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Granulocyte–macrophage and macrophage colony-stimulating factors differentially regulate αv integrin expression on cultured human macrophages

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ABSTRACT **The colony-stimulating factors (CSFs) greatly influence mature macrophage function *in vitro*: macrophage (M)-CSF induces maturation of monocytes and enhances differentiated cell function; granulocyte–macrophage (GM)-CSF stimulates a variety of antimicrobial functions. *In vivo* M-CSF is thought to promote differentiation, and GM-CSF is thought to potentiate the inflammatory response. One mechanism by which these differential effects may be achieved is through the receptor-mediated interaction of macrophages with their extracellular matrix. Here we show that M-CSF induces specifically the expression of the αvβ5 integrin receptor, whereas GM-CSF rapidly induces mRNA and surface expression of the αvβ3 integrin. The M-CSF-treated cells acquire a flattened epitheloid phenotype, and on vitronectin the αvβ5 is located in adhesion plaques. These cells do not bind collagen or laminin. In contrast, cells treated with GM-CSF adopt an elongated phenotype on a number of substrates, including collagen and laminin, and express αvβ3 at the leading edge of cells on vitronectin. These results suggest that a primary means by which the CSFs exert their individual effects on mature cells may be through regulating integrin expression.**

The hemopoietic growth factors granulocyte–macrophage (GM) and macrophage (M) colony-stimulating factors (CSFs) regulate the proliferation and differentiation of granulocytes and macrophages from their precursor cells (1). *In vitro* studies have shown that these factors also have the capacity to potentiate mature leukocyte function (2); the majority of these responses are rapid, requiring only 30–60 min, and are likely to be mediated through membrane-signaling events (3). Other effects of the CSFs on mature cells include the prolongation of survival and, in the case of M-CSF, the induction of a more differentiated phenotype by cultured human macrophages (4). The influence of the CSFs on mature cell function *in vivo* is also likely to be profound, and GM-CSF in particular appears to play a central role in resistance to infection (5). Transgenic mice constitutively expressing this gene exhibit features indicating greatly enhanced macrophage motility: eye opacity and inflammatory lesions in muscle, accompanied by large numbers of macrophages in the pleural and peritoneal cavities (6, 7). These processes, induction of differentiation by M-CSF and of increased motility by GM-CSF, are likely to involve transcriptional regulation, and studies in other systems indicate that the CSFs can induce gene transcription (8–11).

The extracellular matrix (ECM), made up of collagens, proteoglycans, and glycoproteins such as fibronectin and vitronectin, is known to play a profound role in cell growth and differentiation, as well as in cellular migration (12). That the CSFs may be implicated in regulating matrix production was suggested in experiments described by Elliott *et al.* (13) and by Gamble *et al.* (14) showing distinct phases of adhesion by CSF-stimulated monocytes, which in some cases took several hours and was inhibited by cycloheximide. A number of cytokines can regulate ECM receptor expression (15, 16), and transforming growth factor β is considered to exert its profound and multifaceted effects on cell behavior primarily through its ability to regulate the synthesis of ECM and the appropriate cellular receptors (17). The receptors that have received most study in this regard belong to a large family of αβ heterodimers termed integrins (18). The integrins facilitate adhesion to the ECM and are implicated as being signaling molecules involved in the selective gene induction observed upon cell adhesion to various ECMs; recently, Yurochko *et al.* (19) demonstrated integrins as primary signal transduction molecules regulating induction of the immediate early genes interleukin 1β and MAD-6 by human monocytes. The integrins expressed by leukocytes can be divided into three major subgroups: the very late antigens, defined by the β1 subunit in association with any one of eight α subunits; the leukocyte adhesion molecules, defined by the β2 subunit in association with any of three known α subunits; and the vitronectin receptor subgroups (αv), defined by αv in association with any of four known β subunits (reviewed in ref. 18).

