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Antigenically distinct subpopulations of myeloid progenitor cells (CFU-GM) in human peripheral blood and marrow

(monoclonal antibodies/complement-dependent cytotoxicity/cell sorting/cytofluorimetry/hemopoietic stem cells)

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ABSTRACT **Two types of progenitor cells of the human granulocytic and monocytic lineages (CFU-GM) can be distinguished by using mouse monoclonal antibodies against human hemopoietic cells. Type 1 CFU-GM contribute all of the peripheral blood CFU-GM as well as a small fraction of bone marrow CFU-GM and express surface antigens recognized by "anti-lymphomonocytic" monoclonal antibodies S3-13 and S17-25 but not the antigens recognized by R1B19 and WGHS-29-1 (two monoclonal antibodies that react with all the cells of the granulocytic lineage). Type 2 CFU-GM are present only in the marrow and react with S3-13, R1B19, and WGHS-29-1. Partial reactivity with S17-25 was observed only in the complement-dependent cytotoxicity test. *In vitro* culture of type 1 CFU-GM in liquid medium in the presence of granulocyte-macrophage colony-stimulatory factor (GM-CSF) generates colony-forming cells that have the surface phenotype of type 2 CFU-GM. This finding supports the idea of two different stages of maturation of myelomonocytic progenitor cells represented by type 1 and type 2 CFU-GM.**

The early stages of myeloid differentiation from pluripotent stem cells to morphologically recognizable myeloblasts are not yet completely defined. The ability to grow granulocyte-macrophage progenitor cells (CFU-GM) *in vitro* (1–3) has provided information about both normal and abnormal progenitor cells (4). CFU-GM are considered to be cells committed to the generation of granulocytes and monocytes (5) and to represent an intermediate population between the pluripotent stem cells and morphologically recognizable myeloid cells (6). However, there is evidence to suggest the existence of CFU-GM subpopulations that differ in size (7–9), density (10), stage of cell cycle (8, 9), and responsiveness to different stimulators (7, 11).

Surface antigens of CFU-GM have also been studied by using polyclonal heteroantisera and monoclonal antibodies that recognize antigens related or unrelated to the HLA system (12–15). However, none of these antibodies have detected antigenic differences among subpopulations of these myeloid progenitor cells. We present evidence indicating that at least two antigenically distinct populations of CFU-GM exist at different stages of differentiation, based on the reactivity with a panel of mouse anti-human monoclonal antibodies, and that one population derives from the other.

MATERIALS AND METHODS

Cells. Bone marrow and peripheral blood samples were obtained from normal adult volunteer donors; the use of these samples was approved by the Committee for Protection of Human Subjects of the Children's Hospital of Philadelphia. Low-density cells were separated by centrifugation on a Ficoll-Hypaque gradient (ρ = 1.077 g/ml) (16).

Cells were washed three times in Ca^{2+}- and Mg^{2+}-free 0.15-M phosphate-buffered saline (pH 7.2) and were depleted of monocytes by adherence. In other experiments, peripheral blood cells were depleted of T lymphocytes by the erythrocyte rosetting technique (17).

Monoclonal Antibodies. Mouse monoclonal antibodies R1B19, S4-7, S3-13, and S17-25 were derived in our laboratory, as described, by immunizing mice with acute myeloblastic leukemia cells (18, 19). WGHS-29-1 was generated in the laboratory of H. Koprowski and Z. Steplewski (Wistar Institute, Philadelphia) (20) by immunization with primary gastric adenocarcinoma and was characterized for its reactivity with hemopoietic cells by us.

Antibodies S3-13, S4-7, R1B19, WGHS-29-1, and S17-25 (IgM isotype) all were found to be cytotoxic in the presence of complement. Antibodies WGHS-29-1, R1B19, and S4-7 react with carbohydrate moieties of glycolipids and glycoproteins (ref. 20; unpublished data). Antibody WGHS-29-1 has been shown to react specifically with the oligosaccharide fucopentaose III (20), the antigenic determinant also recognized by anti-SSEA-1 antibody (21). Antibody R1B19 immunoprecipitates glycoproteins of 145 and 105 kilodaltons, similar to those precipitated by antibody S4-7 (unpublished data). S3-13 immunoprecipitates a 29-kilodalton protein (unpublished data); the antigen recognized by S17-25 has not yet been identified.

