Recombinant Human Macrophage Colony-Stimulating Factor in Nonhuman Primates: Selective Expansion of a CD16+ Monocyte Subset With Phenotypic Similarity to Primate Natural Killer Cells


The CD16 receptor (FcyRIII) is found on many tissue macrophages (Mφs), but its expression on circulating monocytes is restricted to a small, phenotypically distinct subset. The number of these CD16+ monocytes may be markedly increased in response to sepsis, human immunodeficiency virus infection, or metastatic malignancy. We have recently shown that the CD16+ monocyte population is selectively expanded by administration of recombinant human macrophage colony-stimulating factor (rhM-CSF). In the current study, we used the highly rhM-CSF-responsive cynomolgus primate model to further characterize this novel monocyte population. Animals treated with rhM-CSF underwent a progressive and essentially complete conversion to the CD16+ monocyte phenotype, with up to a 50-fold increase in the number of CD16+ cells. This increase was paralleled by the emergence of a population of circulating cells that morphologically resembled large granular lymphocytes (LGLs). However, quantitatively, this population corresponded closely to the number of CD16+ monocytes, and fluorescence-activated cell sorting (FACS) confirmed that they were the same. In addition to their LGL-like morphology, many rhM-CSF-induced CD16+ monocytes showed a pattern of size, granularity, and quantitative cell surface marker expression that closely resembled the pretreatment LGL/natural killer (NK) cell population but that did not resemble the pretreatment monocyte population. However, rhM-CSF-induced CD16+ monocytes could be distinguished from LGL/NK cells by fact that they all expressed cell surface receptors for rhM-CSF, and many of them showed reduced but detectable phagocytic and respiratory burst activity. Studies of human subjects treated with rhM-CSF also showed an analogous population of “LGL-appearing” CD16+ mononuclear cells. Thus, our studies reveal a previously unsuspected ability of cells in the monocyte lineage to adopt a phenotype similar to that of LGL/NK cells. The extent of this phenotypic convergence suggests that the two lineages retain access to elements of a similar developmental pathway.

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Complete blood counts (CBCs). Samples from cynomolgus monkeys were analyzed on a Becton 9000 hematology analyzer (Serono-Baker, Allentown, PA) with a veterinary analysis package and discriminators set for monkeys. Differential cell counts were performed on Wright-stained peripheral blood (PB) smears by a hematologist familiar with cynomolgus samples (D.H.M.). The morphological criteria for primate monocytes, lymphocytes, and large granular lymphocytes (LGLs) were those described by Huse25 and corresponded closely to their respective human counterparts. Human specimens for differential cell counts were processed as routine samples in our clinical hematology laboratory. Each sample was analyzed independently in blinded fashion by two or three certified hematology technicians.

Isolation of PB mononuclear cells (PBMCs). Heparinized whole blood was collected from animals under sterile conditions and shipped at room temperature by overnight courier. The blood was diluted 2:1 in sterile RPMI 1640 medium with 3% dextran (Sigma Chemical Co, St Louis, MO) and sedimented twice at unit gravity. The leukocyte-rich plasma was collected, washed, and centrifuged over a hypertonic (320 mOsm) 2-step Percoll (Pharmacia, Piscataway, NJ) gradient of specific gravities 1.064 and 1.079. Platelets were trapped at the first interface, and PBMCs were collected from the second interface. This protocol effectively separated PBMCs from granulocytes and erythrocytes, as confirmed by Wright's stain.

Immuno phenotype analysis. For 3-color analysis, mononuclear cells were isolated as described above and unconjugated monoclonal antibodies (MoAbs) against the following determinants were used in conjunction with a third-color secondary antibody (Red613; Gibco-BRL, Gaithersburg, MD) as previously described:26 CD2, CD1a, CD11b, CD13, CD33, CD57, HLA-DR, and isotype-matched negative controls (all from Amac, Westbrook, ME); CD56 (Coulter Corp, Hialeah, FL); and Fcy-R-II/ID32 and Fcy-R-ICD64 (Medarex, West Lebanon, NH). First-color staining was with fluorescein-conjugated anti-CD14 (clone 3G8; Amac), and second-color staining was with phycoerythrin (PE)-conjugated anti-CD14 (clone M4; Coulter).

