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Macrophage-colony-stimulating factor regulates expression of the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ by murine bone marrow macrophages

(cytokines/hematopoiesis/stromal matrix)

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ABSTRACT **We observed that when monocyte/macrophage precursors derived from murine bone marrow were treated with macrophage-colony-stimulating factor (M-CSF), there was a dose-dependent increase in both the number of adherent cells and the degree to which the cells were highly spread. Attachment was supported by fibronectin, but not by vitronectin or laminin, suggesting that the integrins $\alpha_4\beta_1$ and/or $\alpha_5\beta_1$ might mediate this event. Binding to fibronectin was blocked partially by antibodies to either integrin, and inhibition was almost complete when the antibodies were used in combination. By a combination of surface labeling with ^{125}I and metabolic labeling with [^{35}S]methionine and [^{35}S]cysteine, we demonstrated that M-CSF treatment led to increased synthesis and surface expression of the two β_1 integrins. Since attachment to fibronectin and/or stromal cells plays an important role in the maturation of other hematopoietic lineages, we propose that the action of M-CSF in the differentiation of immature monocytes/macrophages includes stimulated expression of the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$, leading to interactions with components of the marrow microenvironment necessary for cell maturation.**

Unlike other immature cells of the hematopoietic lineage (1–11), macrophage precursors are nonadherent. This is so both for authentic murine bone marrow precursors (12) and for transformed human cell lines, such as HL-60 and U-937, which adhere to the substrate when stimulated with 1α,25-dihydroxyvitamin D_3 (13–15), an agent which stimulates macrophage differentiation. The detailed mechanism(s) by which the cells attach is not known, but may involve integrins, a family of heterodimeric integral membrane proteins which mediate in cell–matrix interaction (16, 17). Hemonectin, a marrow-specific protein (18), supports attachment of granulocyte precursors (19), whereas fibronectin acts as a ligand for a wide range of cells in the hematopoietic lineage (1–8, 10, 11). As individual cell types differentiate, their capacity to bind to matrix proteins changes (1, 7–9, 12).

There are few reports on the binding of monocytic precursors to marrow components. These cells differentiate under the control of macrophage-colony-stimulating factor (M-CSF), a cytokine which is also needed for their survival and proliferation (20). Here we show that M-CSF regulates synthesis and surface expression of the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ by immature monocytes, resulting in increased attachment to fibronectin. Thus, our data provide evidence for a pathway by which M-CSF stimulates macrophage differentiation.

MATERIALS AND METHODS

Cell Isolation and Culture. Bone marrow macrophages were prepared as described (21) and cultured overnight in α minimal essential medium (α-MEM) containing 15% fetal bovine serum, with the addition of various concentrations of pure murine M-CSF (see below). Nonadherent cells were collected and layered on ice-cold horse serum for 15 min, at which time the top layer of cells was collected, layered again on horse serum, and centrifuged at 1000 × *g* for 7 min. The isolated macrophages were resuspended at 5×10^5 cells per ml in α-MEM with 15% fetal bovine serum and cultured in Teflon beakers for 24–72 hr, during which time they were exposed to a constant concentration of pure M-CSF in the range 1–1000 units/ml (1 unit = 0.44 pmol; ref. 22). The cells were centrifuged onto slides, fixed, and stained for nonspecific esterase activity (Sigma).

Cell Attachment Assay. Flat-bottom 96-well plates were coated overnight with human fibronectin or mouse vitronectin or laminin. Plates were rinsed with phosphate-buffered saline (PBS) and blocked with 10% denatured bovine serum albumin for 30 min. Equal numbers of cells, removed from the appropriate Teflon beaker and rinsed with α-MEM, were added to each well. After 45 min at 37°C in a CO_2 incubator, nonadherent cells were rinsed off and adherent cells were fixed with 2% formaldehyde for 20 min and stained with 1% methylene blue for 10 min. The stained cells were solubilized in 0.1 M HCl and the absorbance of the wells was read at 650 nm in a Titertek Multiscan ELISA reader (23). For the inhibition studies, cells cultured for 48 hr in Teflon beakers with M-CSF at 250 units/ml were isolated by centrifugation, rinsed, preincubated at 37°C for 30 min with nonimmune mouse IgG at 100 μg/ml (to block Fc receptors), and then incubated with antibodies to integrins $\alpha_4\beta_1$ and/or $\alpha_5\beta_1$ for a further 30 min. After washing with PBS, equal numbers of cells were added to the microwells and treated as described above.