Cytofluorimetry and Cell Sorting. Peripheral blood leukocytes (PBL) or bone marrow low-density cells (5×10^6) were incubated with a saturating concentration of each monoclonal antibody and then, after three washings, with a fluorescinated $F(ab)_2$ goat anti-mouse antibody. The negative control was incubated with medium and then with the fluoresceinated second antibody. Cells were separated by using an Ortho Cytofluorograf 50 HH cell sorter, as described, and analyzed for forward- and right-angle scatter and intensity of fluorescence (18). Fluorescence intensity threshold was set such that 99% of control cells were negative. One to 2 hr was required for sorting positive and negative cells from each sample. For the morphological identification of fluorescent and nonfluorescent cells, cytocentrifuge slides were prepared and stained with May–Grunwald–Giemsa stain. Positive (fluorescent) and negative (nonfluorescent) cells of each bone marrow sample were tested separately for the growth of CFU-GM. The number of CFU-

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Abbreviations: CFU-GM, granulocyte-macrophage progenitor cells; GM-CSF, granulocyte-macrophage colony-stimulatory factor; PBL, peripheral blood leukocytes.

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GM in each fraction was expressed as a percentage of the total population of colony- and cluster-forming cells present in the unfractionated bone marrow.

Complement-Dependent Cytotoxicity Test. Peripheral blood ($4–6 \times 10^6$) or bone marrow ($4–6 \times 10^5$) cells were suspended in 0.2 ml of McCoy's 5A medium containing 10% fetal bovine serum and incubated at 4°C with an equal volume of a predetermined optimal concentration of each monoclonal antibody. After 45 min, 0.4 ml of rabbit complement (Low TOX H, Accurate Chemical, Westbury, NY), diluted 1:4 with medium, was added. Incubation was continued at 37°C for 90 min. In some experiments, cells were incubated at 4°C for 30 min with antibody, washed, and incubated at 37°C for 45 min with complement. Identical results were obtained with the two different treatments.

Control samples were: (*a*) cells incubated with McCoy's 5A medium only; (*b*) cells incubated first with medium and then with complement; and (*c*) cells incubated for 2 hr with antibodies and then washed.

CFU-GM Assay. At the end of the incubation with complement, the cells of each sample were washed and cultured in McCoy's 5A medium, modified according to Pike and Robinson (3), containing 20% fetal bovine serum, 0.3% agar (Difco), and 10% conditioned medium as a source of granulocyte-macrophage colony-stimulatory factor (GM-CSF). Conditioned medium was obtained by culturing normal PBL (22) in McCoy's medium containing 10% fetal bovine serum and 10% autologous human plasma for 5 days. In some experiments, medium conditioned by the GCT cell line (23) (GIBCO) was used and identical results were obtained. Cultures were seeded in 35-mm Petri dishes, each containing 1×10^5 low-density nonadherent bone marrow cells or 1.5×10^6 low-density nonadherent PBL. Colonies (aggregates containing 40 or more cells) and clusters (4–39 cells) from bone marrow cultures were scored on days 7 and 14 of culture (9). Peripheral blood colonies and clusters were scored after 10 days of culture and, in some experiments, also after 14 days.

For morphological assessment of colonies, the whole agar layer was fixed with 2% glutaraldehyde, detached from the dish, and dried on a glass slide as described by Salmon and Buick (24). Colonies were stained either with May–Grunwald–Giemsa or with cytochemical stains for specific and nonspecific esterase (25).

Suspension Culture of PBL. Peripheral blood, low-density, nonadherent cells were cultivated at 1.5×10^6/ml in Iscove's modified Dulbecco's medium (GIBCO) containing 20% fetal bovine serum and either 10% GCT-conditioned medium or 10% leukocyte-conditioned medium as sources of GM-CSF. Aliquots of cells were taken at day 0 and at intervals of 2 to 3 days. The concentration of CFU-GM in the cell suspension was determined and their reactivity with monoclonal antibodies was measured by the complement-dependent cytotoxicity test.

RESULTS

Fig. 1 summarizes the reactivity of the five antibodies with hemopoietic cells as determined by cell sorting. R1B19 and WGHS-29-1 react with all cells of the granulocytic lineage and S4-7 also reacts with monocytes (18, 19). Antibodies S3-13 and S17-25 react with blast cells and with lymphocyte and monocyte subsets. Separation of the lymphocytic population into T and non-T lymphocytes by double erythrocyte rosetting (data not shown) indicates that S3-13 also binds to a large subset of T lymphocytes (unpublished data), whereas S17-25 binds to both T and non-T lymphocytes.