For direct 2-color analysis, fluorescein-conjugated antibodies against CD16, CD11b and Fcγ-R-II were used with PE-conjugated CD14. PE-conjugated CD56 was used with fluorescein-conjugated CD14. Staining was performed using a commercial single-step, whole blood lysis technique (FACS-Lyse; Becton Dickinson, San Jose, CA).

Standard criteria of forward and right-angle light scatter were used to establish expected analysis gates for monocytes and lymphocytes as previously described.26 In untreated animals, cells classified as monocytes by light scatter criteria expressed high levels of CD14 and showed typical monocyte morphology when isolated by cell sorting (see Results). Cells identified as LGLs by light scatter were predominantly CD16- and uniformly CD14+ and showed the expected morphology of lymphocytes after sorting. After rhM-CSF treatment, CD16+ mononuclear cells formed a discrete population overlapping the lower range of the monocyte gate and the LGL gate (thus, a combined gate was used for rhM-CSF–treated animals). Light scatter gates were used only to confirm the size and granularity properties of the various populations.

The absolute number of CD16+CD14+ cells was calculated as the percentage of total leukocytes that were CD16+CD14+ by fluorescence-activated cell-sorting (FACS) analysis multiplied by the white blood cell count on CBC from the same sample.

A panel of 11 MoAbs against the human CD56 antigen, contributed by various investigators to the Fifth International Leukocyte Typing Workshop,26 was the generous gift of J. Ritz (Dana Farber Cancer Institute, Boston, MA). Eight of these MoAbs were found in our hands to cross-react with primate cells, and each of these confirmed the expression of CD56 on both monkey NK cells and monocytes. To facilitate reproduction by other investigators, the data presented are those obtained with a commercially available anti-CD56 antibody, NKH-1 (Coulter).

Respiratory burst and phagocytosis assays. Sorted CD16+CD14+ cells were placed in tissue culture wells and activated with 100 ng/mL phorbol myristate acetate (PMA; Sigma) for 30 minutes in the presence of 0.5 mg/mL nitroblue tetrazolium (NBT; Sigma), as previously described.21 Cells were scored for the presence or absence of formazan reaction product using an inverted microscope. To correlate respiratory burst with immunophenotype, in other experiments fresh PBMCs were incubated for 15 minutes at 37°C with dihydrothrombin-123 (DHR-123; 10 μg/mL; Molecular Probes, Eugene, OR), a substrate that becomes fluorescent when oxidized by superoxide anion. This probe has been used to measure respiratory burst in human and murine granulocytes and Mφs.30 DHR-123–labeled cells were counterstained with CD14-PE and analyzed by flow cytometry.

To assess phagocytosis, sorted CD16+CD14+ cells were incubated overnight with antibody-opsonized iron microparticles (Advanced Magnetics, Cambridge, MA). Cells were harvested for cytocentrifuge preparations, and ingested iron was shown by Prussian-Blue iron stain (Sigma Diagnostics). The intense blue reaction product generated by this stain allowed ready and unambiguous detection of ingested particles. Using this assay, greater than 90% of human monocytes showed phagocytosis. Two experimental artifacts potentially interfered with this assay in the primates, ie, the fact that (1) sorted cells tended to lose viability in culture, as described above, and (2) phagocytosis of large numbers of iron particles rendered the cells fragile and liable to lysis during the cytocentrifuge process. However, because both of these artifacts would tend to reduce rather than increase the measured phagocytosis, we report the data as a real, if probably conservative, estimate of the presence of phagocytic activity.

Binding of fluorescein-labeled rhM-CSF. Fluoresceinated rhM-CSF was prepared by incubating purified rhM-CSF with a 10:1 molar ratio of fluorescein 5-isothiocyanate (Molecular Probes) for 30 minutes in 0.1 mol/L NaH2CO3 buffer, pH 8.35. Fresh whole blood was incubated with 1 μg/mL fluoresceinated rhM-CSF for 60 minutes at 4°C and then immediately fixed with 0.5% paraformaldehyde to prevent internalization of bound receptors. PBMCs were isolated over a percoll gradient as described above and were analyzed by flow cytometry. To show specificity of staining, duplicate samples were incubated with fluoresceinated rhM-CSF in the presence of a 100-fold molar excess of unlabeled rhM-CSF, which completely abrogated binding of labeled ligand.

