Immunologic Selection of Hemopoietic Precursor Cells Utilizing Antibody-Mediated Plate Binding ("Panning")

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We utilized the property of antibody adherence to plastic to separate and obtain enriched fractions of human myeloid (CFU-GM), erythroid (BFU-E) and pluripotent (CFU-GEMM) hemopoietic precursor cells. Nonadherent buoyant human marrow cells coated with mouse anti-human HLA-DR monoclonal antibody (Mc ab), an anti-pan T lymphocyte Mc ab (Leu 1/17F12) or a granulocyte–monocyte-specific Mc ab (MCS2) were incubated on polystyrene Petri plates coated with affinity purified goat anti-mouse immunoglobulin G (IgG). Cells bound to the coated plates and nonbound cells were separately recovered ("panned") by differential elution. Analysis of the nonadherent buoyant marrow cells demonstrated 12% to possess HLA-DR, 6% T, 40% MCS2 antigens on their surface by indirect immunofluorescence (IMF). After panning, 15% ± 8%, 14% ± 4% and 8% ± 6% cells were plate-bound by their respective antibodies, demonstrating differing binding efficiencies. A substantial degree of purity of the recovered cell fractions was shown for bound 74% ± 6% and 75% ± 5% IMF positive cells) and nonbound cells (3% ± 1% and 0.1% ± 0.8% positive cells) coated with anti-HLA-DR or anti-T Mc ab respectively, with lesser purity for MCS2 panned cells. Seventy-three percent to 126% CFU recovery was noted, with a sevenfold enrichment of the HLA-DR bound cells for CFU-GM and CFU-GEMM, and 3.5-fold enrichment for BFU-E. Sequential panning, obtaining T nonbound-DR bound-surface immunoglobulin nonbound fractions, resulted in tenfold CFU-GM enrichment (107/104 cells, ± 1/100). Anti-MCS2 antibody was ineffective for panning, but use of this antibody in fluorescence-activated cell sorting (FACS) indicated the absence of the MCS2 antigen on the vast majority of CFU-GM. This study describes a relatively rapid and inexpensive method for obtaining enriched antigenically defined hemopoietic precursors in high yield. These techniques should prove useful for more clearly evaluating cellular and humoral interactions with hemopoietic precursor cells.

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IMMUNOLOGIC METHODS Provide useful dimensions for characterizing and separating hemopoietic cells. The ability to fractionate lymphocytes on the basis of surface phenotype has been a major technical advance in the study of the functional diversity of these cells. Many of the methods currently used exploit antibody specificity to separate cells of one type from a mixed population.1,2 Recent studies have demonstrated an effective and relatively inexpensive technique for selectively enriching lymphocyte populations from murine spleen cells or human peripheral blood for study of their immunologic potential.3,5 This method, using immune adsorption (termed "panning"), is based on the observation that antibody molecules adsorb onto polystyrene Petri plates and bind specific antigens on cell surfaces.6 Utilizing monoclonal antibodies, we and others have demonstrated that human myeloid, erythroid, and pluripotent colony-forming cells (CFU-GM, BFU-E, and CFU-GEMM) have cell surface phenotypes possessing receptors for HLA-DR and lacking a pan-T lymphocyte antigen (Leu 1)1,7.9 In order to evaluate more precisely interactions between hemopoietic regulatory cells and substances and their precursor target cells, we have developed the immune adsorption technique to obtain relatively homogeneous hemopoietic cell populations from human bone marrow. We have demonstrated that this method could be used for either specific cell selection or elimination, that the fractionated cells were recovered in high yield with lower levels of contamination by other cell types, and that enriched fractions of hemopoietic precursor cells that retained biologic function were obtained.