Phenotypic Analysis of Bone Marrow CFU-GM. Table 1 shows the reactivity of bone marrow CFU-GM with the five monoclonal antibodies as determined by the complement-mediated cytotoxicity test. More than 90% of the myeloid progenitors that formed colonies after 7 days of culture (day 7 CFU-GM) were killed by treatment with R1B19, WGHS-29-1, S4-7, or S3-13, and more than 90% of day 14 CFU-GM were killed by treatment with S3-13. However, day 14 CFU-GM were less sensitive to treatment with R1B19, WGHS-29-1, and S4-7, which inhibited an average of 57%, 68%, and 70% of these cells, respectively. S17-25 antibody, by contrast, inhibited only some of day 7 CFU-GM but inhibited almost all day 14 CFU-GM. However, the difference in reactivity between day 7 and day 14 CFU-GM was less evident when high concentrations of S17-25 antibody were used (ascitic fluid at dilutions <1:200). In these experiments, 70–75% of day 7 CFU-GM evidenced reactivity. This might indicate that most day 7 CFU-GM actually express the antigen recognized by S17-25 antibody but probably in a smaller amount than day 14 progenitors, so that higher concentrations of antibody are required to effect complement activation. Inhibition by S17-25 was greater on colony-forming cells than on clusters. No differences were detected in the degrees of inhibition between colonies and clusters with other antibodies tested on any of the scoring days. Morphological analysis showed that neutrophilic, macrophagic, eosinophilic, and mixed colonies were inhibited to a similar degree by R1B19, WGHS-29-1, S4-7, and S3-13 antibodies. However, S17-25 reacted with 40–60% of day 14 macrophagic CFU compared with 95–100% of day 14 granulopoietic progenitors. The growth pat-

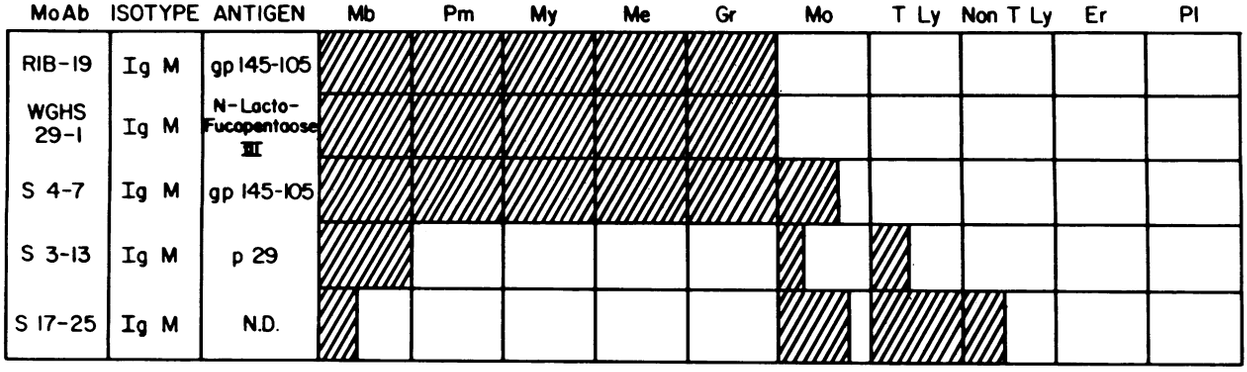

FIG. 1. Reactivity of monoclonal antibodies (MoAb) with peripheral blood and bone marrow cells. The cell types reactive with the indicated antibodies were identified morphologically after sorting of the positive and negative populations. The percentage of positive cells in each subpopulation is indicated by the extent of filling of each rectangle. Mb, myeloblasts; Pm, promyelocytes; My, myelocytes; Me, metamyelocytes; Gr, granulocytes; Mo, monocytes; T Ly, T lymphocytes; Non T Ly, non-T lymphocytes; Er, erythroid cells; Pl, platelets. N.D., not determined.