The approach described, which is based on that used by Byrne et al31 to study M-CSF receptor expression, was chosen over immunofluorescent staining with MoAb against the M-CSF receptor (c-fms) because: (1) the labeled–cytokine technique was significantly more sensitive in our hands; (2) in preliminary experiments, we were unable to detect cynomolgus monkey c-fms using MoAb raised against the human protein; and (3) the use of unlabeled-competitor controls with fluoresceinated rhM-CSF provided an important functional confirmation that the signal generated by our assay reflected true physiological binding of rhM-CSF.
rhM-CSF treatment induces a morphologically ambiguous population of mononuclear cells. Cynomolgus monkeys were treated for 14 days with rhM-CSF. Monocytes, as defined by classical morphological criteria on PB smears, reached a peak on days 5 to 7 of treatment and then rapidly decreased to baseline values (Fig 1A). In previous studies, we had attributed the transient nature of this monocytosis to tachyphylaxis, but we now noted that the reduction in monocytes was offset by the emergence of a new population of mononuclear cells (Fig 1B). These cells, which we had initially classified as atypical lymphocytes and LGLs, were found on closer inspection to possess an ambiguous morphology, showing a range of features between the morphologies of LGLs and monocytes (Fig 2). Because this population remained elevated throughout rhM-CSF treatment, we speculated that these cells might be of monocytic rather than lymphoid origin.

Cells with ambiguous morphology correspond to a population of CD16+/CD14- cells. In untreated animals, the markers CD14 and CD16 identified two distinct mononuclear cell populations: CD14+ cells that were CD16- and fell within the monocye gate by light scatter (see Materials and Methods for gating criteria), and CD16+ cells that were CD14- and fell within the LGL gate by light scatter. When these two populations were isolated by FACS sorting, the CD16+ cells showed the expected morphology of monocytes, whereas the CD16- cells were large lymphocytes (see Fig 3, R1 and R2).

However, in animals treated with rhM-CSF, we observed a transitional pattern of CD14 and CD16 expression. This intermediate pattern was clearly evident after 7 days of treatment (Fig 3, day 7) and by day 14 had progressed to the point that virtually all of the classically monocytic CD14+ cells had been replaced by CD16+/CD14- and CD16+/CD14+ cells. When we compared the absolute number of CD16+ cells in rhM-CSF–treated animals with the morphologically ambiguous cells identified on PB smears (Fig 1), we found that the two cell types corresponded closely in 12 of 12 samples analyzed simultaneously by FACS and CBC (correlation coefficient, r = .94 by Pearson's product moment; linear relationship, slope = 1.2; P < .001). This was true both for serial samples from individual animals and for the pooled data from all animals.

When the CD16+/CD14- cells were sorted, they were found to show intermediate morphological characteristics, ranging from cells with monocytoid features to those with a classically lymphoid appearance, with many cells showing a mixture of features (Fig 3, day 14 sorted R3). Because monocytes and lymphocytes had been quite distinct morphologically before rhM-CSF treatment, the morphology of the sorted CD16+ cells further confirmed that they represented the ambiguous cells observed on PB smears.

Immunophenotypic resemblance of rhM-CSF–induced monocytes to LGL/NK cells. To characterize the immunophenotype of the rhM-CSF–induced CD16+ cells, we turned to 3-color flow cytometry. We first established the phenotype of the monocyte and LGL/NK populations before treatment with rhM-CSF. This was performed by separately gating the CD14+/CD16- (monocyte) and CD14-/CD16+ (NK/LGL) cells based on 2-color immunofluorescence and then analyzing additional antigens in a third color. As shown in Table 1, the two populations differed significantly in the expression of a number of markers. In addition to the differences in CD14 and CD16 already described, pretreatment monocytes expressed CD11b, FcyR-II, and HLA-DR at high levels, whereas pretreatment CD16+ LGL/NK cells did not express FcyR-II and expressed CD11b and HLA-DR at levels an order of magnitude lower than those for monocytes. Many LGL/NK cells were also found to express the NK surface antigen CD56 and the lymphoid marker CD2, a pattern similar to the phenotype of human NK cells.