MATERIALS AND METHODS

Cell Preparation

Normal human marrow cells, obtained by aspiration, were washed and suspended in Iscove's medium containing 15% fetal calf serum (FCS). Light-density (<1.077 g/cc) nonadherent marrow cells were obtained by placing buoyant mononuclear cells from Ficoll-Hypaque centrifugation on plastic tissue culture dishes in Iscove's medium-7.5% FCS and recovering the nonadherent cells after 60-minute incubation at 37 °C, as previously described.9 Peripheral blood polymorphonuclear leukocytes (PMNs) were obtained by recovering the cell pellet after Ficoll-Hypaque sedimentation. The cells in the supernatant (95% PMNs) were washed and resuspended in phosphate-buffered saline (PBS) for assessment of immunofluorescence.

Antibodies

Monoclonal mouse anti-human HLA-DR antibody (2.06), anti-T lymphocyte antibody (Leu-1/17F12), and anti-granulocyte anti-
body (MCS2), were obtained by somatic cell hybridization, as previously reported.11 15 The cloned hybridomas produced monoclonal IgG antibodies of defined specificities. The anti-HLA-DR antibody reacts in radioimmune binding and cytotoxicity assays with human B cell lines, peripheral B cells, and macrophages, but not with the T cell lines or peripheral T cell populations.15 17 The anti-T lymphocyte antibody detects a 67,000 g mol. wt protein antigen common to virtually all peripheral T lymphocytes, most medullary thymocytes, and T-acute lymphoblastic leukemia cells, but is absent from B cells, B cell lines, monocytes, erythrocytes, platelets, mature granulocytes, and nonrosetted lymphocytes.5 16 The MCS2 antibody, prepared by Drs K. Sagawa, E. Tatsumi, and J. Minowada, Roswell Park Memorial Institute, detects an antigen present on granulocytic cell at all stages of differentiation, myeloblasts through neutrophils, acute myeloid leukemia (AML) blasts, and myeloid leukemia cell lines, but is absent from lymphocytes, lymphoid cell lines, erythroid cells, or platelets.5 Fluoresceinated (FITC) affinity purified multivalent rabbit anti-human immunoglobulin (Ig) (Tago Immunodiagnostics, Burlingame, Calif) was utilized to detect surface immunoglobulin (SIg) and the non-FITC compound used to deplete B lymphocytes. Affinity purified goat anti-mouse IgG, used for coating the Petri plates, and the FITC-conjugated compound, used for immunofluorescence, were obtained from Tago Inc. FITC-conjugated rabbit anti-goat IgG was obtained from Cappell Labs (Cochraneville, Pa).

**Panning Technique**

Two panning methods were utilized—indirect and direct. For indirect panning (used with HLA-DR and Leu 1), polyurethene Petri plates (Fisher, 100 x 15 mm) were coated with 100 μg of goat anti-mouse IgG (10 mg/ml, in 0.05 M TRIS buffer, pH 9.5) for 40 minutes at 23°C, as previously described.5 15 Prior to use, the solution was decanted and the plates were washed four times with phosphate buffered saline (PBS), pH 7.4, then exposed to PBS 1% FCS for 15 minutes at 4°C. Nonadherent buoyant (NAB) mononuclear cells were coated with 75 μL of a 1:10 dilution of the murine monoclonal HLA-DR or Leu 1 antibodies (65 μg per 2 x 10^6 cells) for 20 minutes at 4°C. The cells were washed with cold PBS and suspended in 3 ml 5% FCS-PBS. Monoclonal antibody-coated cells, 2 x 10^6, kept at 4°C, were poured onto the goat anti-mouse IgG-coated plates. The cells were incubated on a level surface at 4°C for 70 minutes, swirling the plate once midway through the incubation period to redistribute attached cells. After incubation, the nonbound cells were removed by swirling the plate, decanting the supernatant, and gently rinsing the plates five times with 1% FCS-PBS. To recover the bound cells the entire surface of each plate was vigorously flushed with 1% FCS-PBS using a Pasteur pipette. As assessed by direct visualization, all of these bound cells were thus removed. The cells were separately recovered and counted on days ten and 14. Placental CM was used to assess formation was noted for the NAB or panned cells.