Table 1. Reactivity* of mouse anti-human monoclonal antibodies on bone marrow CFU-GM

Pretreatment	Day 7		Day 14	
	Colonies†	Clusters‡	Colonies†	Clusters‡
Complement (C) only	98 ± 5	99 ± 4	96 ± 6	103 ± 8
R1B19 + C	6 ± 6	13 ± 10	41 ± 26	52 ± 21
WGHS-29-1 + C	8 ± 4	11 ± 6	31 ± 12	39 ± 20
S4-7 + C	9 ± 5	2 ± 2	29 ± 18	30 ± 9
S3-13 + C	2 ± 2	3 ± 2	3 ± 3	4 ± 2
S17-25 + C	49 ± 4	76 ± 3	9 ± 2	34 ± 6
Antibodies only	101 ± 7	98 ± 4	102 ± 3	98 ± 5

* Growth as a percentage of control cultures (100%). Values are means ± SEM of at least three experiments with different bone marrow samples. The number of colonies in control cultures (mean ± SEM of at least three experiments with different bone marrows) was 140 ± 35 on day 7 and 128 ± 32 on day 14. The number of clusters was 220 ± 45 and 92 ± 28 on days 7 and 14, respectively.
† Aggregates containing 40 or more cells.
‡ Aggregates containing 4–39 cells.

terns of cells treated with complement alone, with antibodies alone, or with medium alone were similar.

Further characterization of the bone marrow CFU-GM phenotype was carried out with the five antibodies by using immunofluorescence and flow cytofluorimetry. Positive (fluorescent) and negative (nonfluorescent) cells were separated and collected sterilely, and their colony-forming ability was determined. The percentage of contaminating negative cells in the positive fraction was always <7%. The results of this experiment (Table 2) confirm the results obtained by the complement-mediated cytotoxicity test with R1B19, WGHS-29-1, S4-7, and S3-13. Essentially all the day 7 and day 14 marrow CFU-GM were detected by S3-13, but only a fraction of the day 14 marrow CFU-GM were recognized by R1B19, S4-7, or WGHS-29-1. In the sample treated with S17-25, almost all day 7 and day 14 colony-forming cells were found in the nonfluorescent cell fraction. This finding is at variance with the results in the cytotoxicity assay in which S17-25 reacted with almost all day 14 CFU-GM.

Table 2. Marrow CFU-GM after fluorescence-activated cell sorting

Fraction	Day 7		Day 14	
	% colonies	% clusters	% colonies	% clusters
R1B19:				
Positive cells	94 ± 6	86 ± 13	55 ± 30	61 ± 19
Negative cells	6 ± 6	14 ± 13	45 ± 30	39 ± 19
WGHS-29-1:				
Positive cells	98 ± 2	90 ± 10	70 ± 10	76 ± 9
Negative cells	2 ± 2	10 ± 10	30 ± 10	24 ± 9
S4-7:				
Positive cells	99 ± 1	93 ± 7	72 ± 17	75 ± 20
Negative cells	1 ± 1	7 ± 7	28 ± 17	25 ± 20
S3-13:				
Positive cells	91 ± 9	89 ± 10	95 ± 4	92 ± 7
Negative cells	9 ± 9	11 ± 10	5 ± 4	8 ± 7
S17-25:				
Positive cells	5 ± 2	8 ± 4	9 ± 3	12 ± 4
Negative cells	95 ± 2	92 ± 4	91 ± 3	88 ± 5

Results are given as percentage of CFU-GM present in the unfractionated population that are positive or negative when tested with the indicated antibody. Values are means ± SD of three experiments with different bone marrows. On day 7, the number of CFU-GM ranged between 105 and 196 per 10^5 light-density, nonadherent cells in the unfractionated population for colonies and between 147 and 285 for clusters. On day 14, colonies ranged between 52 and 114 and clusters between 66 and 129. The percentage of CFU-GM lost during the passage of cells through the cell sorter was 20 ± 16% for day 7 CFU-GM and 35 ± 12% for day 14 CFU-GM.

This discrepancy might be explained by the greater sensitivity of the cytotoxicity assay compared to the fluorescent assay and suggests that the antigenic determinant recognized by S17-25 is not abundant on CFU-GM cells.

