Granulocyte-Macrophage Colony-Stimulating Factor Receptor: Stage-Specific Expression and Function on Late B Cells

By K.J. Till, J. Burthem, A. Lopez, and J.C. Cawley

Granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors (GMR) are expressed on myeloid cells throughout their maturation sequence. During myelopoiesis, GM-CSF induces the proliferation of precursors and has multiple effects on more mature cells; such effects include induction of maturation and priming for subsequent stimulation. GMR is expressed on a range of other cell types including acute leukemia blasts of myeloid and lymphoid lineage, but has been little studied on more mature lymphoid cells. Using sensitive triple-layer immunophenotypic techniques, we show here that both the alpha and beta chains of the GMR are expressed on hairy cells (HCs) and myelomatosous plasma cells (PCs), but not on chronic lymphocytic leukemia (CLL) or prolymphocytic leukemia (PLL) lymphocytes. The receptor was demonstrable on normal PCs in tonsil, but not on either activated or resting tonsillar B cells or on circulating normal B lymphocytes. The expression of the receptor is therefore stage specific, rather than a feature of activation. Perhaps, surprisingly, in view of its effects on myeloid cells, GM-CSF did not stimulate the proliferation or differentiation of HCs and did not protect them from apoptosis. However, the cytokine had a profound effect on the interaction of the HC with its environment. Thus, the cytokine caused a major cytoskeletal reorganization resulting in the inhibition of motility and loss of adherence to cellular and matrix ligands. These studies indicate the importance of GM-CSF outside myelopoiesis and demonstrate a previously unrecognized stage specific role for the cytokine in B-cell biology. Taken together with our previous report that M-CSF enhances B-cell motility, the present findings indicate that myeloid growth factors act in concert to facilitate the controlled migration of certain B cells into and within tissues.

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MATERIALS AND METHODS

Cell Sources

Patients. Cells from six patients with hairy cell leukemia (HCL) were examined: all the patients had entirely typical disease morphologically, histologically, and immunophenotypically. Peripheral blood mononuclear (PBM) cells were used from four of the cases, whereas in the other two, splenic lymphocytes were employed.

Peripheral blood was examined from eight cases of chronic lymphocytic leukemia (CLL), two cases of prolymphocytic leukemia (PLL), and one case of plasma-cell leukemia; in addition bone marrow was used from four cases of multiple myeloma. Normal B lymphocytes were derived from peripheral blood (n = 3) or tonsil (n = 4).

Cell Preparation

PBM cells were isolated by ficoll-hypaque (F/H) density gradient centrifugation. Tissue from spleen and tonsil was dispersed through a wire mesh before F/H centrifugation. Where T cells formed >5% of PBM, they were removed before culture using 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocyte (SRBC) rosetting.

Normal B lymphocytes were purified from tonsil and peripheral blood by two rounds of SRBC rosetting to remove T lymphocytes; monocytes were then removed by plastic adherence. The resulting populations contained >95% CD19-positive B lymphocytes. The tonsil B cells were then separated further into their low-density (activated; CD25 > 80%) and high density (resting; CD25 < 10%) components by density gradient centrifugation over 60% percoll.

Plasma cells were purified from tonsil mononuclear cells using one round of SRBC rosetting followed by depletion of remaining CD3-, CD14-, and CD19-positive cells using magnetic beads coated with sheep antimouse immunoglobulin. Plasma-cell populations purified in this way were >95% CD38 positive.

Human endothelial cells (HUVE) were obtained from umbilical cords. Briefly, the umbilical vein was filled with phosphate-buffered saline (PBS) containing 0.17% trypsin and detached endothelial cells removed by flushing with PBS. The HUVE were then washed and cultured as described below.