**Indirect immunofluorescence.** For indirect immunofluorescence, 50 μL of the monoclonal antibody (at the appropriate dilution) was added to the cell pellet (1 x 10^6 cells) for 20 minutes at 4°C. The cells were washed with PBS and spun at 1,200 rpm for ten minutes. The antibody-coated cell pellet was incubated for 20 minutes at 4°C with fluoresceinated goat anti-mouse IgG, diluted 1:10 with PBS (in 0.02% sodium azide to prevent capping). The cells were washed three times in PBS, resuspended, and the proportion of cells possessing cell membrane fluorescence was determined with a Leitz (Rockleigh, NJ) Ortholux II fluorescence microscope. Normal goat or human sera were used as negative controls.

**Direct immunofluorescence.** For direct immunofluorescence, 50 μL of the fluoresceinated rabbit anti-human Ig was added to the cell pellet (1 x 10^6 cells) for 20 minutes at 4°C. The cells were then washed, and the proportion possessing surface membrane fluorescence was assessed as above.

**Morphology**

Cells obtained from bound and nonbound fractions were examined for morphology after Wright’s-Giemsa staining of cyt centrifuge preparations.

**Colony Formation by Granulocyte Macrophage (CFU-GM), Erythroid (BFU-E), and Pluripotent (CFU-GEMM) Progenitors**

Nonadherent buoyant (NAB) marrow mononuclear cells and cells obtained from panned fractions were cultured essentially as previously described,16 in Iscove’s medium, 30% FCS, 1 unit purified human urinary erythropoietin (1,100 μg/mg protein, obtained from the National Heart, Lung, and Blood Institute, National Institutes of Health), 1% T cell line-conditioned medium (MoCM), 5 x 10^-5 mol/L 2-mercaptoethanol in a final concentration of 1.1% methylcellulose in 35-mm tissue culture dishes (Lux, Miles Labs, Naper ville, III). The MoCM was kindly provided by Dr David Golde, UCLA Medical Center.14 After 14 days of incubation at 37°C in a moist atmosphere containing air with 5% CO₂, erythroid bursts (BFU-E) and pluripotent (CFU-GEMM) mixed hemopoietic colonies were counted using an inverted microscope at 30x. Representative colonies (10% to 20% of CFU-GMs, BFU-Es, and most CFU-GEMMs) were picked and cyt centrifuge preparations made for Wright’s-Giemsia staining to assess their morphology. To determine CFU-GM, cells were also plated as above but with 15% human placental conditioned medium as a source of colony stimulating factor (CSF) rather than using MoCM and erythropoietin and with 15% rather than 30% FCS in the nutrient medium. Myeloid colonies were counted on days ten and 14. Placental CM was used to assess CFU-GM as its GM-CSF activity was greater than that in MoCM. In the absence of MoCM, erythropoietin, or placental CM, no colony formation was noted for the NAB or panned cells.

**Cytotfluorographic Analysis and Cell Separation**

MCS2 antigens on the cell surface were identified by the binding of anti-MCS2 monoclonal antibody detected by indirect immunofluorescence. One million cells were placed in a plastic tube and incubated with 100 μL of a 1:10 dilution of anti-MCS2 antibody at 4°C in PBS plus 0.02% sodium azide to remove excess antibody and incubated for 20 minutes at 4°C with 100 μL of FITC goat anti-mouse Ig. The cells were washed twice, fixed in 1% formalde-
antibody coating of 2 x 10^6 nonadherent buoyant cells bound) occurred with 65 to 260 μg monoclonal antibody. The cell populations enriched for MCS2+ or MCS2- cells were obtained by aseptic separation of these labeled cells using the FACS IV, and were then plated and assessed for CFU-GM growth in vitro. Prior to sorting, the cells were resuspended in cold RPMI 1640 medium plus Pen-strep (GIBCO, Santa Clara, Calif), and the sorted populations were kept on ice. In pilot experiments to determine whether antigenic modulation would interfere with cell separation, the cells labeled for analysis in azide-containing reagents and buffers were compared for fluorescence intensity with those prepared for sorting with azide omitted. The fluorescence histograms obtained were superimposable, permitting us to perform the separation experiments in the absence of azide (which is toxic for hemopoietic progenitor cells).

