninduced fetal liver clones secreted detectable levels of IgM (Table 3) in agreement with our Southern analysis (Fig. 4) and our inability to biosynthetically label and immunoprecipitate Ig from any of the uninduced clones (unpublished results). Another distinguishing feature of the B-cell maturation was the time-course required for maximal production of IgM-secreting (mature) B cells in these assays. Fetal liver clones produced the maximum number of IgM-secreting cells on days 9–11 postinduction, whereas normal adult bone marrow or adult spleen peaked 2–5 days earlier. Table 3 contains results from days 7 and 10 postinduction.

The clonal heterogeneity of fetal liver B-cell cultures was also analyzed by using several different chemically modified

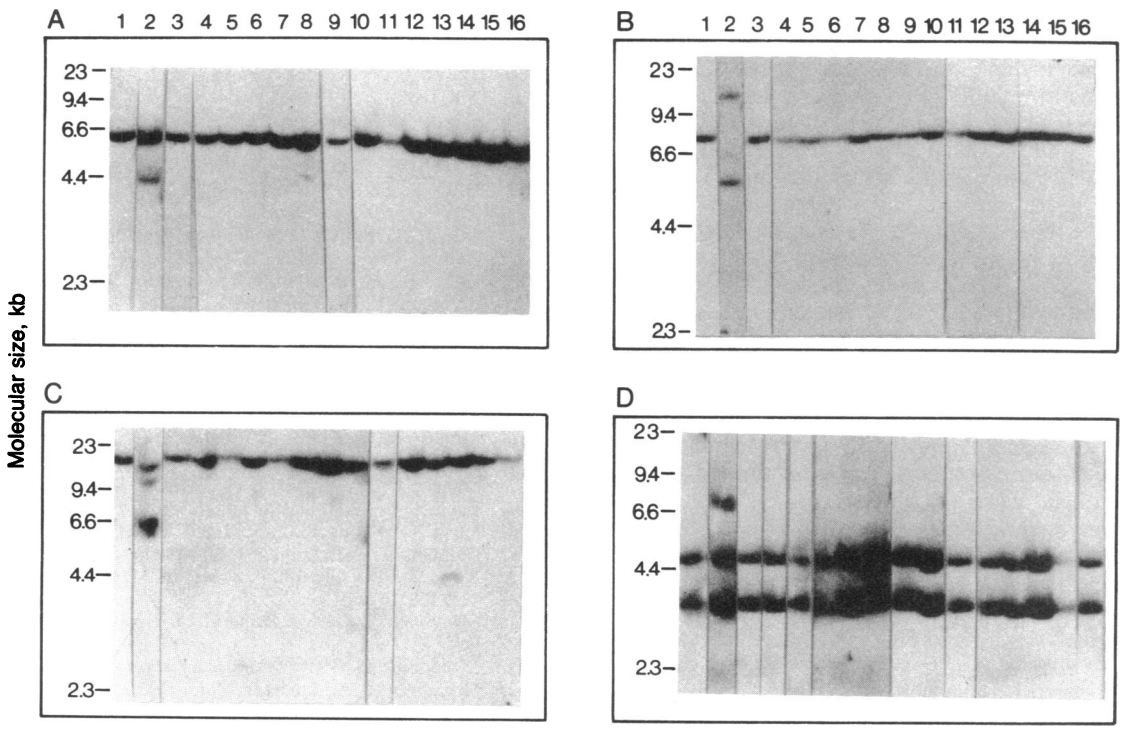

FIG. 4. Ig gene configuration among fetal liver clones. Southern blot analysis of *Eco*RI- or *Bam*HI-digested DNA from factor-dependent fetal liver cell lines was performed with J_H-specific (*A* and *B*), c_κ-specific (*C*), or V_λ-specific (*D*) probes. DNA samples in *A* and *D* were digested with *Eco*RI, whereas samples in *B* and *C* were digested with *Bam*HI. Each DNA sample (10 μg) was loaded into the following lanes: 1, BALB/c adult liver; 2, Sp6 B-cell hybridoma 1; 3, FL1; 4, FL1.2; 5, FL1.6; 6, FL2; 7, FL2.3; 9, FL3; 10, FL3.1; 11, FL3.3; 12, FL4; 13, FL4.7, FL5; 15, FL5.5; and 16, FL5.12. Control sample in lane 2 of *D* was Sp2/0 B-cell hybridoma DNA used to detect λ chain gene rearrangement. *Hin*dIII digests of λ phage DNA were used as molecular size markers.