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**Cell Culture**

Lymphocyte cultures were performed in RPMI 1640 medium containing 100 U/mL penicillin/streptomycin, 100 μg/mL L-glutamine and 10% fetal calf serum (complete medium) and incubated at 37°C in 5% CO₂ in air. HL60 cells were kept in continuous culture in complete medium and subcultured twice a week. HUVE were cultured to confluence in Iscove’s modified minimal essential medium (MEM) containing 20% fetal calf serum, 100 U/mL penicillin/streptomycin, and 100 μg/mL L-glutamine. HUVE were used at first passage.

All B-cell cultures were performed in complete medium at a cell concentration of 2 x 10⁶/mL (except where stated).

**Cytokines and MoAbs**

The following cytokines were used: GM-CSF; IL-3, IL-6 (Sandoz, Leeds, UK); tumor necrosis factor α (TNFα; Knoll, Munich, Germany); IL-2 (Sigma, Poole, UK); and M-CSF (Eurocetus; Harrowfield, UK). In certain experiments, the polyclonal B-cell activator SAC (Sigma) was also employed.

MoAbs against the following antigens were used: GMR; α2, αβ, and β2; α2 and αβ chains; these MoAbs do not block GM-CSF binding) CD11b; CD19; CD25; CD38; light-chain immunoglobulin (κ, λ; Becton Dickinson, Oxford, UK); RF6D (Dako, High Wycombe, UK); and the α4, α5, β1, and β3 integrin chains (HCs express α4β1, α5β1, and α6β3 integrin heterodimers) In addition, blocking MoAbs to GM-CSF and IL-1β (Serotec, Oxford, UK) were used.

**Immunophenotyping**

Fluorescence-activated cell sorter. Because levels of cytokine receptor expression are generally low, three-layer immunofluorescence was used to identify cells expressing GMR. Briefly, cells were incubated with the GMR α and β, MoAbs at 100 μg/mL, followed by donkey antimouse biotin at 50 μg/mL (Vector Labs, Petersborough, UK), and finally with streptavidin-phycocerythrin diluted 1:5 (Becton Dickinson). These antibody concentrations had been found to produce optimal staining on control HL60 cells.

Other surface antigens were detected with indirect immunofluorescence employing goat antimouse fluorescein isothiocyanate (FITC) as second layer. Both first- and second-layer antibodies were added at saturating concentrations. Cells were analyzed on a FACS analyzer using a Consort 30 computer (Becton Dickinson).

Alkaline phosphatase anti-alkaline phosphatase (APAAP). In selected experiments, this technique was used to demonstrate GMR positivity. Also, the method allowed morphological identification of GMR-positive cells and confirmation of the plasma-cell nature of certain positive cells in tonsil (using a MoAb to the rough endoplasmic reticulum, RF6D).

For all three staining methodologies, the appropriate class-specific control IgG1 and IgG2 were used.

**Binding Studies**

To confirm the antibody data for GMR expression, binding studies using radiolabelled (125I) GM-CSF were performed according to the method of Woodcock et al. Briefly, HCs were incubated for 2 hours with 0.1, 0.5, 1, 5, or 10 ng/mL of (125I-GM-CSF (DuPont, Wilmington, DE) in the presence or absence of 1 μg/mL of unlabeled GM-CSF. Cell-associated radioligand was separated from free radioligand by overlaying the cell suspension on phthalate oil and centrifuging for 2 minutes at 13,000 rpm in a microfuge. The cell pellet was then removed by cutting, and radioactivity was measured using a gamma counter. Receptor number and dissociation constants were determined using Scatchard analysis.

**Effect of Cytokines on GMR Expression**

GMR expression by HCs was measured after 4, 24, and 48 hours of culture in the presence of GM-CSF at 0.1, 1, and 10 ng/mL, or IL-2 at 100 U/mL. Normal tonsil B cells were stimulated with 0.001% SAC plus 100 U/mL IL-2; GMR expression was examined on these cells at 4, 24, and 48 hours.