In previous studies, we have reported that human monocytes in culture acquire surface expression of αvβ3 (20) and upon prolonged culture begin to express αv in association with an undetermined β subunit termed β3b (21). Since the αv subfamily has recently been recognized as being intimately involved in cell migration (22–24), we sought to determine whether CSFs were involved in regulating expression of these integrins. We show here that GM-CSF and M-CSF differentially regulate the αv integrins expressed by cultured human macrophages and suggest that this event may be critical to an understanding of the physiological roles of these cytokines.

MATERIALS AND METHODS

Monocyte Purification. Human monocytes were isolated from peripheral blood of normal volunteers after dextran sedimentation (dextran T500; Pharmacia) and density gradient centrifugation on a Lymphoprep cushion (Nyegaard, Oslo). Cells within the mononuclear fraction were aspirated and washed four times with 250 ml of Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution with 0.06% EDTA to reduce platelet contamination. Cells were resuspended in RPMI 1640 supplemented with 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM glutamine, 10 mM Hepes, 0.075% $NaHCO_3$, gentamycin (160 mg/liter), and 20% (vol/vol) fetal calf serum (FCS; heat inactivated) and allowed to adhere to polystyrene dishes (Nunc) for 60 min at 37°C in 10% CO_2. Nonadherent cells were then

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Abbreviations: ECM, extracellular matrix; CSF, colony-stimulating factor; GM-CSF, granulocyte–macrophage CSF; M-CSF, macrophage CSF; mAb, monoclonal antibody; FCS, fetal calf serum.
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removed by gentle washing, and adherent monocytes were maintained in RPMI 1640 containing 10% FCS. Within a 12-hr period of culture, the monocytes detach from the dishes and remain in a nonadherent state for the duration of experimentation. The method outlined above consistently yielded >95% pure macrophages by morphological and cytochemical criteria as described (21), and >94% cells were strongly positive for the CD14 monocyte lineage marker as determined by flow cytometric analysis.

Cytokine Stimulation. Nonadherent (12 hr postpurification) monocytes were stimulated in the continuous presence of the following human recombinant cytokines: GM-CSF and M-CSF (purified cytokine derived from transfected COS cell supernatant) at 50 ng/ml (Genetics Institute, Cambridge, MA), interleukin 3 at 50 ng/ml (Immunex, Seattle), and transforming growth factor β at 10 ng/ml (Sigma) for various time periods as indicated.

Endotoxin Levels. Bacterial endotoxin is a potent regulator of leukocyte function (25). To minimize its contamination, all monocyte culturing was undertaken using sterile disposable plasticware, and only reagents of the highest quality were used. FCS (Cytosystems, Sydney, Australia) was screened using the limulus amoebocyte lysate assay and selected for low endotoxin content (25 pg/ml) and the inability to support monocyte survival in the absence of exogenous stimuli; the CSFs were also shown to contain <25 pg of endotoxin per ml by this assay. Water, RPMI 1640, and Hanks' balanced salt solution (Cytosystems) contained <60 pg of endotoxin per ml as shown by the manufacturers.

Antibodies. Monoclonal antibodies (mAbs) 13C2 and 23C6 recognize the αv subunit and αvβ3 complex, respectively (26). SZ-21 has specificity for the β3 subunit (20), whereas A-1A5 is directed to the β chain of the (β1) very late antigen family (27). The anti-β5 serum was raised to a synthetic peptide corresponding to the predicted intracytoplasmic domain of the β5 subunit (28).

Labeling and Immunoprecipitations. Cultured macrophages were labeled at the cell surface by lactoperoxidase-catalyzed iodination as described (21). Cells were solubilized in 1% Nonidet P-40 lysis buffer and precleared. Immunoprecipitations were carried out by using either mAbs directly coupled to Sepharose beads or polyclonal serum followed by protein A-Sepharose. Labeled integrin subunits were separated by using SDS/7.5% polyacrylamide gels under reducing conditions and analyzed by autoradiography as described (21).