During treatment with rhM-CSF, the pattern of antigen expression on the transitional CD14+/CD16+ cells progressively diverged from that of pretreatment monocytes, coming eventually to closely resemble the pretreatment pattern of LGL/NK cells (Table 1). This was true for all informative markers tested except for the lymphocyte-specific marker CD2, which was not expressed on the rhM-CSF–induced cells. This shift toward an immunophenotype resembling LGL/NK cells was a coordinated process, affecting multiple antigens simultaneously. As shown in Fig 4, after rhM-CSF treatment, the few remaining CD14+ cells also showed the levels of CD16, CD11b, and FcyR-II characteristic of those for pretreatment monocytes. In contrast, the population of cells expressing reduced levels of CD14 showed a coordinated shift in CD16, CD11b, and FcyR-II, in each case toward levels identical to those for pretreatment LGL/NK cells. This change was progressive over the course of rhM-CSF treatment, and at each time point there was a transitional population linking the CD14+ cells to the remaining CD14+ cells.

CD56 expression on monocytes and LGL/NK cells. In humans, the CD56 antigen is a useful marker for NK cells because it is not expressed on other lymphoid or myeloid cells. In cynomolgus monkeys, however, we found that
CD56 was constitutively expressed on both LGL/NK cells and monocytes (Fig 5), paradoxically at even higher levels on monocytes. Although CD56 was therefore not specific for NK cells in the primate system, there was a clear difference between the levels characteristic of monocytes and those of LGL/NK cells. Consistent with the patterns shown in Fig 4, after rhM-CSF treatment the few residual CD14<sup>+</sup> monocytes retained their high level of CD56 expression, whereas the rhM-CSF–induced CD16<sup>+</sup>/CD14<sup>−</sup> population now expressed CD56 at the level characteristic of pretreatment LGL/NK cells (Fig 5).

Many rhM-CSF–induced CD16<sup>+</sup> cells are phagocytic and show respiratory burst activity. The preceding immunophenotype data, although suggestive, did not definitively establish the lineage derivation of the CD16<sup>+</sup> cells. Therefore, we assessed additional functional properties characteristic of monocyctic cells. As shown in Fig 6, we found that a large subset of the rhM-CSF–induced cells were capable of oxidizing the indicator dye DHR-123 after activation with PMA, reflecting respiratory burst activity. The presence of a respiratory burst was independently confirmed by performing NBT-reduction assays on sorted cell populations. Before rhM-CSF treatment, essentially all viable CD16<sup>+</sup>/CD14<sup>+</sup> monocytes were NBT-positive, whereas fewer than 1% of CD16<sup>+</sup>/CD14<sup>−</sup> NK cells were positive. After rhM-CSF, 48% to 54% of CD16<sup>+</sup>/CD14<sup>−</sup> cells reduced NBT, which is consistent with the DHR-123 data. In both assays, the response was somewhat attenuated compared with that for pretreatment monocytes. We obtained similar results with a second rhM-CSF-induced CD16<sup>+</sup>/CD14<sup>−</sup> population now expressed CD56 at the level characteristic of pretreatment LGL/NK cells of the monocytic lineage<sup>**</sup> and a small subset of rhM-CSF-induced CD16<sup>+</sup>/CD14<sup>−</sup> NK cells (Fig 5).