**Functional Effects of GM-CSF on HCs**

**Proliferation.** HCs were incubated in the presence and absence of GM-CSF at 1, 10, and 100 ng/mL, with or without the following cytokines: IL-3 (1, 10, and 100 ng/mL), IL-6 (1, 50, and 100 U/mL), M-CSF (100 and 1,000 U/mL), and TNFα (100 and 1,000 U/mL). IL-6 was used because studies on myeloma have shown that proliferation induced by IL-6 was enhanced by GM-CSF. M-CSF and TNFα were used because they synergize with GM-CSF on myeloid cells and because TNFα is a known growth factor for HCs. IL-3 was used because it shares the β chain with GM-CSF. Proliferation, measured by tritiated thymidine incorporation, was examined at 3, 6, 9, and 12 days.

**Differentiation.** As a measure of differentiation, immunoglobulin secretion by HCs was determined. Supernatants were collected from HCs cultured for 10 days in the presence or absence of GM-CSF at 1, 10, and 100 ng/mL. Levels of secreted IgG and IgM were then measured using enzyme-linked immunosorbent assay (ELISA). Tonsil B cells were incubated with SAC (0.001%) plus IL-2 (100 U/mL) as a positive control for the detection of immunoglobulin secretion.

**Antigen expression.** HCs were incubated in the presence or absence of GM-CSF at 0.1, 1, and 10 ng/mL and examined at 30 minutes, 1, 4, 24, and 48 hours, using MoAbs against the following antigens; CD11b, CD19, CD25, κ and λ. Justification for the use of these antibodies is given in the Results.

**Apoptosis.** The survival of HCs in vitro is dependent on cell number, with HCs undergoing markedly increased apoptosis at densities below 1 x 10⁶/mL. HCs were therefore seeded at a range of concentrations between 2 x 10⁴ and 2 x 10⁶/mL in complete medium in the presence and absence of GM-CSF at 1, 10, and 100 ng/mL for 2 days. HCs were then harvested and propidium iodide (PI) (a vital dye) added. HCs were then assayed for apoptosis by fluorescence-activated cell sorter (FACS) analysis; dead cells formed a population of cells distinguishable by their increased uptake of PI and their decreased size. To show that cell death was indeed due to apoptosis, HCs were incubated as above and stained using hypotonic PI. Apoptotic HCs formed a marked sub-G1 population on the FACS histogram.

**Cell motility.** Briefly, glass cover slips were coated overnight with either vitronectin or fibronectin at 20 μg/mL and washed. The coverslips were then placed in 24-well plates together with 1 x 10⁶ HCs in RPMI and incubated for 90 minutes. GM-CSF (10 ng/mL) was then added for 1, 5, and 10 minutes. The effects of the cytokine were arrested by adding glutaraldehyde, and the coverslips thoroughly washed, allowed to dry, and stained with Harris’ hematoxylin.

The cells were examined microscopically and then analyzed in an objective, quantitative manner by image analysis. Area, length, circularity, and perimeter of HCs proved useful measures of the changes observed in the presence GM-CSF. The statistical significance of these differences observed in treated and control cultures was then calculated using analysis of variance.

**Chemotaxis/chemokinesis.** Nitrocellulose filters were coated overnight with 20 μg/mL of vitronectin or fibronectin and washed in PBS. GM-CSF at 0.01, 0.1, 2, 10, and 100 ng/mL was added to the HCs in the top chamber and the chamber incubated at 37°C for 90 minutes. The filter was then fixed with paraformaldehyde, stained...