RESULTS

The cell surface antigens present on peripheral blood and marrow cells, determined by immunofluorescence assays are shown in Table 1. These findings correlated well with those expected by specificities claimed for the antibodies (see preceding) and prior studies regarding the cellular composition of marrow and peripheral blood. To determine the specificity of the panning procedure for cell selection, a variety of conditions were evaluated. As shown in Table 2 using anti-HLA-DR, indirect panning appeared to be somewhat better than the direct method regarding the proportion of cells bound to the plates. Nonspecific sticking did not occur, nor was there evidence for Fc receptor binding. Optimal conditions (13% marrow cells bound) occurred with 65 to 260 μg monoclonal antibody coating of 2 x 10^6 nonadherent buoyant marrow cells. Similar data (not shown) was found for Leu 1. In contrast, essentially no binding of the marrow cells with MCS2 was found by the indirect method, whereas 8% of the cells were bound by the direct technique. Thus, indirect panning was subsequently used for HLA-DR and Leu 1 and direct panning for MCS2 (direct panning was also found to be better for Ig and was therefore used). After the initial incubation of the Petri plates with 100 μg of goat anti-mouse IgG, pouring residual antibody onto two subsequent plates permitted them still to be effective for panning. This finding is in agreement with prior data indicating that approximately 30 μg of IgG binds to 100-mL Petri plates.

In order to determine which antibodies remained bound to the cells after indirect Leu 1 panning, the bound and nonbound cells were coated with FITC-conjugated antibodies directed against mouse or goat IgG with or without prior recoating of the cells with anti-Leu 1 or goat anti-mouse IgG. Results shown in Table 3 (line 2) indicate that 40% to 57% of the initially positive-bound cells retained the mouse anti-human antibody after the panning procedure. None of the goat anti-mouse antibody used for coating the Petri plates was detected on the bound or nonbound cells (line 4). As a positive control for the FITC-conjugated rabbit anti-goat IgG antibody, triple-antibody immunofluorescence for Leu 1-bound cells (line 5) was shown to be similar to that obtained with two antibodies (line 1).

Purity of Fractions

Separation of the cells into bound and nonbound fractions was achieved by panning with anti-HLA-DR and Leu 1 antibodies, showing good agreement with values expected by immunofluorescence (Fig 1). However, only 8% were bound with MCS2, indicating differential effectiveness of these IgG antibodies for panning (and the poor ability to pan for MCS2 positive
cells). This was further demonstrated when the purity of the bound and nonbound fractions was determined by immunofluorescence. For HLA-DR, Leu 1, and MCS2, the bound fractions were 74%, 77%, and 65% positive for their respective antibodies (Fig 1). The nonbound fractions were contaminated with 2.6%, 0.9%, and 11% positive cells.

Sequential panning was performed in which cells in the Leu 1 nonbound fractions were coated with HLA-DR and repanned. Fourteen percent of the Leu 1 nonbound cells were positive for HLA-DR by immunofluorescence; 11% of the Leu 1 nonbound cells bound to the new plates, of which 80% were positive for HLA-DR by immunofluorescence (Fig 1). Sequential panning was also performed with the HLA-DR bound cells by coating the cells with MCS2 and repanning. Fourteen percent of these cells bound, of which 75% were positive for MCS2. It should be noted, however, that in the sequentially panned cells (which were initially bound, and thus had potentially been coated with two monoclonal antibodies) the fluorescence could have been related to either the first or second monoclonal antibody exposure, or both combined. For triply panned cells (Leu 1 nonbound-DR bound exposed to anti-human Ig), bound and nonbound cells were highly separated with regard to Ig positivity (74% vs 2%).