RNA Blot Analysis. Northern hybridization of integrin mRNA levels was done using total cellular RNA (10 μg per lane) prepared by the guanidine isothiocyanate procedure (29). RNA was fractionated in 1% agarose gels containing formaldehyde, transferred onto Hybond N^+ (Amersham) nylon membrane filters, and hybridized with probes labeled using the random prime technique. Blots were washed in 2× standard saline citrate/0.1% SDS at 65°C, followed by two washes in 0.2× standard saline citrate/1% SDS at 65°C. Prior to autoradiography, filters were scanned, and relative cpm were quantitated using the Ambis radioanalytic imaging system (Automated Microbiology Systems, San Diego). β3 message was detected by using a 2.3-kb *Eco*RI cDNA insert (30), whereas the 250-bp β5 probe was generated by PCR (16) and verified by sequence analysis.

Attachment to Vitronectin. Vitronectin was purified from expired human serum according to the method of Yatohgo *et al.* (31), and its purity was determined by SDS/PAGE and silver staining. Glass coverslips (30 mm) were placed into 24-well plates (Linbro) and coated at 4°C overnight with human vitronectin [0.3 ml at 50 μg/ml in phosphate-buffered saline (PBS)]. Cultured macrophages were harvested, washed twice with PBS, and resuspended in RPMI 1640 containing 1 mM $CaCl_2$ and bovine serum albumin at 1 mg/ml; cells (10^5 per well) were allowed to adhere for 90 min at 37°C in 10% CO_2. Nonadherent cells were removed by gentle washing, and visual and photographic assessment of cell attachment and spreading were performed after fixation and staining using Coomassie blue (0.1% Coomassie blue in 10% acetic acid/40% methanol). Attachment to human fibronectin, fibrinogen, mouse laminin, and collagen type I (Collaborative Research) was as described above.

Indirect Immunofluorescence. Macrophages were allowed to attach and spread on vitronectin-coated glass coverslips and stained by indirect immunofluorescence as described by Larjava *et al.* (32). In brief, cells were fixed with 4% formaldehyde with 5% sucrose in PBS for 10 min and permeated with 0.5% Triton X-100 in PBS for 10 min; nonspecific binding sites were blocked by using 3% bovine serum albumin in PBS containing 0.1% glycine and a 1:10 dilution of nonimmune rabbit serum for 90 min. Cells were incubated for 1 hr with the indicated concentrations of mAb in PBS containing bovine serum albumin at 1 mg/ml and then counterstained with fluorescein-conjugated sheep anti-mouse IgG. Coverslips were mounted in Gel-mount (Biomedia, Foster City, CA) containing *p*-phenylenediamine (Sigma) at 1 mg/ml to inhibit photobleaching. Fluorescence was detected with a photomicroscope (Axioplan; Zeiss) fitted with epifluorescence.

RESULTS

M-CSF but Not GM-CSF Induces mRNA for the Integrin β5 Subunit. To determine whether M-CSF or GM-CSF was able to induce β5 expression, cultured human macrophages were stimulated with the indicated cytokines for a period of 6 hr and then probed for β5 mRNA by Northern blot analysis (Fig. 1*A*). Since interleukin 3 has similar physiological properties to GM-CSF and TGF-β has been reported to regulate the expression of several integrin subunits, we also tested these cytokines in the system. Of these only M-CSF was found to induce β5 mRNA at this early time point. We sought then to test whether the cells could be restimulated with M-CSF, and as is shown in Fig. 1*A*, cells previously stimulated with M-CSF and then rendered quiescent reexpressed abundant mRNA for the β5 subunit when restimulated with M-CSF.

To further examine the kinetics of integrin β-subunit induction by the CSFs, macrophages were stimulated with either M-CSF or GM-CSF for time points up to and including 24 hr, and mRNA levels were detected by Northern blot analysis. When these filters were probed for the β5 subunit, it was found that in cells stimulated with M-CSF, peak expression was obtained after 6 hr in culture, with levels returning to baseline by 24 hr (Fig. 1*B*). In GM-CSF-stimulated cells, there was no β5 expression detected for the first 12 hr; however, expression became apparent at the 24-hr point (Fig. 1*B*). Previously Horiguchi *et al.* (8) had demonstrated GM-CSF *in vitro* to stimulate M-CSF expression in human monocytes. In light of this observation, we reprobed the same filters for M-CSF production and found that the cells stimulated with GM-CSF were induced to generate M-CSF mRNA, peaking at 6 hr (data not shown). It can be suggested therefore that the delayed time course of β5 mRNA expression in cells stimulated with GM-CSF is due to endogenous M-CSF production.