Table 1. GMR Expression by Various Neoplastic B-Cell Types

<table>
<thead>
<tr>
<th>Disease</th>
<th>GMRA % Positive</th>
<th>MFI*</th>
<th>No.</th>
<th>GMRB % Positive</th>
<th>MFI*</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCL</td>
<td>100t</td>
<td>25 ± 6</td>
<td>6</td>
<td>100t</td>
<td>18 ± 5</td>
<td>3</td>
</tr>
<tr>
<td>CLL</td>
<td>0t</td>
<td>5 ± 5</td>
<td>8</td>
<td>0t</td>
<td>5 ± 5</td>
<td>2</td>
</tr>
<tr>
<td>PLL</td>
<td>0t</td>
<td>5 ± 5</td>
<td>2</td>
<td>0t</td>
<td>5 ± 5</td>
<td>2</td>
</tr>
<tr>
<td>Plasma cell leukemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>1</td>
<td>1</td>
<td>91</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>90 ± 10</td>
<td>18 ± 10</td>
<td>2</td>
<td>90 ± 8</td>
<td>15 ± 9</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
* The mean fluorescence of positive cells expressed in arbitrary units.
† Because the reactivity of all the HCs was shifted to the right relative to the control (Fig 1), they were considered to be 100% positive.
‡ The FACS peaks for these cells could be superimposed on the isotypic control peaks.
§ In these patients, myeloma PCs could only be enriched to a maximum of 80% purity and, because contaminating cells were not distinguishable on the basis of volume/light scatter, examination was performed by APAAP rather than by FACS. PCs were identified by both morphology and by staining for the PC antigen RFD6.
¶ In these patients, highly pure (>95%) PCs were obtained, and the analysis was therefore performed by FACS. In both FACS and APAAP preparations, a minority of PCs were clearly negative (cf. HC populations).

Results

HCs Possess Receptors for GM-CSF

HCs were shown to express both the α and β chains of the GMR in all six cases studied (Table 1). The fact that there was a unimodal distribution of fluorescent HCs, which was shifted to the right relative to the isotypic control, indicates that all HCs were positive (Fig 1); this was confirmed by APAAP staining (n = 3, data not shown). The intensity of staining of HCs (mean fluorescence intensity [MFI], 25 ± 6) was similar to that of positive control cells (for HL60, MFI 30 ± 2; monocytes, MFI 31 ± 1). In addition, binding studies confirmed that HCs specifically bound GM-CSF, with approximately 300 molecules per cell, and a kD of 44 pmol/L (n = 1).

To determine whether the expression of receptor by HCs is a feature of differentiation or activation, we examined a range of other lymphoproliferative disorders of differing maturity, together with resting and activated normal B cells.

GMR Expression by Other Neoplastic B-Cell Types

Expression of the GMR was not seen on the B cells of either CLL (eight cases) or PLL (two cases) (Table 1). Expression of both chains of the GMR was, however, consistently demonstrable on the plasma cells of multiple myeloma (six of six positive) and of plasma cell leukemia (one case) (Table 1); positivity was observed by both FACS and APAAP.

GMR Expression by Normal B Cells

Purified peripheral blood B cells did not express GMR (three of three cases; data not shown). In purified tonsil B-cell preparations, a variable but small number of cells (up to 10%) were positive, while most lacked receptor (data not shown).

Adhesion. 35S-methionine–labeled HCs were incubated on fibronectin- or vitronectin-coated plates or on HUVE for 90 minutes. GM-CSF (10 ng/mL) was added and the cells incubated for a further 1, 10, or 30 minutes. After washing, the cells were trypsinized to ensure all HCs were harvested. The harvested radioactivity provided a measure of adherent cells.

Actin polymerization. HCs were incubated with and without GM-CSF for 30 seconds, 1, 2, 5, 10, and 20 minutes. HCs were then fixed in paraformaldehyde containing lysophosphatidylcholine and NBD-phallacidin for 15 minutes. Lysophosphatidylcholine permeabilizes the cells, and NBD-phallacidin stains polymerized actin. The reaction was stopped by adding an excess of FACS buffer (PBS containing 1% bovine serum albumin [BSA] plus 0.1% azide), and the fluorescence measured by FACS analysis.

Fig 1. GMRA expression by HCs and HL60 cells. (—) Isotypic control; (---) α; (-----) β. Both HCs and HL60 cells can be seen to be positive with respect to the negative control.
Regulation of both the examined. Culture of HCs with the cytokine caused down-regulation of GMR on HCs in response to GM-CSF was observed, despite marked increases in the levels of CD25 (Table 2).