Reactivity of Peripheral Blood CFU-GM with Monoclonal Antibodies. The phenotype of peripheral blood CFU-GM was markedly different from that of bone marrow CFU-GM (Table 3). Peripheral blood CFU-GM were not affected by treatment with either R1B19 or WGHS-29-1 in a complement-dependent cytotoxicity assay. However, 40–60% of these cells were killed by treatment with S4-7 and an even greater inhibition of colony growth (90–95%) was observed by treatment with S3-13 or S17-25. Treatment with complement alone or with antibodies alone did not inhibit the growth of day 10 peripheral blood CFU-GM. (Peripheral blood colonies could not be scored on day 7 of culture because they became evident only after 10 days). No significant differences were observed in the inhibition of colonies scored at day 10 and at day 14. Morphological examination revealed that almost all (95–98%) peripheral blood CFU-GM produced granulocytic colonies. The possibility that the phenotype of peripheral blood CFU-GM represents peculiar characteristics of granulocytic progenitors is ruled out by the observation that bone marrow progenitors, which give rise to granulocytic colonies, react with R1B19, WGHS-29-1, and S4-7 monoclonal antibodies to the same extent as do bone marrow CFU-GM, which generate mixed or macrophagic colonies.

Because antibodies S3-13 and S17-25 also react with T lymphocytes (unpublished data), we investigated whether or not inhibition of CFU-GM growth could be ascribed to the killing of accessory T lymphocytes. PBL were depleted of granulocytes (16), of monocytes (26), and of T cells (17). The depletion of T lymphocytes was done by erythrocyte rosetting twice; <1% of rosetting cells remained in the preparation. The depleted leukocytes were tested in a CFU-GM assay system after treatment with the various antibodies in the presence of complement. The results in Table 3 indicate that antibodies S3-13 and S17-25 almost totally inhibited CFU-GM colony formation even in the absence of T cells.

Evolution of the CFU-GM Phenotype *in Vitro*. Experiments were done to determine whether peripheral blood CFU-GM

Table 3. Reactivity of mouse anti-human monoclonal antibodies on peripheral blood CFU-GM before and after removal of T lymphocytes

Pretreatment	With T lymphocytes		No T lymphocytes	
	Colonies	Clusters	Colonies	Clusters
Complement (C) only	94 ± 15	93 ± 14	100 ± 8	113 ± 13
R1B19 + C	97 ± 5	94 ± 6	ND	ND
WGHS-29-1 + C	102 ± 20	104 ± 7	ND	ND
S4-7 + C	37 ± 25	49 ± 28	61 ± 12	57 ± 13
S3-13 + C	8 ± 7	10 ± 10	2 ± 1	3 ± 2
S17-25 + C	1 ± 1	1 ± 1	21 ± 15*	18 ± 16*

Colonies and cluster growth, scored 10 days after plating, as a percentage of control cultures. Values are means ± SEM of at least three experiments with different blood samples. The number of colonies and clusters in control cultures (mean ± SEM of at least three different blood samples) was 56 ± 10 and 47 ± 12, respectively, for total mononuclear leukocytes and 132 ± 13 and 113 ± 12, respectively, for T-depleted leukocytes. ND, not determined.
* The high values were generated in one experiment in which the extent of inhibition was markedly less than that in the other two experiments.

with a surface phenotype S3-13$^+$, S17-25$^+$, S4-7$^\pm$, R1B19$^-$, and WGHS-29-1$^-$ could give rise to myeloid progenitor cells with phenotype S3-13$^+$, S17-25$^\pm$, S4-7$^+$, R1B19$^+$, and WGHS-29-1$^+$, similar to that found in the marrow. PBL depleted of granulocytes and monocytes were cultivated in suspension cultures for several days in the presence of GM-CSF.

The total number of CFU-GM (colony-forming plus cluster-forming cells) per ml of suspension culture increased during the first 5 days of culture to a peak level of about 240% of the initial value (Fig. 2*A*). The increment was more evident in cluster-forming than in colony-forming cells, the latter increasing up to 160% of the initial value. After 5 days, a decrease was observed in the concentration of both colony-forming and cluster-forming cells. Morphologically, the colonies did not differ significantly from colonies derived from cells plated on day 0.

The complement-mediated cytotoxicity assay with monoclonal antibodies showed that most of the peripheral blood CFU-GM could acquire *in vitro* the phenotype expressed by bone marrow CFU-GM (Fig. 2*B*). After 7 days of culture, >90% of myeloid progenitor cells reacted with S4-7 and WGHS-29-1 and 67% reacted in addition with R1B19. The proportion of CFU-GM recognized by S17-25 gradually decreased from 96% on day 0 to 43% on day 7. No significant differences could be detected in the surface phenotype of colony-forming and cluster-forming cells, and results were similar when leukocyte- or GCT-conditioned medium was used as a source of GM-CSF.