In contrast to the results obtained for β5, when the same filters were reprobed for β3 expression, it was found that, whereas M-CSF-treated cells ceased to express any preexisting β3 mRNA, GM-CSF treatment of the cells induced β3 mRNA levels that peaked at 6 hr and returned to baseline by 24 hr after stimulation (Fig. 1*B*).

CSFs Differentially Regulate the Surface Expression of αv Integrins and Cell Phenotype on Vitronectin. To determine whether the results obtained in Northern blots were reflected

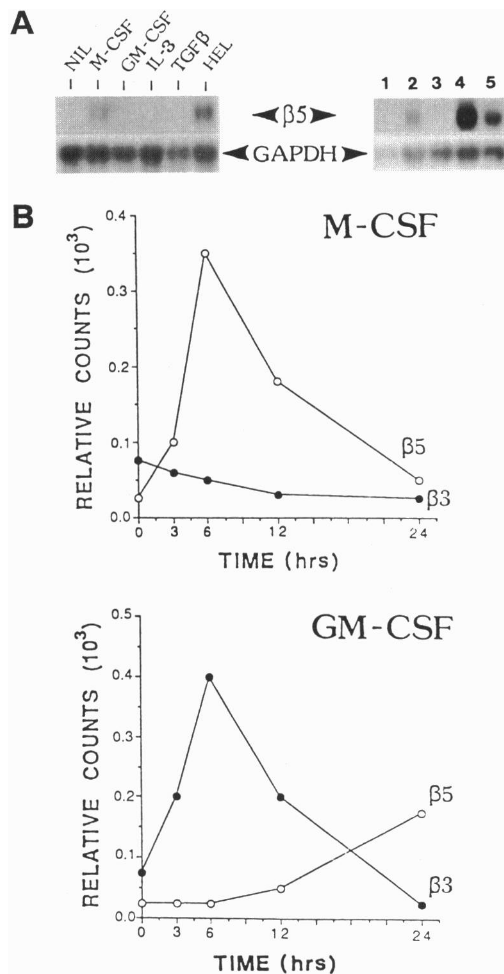

FIG. 1. Effect of different cytokines on the induction of mRNA for integrins $\beta3$ and $\beta5$ by cultured human macrophages. (*A*) Cultured human macrophages were stimulated for 6 hr with the cytokines indicated, and total RNA for $\beta5$ was analyzed by Northern blot. (*Right*) Cultured monocytes were analyzed for $\beta5$ mRNA after no stimulation for 6 hr (lane 1), stimulation with M-CSF for 6 hr (lane 2), stimulation with M-CSF and then maintenance for 48 hr without restimulation (lane 3), and stimulation with M-CSF at days 3, 6, and 9 and then maintenance in 0.1% FCS overnight prior to restimulation with M-CSF for 6 hr (lane 4). In both panels, RNA isolated from human embryonic fibroblasts (HEL in *Left* and lane 5 in the *Right*) served as a control. Filters were stripped and reprobed for GAPDH mRNA as a measure of RNA loading and transfer. (*B*) Relative cpm taken from the Ambis radioanalytic imaging system of Northern blots of cells stimulated with M-CSF (*Upper*) or GM-CSF (*Lower*) and probed for $\beta3$ and $\beta5$ mRNA at the times indicated. The blots were also probed for GAPDH mRNA to confirm equal amounts of mRNA in each sample (not shown). It should be noted that each sample presented in Northern blot experiments represents RNA taken from an individual donor. These experiments were repeated three times with essentially the same results. IL-3, interleukin 3; TGFβ, transforming growth factor β.