Cell Labeling and Immunoprecipitation. After 72 hr of incubation, $3–5 \times 10^6$ cells were surface-radioiodinated as follows. The cells were washed three times and suspended in PBS, 1 mCi (37 MBq) of ^{125}I was added, and the reaction was started by the addition of α-D-glucose, glucose oxidase, and lactoperoxidase. After 10 min, the cells were rinsed three times with PBS and lysed by addition of radioimmunoprecipitation assay (RIPA) buffer [0.01 M Tris, pH 7.4/0.15 M NaCl/1% (wt/vol) sodium deoxycholate/1% (vol/vol) Triton X-100/0.1% (wt/vol) SDS/4 mM phenylmethanesulfonyl fluoride with aprotinin (Sigma catalogue no. A1153) at 16 μg/ml]. After 60 min at 4°C, cell extracts were centrifuged at 12,000 rpm in a microcentrifuge, supernatants were collected, and immune complexes were obtained from aliquots containing equal amounts of trichloroacetic acid-precipitable radioactivity, by incubating the appropriate volume with excess antibodies to $\alpha_4\beta_1$ or $\alpha_5\beta_1$, followed by 100 μl of a 50% suspension of protein G-Sepharose in PBS. The beads were washed twice each with RIPA buffer, PBS/0.5% Tween 20/0.1% ovalbumin, and

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Abbreviation: M-CSF, macrophage-colony-stimulating factor.

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PBS/0.5% Tween 20. Bound proteins were eluted and analyzed by SDS/PAGE in either 6% or 7.5% polyacrylamide gels under reducing or nonreducing conditions. Gels were stained with 0.1% Coomassie blue to confirm equal loading of antibody, dried, and subjected to autoradiography at −80°C with Kodak film.

Metabolic Labeling Studies. Cells obtained after 48 hr of culture were incubated with [^{35}S]cysteine/methionine (molar ratio ≈ 15/85) at 100 μCi/ml in cysteine- and methionine-free α-MEM containing 15% dialyzed fetal bovine serum. After 1 hr the medium was replaced with complete nonradioactive medium, and the incubation was continued for up to a further 12 hr, at which time the cells were lysed with 1 ml of RIPA buffer. Immune complexes were isolated from lysates containing equal amounts of cell protein as described above. After electrophoresis, the gels were treated with En^{3}Hance (New England Nuclear) and radioactivity was detected by fluorography at −80°C.

Antibodies. Nonimmune rat IgG was obtained from The Binding Site (San Diego). A rabbit polyclonal antibody against a fusion protein containing the murine α_5 subunit (24) was used without purification. An immunopurified polyclonal antibody against the cytoplasmic tail of human α_5 chain (provided by John McDonald, Washington University Medical Center, St. Louis) was used in some studies. The rat anti-murine α_4 monoclonal antibody R1-2 (25) was purified by protein G-Sepharose chromatography of medium of the hybridoma provided by Irving Weissman, Stanford University, CA. Mouse vitronectin and laminin were purchased from Telios Pharmaceuticals (San Diego). Human fibronectin was from Collaborative Research.

Miscellany. General reagents were obtained from Sigma, except where stated. Protein content was determined with the micro protein assay kit from Pierce, using bovine serum albumin as a standard. Tran^{35}S-label and [^{35}S]cysteine were from ICN. Murine M-CSF was purified and assayed by the method of Stanley and Guilbert (22).

RESULTS

During studies on the role of M-CSF on macrophage function, we noted that as the amount of M-CSF added was increased, so did the percentage of adherent cells. In addition, the cells exhibited morphological alterations (Fig. 1). Cells treated with low levels of the cytokine were exclusively round, whereas higher levels caused many of the cells to express extensions, resulting in an elongated appearance. The maximal response was seen with M-CSF at 1000 units/ml.

We reasoned that changes in adherence and shape might reflect integrin-mediated events. To identify the matrix proteins involved, we coated microtiter plates with laminin, vitronectin, or fibronectin. Only fibronectin supported attachment of macrophages treated with M-CSF (Fig. 2). This effect was dependent on the fibronectin concentration (Fig. 3*A*) and was maximal at a coating concentration of 10–15 μg/ml. Using fibronectin-coated plates and cells cultured with various amounts of M-CSF, we confirmed that attachment was cytokine-dependent (Fig. 3*B*).

