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Fibroblast-Secreted Macrophage Colony-Stimulating Factor Is Responsible for Generation of Biphenotypic B/Macrophage Cells from a Subset of Mouse B Lymphocytes

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Normal and malignant CD5⁺ B lymphocytes can develop macrophage-like characteristics. One stimulus of this phenotypic shift is culture of normal mouse splenic B lymphocytes with splenic fibroblasts or their conditioned media. These biphenotypic B/macrophage (B/Mφ) cells simultaneously display macrophage characteristics, such as phagocytosis and F4/80 expression, while retaining B cell features, including expression of surface Ig, CD5, B220, and rearranged Ig genes. The present study investigated the macrophage-secreted factor that promotes this phenotypic change from B cell to B/Mφ cell. RT-PCR analysis demonstrated that mRNA for M-CSF is produced by splenic fibroblasts. Recombinant M-CSF (CSF-1) could replace fibroblast-conditioned medium to elicit the development and survival of B/Mφ cells from splenic B lymphocytes. In addition, neutralization of fibroblast-secreted M-CSF with specific mAbs abrogated the ability of conditioned supernatants to promote outgrowth of B/Mφ cells. The transition from B lymphocyte to B/Mφ cell was marked by the kinetic appearance of mRNA for the M-CSF receptor, c-fms, at day 3 following culture initiation. These results demonstrate that M-CSF is important in the development and physiology of mouse B/Mφ cells and potentially in the growth of human biphenotypic hematological malignancies. Interestingly, the presence of IFN-γ in splenic B lymphocyte cultures abrogated the effect of fibroblast-conditioned medium or M-CSF on outgrowth of B/Mφ cells. Furthermore, these findings suggest that a Th1 microenvironment favored by typical macrophages is detrimental to the outgrowth of B/Mφ cells.


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Abbreviations used in this paper: B/Mφ, B/macrophage cell; rh, recombinant human; rm, recombinant mouse; SFCM, splenic fibroblast-conditioned medium.
regulatory mechanism must exist such that B cells do not typically differentiate into B/MΦ cells unless exposed to a cytokine in an appropriate microenvironment requiring macrophage-like effector function. This report demonstrates that M-CSF is a critical factor in the development of biphenotypic B/MΦ cells from their B lymphocyte precursors.

Materials and Methods

B cell purification

Purification of B lymphocytes was performed as previously described (5). Briefly, a single cell suspension was prepared from spleens of 6- to 10-week-old male B6D2F1 mice (The Jackson Laboratory, Bar Harbor, ME) by mechanical disruption. Erythrocytes were lysed by brief incubation in buffered ammonium chloride. The erythrocyte-depleted splenocyte suspension was incubated for 2 h at 37°C in a humidified atmosphere with 7% CO2 in RPMI 1640 (Life Technologies, Gaithersburg, MD) containing 5% FBS (HyClone, Logan, UT), 5 × 10–5 M 2-ME (Eastman Kodak, Rochester, NY), 10 mM HEPES (United States Biochemical, Cleveland, OH), 2 mM L-glutamine (Life Technologies), and 50 μg/ml gentamicin (Life Technologies), which resulted in adherance of macrophages. Nonadherent cells were washed from the plates, pelleted, and resuspended in a mixture of the following hybridoma supernatants: 30H12 (anti-Thy 1.2), GK 1.5 (anti-CD4), and 3.155 (anti-CD8). This suspension was incubated for 30 min on ice. The lymphocytes were depleted by the addition of low toxicity rabbit complement (Accurate Chemicals/Cedarlane, Westbury, NY) and incubation at 37°C for 45 min. These cells are <1% Thy 1.2- and <1% F4/80-positive as measured by indirect immunofluorescence and flow cytometric analysis of 10,000 cells. Furthermore, they are nonresponsive to Con A, nonphagocytic, and nonspecific esterase-negative.

Cell lines

The isolation of normal mouse spleen fibroblasts and their passage in vitro was described previously (12). Line 1, a mouse small cell lung carcinoma was kindly provided by Dr. Edith M. Lord (University of Rochester, Rochester, NY). LADMAC, a bone marrow monocyctoid cell line that is transfected with a form of myc and secretes M-CSF (13), was generously provided by Dr. William S. Walker (St. Jude Children’s Research Hospital, Memphis, TN). Conditioned medium from these cell lines was collected from confluent cultures and passed through 0.2-μm syringe filters (Gelman Sciences, Ann Arbor, MI) before addition to purified B lymphocytes.