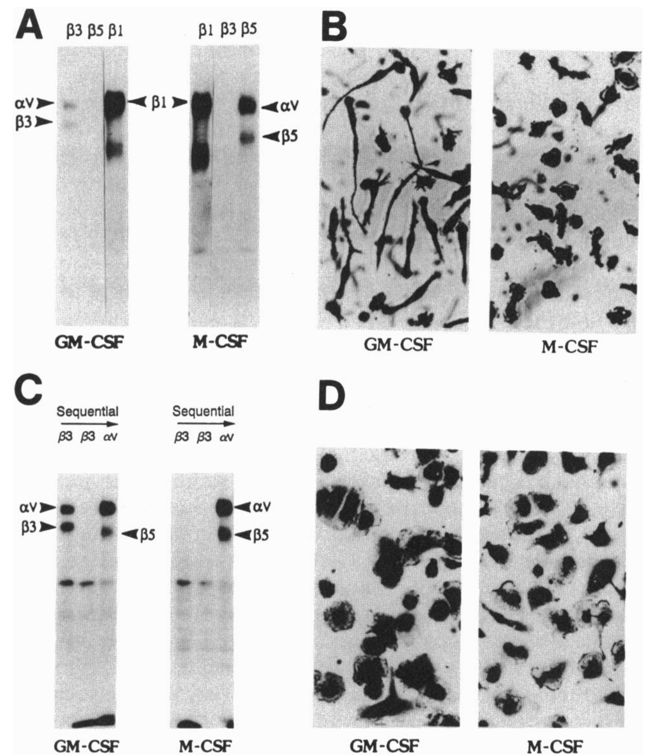

FIG. 2. Regulation of surface integrin expression and macrophage morphology on vitronectin after a 24-hr (*A* and *B*) or 4-day (*C* and *D*) treatment with either GM-CSF or M-CSF. (*A*) Twenty-four-hour CSF-treated macrophages were surface radioiodinated, and lysates were immunoprecipitated with mAb 23C6 ($\beta3$) to the $\alpha v\beta3$ complex, anti-$\beta5$ serum ($\beta5$) to the cytoplasmic domain of the $\beta5$ subunit, and mAb A-1A5 ($\beta1$) to the very late antigen β subunit. (*B*) Morphology of the corresponding cells spread on vitronectin for 90 min in serum-free conditions. (*C*) A 4-day CSF-treated and iodinated macrophage lysate was immunoprecipitated sequentially with mAb 23C6 ($\beta3$) to the $\alpha v\beta3$ complex, followed by mAb 13C2 (αv) to the αv subunit or sequentially with anti-$\beta5$ serum followed by 13C2 (not shown). Labeled integrin subunits were analyzed by SDS/PAGE under reducing conditions. (*D*) Morphologies of the corresponding cells on vitronectin. These results are representative of three other experiments in which similar results were obtained.

at the cell surface, macrophages were radioiodinated at the cell surface, and lysates were immunoprecipitated with antibodies to the αv integrin subunits. At 24 hr after stimulation, it was found that GM-CSF-treated cells expressed $\alpha v\beta3$ but no $\alpha v\beta5$; conversely, the M-CSF-treated cells displayed $\alpha v\beta5$ but no $\alpha v\beta3$ (Fig. 2*A*). By 4 days of culture, the GM-CSF-stimulated cells continued to express $\alpha v\beta3$ but now also exhibited $\alpha v\beta5$ on their surface; the M-CSF cells continued to express only $\alpha v\beta5$ at this time (Fig. 2*C*).

These results for αv-integrin expression were accompanied by great morphological differences when the cells were allowed to attach to a vitronectin substrate: thus at 24 hr, the GM-CSF-treated cells presented an elongated "motile" phenotype, whereas the M-CSF-treated cells appeared flatter and slightly spread (Fig. 2*B*). By 4 days after treatment, the GM-CSF cells assumed a well-spread flattened morphology, which was indistinguishable from cells treated with M-CSF (Fig. 2*D*).