When the tonsil B cells were separated into low- and high-density fractions (respectively containing higher and lower percentages of CD25-positive, activated lymphocytes) both populations contained a similar number (up to 10%) of GMR-positive cells, despite differing greatly in their activation state (Table 2). Furthermore, when the high-density tonsil B cells were activated with SAC plus IL-2 for up to 48 hours, no increased GMR expression was observed, despite marked increases in the levels of CD25 (Table 2).

We next examined the morphology of the GMR-positive cells in APAAP-stained tonsil preparations; the GMR-positive cells were GMR positive by both FACS and APAAP; their plasma cell nature was confirmed morphologically and by APAAP staining for RFD6 (Table 2).

**Table 2. GM-CSF Receptor Expression by Tonsil B Cells**

<table>
<thead>
<tr>
<th>Tonsil B Cells</th>
<th>GM-Rα</th>
<th></th>
<th></th>
<th>βc</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-density fraction*</td>
<td>10 ± 2</td>
<td>24 ± 25</td>
<td>4</td>
<td>5 ± 1</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>High-density fraction†</td>
<td>12 ± 5</td>
<td>21 ± 19</td>
<td>4</td>
<td>5 ± 2</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>High-density fraction stimulated with SAC + IL-2§</td>
<td>6 ± 2</td>
<td>5 ± 5</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Nonfractionated CD19-depleted PCs$</td>
<td>84</td>
<td>25</td>
<td>1</td>
<td>58</td>
<td>9</td>
</tr>
</tbody>
</table>

* >80% CD25+.
† >80% CD25+; results obtained after a 48-hour culture.
§ >90% CD38/RFD6+. PCs examined for GMR and RFD6 by APAAP.

**Regulation of GMR Expression on HCs**

GM-CSF is known to downregulate its receptor on myeloid cells.[30,31] To determine whether or not this was so for B cells and to establish whether the receptor was active, regulation of GMR on HCs in response to GM-CSF was examined. Culture of HCs with the cytokine caused down-regulation of both the α and βc (Fig 2) chains of the GMR (three of three cases). Reduced receptor expression was most marked at 24 hours, and had returned to prestimulation levels by 48 hours (Fig 2).

HCs were incubated with IL-2 to ensure that the down-regulation of GMR expression in response to GM-CSF was specific (HCs express all three chains of the IL-2 receptor).[32] IL-2 at 100 U/mL had no effect on HC GMR expression at 4, 24, and 48 hours (n = 3, data not shown).

**GM-CSF Has no Effect on the Proliferation/ Differentiation/Survival of HCs**

**Proliferation.** Culture of HCs for up to 12 days in the presence of GM-CSF did not induce HC proliferation (three of three cases; data not shown). Because costimulation with other cytokines enhances the proliferation of myeloid cells induced by GM-CSF,[33] the effects on HCs of costimulation with various other cytokines were also investigated. As expected,[26] TNFα induced HC proliferation; however, GM-CSF had no additional effect (three of three cases; data not shown). M-CSF, IL-3, and IL-6 had no influence on HC proliferation; GM-CSF did not synergize with these growth factors (three of three cases; data not shown).

**Differentiation.** This was assessed by both immunoglobulin production and immunophenotypic analysis. After 10 days’ culture, HCs produced only background levels of immunoglobulin secretion (<0.03 ng/mL IgG and IgM). Culture with GM-CSF in the presence and absence of IL-6 had no effect on immunoglobulin secretion (data not shown).

Incubation of HCs with GM-CSF did not induce any consistent changes in the expression of CD11b, CD19, CD25, k or λ during 4 days of culture (data not shown). These antigens were chosen either because they are markers of B-cell differentiation/activation (CD19, CD25, κ/λ) or because GM-CSF is known to alter their expression on myeloid cells (CD11b).[34,35]

**Survival.** HC survival declined after a 48-hour culture, and the morphological features of cell death by apoptosis were observed. Cell death occurred at all the cell densities tested, but was most marked at low seeding concentrations (≤1 × 10⁶/mL). In selected experiments, cell death was formally shown to be the result of apoptosis by FACS analysis of cellular DNA content. GM-CSF, over a range of concentrations, had no effect on survival/apoptosis, regardless of the density of HCs (data not shown).