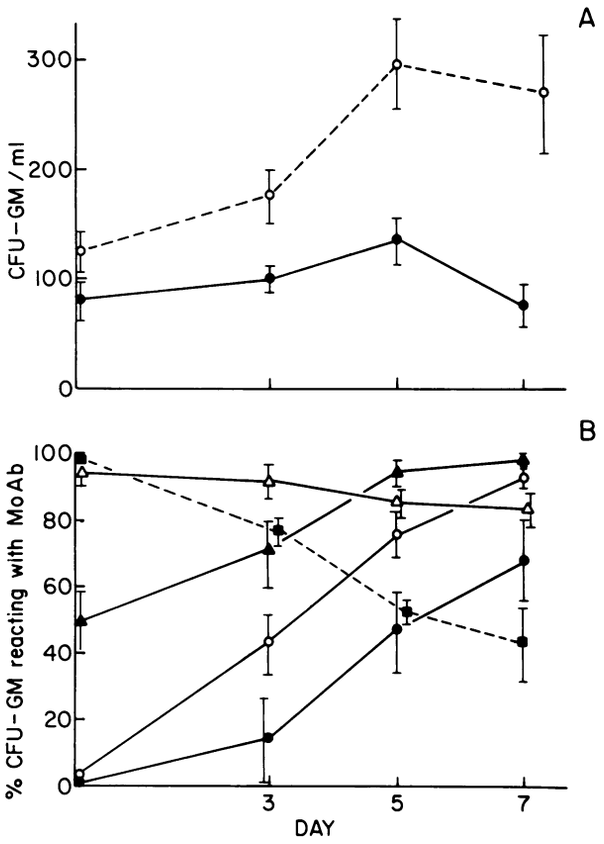

FIG. 2. (*A*) Variation in the concentration of CFU-GM in peripheral blood cell suspension during 7 days of culture in liquid medium. Values are means ± SD of at least three experiments with different blood samples. ●, Colony-forming cells; ○, colony-forming plus cluster-forming cells. (*B*) Percentage of peripheral blood CFU-GM reacting with monoclonal antibodies at different days in liquid medium. Values are mean (± SD) percentage inhibition of colonies plus clusters in cultures treated with antibodies and complement compared to cultures treated with complement alone. ●, R1B19; ○, WGHS-29-1; ▲, S4-7; △, S3-13; ■, S17-25.

DISCUSSION

Mouse anti-human monoclonal antibodies were used to define the phenotype of CFU-GM able to give rise *in vitro* to colonies of granulocytes and monocytes-macrophages. Table 4 summarizes the results of our study. The antigens recognized by antibodies R1B19, WGHS-29-1, and S4-7, which are expressed by morphologically recognizable cells of the myelomonocytic lineage, are also expressed by most bone marrow CFU-GM.

Cell composition of the colonies formed *in vitro* did not correlate with the pattern of reactivity of the antibodies with different cells. In particular, antibodies with different specificities for mature granulocytes and monocytes inhibited the growth of granulocytic and monocytic progenitors to the same extent.

The pattern of reactivity of four monoclonal antibodies to bone marrow and peripheral blood CFU-GM was consistent with the presence of antigenically different subpopulations of myeloid progenitors.

The results of the cell sorting analyses with R1B19, WGHS-29-1, S4-7, and S3-13 confirmed the data obtained in the cytotoxicity assay. However, in contrast to the data obtained in the cytotoxicity test, treatment with S17-25 resulted in only a small proportion of CFU-GM among fluorescent cells. Because antibody S17-25 in the presence of complement kills only a small fraction (about 10%) of marrow cells, which does not include mature myeloid cells, it seems unlikely that the lysis of these cells could secondarily determine the killing of CFU-GM. It is also unlikely that inhibition of colony growth by S17-25 and complement might be determined by the killing of stimulatory lymphocytes or monocytes. In fact, we and others (11) have shown that peripheral blood CFU-GM grow well even after depletion of monocytes and T cells. Moreover, after separation of bone marrow cells with the cell sorter, normal growth of CFU-GM is observed in fractions reactive with R1B19 and WGHS-29-1, which are virtually depleted of both lymphocytes and monocytes. It is possible that the antigen recognized by S17-25, which is not present on more mature granulopoietic cells, is expressed at low levels on the surface of CFU-GM so that few molecules of the antibody bind to these cells. This binding could be sufficient to activate complement but not sufficient to give to CFU-GM a fluorescence of intensity above the threshold of cell sorter.