Of the known integrins, only members of the αv subfamily are recognized as binding vitronectin, and these differences in morphology presumably reflect the differential expression of the αv-associated β subunits induced by GM-CSF and M-CSF. To substantiate this, we performed flow cytometric analysis of cytokine-treated cells at each time point with a panel of mAbs to the known integrin α and β subunits and immunoprecipitation analysis for $\beta1$ (Fig. 2*A*) and $\beta2$ (data not shown). We found that the cultured macrophages consistently expressed $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$ (very late antigens 4, 5, and 6) and $\alpha L\beta2$, $\alpha M\beta2$, and $\alpha X\beta2$ (LFA-1, Mac-1, and p150/95), but the CSFs did not alter the relative or absolute expression of these integrins.

Macrophage $\alpha v\beta3$ and $\alpha v\beta5$ Are Implicated in Determining Distinct Morphological Phenotypes on Vitronectin. Evidence for the functional involvement of integrins in spreading on a particular substrate can be obtained directly by examining the distribution of these receptors. This was accomplished by

permitting 24-hr GM-CSF-stimulated macrophages to attach to vitronectin for 90 min and examining these cells for the distribution of the $\beta3$ subunit by immunofluorescence analysis. As illustrated in Fig. 3*A*, the $\beta3$ staining appeared to decorate the leading edge of triangular-shaped cells, whereas for cells with a more elongated morphology, $\beta3$ staining was identified only in the bulbous processes in apposition to the points of cellular attachment (Fig. 3*A*). In these cells, staining for αv gave a similar pattern (data not shown). To examine the distribution of the $\beta5$ subunit, we chose macrophages cultured in the presence of M-CSF for a period of 4 days because these cells displayed both high levels of $\alpha v\beta5$ and an enhanced capacity to spread on vitronectin as shown above. When the cells were allowed to spread for 90 min on vitronectin (Fig. 3*B*), the αv staining in permeated cells was localized to streaks, both peripheral and within the body of the cytoplasm. In these cells, the streaks could be identified as adhesion plaques by both vinculin staining and interference reflection microscopy (data not shown). However, when we attempted to stain M-CSF-stimulated cells with polyclonal rabbit antibodies to the cytoplasmic domain of the $\beta5$ subunit, no clear fluorescence staining could be identified. Indeed, immunofluorescence staining with this reagent was identical to that seen with nonimmunized rabbit serum used as a control. As a consequence of this result, it was important to demonstrate that the only β subunit found in association with αv in these M-CSF-stimulated macrophages was $\beta5$. This was achieved by preclearing $\beta5$ from a lysate of surface radioiodinated cells and demonstrating that after sequential clearance there was no residual αv (Fig. 3*C*). Thus these data demonstrate that the surface expression of $\alpha v\beta5$ on cultured macrophages is a determining factor in their morphology on vitronectin.

CSFs Influence the Attachment and Morphology of Macrophages on Matrix Glycoproteins and Collagen Type I. Vitronectin provided a convenient substrate to directly contrast the roles of $\alpha v\beta3$ and $\alpha v\beta5$ in cellular adhesion because both integrins function as receptors for this glycoprotein. However, it was important to investigate whether the influences of the CSFs extended to other substrates that would be critical in determining macrophage migration through and beyond the subendothelial matrix. We therefore tested the GM-CSF- and M-CSF-treated macrophages for their attachment to and morphology on fibronectin, fibrinogen, laminin, and collagen type I. The M-CSF-stimulated macrophages attached and spread on fibronectin and fibrinogen, and their morphologies appeared identical to that seen above on vitronectin. These cells did not attach to laminin or collagen type I even when allowed to adhere for 4 hr. In contrast, cells pretreated with GM-CSF for 24 hr adopted an elongated phenotype when plated on all of the substrates tested. It was noticeable, however, that whereas on fibronectin and fibrinogen this process was complete by 90 min, cells on laminin and collagen were attached at this time point, then progressively spread before acquiring the elongated phenotype in a process that required 3–4 hr. This latter event was inhibited by cycloheximide (data not shown), possibly implicating deposition of an endogenous matrix whose identity is unknown. These results suggest that GM-CSF, perhaps by its induction of the $\beta3$ integrin, directly influences the migratory capacity of human macrophages on a variety of substrates.