Fibronectin contains several sites to which integrins can bind. One is the target of the integrin $\alpha_5\beta_1$ and can be blocked by Arg-Gly-Asp (RGD)-containing peptides, while the other is RGD-independent and binds the integrin $\alpha_4\beta_1$ (16). To establish which integrin(s) was involved in attachment, we used a blocking polyclonal antibody to murine $\alpha_5\beta_1$ and a rat monoclonal antibody to murine $\alpha_4\beta_1$. Both antibodies inhibited, in a dose-dependent manner, attachment of M-CSF-treated macrophages to plates coated with fibronectin (Fig. 4). Neither antibody totally blocked binding, but when they were combined at maximal concentration, there was an additive effect, resulting in >90% inhibition.

The above results suggested that both $\alpha_4\beta_1$ and $\alpha_5\beta_1$ were involved in attachment and were regulated by the amount of M-CSF in the culture medium. To confirm this latter hypothesis, equal numbers of macrophages, cultured for 3 days with various concentrations of M-CSF, were surface-labeled and subjected to immunoprecipitation with the same antibodies used in the blocking studies. The rabbit polyclonal antibody recognized a molecule which, under reducing conditions, migrated as a single broad band centered at 140 kDa (Fig. 5*A*). These results correspond to those previously reported for murine $\alpha_5\beta_1$ (24). Similar results were obtained with the immunopurified rabbit polyclonal antibody 33.11, prepared against the cytoplasmic tail of the human α_5 sequence (data

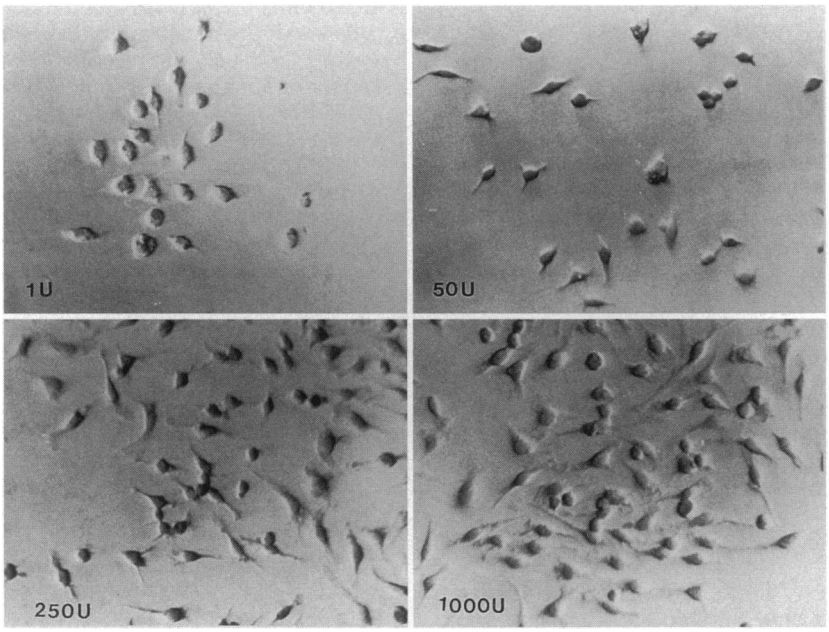

FIG. 1. M-CSF mediates attachment and spreading of immature murine bone marrow macrophage precursors. The mononuclear fraction isolated from marrow was cultured in Teflon beakers with murine M-CSF at 1, 50, 250, or 1000 units/ml. After 2 days, equal numbers of cells were plated on tissue culture plastic, and after 45 min the adherent cells were photographed. M-CSF results in an increase in both the number of adherent cells and the degree of spreading. The maximal effect is achieved at 1000 units/ml.