### Biologic Activity

Cells in the bound and nonbound fractions were assessed for hemopoietic colony formation. As shown in Figs 2 and 3, cells in the prepanned fractions had plating efficiencies of 103 CFU-GM, 156 BFU-E, and 2 CFU-GEMM per 10^6 NAB marrow cells. In the HLA-DR bound fraction, approximately sevenfold enrichments occurred for CFU-GM and CFU-

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**Fig 1.** Purity of the human marrow cell fractions. Cell surface phenotypes of prepanned, bound (B) and nonbound (NB) cell fractions obtained after panning were determined by indirect immunofluorescence using monoclonal antibodies against HLA-DR, Leu 1, and MCS2 antigens, using FITC-conjugated goat anti-mouse IgG as the second antibody. Direct immunofluorescence, utilizing FITC-conjugated goat anti-human IgG, was used to detect surface Ig. Panning was performed on nonadherent buoyant marrow cells as described in the text. Numbers in parentheses indicate the number of separate experiments. Levels of bar graphs indicate mean values obtained from the combined separate experiments, with SD generally being ±10% to 15%. Error bars have been omitted for reasons of clarity.

**Fig 2.** Hemopoietic colony formation of cells selected by the antibody mediated petri plate binding ("panning") technique. Proportions of bound (B) and nonbound (NB) marrow cells are shown for each antibody and for the sequential antibody combinations. The mean incidences of CFU-GM, BFU-E and CFU-GEMM for prepanned, bound (B) and nonbound (NB) fractions are shown. Numbers in parentheses indicate the number of separate experiments used to determine the values. Levels of bar graphs indicate mean values obtained from the combined separate experiments, with SD generally being ±10% to 15%. Error bars have been omitted for reasons of clarity. Methods for colony-forming assays are stated in the text.
GEMM, whereas only a 3.5-fold enrichment occurred for BFU-E. (A seven- to eightfold enrichment was anticipated, considering recovery of total cells or of immunofluorescent phenotypes.) Virtually all of the colony-forming cells were present in the Leu 1 non-bound fraction, and no enrichment was noted, as only a small degree of depletion of nonclonogenic cells (16%) occurred by panning with this antibody (see following). Sequential panning showed that the doubly panned Leu 1-nonbound HLA-DR-bound fractions had an eightfold enrichment of CFU-GM and CFU-GEMM, again with a lesser (3.6-fold) enrichment for BFU-E. In pilot experiments, it was demonstrated that coating marrow cells with the anti-HLA-DR antibody and processing them for panning (but not panning them) did not alter colony formation of CFU-GM, BFU-E, or CFU-GEMM relative to cells that were similarly processed but unexposed to this antibody (data not shown). Sequential triple panning using an Ig pan for Leu 1-nonbound HLA-DR-bound cells led to a tenfold enrichment of CFU-GM (1068/10^9 marrow cells, = 1/100). Adding 1% or 10% autologous T cells (from the Leu 1-bound fraction) to the doubly or triply panned cells did not alter colony formation. Colonial morphology, as determined by Wright's-Giemsa staining, did not differ from that for the plated NAB marrow cells.

In evaluating MCS2 for panning of marrow cells, of interest was the finding that 93% to 95% of the colony-forming cells were in the MCS2 nonbound fraction. However, due to the poor plate-binding characteristics of cells coated with MCS2, FACS was used as an independent technique to evaluate CFU-GM phenotype with respect to MCS2. Thus, NAB marrow cells coated with anti-MCS2 antibody were sorted by FACS, and the positively and negatively stained cells were assayed for in vitro colony formation. Fifty eight percent of the presorted marrow cells stained positively with MCS2 (Fig 4). After FACS separation, the cells were sorted into "positive" and "negative" fractions that contained 99.4% and 8.0% positive cells, respectively (Fig 4). The CFU-GM plating efficiencies for presorted, MCS2+ and MCS2− cells were 92, 12, and 141 per 10^5 cells respectively. These values correspond to 89% of the CFU-GM being recovered in the MCS2− fraction.