rhM-CSF–induced CD16<sup>+</sup> cells express receptors for rhM-CSF. Expression of M-CSF receptors (M-CSFRs) is quite restricted in mature hematopoietic cells, being confined to cells of the monocyctic lineage<sup>2</sup> and a small subset of activated B cells.<sup>23</sup> As shown in Fig 7, after rhM-CSF treatment, essentially all the CD16<sup>+</sup> mononuclear cells expressed M-CSFRs, measured as the ability to bind fluorescein-labeled rhM-CSF. M-CSFR expression was not related to CD14 expression, because the CD14-bright, -dim, and -negative populations all bound M-CSF identically. The preceding immunophenotype data, although suggestive, did not definitively establish the lineage derivation of the CD16<sup>+</sup> cells. Therefore, we assessed additional functional properties characteristic of monocyctic cells. As shown in Fig 6, we found that a large subset of the rhM-CSF–induced cells were capable of oxidizing the indicator dye DHR-123 after activation with PMA, reflecting respiratory burst activity. The presence of a respiratory burst was independently confirmed by performing NBT-reduction assays on sorted cell populations. Before rhM-CSF treatment, essentially all viable CD16<sup>+</sup>/CD14<sup>+</sup> monocytes were NBT-positive, whereas fewer than 1% of CD16<sup>+</sup>/CD14<sup>−</sup> NK cells were positive. After rhM-CSF, 48% to 54% of CD16<sup>+</sup>/CD14<sup>−</sup> cells reduced NBT, which is consistent with the DHR-123 data. In both assays, the response was somewhat attenuated compared with that for pretreatment monocytes. We obtained similar results with a second rhM-CSF-induced CD16<sup>+</sup>/CD14<sup>−</sup> population now expressed CD56 at the level characteristic of pretreatment LGL/NK cells (Fig 5).

Phenotype of rhM-CSF–induced monocytes in humans. We have previously shown that human subjects treated with rhM-CSF show a pattern of CD14 and CD16 expression very similar to that of primates.<sup>12</sup> Therefore, we examined materials from our clinical trials of rhM-CSF and asked whether there were mononuclear cells in these subjects that had a morphology analogous to those observed in primates. We found that morphologically ambiguous cells comprised up to 60% of the mononuclear cells on CBCs from humans treated with rhM-CSF (Fig 8). Furthermore, in 80% of the samples analyzed, enumeration of monocytes by conventional morphological criteria (CBC and differential cell count) failed to detect up to 65% of the true monocyte number, as shown by FACS analysis on the same sample (P < .0001 by Wilcoxon signed rank test; n = 24). FACS sorting of the CD16<sup>+</sup>/CD14<sup>−</sup> cells in humans confirmed their ambiguous morphology, as it had in the primates (data not shown). Thus, the unusual features of the rhM-CSF–induced CD16<sup>+</sup> population was not a peculiarity of the cynomolgus system.

In the human system, it was possible to perform more extensive immunophenotyping, because more antibodies were available and the pattern of markers for human monocytes and NK cells is well established. As shown in Table 2, the immunophenotype of the CD16<sup>+</sup>/CD14<sup>−</sup> cells in humans clearly suggested a monocyte origin (CD14<sup>+</sup>, CD13<sup>−</sup>, CD33<sup>+</sup>, FCγR-I<sup>+</sup>, FCγR-II<sup>−</sup>, HLA-DR<sup>−</sup>, CD56<sup>+</sup>, CD57<sup>−</sup>) and was distinct from the true NK cells present in the same samples.

**DISCUSSION**

It is only recently that CD16<sup>+</sup> monocytes have been recognized as a distinct subset with unique phenotypic and functional attributes.<sup>6,7</sup> The biological role of these cells is not yet clear, but the fact that they are preferentially expanded during overwhelming sepsis, while remaining at normal levels during monocytosis resulting from less acute disorders<sup>10,12</sup> suggests that they may be associated with specific states of pathophysiological stress. Consistent with this possibility, CD16<sup>+</sup> monocytes have recently been shown to produce the proinflammatory cytokine tumor necrosis factor after stimulation but not the anti-inflammatory mediator interleukin-10, suggesting that they may be primed for inflammatory responses.<sup>9</sup> We have recently reported that the presence of metastatic malignancy is, of itself, sufficient to induce a marked, constitutive elevation in CD16<sup>+</sup> monocytes, often to levels greater than those observed in sepsis.<sup>12</sup> Thus, the CD16<sup>+</sup> monocyte population may constitute a novel and previously unrecognized marker for the spontaneous host response to malignancy.