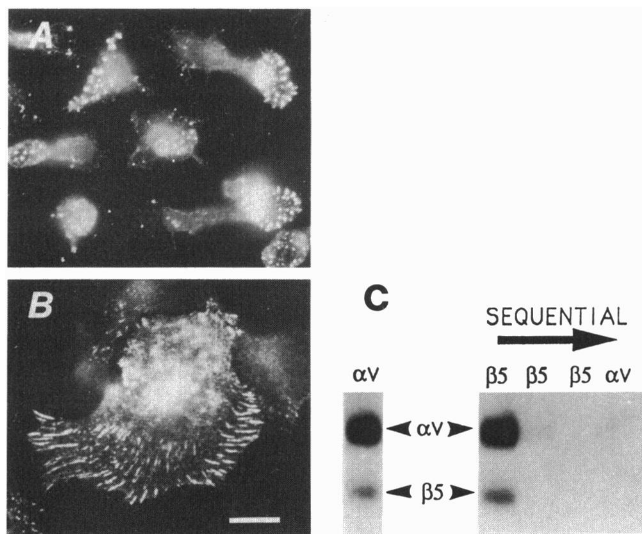

FIG. 3. Localization of $\alpha v\beta3$ and $\alpha v\beta5$ integrins by indirect immunofluorescence. (*A* and *B*) CSF-treated macrophages were allowed to attach and spread on vitronectin in serum-free conditions for 90 min and then permeated and stained with mAb SZ-21 (10 μg/ml) to the $\beta3$ subunit or mAb 13C2 (10 μg/ml) to the αv subunit. (*A*) Photomicrograph of $\beta3$ staining of 24-hr GM-CSF-treated macrophages. (*B*) αv staining of 4-day M-CSF-treated macrophages. (Bar = 20 μm.) (*C*) Four-day M-CSF-treated macrophages were surface radioiodinated, and αv-associated integrins were analyzed by sequential immunoprecipitation and SDS/PAGE under reducing conditions. In the first lane (αv), one-half of the lysate was precipitated with mAb 13C2 to the αv subunit. The remaining lysate was sequentially precipitated three times with anti-$\beta5$ serum ($\beta5$) and then reprecipitated with the 13C2 mAb (αv).

DISCUSSION

In the present study we investigated the mechanisms by which GM-CSF and M-CSF influence the function of mature peripheral blood monocytes; in particular, we analyzed the role of these cytokines in inducing changes within members of the integrin family of receptors. We show that M-CSF rapidly induces the expression of $\alpha v\beta5$ on the surface of cultured human macrophages. Treatment of macrophages with GM-CSF, in contrast, stimulates the cells to express $\alpha v\beta3$ and to display a migratory phenotype. These results are likely to contribute to the differentiating effect of M-CSF and to the role of GM-CSF as a mediator of inflammation.

M-CSF *in vitro* promotes the growth and particularly the differentiation of immature cells of the macrophage lineage. In mature cells this cytokine enhances the survival of monocytes and potentiates the ability of mature cells to perform their differentiated functions (33, 34). Young *et al.* (4) reported that human monocytes cultured in M-CSF acquired many features characteristic of tissue macrophages, suggesting that, at least *in vitro*, this may be a function of M-CSF. How this is achieved is not known, but in many systems it is now apparent that interactions between cells and their ECM are determining factors in differentiation. Further, differentiating signals appear in some cases to be transmitted by specific integrin receptors (12, 16, 17, 35, 36). We show here that M-CSF specifically induces the expression of $\alpha v\beta5$ and that this integrin is involved in the maintenance of a well-spread phenotype of the cells on vitronectin. Whether this receptor can transduce specific differentiative signals in response to vitronectin is not yet known, but these data provide one possible route for M-CSF to induce differentiation.