Table 3. Maturation of fetal liver clones into Ig-secreting cells

BALB/c (*Igh-C^a*)-derived cells	C.AL 20 (*Igh-C^o*)-derived cells	IgM-secreting cells per culture									
		Uninduced*		Day 7		Day 10					
		Igh-C^a	*Igh-C^{a+o}*	*Igh-C^a*	*Igh-C^{a+o}*	*Igh-C^a*	*Igh-C^{a+o}*	TNP-SRBC	FITC-SRBC	NIP-SRBC	SRBC
FL1	B220$^-$ cells	0	0	40	220	970	2,080	—	—	—	—
FL1.2		0	0	—	—	5,125	6,560	694	34	302	14
FL1.6		0	0	—	—	4,215	4,990	510	36	268	10
FL2		0	0	110	290	450	1,425	—	—	—	—
FL2.1		0	0	—	—	3,510	4,815	494	46	278	28
FL2.3		0	0	—	—	2,955	3,765	520	76	306	48
FL3		0	0	185	425	2,155	3,835	—	—	—	—
FL3.1		0	0	795	725	4,210	6,120	580	90	700	72
FL3.3		0	0	110	275	7,610	8,390	444	36	256	6
FL4		0	0	430	1,540	620	1,990	—	—	—	—
FL4.7		0	0	230	485	5,620	4,815	582	26	312	18
FL5		0	0	260	610	875	1,695	—	—	—	—
FL5.5		0	0	210	355	6,650	7,195	568	28	272	12
FL5.12		0	0	395	605	6,090	7,610	334	34	348	16
—		—	—	35	200	200	1,300	86	4	48	4
Bone marrow	—	—	—	19,975	21,050	7,290	6,970	252	92	224	10
—	Bone marrow	—	—	750	13,075	160	4,000	376	158	152	26

Culture conditions and assay procedures were performed as described. Cultures of fetal liver cells and/or bone marrow cells that lacked mitogens did not contain significant numbers of IgM-secreting cells (<50 per culture). Results are expressed as the mean IgM-secreting cells per culture obtained in two or three experiments. TNP, 2,4,6-trinitrophenyl; FITC, fluorescein isothiocyanate; NIP, nitroiodophenyl.
*Uninduced BALB/c fetal liver cell cultures were measured under two conditions: (*i*) fetal liver cells alone or (*ii*) fetal liver cells with mitogens for 7–10 days. Both conditions generated the results shown above.

SRBC indicator cells. Quantitation of Ig-secreting cells that produce antibodies specific for trinitrophenyl-, fluorescein isothiocyanate-, and nitroiodophenyl-conjugated SRBC and unconjugated SRBC was used to measure multiple B-cell (Ig) specificities. All nine of the fetal liver clones were able to generate polyclonal B-cell responses (Table 3).

In summary, experiments described in this report demonstrate that a single-step separation technique can select a fetal liver cell subpopulation that contains a 50- to 100-fold higher frequency of multipotential and restricted precursor cells of both erythroid/myeloid and lymphoid lineages. From this population, we were able to establish cloned cell lines that can be maintained long-term when provided with WEHI-3-CM or purified IL-3. These clones proliferated in methylcellulose cultures but could not be induced to differentiate along the erythroid or myeloid lineages (unpublished results). Since these clones were propagated in the absence of accessory cells we could establish their cell surface phenotype as AA4$^+$, GF1$^+$, LGP 200$^+$, class I MHC "dull" (with little or no IgM, κ light chain, B220, Thy-1, Lyt-1, Lyt-2, class II MHC, or J11d antigens). Genes encoding Ig heavy chains or light chains were found to be in embryonic configuration. However, these clones could be induced to generate mature IgM-secreting B cells *in vitro*. These responses required approximately 10 days to reach maximal levels, and each clone produced B cells committed to several different variable region specificities.