In previous studies, we have shown that rhM-CSF is a potent pharmacological inducer of CD16<sup>+</sup> monocytes both in humans<sup>12</sup> and in nonhuman primates.<sup>11</sup> The nonhuman primates in particular appeared highly responsive to rhM-CSF, undergoing a rapid and essentially complete shift to the CD16<sup>+</sup> phenotype during treatment. Using this primate model, we have now identified a novel phenotypic link between CD16<sup>+</sup> monocytes and cells of the LGL/NK lineage. The degree to which this phenotypic overlap can extend had not been previously appreciated in our human studies, in which the effects of rhM-CSF were more gradual and less complete (although a retrospective analysis of samples from these trials make it evident that the human CD16<sup>+</sup> monocytes closely resemble their primate counterparts both in morphology and CD14/CD16 antigen expression).

The phenotypic overlap between rhM-CSF–induced CD16<sup>+</sup> monocytes and NK cells was surprisingly extensive. In appearance, rhM-CSF–induced monocytes closely resembled their primate counterparts both in morphology and immunophenotype induced by rhM-CSF occurred coordinately.

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Fig 2. Morphology of mononuclear cells from animals receiving rhM-CSF. (A) Typical monocyte morphology (pretreatment sample), (B) typical large lymphocyte (pretreatment), and (C) and (D) rhM-CSF–induced cells with ambiguous morphology (day 14 of rhM-CSF) are shown. These cells constituted up to 60% of total mononuclear cells on day 14 and were present in 8 of 8 courses of rhM-CSF in four animals. Wright-stained PB smears, original magnification ×750.

Fig 3. Morphology of rhM-CSF–induced CD16⁺ mononuclear cells. Treatment with rhM-CSF led to a progressive shift from the CD16⁻/CD14⁺ monocyte phenotype to the CD16⁺/CD14⁻ phenotype, as shown in the upper panels. The individual populations shown in R1, R2, and R3 were FACS-sorted, and cytocentrifuge preparations were stained for morphology (lower panels). All populations were reanalyzed immediately after sorting and were greater than 95% pure (data not shown). Data from one of four representative experiments are shown. Wright stain, original magnification ×750.

Fig 8. Human subjects treated with rhM-CSF have circulating mononuclear cells with ambiguous morphology. Human subjects showed rhM-CSF–induced cells with the morphology of classical monocytes (*), but there were also many cells (up to 60% of total mononuclear cells) that were morphologically similar to those observed in the rhM-CSF–treated monkeys. Wright-stained PB smear, original magnification ×750.
shifting together from a pattern characteristic of pretreatment monocytes to a pattern closely resembling pretreatment NK cells. It would seem improbable that these multiple, coordinated changes would occur as unrelated events.

There are two possible interpretations of these data; either rhM-CSF induced monocyte precursors to follow a more "NK-like" differentiation pathway, or, alternatively, treatment with rhM-CSF caused NK cell precursors to adopt monocyteic features. Either of these hypotheses is tenable. On balance, we are inclined to favor the view that the M-CSF-induced cells are monocyteic in origin for the following reasons: (1) there was a progressive and gradual transition during treatment from a monocyteic to an "NK-like" phenotype; (2) many of the cells retained respiratory burst and phagocytic activity; (3) they expressed M-CSFRs, which has not been described in NK cells; and (4) the human counterparts of these cells were recognizably monocyteic. However, the morphology of many of the M-CSF-induced cells in our study closely resembled that of large agranular lymphocytes, which are the precursors of NK cells. Therefore, it remains possible that rhM-CSF might act, directly or indirectly, on both monocyte and NK cell precursors, and, in fact, the CD16+ population observed in the monkeys represented a mixture of cells derived from both lineages.

A connection between monocytes and NK cells has been proposed previously. However, the suggestion sometimes advanced in earlier studies that NK cells arise from the monocyteic lineage has been disproved by the identification of a common T-cell/NK-cell progenitor in fetal thymus, and the preponderance of recent evidence clearly links the NK lineage to that of T cells. 34-36 We agree with this assignment of NK cells to the lymphoid lineage, and we do not propose that rhM-CSF causes monocytes to "become" NK cells. However, our data do suggest that, under specific circumstances, cells of the monocyteic lineage retain access to a differentiation pathway similar to that of NK cells. The possibility of a developmental link between monocytes and NK cells is not a novel suggestion. Both cell types arise in the bone marrow and, at some point, are known to share a common precursor. 34 Recently, a subset of circulating NK cells has been shown to express both NK markers and the myelomonocytic antigen CD33, and a CD33+ NK progenitor cell has been shown in human bone marrow. 36 These observations reinforce the link between myeloid and NK lineages early in hematopoiesis, even though, similar to T and B cells, NK cells subsequently diverge into a separate lineage.