Our findings are somewhat at variance with those of Wayner *et al.* (37). Those authors reported that the M21 human melanoma cells, as well as H2981 and UCLA-P3 lung carcinoma cells, spread on vitronectin but that $\alpha v\beta5$ was not localized to adhesion plaques, even though αv localized to these areas in M21 cells and a mAb to $\alpha v\beta5$ blocked attachment of UCLA-P3 cells to this substrate. We could not detect $\beta5$ in the αv-containing adhesion plaques seen in macrophages plated on vitronectin using an antiserum raised to a $\beta5$ cytoplasmic peptide, despite the fact that this is a good

immunoprecipitating reagent. The reason for this is not known; possibly the (cytoplasmic) epitope is sequestered in complexes, but this is the same antiserum used by Wayner *et al.* (37). With this reagent, in a series of experiments, we could identify no immunofluorescence staining that differed from the nonimmunized rabbit serum used as a control. Since we showed that M-CSF-treated macrophages expressed only $\beta 5$ in association with αv (Fig. 3*C*), we concluded that the αv seen clearly in adhesion plaques (Fig. 3*B*) was the integrin receptor $\alpha v \beta 5$. Whether these conflicting conclusions are a result of using different cell types (transformed cell lines versus normal cultured macrophages) awaits the development of good anti-$\beta 5$ mAbs.

GM-CSF is well recognized as an inflammatory cytokine, and transgenic mice constitutively expressing this gene suffer from inflammatory lesions and large increases in the numbers of peritoneal and pleural macrophages (6, 7). Hence the direct implications of our findings appear compelling. We show that GM-CSF causes macrophages to adopt an elongated "fibroblastoid" morphology characteristic of migratory cells (38) and rapidly induces the production and surface expression of $\alpha v \beta 3$ by these cells. Further, this integrin is expressed at the leading edge of cells plated on vitronectin. A number of recent reports have established a fundamental role for $\alpha v \beta 3$ in cellular migration both *in vitro* and *in vivo*. In a study of melanoma (22), a direct correlation between melanoma cell invasiveness and $\alpha v \beta 3$ expression was obtained by histochemical examination of integrin distribution in skin sections, and Seftor *et al.* (23) recently suggested that signaling through the $\alpha v \beta 3$ receptor accounted for the enhanced capacity of melanoma cells to invade basement matrices *in vitro*. In addition, transfection of $\beta 3$ into human pancreatic carcinoma cells resulted in transfectants acquiring the ability to spread and migrate on both vitronectin and fibrinogen substrates (24), and a recent report (39) found that migration, but not attachment, of vascular smooth cells on a variety of matrix glycoproteins was significantly inhibited by a specific polyclonal antibody directed to the $\alpha v \beta 3$ receptor. Taken together these data suggest an exclusive migratory role for the $\alpha v \beta 3$ receptor. It can be suggested therefore that the surface expression of $\alpha v \beta 3$ induced by GM-CSF treatment of monocytes contributes directly to the migratory phenotype.

The inflammatory response is characterized by a massive infiltration of leukocytes from the circulation into the tissues. As binding to the vascular endothelium is an initiating event in monocyte recruitment to the localized site of inflammation, this process has received great attention (40). However, the equally important migratory events subsequent to this process have received scant attention. Our concept of the role played by CSFs in modulating macrophage function *in vivo* is that GM-CSF would be the first cytokine encountered by circulating monocytes since endothelial cells release this cytokine upon stimulation or damage (34). As shown here, GM-CSF rapidly induces macrophages to express $\alpha v \beta 3$ and hence the capacity to migrate on subendothelial and interstitial matrix components. If there is a concurrent site of deep-seated infection, bacterial products (25) and GM-CSF produced by fibroblasts (34) would provide a chemotactic stimulus. Within a few hours, when these cells have reached the site of infection, the autocrine loop (8) will initiate endogenous M-CSF production. At this stage the cells are expressing both $\alpha v \beta 3$ and $\alpha v \beta 5$ (Fig. 2*C*), and in such a setting the $\alpha v \beta 3$ integrin may play a role in clearing cellular debris, since it has recently been reported as a primary receptor mediating phagocytosis of apoptotic neutrophils (41). In addition, the effects of endogenous M-CSF will be to localize cells to the site, to enhance phagocytosis, and to initiate wound repair by production of other factors such as thrombospondin (M.O.D.N., unpublished data), which is a comitogen for smooth muscle cells and is involved in the protection of newly deposited matrix glycoproteins.

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