**GM-CSF Inhibits the Motility of HCs**

HCs respond differently to different substrata; on vitronectin, they adopt a polarized morphology indicative of cell movement.[22] GM-CSF caused HCs to lose this polarity and, within 1 minute, to take on a rounded, unspread appearance. These morphological changes were confirmed by image analysis, where the rounding up was reflected in increased cell circularity and decreased cell area, length, and perimeter (P < .01) (Fig 3). A neutralizing anti-GM-CSF MoAb abrogated these effects, whereas treatment with an irrelevant MoAb (against IL-1β) had no effect (data not shown).

HCs migrate spontaneously into filters coated with vitronectin or fibronectin.[29] GM-CSF inhibited this movement, whether present on either or both sides of the filter; this inhibition was dose-dependent (Table 3). These results indicate that the cytokine inhibits cell movement and is not chemotactic.

**GM-CSF Inhibits the Adhesion of HCs**

HCs adhere to both fibronectin- and vitronectin-coated surfaces. On fibronectin, they display a spread (nonpolar), adherent appearance.[23] GM-CSF caused the HCs to become less spread and, within 10 minutes, many of the cells had detached. These morphological changes were confirmed by image analysis (data not shown), and the effects were blocked with a neutralizing anti-GM-CSF MoAb.
GMR EXPRESSION AND FUNCTION ON LATE B CELLS

Fig 2. Effect of GM-CSF (10 ng/mL) on HC GMR and βc expression. GM-CSF induced a transient reduction in the levels of both receptor chains. Similar results were obtained with 0.1 and 1 ng/mL of GM-CSF.

In an adherence assay, addition of GM-CSF to HCs adherent to both fibronectin or vitronectin caused a rapid (within 1 minute) reduction in adherence, and the cells did not reattach after up to 30 minutes of culture (Table 4).

HCs also adhere to endothelial cells in vivo and in vitro, and this adhesion provides a proliferative signal. Therefore, we investigated the effects of GM-CSF on HC adhesion to endothelium. After a 10-minute incubation with the cytokine, the number of HCs adherent to HUVE was markedly reduced (control 100%, GM-CSF 32% ± 3%; n = 3).

Changes Induced by GM-CSF Are Accompanied by Cytoskeletal Reorganization, but not by Altered Integrin Expression

The decrease in HC adhesion to HUVE and matrix proteins was not due to changes in integrin expression. Thus, both short (1 to 30 minutes) and longer term (4 to 24 hours) culture with GM-CSF did not alter the expression of the α4, α5, αM, β1, and β3 integrin chains on HCs (data not shown).

GM-CSF, however, rapidly reduced the amounts of polymerized actin in HCs (Fig 4). This decrease was evident after 1 minute of incubation with the cytokine and persisted for 5 minutes; levels returned to those of the control HCs by 10 minutes. No further changes were seen after this time point.

Table 3. Influence of GM-CSF on HC Movement

<table>
<thead>
<tr>
<th>GM-CSF Above Filter (ng/mL)</th>
<th>0</th>
<th>0.01</th>
<th>0.1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF below filter (ng/mL)</td>
<td>0</td>
<td>165 ± 11</td>
<td>159 ± 20</td>
<td>117 ± 9*</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>174 ± 20</td>
<td>144 ± 16</td>
<td>125 ± 9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>143 ± 17</td>
<td>144 ± 16</td>
<td>125 ± 9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>139 ± 9</td>
<td>139 ± 9</td>
<td>112 ± 10*</td>
</tr>
</tbody>
</table>

Checkerboard analysis of the effect of GM-CSF on the spontaneous migration of HCs into fibronectin-coated filters. Values of migration are in bold and represent the distance moved by second leading front cells (mean [μm] ± SD of 10 readings from duplicate filters).