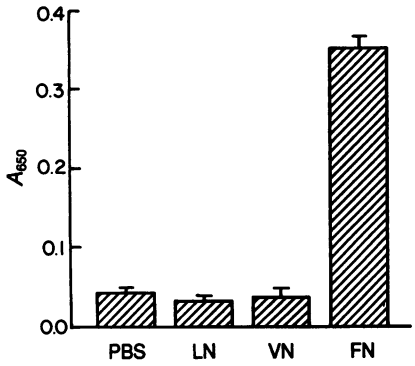

FIG. 2. Macrophage precursors treated with M-CSF adhere to fibronectin, but not other matrix proteins. Equal numbers of cells treated for 2 days with M-CSF at 250 units/ml were added to plates coated with phosphate-buffered saline alone (PBS) or containing laminin (LN), vitronectin (VN), or fibronectin (FN) and the adherent cells were quantitated.

not shown). The amount of $\alpha_5\beta_1$ on the surface was clearly correlated with the concentration of M-CSF used to grow the cells. Fig. 5*B* shows the result of immunoprecipitation with the antibody R1-2, made against the Peyer's patch adhesion molecule present on murine lymphocytes, which was shown subsequently to be the integrin $\alpha_4\beta_1$ (26). Once again, there was a M-CSF-dependent increase in the amount of two bands at 70 and 80 kDa. These proteins have been identified as the posttranscriptionally cleaved fragments of the α_4 subunit (27). The failure to coimmunoprecipitate the β_1 subunit with the

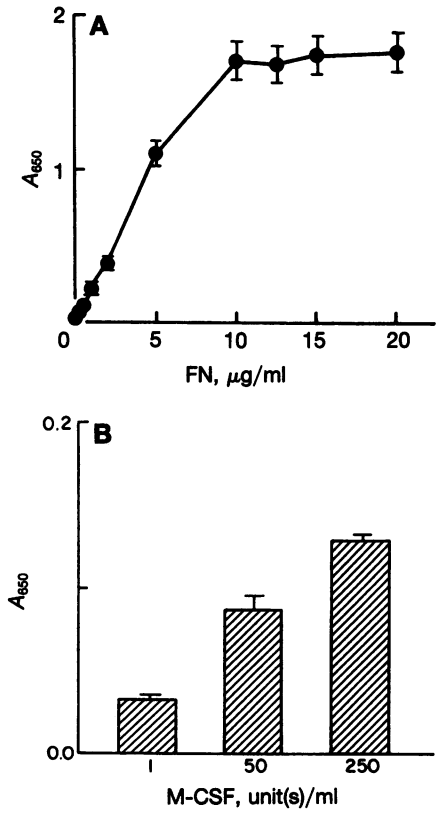

FIG. 3. Attachment to fibronectin is dose-dependent with respect to both matrix protein and M-CSF treatment of cells. (*A*) Equal numbers of cells treated with M-CSF at 250 units/ml were added to plates coated with a range of concentrations of fibronectin (FN). (*B*) Cells treated with various concentrations of M-CSF were plated onto plates coated with fibronectin at 15 μg/ml (binding to fibronectin increases over the range 0.5–12.5 μg/ml).

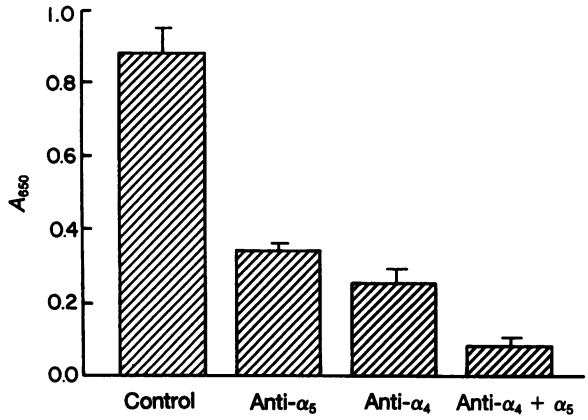

FIG. 4. Antibodies to the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ inhibit attachment to fibronectin. Cells grown with M-CSF at 250 units/ml were pretreated with excess nonimmune IgG, followed by antibodies to either $\alpha_4\beta_1$ and/or $\alpha_5\beta_1$. Cells were then added to plates coated with fibronectin at 15 μg/ml. The numbers of adherent cells were determined after 45 min. Control, nonimmune IgG only.

anti-α_4 antibody arises from the facile dissociation of the subunits of this integrin (28).

We wished to determine whether alterations in protein synthesis were responsible for the increased surface expression of the two integrins. In initial experiments, we established the time course of incorporation of label into the integrin $\alpha_4\beta_1$. When cells grown in medium with M-CSF at 250 units/ml were pulsed with [^{35}S]methionine/cysteine for 1 hr and chased for 12 hr in medium containing unlabeled methionine and cysteine, there was a large, dose-dependent increase in the amount of label incorporated into both the α and β chains of both integrins (Fig. 6). When cells were pulse-labeled in the standard manner and aliquots were immunoprecipitated in parallel with excess amounts of the antibodies R1-2 (anti-$\alpha_4\beta_1$) and 33.11 (anti-$\alpha_5\beta_1$), the amount of $\alpha_5\beta_1$ labeled was much greater than that of $\alpha_4\beta_1$ (data not shown), suggesting that the rates of synthesis of the two integrins are quite different.