Culture of B lymphocytes to elicit B/MΦ cells

A total of 3 × 106 purified B lymphocytes was incubated in 50% spleen fibroblast-conditioned medium (SFCM), which was harvested from confluent cultures of normal mouse spleen fibroblasts as described above. As indicated, recombinant human (rh)M-CSF (Genzyme, Cambridge, MA) or recombinant mouse (rm)M-CSF (R&D Systems, Minneapolis, MN) was added as a substitute for 50% fibroblast-conditioned medium. Two units (10 ng/ml) of rhM-CSF was comparable to 2 ng/ml mM-CSF in its ability to elicit B/MΦ cell outgrowth (shown in Fig. 3D). In some experiments, rmFlt3L or rmM-CSF (both from Genzyme) was added to the B cell cultures. Cells were incubated at 37°C for at least 6 days before analysis of B/MΦ cell outgrowth. At the time points indicated in the figure legends (ranging from 6–21 days), nonadherent dead conventional B lymphocytes were vigorously washed from the adherent B/MΦ cell monolayer with two changes of medium. PBS containing 0.1% EDTA was added, and plates were incubated 10 min at room temperature to promote detachment of B/MΦ cells from the surface. After gentle tapping of the culture plates, cell scrapers were used to detach firmly adherent cells and did not result in decreased viability. B/MΦ cells harvested in this manner are morphologically and functionally identical to those previously described (5), coexpressing surface B220 and F4/80, and are phagocytic.

RT-PCR assays

Total RNA was isolated from cells using Tri-Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s instructions. RNA was reverse transcribed using a modification of the method of Dallman et al. (14). Briefly, oligo(dt) (2 μg; Pharmacia, Piscataway, NJ) annealing to RNA was accomplished by a 60°C incubation for 5 min followed by a 3-min incubation on ice. Thereafter, a RT reaction mix was added that contained 1× reaction buffer (Life Technologies), 200 μM dNTPs (Phar- macia), 40 U RNasin (Promega, Madison, WI), and 200 U Moloney murine leukemia virus-reverse transcriptase (Life Technologies) in a total volume of 40 μl. For each RNA isolate, a control not containing reverse transcrip-

tase was included to insure that bands obtained in the PCR were not a result of DNA contamination. RT was conducted in a Perkin-Elmer (Norwalk, CT) Thermal Cycler for 60 min at 37°C followed by denaturation of the enzyme at 95°C for 10 min. The sample was diluted by adding an equal volume of water. A total of 10 μl cDNA was used in a 100-μl PCR reaction containing 200 μM dNTPs, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl, 1 μM each primer, and 2.5 μl Taq polymerase (Boehringer-Mannheim, Indianapolis, IN). Primer sequences for M-CSF analysis were: 5′-CCTG CAGCAGTTGATCGCAGAC and 3′-CAGCTTGGTCGACCATCCT. The expected PCR product size for M-CSF cDNA using these oligonucleotides is 413 bp. M-CSF receptor (c-fms) cDNA was amplified using primers: 5′-ACTCCTCAACCTGATCAGGCT and 3′-GTCCACAGCGTT GAGACTGAG. These primers are expected to yield a 756-bp product. Primers used to detect IFN-γ cDNA were: 5′-GGTTACTGCGACCCGGA CAGTGC and 3′-TCCGCTCCTCTGAGGACATTG, which amplify a 595-bp product. All primers were synthesized by Genosys Biotechnologies (The Woodlands, TX). PCR was conducted using an initial denaturation at 94°C for 2 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and polymerization at 72°C for 3 min. A total of 1 μl of each PCR reaction was diluted in loading buffer and electrophoresed in a 2% agarose gel in a chamber containing 1X Tris borate EDTA (TBE) with ethidium bromide. Gels were photographed under UV illumination.