Recovery

After a single panning with Leu 1 or HLA-DR antibodies, approximately 85% of the marrow cells were recovered (Fig 3). The cells were recovered in the bound and nonbound fractions in proportions expected by immunofluorescence determination of cell surface phenotypes. Regarding colony-forming cells, after single panning with these antibodies, the recovery values were 126% and 121% for CFU-GM, 92% and 73% for BFU-E, and 85% and 95% for CFU-GEMM, respectively. The vast majority of the colony-forming cells recovered were segregated into the Leu1-nonbound or HLA-DR−bound fractions (Fig 3). Of the cells recovered after HLA-DR panning, 15% of the NAB marrow cells, 79% of the CFU-GM, 85% of the CFU-GEMM, but only 63% of the BFU-E were recovered in the HLA-DR bound fraction (Fig 3). After sequential double panning with these antibodies, approximately 75% of the CFU-GM, 61% BFU-E, and 81% CFU-GEMM were present within the cell population con-
taining about 7% of the initial cells (Leu 1 nonbound, HLA-DR bound). Triple panning (Ig pan of Leu 1 nonbound-HLA-DR bound cells) led to 6% recovery of the initial cells plated (86% of the previous pan’s cells) and 23% to 41% of the colony-forming cells. After this procedure, virtually all of the colony-forming cells were recovered in the Ig nonbound fraction. For the MCS2 antibody, 80% of the marrow cells and 50% to 73% of the colony-forming cells were recovered after single panning (Fig 5).

**Morphology**

Selective enrichment of marrow cells occurred after panning with different antibodies. Utilizing Leu 1, marked homogeneity (90%) of lymphocytes was present in the bound fraction, with only 5% lymphocytes in the nonbound fraction. As demonstrated by immunofluorescence, 77.0% and 0.9%, respectively, of these cells were T lymphocytes. In the HLA-DR bound fraction, enrichment of myeloblasts, promyelocytes, and small lymphocytes occurred (ie, comprised 83% of the cells). In the MCS2 bound fraction, only granulocytic cells were recovered, virtually all (97%) being intermediate and late (myelocytes to PMNs) in maturation. After sequential panning assessing, Leu 1 nonbound cells, enrichment of immature myeloid cells, and lymphocytes occurred in the HLA-DR bound fraction (ie, comprised 80% of the cells), whereas a striking increase in myelocytes and metamyelocytes (77% of the cells) combined with a near absence of lymphocytes was noted in the HLA-DR nonbound fraction. After triple sequential panning assessing Leu 1 nonbound HLA-DR–bound cells on an anti-human Ig pan, further enrichment of myeloblasts, promyelocytes, and large lymphocytes (70%) occurred in the Ig nonbound fraction, whereas predominantly lymphocytes (81%) were present in the Ig-bound fraction.

**DISCUSSION**

These data indicate that the antibody-mediated polystyrene Petri plate binding technique described (immune adherence or “panning”) was capable of recovering 81% to 86% of the human marrow cells and 73% to 126% of the hemopoietic colony-forming cells. Fractional separation of these cells using monoclonal antibodies correlated well with their demonstrated cell surface phenotypes when using HLA-DR and Leu 1 antibodies. In contrast, the MCS2 antibody showed relatively poor plate-binding affinity for the myeloid cells and essentially none for the CFU-GM or other hemopoietic progenitor cells. However, since a large percentage of the marrow cells were MCS2 positive by indirect immunofluorescence and FACS analysis and only a small percentage bound to panning dishes, it is apparent that panning for MCS2+ cells is not effective. Thus, to determine whether CFU-GM expressed the MCS2 antigen, we obtained highly purified MCS2+ and MCS2− cells by FACS separation (Fig 4). This method demonstrated that the vast majority (89%) of the CFU-GM were in the MCS2− fraction (ie, most CFU-GM lacked the antigen defined by this antibody).