DISCUSSION

Macrophages and monocytes derive from immature bone marrow precursors by a process involving hematopoietic growth factors acting in a hierarchical manner. While a number of cytokines are responsible for proliferation and differentiation of the various well-defined hematopoietic lineages, M-CSF is specific for macrophage survival and maturation (20) and for generation of mature osteoclasts (29) arising by fusion of monocyte/macrophage precursors (30). Finally, in addition to modulating precursor proliferation and differentiation, M-CSF targets mature monocytic cells, enhancing their phagocytic capacity (26, 31).

Adherence to substrate is characteristic of monocytic differentiation. Integrins are pivotal to cell–matrix recognition, an event associated with cell anchorage, extravasation, and migration (16). Integrins also transmit intracellular signals (e.g., ref. 32; reviewed in ref. 33), and these receptors are involved in cell growth (34, 35). Integrin-mediated matrix attachment is specific, depending on recognition of amino acid sequences in target proteins (16). Mature monocytes express members of the β_1, β_2, and β_3 integrin families and thus these cells can bind to a range of matrix proteins, including laminin, fibronectin, and vitronectin.

We show here that M-CSF stimulates expression of integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ by immature macrophages, resulting in their binding to fibronectin, a major matrix protein in the marrow microenvironment. Although a number of studies have addressed regulation of integrin expression by transformed

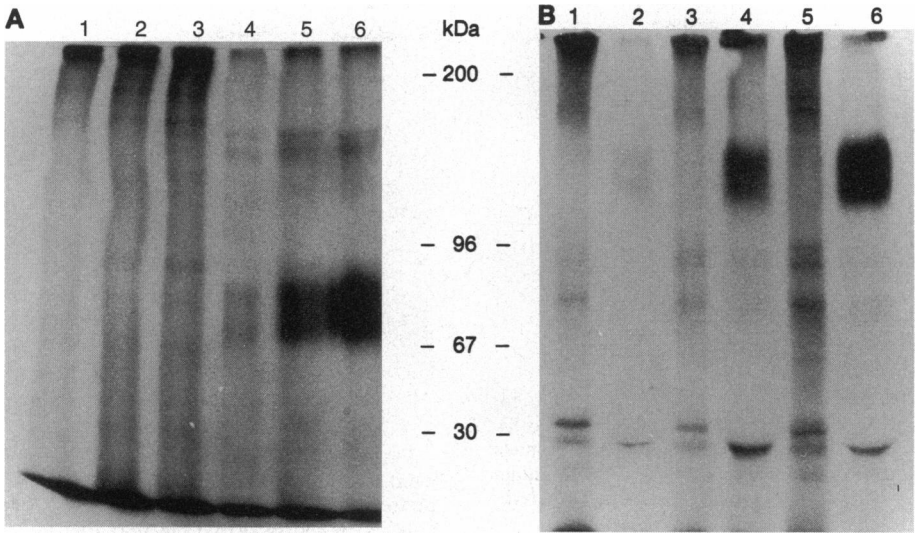

FIG. 5. M-CSF stimulates surface expression of the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ in a dose-dependent manner. After growth for 3 days with M-CSF at 1, 50, or 250 units/ml, equal numbers of cells were surface-labeled with ^{125}I and lysates were subjected to immunoprecipitation with antibodies to $\alpha_4\beta_1$ and $\alpha_5\beta_1$. Equal amounts of trichloroacetic acid-precipitable radioactivity were analyzed by SDS/6% PAGE and autoradiography. Both $\alpha_4\beta_1$ (*A*) and $\alpha_5\beta_1$ (*B*) expression increased dose-dependently with the concentration of M-CSF. (*A*) Lanes 1–3, immunoprecipitation with nonimmune rat IgG_1; lanes 4–6, immunoprecipitation with rat monoclonal anti-$\alpha_4\beta_1$. (*B*) Lanes 1, 3, and 5, immunoprecipitation with nonimmune rabbit serum; lanes 2, 4, and 6, immunoprecipitation with rabbit polyclonal antibody to $\alpha_5\beta_1$. In *A*, lysates used in lanes 1 and 4, 2 and 5, and 3 and 6 were obtained from cells treated, respectively, with M-CSF at 1, 50, or 250 units/ml. In *B*, lysates in lanes 1 and 2, 3 and 4, and 5 and 6 were obtained from cells treated, respectively, with M-CSF at 1, 50, or 250 units/ml.