Flow cytometric analysis

B/MΦ cells were resuspended in PBS containing 0.1% azide and 1% BSA (PAB) for staining. To block FcR, samples were incubated in a 1:20 dilution of normal rat serum (Jackson ImmunoResearch, West Grove, PA) for 20 min on ice. Primary Abs or isotype-matched controls were then added for 30 min at a concentration of 1 μg per 1 × 106 cells. FITC-conjugated rat anti-mouse CD45R (B220, clone RA3-68B2) and biotin-recombinant conjugated rat anti-mouse F4/80 (Caltag Laboratories, Burlingame, CA) or FITC-rat IgG2a and biotin-rat IgG2b were employed for two-color flow cytometry (PharMingen, San Diego, CA). After three washes with PAB, 0.4 μg streptavidin-TRicolor (Caltag) was added to each sample and incubated for 30 min on ice. Samples were washed and analyzed for simultaneous expression of B220 and F4/80 using an Epics Elite-ESP flow cytometer (Coulter Electronics, Hialeah, FL). Ten thousand events were examined based on gates set according to the negative controls.

mAb preparation

The 5A1 hybridoma, which produces a rat IgG1-neutralizing Ab reactive with mouse M-CSF, was generously provided by Dr. Hsue-San Lin (Washington University, St. Louis, MO) (15). The hybridoma was grown in complete RPMI medium described above until confluence. The culture supernatant was first concentrated using the Millipore (Marlboro, MA) Minitan System. Affinity purification was done by passing the concentrated supernatant over an anti-rat IgG agarose column (Sigma, St. Louis, CA) followed by elution with 100 mM glycine (pH 3.0). The purified Ab was concentrated and dialyzed using Centricon-10 Microconcentrators (Amicon, Beverly, MA).

Results

Fibroblasts and their secreted products elicit B/MΦ cells

Highly purified splenic B lymphocytes, when cultured in RPMI medium alone, remain nonadherent and die rapidly in the absence of stimulation. However, when viable but not fixed splenic fibroblasts are present, adherent B/MΦ cells are found in the B cell cultures (5). Similar B/MΦ cells are elicited when B lymphocytes are cultivated in the presence of 50% SFCM, as described in the Materials and Methods. To determine whether the development of B/MΦ cells was stimulated by fibroblast-specific cytokines, or a cytokine potentially produced by many cell types, the ability of other cell culture supernatants to support B/MΦ cell outgrowth was tested. As shown in Table I, LADMAC, a monocyctoid line secreting high amounts of M-CSF (13), could replace 50% SFCM to promote B/MΦ cell outgrowth at numbers comparable to fibroblast supernatant. Supernatant from line 1, a mouse small cell lung carcinoma line, also possessed this property; however, B/MΦ cell yield was reduced ~3-fold compared with fibroblast-conditioned...
Cytokine mRNA profiles for cells whose culture supernatants were permissive and nonpermissive for B/Mφ cell growth. To further determine whether permissive supernatants express a common cytokine pattern, cytokine mRNA profiles were obtained by RT-PCR analysis of the cells that supported or did not support B/Mφ outgrowth (Fig. 1). Candidate cytokines important for expansion of B/Mφ cells in vitro would likely be members of the colony-stimulating family, known to participate in the differentiation of typical Mφ. Splenic fibroblasts did not express IL-3 by RT-PCR; therefore, that cytokine was not pursued in this study (data not shown). While splenic fibroblasts and other permissive cell types produced mRNA for M-CSF (Fig. 1) and GM-CSF (data not shown), the nonpermissive, Con A-treated spleen cells contained M-CSF plus IFN-γ mRNA (Fig. 1). Line 1 carcinoma cells also displayed a faint band amplified by IFN-γ-specific primers. These data, along with results obtained in Table I, suggest that the relative concentration of IFN-γ in culture supernatants either will not support outgrowth of B/Mφ cells, or will support outgrowth of these cells at a lower cell number (as occurs using supernatant from line 1 cells). The question of whether or not IFN-γ was an inhibitor of B/Mφ outgrowth was assessed next. The addition of 10–100 U/ml rmIFN-γ to cultures of purified B lymphocytes in 50% SFCM inhibited the development of B/Mφ cells (Fig. 2), and doses of 150 U/ml completely abrogated this process (data not shown). 50% line 1-conditioned medium produced fewer B/Mφ cells than SFCM, perhaps due to the production of very low levels of IFN-γ protein, which are undetectable in an ELISA with a sensitivity of 0.5 ng/ml (Dr. E. M. Lord, unpublished observations). Addition of exogenous IFN-γ to line 1 supernatant also prevented the expansion of B/Mφ cells from splenic B lymphocytes (Fig. 2). Thus, conditioned media lacking IFN-γ support the optimal outgrowth of B/Mφ cells from their normal splenic B lymphocyte precursors.