The fractional purity of the cells obtained by Leu 1 or HLA-DR panning was substantial. Seventy-four percent to 77% of the bound cells were positive by immunofluorescence, whereas the nonbound cells were contaminated with only 0.9% to 2.6% positive cells. After indirect panning, a proportion (40% to 57%) of the murine monoclonal antibody remained on the bound cells, whereas none of the goat anti-human IgG antibody was detected (Table 3). These findings indicate the need to recoat the cells with the monoclonal antibody prior to immunofluorescence assay and also demonstrate the necessity for further processing if it is desired to remove residual monoclonal antibodies from the selected cells. Of importance was the finding that biologic activity was retained and high recovery of
colony-forming cells was demonstrated following panning with these antibodies. For CFU-GM and CFU-GEMM, an approximately sevenfold enrichment occurred with HLA-DR panning, whereas this was only 3.5-fold for BFU-E. Further enrichment occurred after double (T-nonbound HLA-DR–bound fractions) and triple (T-nonbound HLA-DR–bound Ig-non-bound fractions) panning. However, the degree of enrichment of colony-forming cells achieved by sequential panning was somewhat less than expected, possibly related to cell damage by multiple manipulations during processing. The relatively low degree of enrichment of BFU-E after HLA-DR panning, associated with the diminished proportion of BFU-E bound to the plates (63%), suggests that differing binding characteristics for HLA-DR exist for erythroid progenitor cells compared to the other precursors assayed. In this regard, prior studies from our lab demonstrated a slightly lower degree of HLA-DR dependent complement-mediated cytotoxicity of BFU-E relative to CFU-GM with our monoclonal HLA-DR antibody. Further, a recent investigation has demonstrated lineage-restricted variation in expression of HLA-DR encoded molecules, in which certain of these antigens on lymphoid cells differ from those on myeloid precursors. Our current experiments indicated that coating the HLA-DR receptors of erythroid precursors during cell processing did not interfere with stimulatory signals for their clonal proliferation. The lack of enrichment for BFU-E in the sequentially panned cells also did not appear to be due to T cell depletion, as addition of autologous T cells did not enhance colony formation. Further, no alteration of the plating efficiency of the hemopoietic precursors occurred after depletion of marrow T lymphocytes (ie, in the Leu 1 nonbound fraction). Although controversy exists regarding the need for T cells to support erythropoiesis in vitro, this finding substantiates recent extensive studies from our laboratory and those of others depleting T cells by various methods (complement-mediated cytotoxicity, sheep erythrocyte rosetting) that showed no alteration of colony formation after T cell removal.

Morphologic assessment of the panned fractions correlated well with the values expected from consideration of antibody specificities. Enrichment for selected cell lineages or stages in their differentiation were obtained by this method. In the triply panned fraction with the highest concentration of hemopoietic precursors (about 1 CFU-GM/100 cells in the T nonbound-DR bound Ig nonbound fraction) morphologic enrichment was mainly evident for myeloblasts and large lymphoid cells. These findings are consistent with prior findings using other enrichment techniques to morphologically define the stem cell compartment.

These investigations indicate the utility of this technique for obtaining relatively homogeneous human marrow cell populations to perform preparative and analytic studies to characterize the biology of these cells. Expenditures of time and cost for this procedure to obtain sterile, antigenically defined and enriched populations of human hemopoietic progenitor cells with high recovery compare favorably with use of the fluorescence-activated cell sorter. A major advantage of panning over cell sorting is the number of cells that can be conveniently processed and recovered by panning (multiple plates each containing 2 x 10^7 cells can readily be “panned” within 70 minutes, compared to approximately 10^7 cells processed per hour with only about 30% recovery using current models of cell sorters). Recent studies have demonstrated enrichment of hemopoietic progenitor cells from mouse marrow by panning, although with relatively high cell loss. Another useful method for myeloid precursor enrichment has utilized an antibody-bound erythrocyte rosetting technique to selectively obtain CFU-GM from peripheral blood of patients with chronic myeloid leukemia (CML). Although this method was less effective for normal marrow cells, findings with CML peripheral blood CFU-GM were similar to ours with regard to binding characteristics of these cells to anti–HLA-DR, anti-T, and anti-myeloid antibodies. We are currently evaluating other antibodies to further purify hemopoietic precursors and their regulating cells and are exploring methods to scale up the procedure for clinical purposes. These studies should permit further analysis of cellular and humoral interactions with relatively homogeneous populations of hemopoietic precursors in order to better understand the modulating influences of these cells for human hemopoiesis.

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Immunologic selection of hemopoietic precursor cells utilizing antibody-mediated plate binding ("panning")

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