monocytic cells, in only a few reports was the target population authentic monocyte/macrophage precursors. Human hematopoietic stem cells ($CD34^+$, DR^-, $CD15^-$) with multilineage capacity fail to attach to fibronectin but, by an undefined mechanism, adhere to thrombospondin (36). In a related study, a mixed population of more differentiated cells committed to the myeloid lineage ($CD34^+$, DR^+) expressed $\alpha_4\beta_1$ and $\alpha_5\beta_1$, but the subset most capable of forming granulocyte/macrophage-colony-forming units had low levels of both integrins. In differentiating polymorphonuclear leukocyte precursors, $\alpha_4\beta_1$ is widely expressed up to the band stage, whereas $\alpha_5\beta_1$ is largely absent in both metamyelocytes and myelocytes. Compared with granulocytic progenitors, mature polymorphonuclear leukocytes have less $\alpha_4\beta_1$ and $\alpha_5\beta_1$. Induction of granulocytopoiesis by treatment of $CD34^+$ cells with granulocyte-colony-stimulating factor mirrors this observation, as, contrasted with macrophage precursors, $\alpha_4\beta_1$ and $\alpha_5\beta_1$ disappear with time (37).

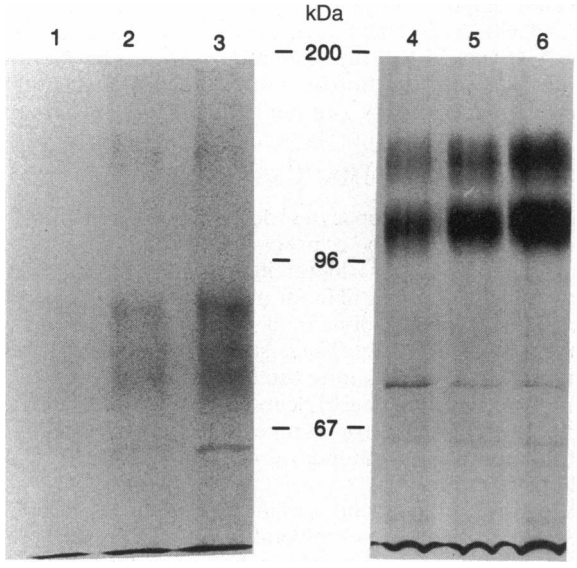

FIG. 6. M-CSF increases synthesis of the integrins $\alpha_4\beta_1$ and $\alpha_5\beta_1$ in bone marrow macrophage precursors in a dose-dependent manner. Equal numbers of cells treated with M-CSF at 1, 50, or 250 units/ml were pulse-labeled with [^{35}S]cysteine/methionine for 1 hr in medium lacking methionine and cysteine and then incubated for 12 hr in complete medium. The amount of newly synthesized integrins was determined by immunoprecipitation analysis. Equal amounts of trichloroacetic acid-precipitable protein were used for the analysis, and all following steps were identical to those described in Fig. 5, except for the use of nonreducing conditions for electrophoresis. Lanes 1–3, immunoprecipitation with rat monoclonal anti-$\alpha_4\beta_1$; lanes 4–6, immunoprecipitation with rabbit polyclonal antibody to $\alpha_5\beta_1$. In lanes 1 and 4, 2 and 5, and 3 and 6, lysates were obtained from cells treated, respectively, with M-CSF at 1, 50, or 250 units/ml.