It had been reported that bone marrow CFU-GM that form colonies after 7 days of culture and those requiring 14 days to grow and terminally differentiate derive from different progenitors cells (9, 27). In those studies it was shown that day 7 CFU-GM have a higher sedimentation rate (6.4–8.2 mm/hr) (9, 27) and higher proliferative activity (about 50% in S phase) than day 14 CFU-GM (sedimentation rate, 5.5–6.4 mm/hr; about 20% of cells in S phase) (9). The rapidly sedimenting CFU-GM are also more sensitive to stimulation by GM-CSF (27). It has

Table 4. Reactivity* of human CFU-GM to mouse anti-human monoclonal antibodies

Antibody	CFU-GM†		
	Peripheral blood	Bone marrow day 14	Bone marrow day 7
R1B19	−	±	+
WGHS-29-1	−	±	+
S4-7	±	±	+
S3-13	+	+	+
S17-25	+	±(−)	±(−)

* As determined by complement-mediated cytotoxicity and by cell sorting analysis.

† Data are given as: +, >80% reactivity; ±, 20–80% reactivity; −, <20% reactivity. Only the discordant results obtained by cell sorting analysis are given in parentheses.

been suggested that day 7 CFU-GM derive from day 14 progenitor cells (28). Peripheral blood CFU-GM have an even lower sedimentation rate (peak at 4 mm/hr) and proliferative activity (<10% in S phase) (8).

On the basis of the surface phenotype, we have now distinguished two subpopulations of myeloid progenitors. Type 1 CFU-GM represent the entire population of circulating CFU-GM with phenotype S3-13$^+$, S4-7$^\pm$, R1B19$^-$, WGHS-29-1$^-$, S17-25$^+$. Type 2 CFU-GM, with phenotype S3-13$^+$, S4-7$^+$, R1B19$^+$, WGHS-29-1$^+$, S17-25$^\pm$, represent all of the bone marrow day 7 colony-forming cells. Bone marrow day 14 CFU-GM, with phenotype S3-13$^+$, S4-7$^\pm$, R1B19$^\pm$, WGHS-29-1$^\pm$, S17-25$^\pm$, probably represent a mixture of the two populations. Because some day 7 colonies reportedly survive until day 14 of culture (27), they could be scored as deriving from the day 14 progenitors and could also contribute to the heterogeneity of the day 14 CFU-GM phenotype. Because type 1 CFU-GM lack antigens expressed by all differentiating myeloid cells, they most likely represent a population of more immature progenitors, an idea supported by the finding that type 1 CFU-GM can acquire the phenotype of type 2 CFU-GM when grown in suspension culture in the presence of GM-CSF. It was possible to demonstrate that, in those conditions, CFU-GM undergo proliferation with no loss of colony-forming ability upon transfer to semisolid medium. During this *in vitro* proliferation, almost all peripheral blood CFU-GM gradually acquired the same surface phenotype as bone marrow type 2 CFU-GM.

The changes in surface markers of peripheral blood CFU-GM during the 7 days of suspension culture also could be explained by the selective growth in this culture of a population of type 2 CFU-GM present on day 0 but too small (<10% of CFU-GM) to be detected in the cytotoxicity test. However, in that case, one would have to hypothesize a capability of type 2 CFU-GM for extensive proliferation (at least four or five divisions) with no loss of colony-forming ability upon transfer to agar because the total number of CFU-GM increased during the first 5 days of culture. In addition, because the type 2 CFU-GM coordinately express each of the three antigens recognized by antibodies R1B19, WGHS-29-1, and S4-7, a synchronous increase in the reactivity should signal the emergence of such a population in culture of these antibodies. However, the reactivity of antibodies S4-7 and WGHS-29-1 with CFU-GM increased earlier than that of R1B19, which never reacted with these myeloid progenitors to the same extent as did S4-7 and WGHS-29-1. Although not definitive, the results do strongly suggest that the changes in the reactivity of these monoclonal antibodies to peripheral blood CFU-GM cultivated in liquid medium are determined by a process of differentiation rather than of selection.

We suggest therefore that type 1 CFU-GM represents a more immature myeloid progenitor circulating in the peripheral blood and constituting a small, variable fraction of the total bone marrow pool of CFU-GM (15–65% of day 14 CFU-GM). These progenitor type 1 CFU-GM differentiate into type 2 CFU-GM which no longer circulate and which constitute the majority of the marrow pool of CFU-GM—i.e., a proportion of day 14 and all of day 7 colony-forming cells.

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