There is also at least a limited precedent for the concept of different lineages "converging" on a similar phenotype. B cells and M&d are known to possess a common bipotential progenitor cell, activated B cells can express both CD14  37
Fig 5. Expression of CD56 on primate LGLs and monocytes. Before treatment with rhM-CSF, cells falling in the light-scatter gate for monocytes were CD14^lo_ and coexpressed high levels of CD56. Pretreatment cells in the LGL light-scatter gate expressed lower levels of CD56 and were CD14^lo_. After treatment with rhM-CSF, a transitional pattern of CD56 expression was observed, similar to that of the antigens shown in Fig 4.

and functional M-CSF receptors, and murine splenocytes have been identified that simultaneously coexpress certain features of both B cells and M6s. However, to our knowledge the current report provides the most extensive example to date of convergent differentiation during hematopoiesis, in which cells from clearly separate lineages come to show similar or even identical phenotypic characteristics.

One prominent feature of the rhM-CSF-induced monocyte phenotype was the reduction in CD14 expression, an attribute that has also been described in human CD16^+ monocytes. Either of two mechanisms might be invoked to account for the change in CD14, constitutive down-regulation of the gene or shedding of CD14 protein from the cell surface. In our study, the coordinate changes in multiple surface antigens in addition to CD14 suggested that the reduction in expression was more likely to be part of an overall shift in phenotype, rather than simply a shedding of the antigen. This interpretation is consistent with work by Ziegler-Heitbrock et al in the human system indicating that CD16^+ monocytes show constitutively reduced levels of CD14 mRNA.

In our cynomolgus monkey model, we found that CD56 was expressed on both NK cells and monocytes. Although this was unexpected, it was unambiguously confirmed using eight different anti-CD56 antibodies. In untreated animals, the level of CD56 on monocytes was found to be 5- to 10-fold greater than that on NK cells. After rhM-CSF administration, those monocytes adopting the CD16^+/CD14^lo_ phenotype expressed CD56 at the levels characteristic of pretreatment NK cells, even though this represented a paradoxical downmodulation. Thus, in our study, the behavior of CD56 served both to emphasize the number of markers shared between the monocytic and NK lineages and also to reinforce the observation that CD16^+ monocytes expressed...
these genes at levels characteristic of NK cells rather than of monocytes.

We do not yet know whether the phenotypic resemblance between CD16+ monocytes and NK cells extends to functional activity as well. We have performed preliminary experiments using unfractionated PBMCs that show that administration of rhM-CSF for 14 days results in an approximately fourfold increase in antibody-independent (ie, NK-like) killing of human tumor cells (D.H. Munn, A.G. Bree, and A.C. Beall, unpublished observations). However, the inability of conventional FACS sorting techniques to isolate the CD16+ population in a long-term viable form (see Materials and Methods) made it impossible to determine whether the CD16+ monocytes were directly responsible for this cytotoxicity. Optimized isolation methods will be required before meaningful functional studies can be performed.

We propose that the similarity that we describe between rhM-CSF–induced monocytes and cells of the NK lineage is significant in three respects. First, given the potential for confusing the two phenotypes on routine hematologic analysis, our observations are of practical significance for the monitoring of clinical trials of rhM-CSF. More importantly, our data provide a novel and compelling example of the ability of two separate hematopoietic lineages to converge on a single developmental program. Finally, given the spontaneous elevation of CD16+ monocytes observed in response to human malignancy, our data raise the question of whether these monocytes may contribute to the host antitumor cytotoxic response in a fashion analogous to that of the NK cells that they resemble.

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Recombinant human macrophage colony-stimulating factor in nonhuman primates: selective expansion of a CD16+ monocyte subset with phenotypic similarity to primate natural killer cells

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