**rM-CSF elicits B/Mφ cells from B lymphocytes**

The mRNA data obtained in Fig. 1 suggest that a cytokine common to permissive supernatants is M-CSF. To further characterize the B/Mφ-promoting effects of the CSFs, rhM-CSF (2.0 U/ml) was added to purified B lymphocyte cultures, and, at day 13, the resultant spindle adherent cells were compared with those control B/Mφ cells elicited by 50% SFCM. Since B/Mφ cells have a striking morphology that differs from typical macrophages with reniform nuclei, cytocentrifuge preparations of B/Mφ cells differentiated with 50% SFCM (Fig. 3A) or rM-CSF (Fig. 3B) were stained with Wright-Giemsa solution. In both samples, enlarged vacuolar granular cells with eccentric round nuclei were present that were morphologically identical to B/Mφ cells (5). Furthermore, this morphologic analysis showed that the B/Mφ cells retrieved after culture in SFCM or rM-CSF were homogeneous and did not contain any typical B lymphocytes or conventional macrophages. Next, adherent cells were harvested from the M-CSF cultures and stained for expression of B220 and F4/80, a hallmark of B/Mφ cells growing in vitro. Fig. 3C demonstrates that M-CSF was the only exogenous factor required to promote B/Mφ development in purified B lymphocyte cultures, as adherent B/Mφ cells elicited by rhM-CSF expressed B220 as well as F4/80. While GM-CSF did promote proliferation of B/Mφ cells already differentiated from B lymphocytes with 50% SFCM, it did not promote their differentiation when added at the onset of B cell culture (data not shown). Thus, M-CSF is sufficient to promote B/Mφ differentiation from splenic B lymphocytes in vitro. The effect of rM-CSF was antagonized by rmIFN-γ (Fig. 3D), as was shown for unpurified conditioned cell supernatants (Fig. 2). Even at high doses of rm-M-CSF.

**FIGURE 2.** IFN-γ inhibits the development of B/Mφ cells. rmIFN-γ was added at the indicated concentrations to the permissive splenic fibroblast or line 1-conditioned media. B/Mφ cells were harvested from cultures and counted by trypan blue exclusion at day 9 following culture initiation. For comparison, in the same experiment, LADMAC supernatant yielded 9 × 10^5 B/Mφ cells, while Con A spleen supernatant or RPMI medium only did not yield any detectable adherent cells.

**FIGURE 1.** Cytokine mRNA profiles for cells whose culture supernatants were permissive and nonpermissive for B/Mφ cell growth. RT-PCR was performed as described in Materials and Methods. **Lane 1**, LADMAC; **lane 2**, Con A-stimulated splenocytes; **lane 3**, line 1 cells; **lane 4**, SPL 1217 splenic fibroblasts. Reverse transcriptase negative controls did not contain any amplified bands (data not shown). The products for M-CSF were 413 bp; IFN-γ, 395 bp; β-actin, 568 bp.