Values indicated by * are statistically significant compared with control values (Wilcoxon sign rank sum test P < .05). In similar experiments involving three patients, statistically significant inhibition of migration into both fibronectin- and vitronectin-coated filters was observed (P < .05 at 2 ng/mL).
DISCUSSION

The present study clearly demonstrates that HCs express both the α and β chains of the GMR. Some of the features of HCs reflect their stage of maturation, while others relate to their state of activation.36-39 We therefore examined GMR expression on other mature and activated B-cell types. These studies indicated that the GMR expression of HCs is likely to reflect their late stage of B-cell development rather than their intrinsic activation. Thus, CLL, PLL, and normal circulating B cells all lacked GMR, while normal and myelomatous plasma cells possessed the receptor. Furthermore, both resting and activated (both in vivo and in vitro) tonsil B cells lacked GMR.

We next examined potential functions of the GMR on HCs in relation to what is known for myeloid cells.9,10,31,33,34,39,41 GM-CSF had no effect on the proliferation or differentiation of HCs, even when tested in combination with factors known to influence these functions. Furthermore, the cytokine did not influence the survival of HCs in vitro.

We have recently shown that M-CSF stimulates HCs motility without affecting proliferation or differentiation.29,42 This prompted us to investigate the effects of GM-CSF on HC motility. To do this, we examined the effect of the cytokine on HC stimulated to become motile by interaction with vitronectin.25 GM-CSF caused a dramatic change in the appearance of the cells such that they became nonpolar, rounded, and detached from the vitronectin substratum; the changes were confirmed objectively by image analysis. These observations prompted us to look at the effects of GM-CSF on HC chemokinesis/chemotaxis and adhesion. The cytokine was clearly not chemotactic and inhibited chemokinesis. Regarding adhesion, GM-CSF caused HCs adherent to fibronectin to round up and detach. This anti-adhesive effect of the cytokine was confirmed in formal adhesion assays of HCs adherent to vitronectin or fibronectin. The experiments with endothelial cells35 also suggested an anti-adhesive effect of GM-CSF on HCs (although an effect of the cytokine on endothelial cells was not excluded by our experimental design). These effects differ from those of the cytokine on other cell types, where both motility and adhesion are generally enhanced.9,44-46

Finally, we investigated the mechanism(s) of these anti-motility/adhesion effects. We first considered the relevant integrins; αβ3 is involved in HC motility,25 while α4/α5β1 and αM/αXβ2 are important in interactions with fibronectin33 and endothelium.20 We clearly showed that levels of expression of these integrins were not affected by exposure to GM-CSF. Next, because the observed effects of the cytokine on HCs all involved the cytoskeleton, we examined the effect of GM-CSF on actin polymerization. Rapid changes in F-actin were demonstrated, indicating cytoskeletal reorganization. We therefore conclude that the effects of GM-CSF are mediated by the induction of cytoskeletal changes, although cytokine-induced changes in integrin function, independent of levels of expression, cannot be ruled out.47

Our present work has indicated that the migration/localization of HCs into and within tissues is influenced by both integrin/extracellular matrix interactions and local cytokine activity.25,29 For example, we have shown that M-CSF stimulates the migration of HCs on vitronectin. The present findings indicate that GM-CSF has the opposite effect. Thus, the tissue migration/localization of HCs is under both positive and negative control by cytokines. Many of the characteristics of HCs do not reflect their malignant nature, but rather are shared by normal activated B cells of a comparable stage of differentiation.32,37,47,49 It seems likely, therefore, that the present findings indicate that myeloid growth factors act in concert to facilitate the controlled migration of certain B cells into and within tissues.

Finally, the present results may have therapeutic relevance, as GM-CSF is currently being used in lymphoproliferative disorders and myeloma to rescue patients from chemotherapy-induced cytopenia.13-15

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