These experiments demonstrate the ability to enrich for restricted and multipotential erythroid/myeloid precursors cells as well as clones that represent multipotential B-lymphoid precursors. This approach will undoubtedly facilitate direct analysis of early phases of hematopoiesis.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann–La Roche Ltd.

1. Iscove, N. N. (1985) in *Leukemia*, ed. Weissman, I. L. (Springer, Berlin), Vol. 30, pp. 5–20.
2. Kincade, P. W., Lee, G., Scheid, M. P. & Blum, M. D. (1980) *J. Immunol.* **124**, 947–953.
3. Ihle, J. N., Keller, J., Oroszlan, S., Henderson, L. E., Copeland, T. D., Fitch, F., Prystowsky, M. B., Goldwasser, E., Schrader, J. W., Palaszynski, E. W., Dy, M. & Lebel, B. (1983) *J. Immunol.* **131**, 282–287.
4. Iscove, N. N., Roitsch, C. A., Williams, N. & Guilbert, L. J. (1982) *J. Cell. Physiol.*, Suppl. 1, 65–78.
5. Clark-Lewis, I. & Schrader, J. W. (1981) *J. Immunol.* **127**, 1941–1947.
6. Williams, N., Eger, R. R., Jackson, H. M. & Nelson, D. J. (1982) *J. Cell. Physiol.* **110**, 101–104.
7. Ihle, J. N., Keller, J., Greenberger, J. S., Henderson, L., Yetter, R. A. & Morse, H. C. (1982) *J. Immunol.* **129**, 1377–1383.
8. Hapel, A. J., Lee, J. C., Farrar, W. L. & Ihle, J. N. (1981) *Cell* **25**, 179–186.
9. Dexter, T. M., Garland, J. M., Scott, D., Scolnick, E. & Metcalf, D. (1980) *J. Exp. Med.* **152**, 1036–1047.
10. Stanley, E. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2969–2973.
11. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120**, 2027–2032.
12. Ihle, J. N., Keller, J., Henderson, L., Klein, F. & Palaszynski, E. (1982) *J. Immunol.* **129**, 2431–2436.
13. Gillis, S., Mochizuki, D. Y., Conlon, P. J., Hefeneider, S. H., Ramthun, C. A., Gillis, A. E., Frank, M. B., Henney, C. S. & Watson, J. D. (1982) *Immunol. Rev.* **63**, 167–209.
14. McKearn, J. P., Baum, C. W. & Davie, J. M. (1984) *J. Immunol.* **132**, 332–339.
15. McKearn, J. P. & Rosenberg, N. (1985) *Eur. J. Immunol.* **15**, 295–298.
16. Springer, T. J., Galfre, G., Secher, D. S. & Milstein, C. (1978) *Eur. J. Immunol.* **8**, 539–551.
17. Smilek, D. E., Boyd, H. C., Wilson, D. B., Zmijewski, C. M., Fitch, F. W. & McKearn, T. J. (1980) *J. Exp. Med.* **151**, 1139–1150.
18. Fagg, B. & Roitsch, C. A. (1985) *J. Cell. Physiol.*, in press.
19. Quintans, J. & Lefkovitz, I. (1973) *Eur. J. Immunol.* **3**, 392–397.
20. Kincade, P. W., Ralph, P. & Moore, M. A. S. (1976) *J. Exp. Med.* **143**, 1265–1270.
21. Grützman, R. (1981) Dissertation (Univ. of Cologne, Cologne, F.R.G.).
22. Lieberman, R. (1978) *Springer Semin. Immunopathol.* **1**, 7–30.
23. McCubrey, J. A. & Risser, R. G. (1983) *J. Virol.* **45**, 950–955.
24. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
25. Marcu, K., Banerji, J., Penncavage, N. A., Lang, R. & Arnheim, N. (1980) *Cell* **22**, 187–196.
26. Zhu, D., Lefkovitz, I. & Köhler, G. (1984) *J. Exp. Med.* **160**, 971–981.
27. Blomberg, B., Traunecker, A., Eisen, H. N. & Tonegawa, S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3765–3769.