In support of our hypothesis that appearance of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ is an event accompanying macrophage differentiation, mature monocytes express an abundance of both these integrins (21, 36). However, little is known about regulation of integrin expression and its functional consequences as macrophage precursors differentiate. Although short-term exposure of mature monocytes to M-CSF was found to enhance α_5 and β_1 mRNA levels, in contrast to the present data, the translational and functional consequences of this event were not explored (38). Furthermore, and dissimilar to our results with macrophage precursors, prolonged M-CSF treatment of mature human monocytes was not accompanied by surface expression of β_1 integrins (39). Thus, the effect of M-CSF on macrophage β_1 integrins has been explored previously with only mature monocytes, which fail in this circumstance to alter their level of expression. Furthermore, in none of these reports were studies performed to determine the basis for regulation of integrin expression. Surface expression of β_1 integrins may be enhanced by accelerated synthesis of rate-limiting α subunits (ref. 40 and references quoted therein). Alternatively, a variety of activators, including platelet-derived growth factor, tumor necrosis factor α, and leukotriene B4, mobilize β_2 integrins from an intracellular vesicular compartment in monocytes, without *de novo* synthesis (41). In light of our surface and metabolic labeling data, binding of M-CSF-stimulated macrophage precursors to fibronectin reflects increased surface expression of $\alpha_4\beta_1$ and $\alpha_5\beta_1$ as a result of synthesis of both heterodimers.

This study provides direct evidence that, in contrast to their mature counterparts, monocyte/macrophage precursors respond to M-CSF by increasing their capacity to interact with the stromal microenvironment, an event fundamental to macrophage differentiation. Given evidence from other members of the hematopoietic family (42–44), it is likely that these adhesion molecules mediate targeting of macrophage precursors to the stromal microenvironment during maturation. Interactions with specific matrix components and/or growth factors bound to matrix proteoglycans (45, 46) are likely to influence such events.

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1. Coulombel, L., Vuillet, M. H., Leroy, C. & Tchernia, G. (1988) *Blood* **71,** 329–334.
2. Lemoine, F. M., Dedhar, S., Lima, G. M. & Eaves, C. J. (1990) *Blood* **76,** 2311–2320.
3. Rosemblatt, M., Vuillet-Gaugler, H. M., Leroy, C. & Coulombel, L. (1991) *J. Clin. Invest.* **87,** 6–11.
4. Ryan, D. H., Nuccie, B. L., Abboud, C. N. & Winslow, J. M. (1991) *J. Clin. Invest.* **88,** 995–1004.
5. Simmons, P. J., Masinovsky, B., Longenecker, B. M., Berenson, R., Torok-Storb, B. & Gallatin, W. M. (1992) *Blood* **80,** 388–395.
6. Teixidó, J., Hemler, M. E., Greenberger, J. S. & Anklesaria, P. (1992) *J. Clin. Invest.* **90,** 358–367.
7. Tsai, S., Patel, V., Beaumont, E., Lodish, H. F., Nathan, D. G. & Sieff, C. A. (1987) *Blood* **69,** 1587–1594.
8. Verfaillie, C. M., McCarthy, J. B. & McGlave, P. B. (1991) *J. Exp. Med.* **174,** 693–703.
9. Vuillet-Gaugler, M. H., Breton-Gorius, J., Vainchenker, W., Guichard, J., Leroy, C., Tchernia, G. & Coulombel, L. (1990) *Blood* **75,** 865–873.
10. Weinstein, R., Riordan, M. A., Wenc, K., Kreczko, S., Zhou, M. & Dainiak, N. (1989) *Blood* **73,** 111–116.
11. Kerst, J. M., Sanders, J. B., Slaper-Cortenbach, I. C. M., Doorakkers, M. C., Hooibrink, B., van Oers, R. H. J., von dem Borne, A. E. G. K., & van der Schoot, C. E. (1993) *Blood* **81,** 344–351.
12. Bartelmez, S., Sacca, R. & Stanley, E. R. (1985) *J. Cell. Physiol.* **122,** 362–369.
13. Kim, Y. R., Abraham, N. G. & Lutton, J. D. (1991) *Leuk. Res.* **15,** 409–418.
14. Polla, B. S., Healy, A. M., Byrne, M. & Krane, S. M. (1987) *J. Clin. Invest.* **80,** 962–969.
15. Reitsma, P. H., Rothberg, P. G., Astrin, S. M., Trial, J., Bar-Shavit, Z., Hall, A., Teitelbaum, S. L. & Kahn, A. J. (1983) *Nature (London)* **306,** 492–494.
16. Hynes, R. O. (1992) *Cell* **68,** 11–25.
17. Springer, T. A. (1990) *Nature (London)* **346,** 425–434.
18. Peters, C., O'Shea, K. S., Campbell, A. D., Wicha, M. S. & Long, M. W. (1990) *Blood* **75,** 357–364.
19. Campbell, A. D., Long, M. W. & Wicha, M. S. (1987) *Nature (London)* **329,** 744–746.
20. Tushinski, R. J., Oliver, I. T., Guilbert, L. J., Tynan, P. W., Warner, J. R. & Stanley, E. R. (1982) *Cell* **28,** 1–81.
21. Clohisy, D. R., Bar-Shavit, Z., Chappel, J. C. & Teitelbaum, S. L. (1987) *J. Biol. Chem.* **262,** 15922–15929.
22. Stanley, E. R. & Guilbert, L. J. (1981) *J. Immunol. Methods* **42,** 253–284.
23. Bar-Shavit, Z., Kahn, A. J. & Teitelbaum, S. L. (1983) *J. Clin. Invest.* **72,** 526–534.
24. Holers, V. M., Ruff, T. G., Parks, D. L., McDonald, J. A., Ballard, L. L. & Brown, E. J. (1989) *J. Exp. Med.* **169,** 1589–1605.
25. Holzmann, B., McIntyre, B. W. & Weissman, I. L. (1989) *Cell* **56,** 37–46.
26. Sampson-Johannes, A. & Carlino, J. A. (1988) *J. Immunol.* **141,** 3680–3686.
27. Bednarczyk, J. L., Szabo, M. C. & McIntyre, B. W. (1992) *J. Biol. Chem.* **267,** 25274–25281.
28. Hemler, M. E., Huang, C., Takada, T., Schwarz, L., Strominger, J. L. & Clabby, M. L. (1987) *J. Biol. Chem.* **262,** 11478–11485.
29. Kodama, H., Nose, M., Niida, S. & Yamasaki, A. (1991) *J. Exp. Med.* **173,** 1291–1294.
30. Shioi, A., Teitelbaum, S. L., Ross, F. P., Welgus, H. G., Ohara, J., Suzuki, H. & Lacey, D. L. (1991) *J. Cell. Biochem.* **47,** 1–6.
31. Ralph, P. & Nakoinz, I. (1987) *Cell. Immunol.* **105,** 270–279.
32. Miyauchi, A., Alvarez, J., Greenfield, E. M., Teti, A., Grano, M., Colucci, S., Zambonin-Zallone, A., Ross, F. P., Teitelbaum, S. L., Cheresh, D. & Hruska, K. A. (1991) *J. Biol. Chem.* **266,** 20369–20374.
33. Juliano, R. L. & Haskill, S. (1993) *J. Cell Biol.* **120,** 577–585.
34. Dalton, S. L., Marcantonio, E. E. & Assoian, R. K. (1992) *J. Biol. Chem.* **267,** 8186–8191.
35. Symington, B. (1992) *J. Biol. Chem.* **267,** 25744–25747.
36. Long, M. W., Briddell, R., Walter, A. W., Bruno, E. & Hoffman, R. (1992) *J. Clin. Invest.* **90,** 251–255.
37. Kerst, J. M., Sanders, J. B., Slaper-Cortenbach, I. C. M., Doorakkers, M. Ch., Hooibrink, B., van Oers, R. H. J., von dem Borne, A. E. G. K. & van der Schoot, C. E. (1993) *Blood* **81,** 344–351.
38. Mufson, R. A. (1990) *J. Immunol.* **145,** 2333–2339.
39. De Nichilo, M. O. & Burns, G. F. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2517–2521.
40. Sheppard, D., Cohen, D. S., Wang, A. & Busk, M. (1992) *J. Biol. Chem.* **267,** 17409–17414.
41. Miller, L. J., Bainton, D. F., Borregaard, N. & Springer, T. A. (1987) *J. Clin. Invest.* **80,** 535–544.
42. Nojima, Y., Humphries, M. J., Mould, A. P., Komoriya, A., Yamada, K. M., Schlossman, S. F. & Morimoto, C. (1990) *J. Exp. Med.* **172,** 1185–1192.
43. Miyake, K., Weissman, I. L., Greenberger, J. S. & Kincade, P. W. (1991) *J. Exp. Med.* **173,** 599–607.
44. Williams, D. A., Rios, M., Stephens, C. & Patel, V. P. (1991) *Nature (London)* **352,** 438–441.
45. Gordon, M. Y., Riley, G. P., Watt, S. M. & Greaves, M. F. (1987) *Nature (London)* **326,** 403–405.
46. Roberts, R., Gallagher, J., Spooncer, E., Allen, T. D., Bloomfield, F. & Dexter, T. M. (1988) *Nature (London)* **332,** 376–378.