**FIGURE 3A.** Splenic Fibroblast Conditioned Medium

**FIGURE 3B.** Line 1 Conditioned Medium

**TABLE I.** Permissive and nonpermissive culture supernatants for B/Mφ cell growth

<table>
<thead>
<tr>
<th>Conditioned Medium</th>
<th>Outgrowth of B220&lt;sup&gt;a&lt;/sup&gt; F4/80&lt;sup&gt;b&lt;/sup&gt; B/Mφ Cells</th>
<th>Cell Yield (×10&lt;sup&gt;-7&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic fibroblasts</td>
<td>Yes</td>
<td>8.3</td>
</tr>
<tr>
<td>LADMAC</td>
<td>Yes</td>
<td>9.0</td>
</tr>
<tr>
<td>Line 1</td>
<td>Yes</td>
<td>3.0</td>
</tr>
<tr>
<td>Splenocytes + Con A</td>
<td>No</td>
<td>0.3</td>
</tr>
<tr>
<td>Unconditioned medium</td>
<td>No</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Purified splenic B lymphocytes (4 × 10<sup>6</sup>) were cultured in 6-well plates containing 50% complete RPMI medium and 50% conditioned medium from the indicated sources. After 9 days, the adherent B/Mφ cells were harvested and counted by trypan blue to assess relative cell yield.
A key point in establishing M-CSF as the required B/Mφ neutralizing Ab to block this activity. SFCM was incubated with a dilution series of 5A1 Ab (rat anti-mouse M-CSF) or isotype control Ab 11B11 (rat anti-mouse IL-4) on ice for 45 min before its addition to purified splenic B cells. Fig. 4A shows that cells treated with SFCM only or SFCM and isotype control Ab are tightly adherent and viable with a spindly B/Mφ morphology. By contrast, when the neutralizing 5A1 Ab (50 μg/ml) was present in purified splenic B cell cultures in SFCM, B/Mφ development was blocked and only dead nonadherent B cells were visible. These results are depicted numerically in Fig. 4B, where B/Mφ cells were removed from the cultures with trypsin and counted using a hemacytometer, and there were no detectable B/Mφ cells in wells containing the 5A1 Ab compared with isotype and fibroblast-conditioned medium-only controls.

**B/Mφ cells, but not their precursors, express c-fms mRNA**

The cytokine M-CSF has been shown to interact with a specific receptor believed to be restricted to cells of the monocyte/macrophage lineage (16). The c-fms protooncogene encoding this receptor gives rise to a transmembrane product that contains an intracellular tyrosine kinase domain (reviewed in Ref. 17). To examine whether the established, normal B/Mφ cells expressed mRNA for c-fms, RT-PCR was employed as described in Materials and Methods. Using a malignant B/Mφ-like cell line, P388D1, as a positive control, data shown in Fig. 5 was obtained. Both normal and malignant B/Mφ lines expressed c-fms mRNA. To assess whether precursor B lymphocytes expressed c-fms mRNA, total purified splenic B lymphocyte RNA was used in the RT-PCR assay. As shown in Fig. 6A, the starting B lymphocyte population did not express c-fms message, and this was confirmed by a second round of PCR for a total of 80 cycles (data not shown). Fluorescence-activated cell sorting of the starting population into CD5⁺ and CD5⁻ B lymphocytes followed by RT-PCR for c-fms also failed to demonstrate expression. These results also indicate that the starting B lymphocyte population was pure and did not contain typical macrophages or their precursors that would express c-fms.

If M-CSF is critical for the induction of B/Mφ cells, the precursor cells must, at some point after culture initiation, express the receptor for this cytokine. A preliminary kinetic analysis of only the nonadherent B cells cultured in SFCM showed no evidence of c-fms mRNA expression at any time point (data not shown). To include any adherent intermediates, a kinetic analysis of adherent and nonadherent cells in each culture was performed, and the results are shown in Fig. 6B. While c-fms mRNA is undetectable at days 1 and 2 following input of purified B lymphocytes in 50% SFCM, a faint band was detectable at day 3. Message for c-fms was easily detectable in B cell cultures harvested on days 4–7 following their initiation, which correlated with the appearance of adherent cells with B/Mφ morphology and coexpression of B220 and F4/80. Therefore, other events before the expression of c-fms must take place to render the precursor mature B lymphocytes responsive to M-CSF in the progression from B cell to B/Mφ cell.

**Discussion**

The identity of the cytokine that induced a novel B/Mφ cell phenotype in normal B lymphocytes was elucidated in this study. While splenic fibroblasts and their conditioned medium were initially used to induce B/Mφ cell development from B lymphocytes (5), this activity is not fibroblast-specific, as LADMAC and line 1 tumor cell supernatants had this capability (Fig. 2). Moreover, rM-CSF could replace conditioned media to induce B/Mφ cell differentiation (Fig. 3). This is in contrast to observations of Cumano et al. (18), who found that M-CSF could not replace S17 fibroblasts and IL-7 to differentiate B cells and macrophages from bipotential...
precursors isolated from fetal liver. Since these are earlier precursors and have unrearranged Ig loci, unlike B/Mf cells (5), perhaps the cytokine requirements to allow macrophage differentiation at that stage are more complex than at the level of the mature Ig+ B cell to B/Mf transition. M-CSF is produced by many cell types, including fibroblasts and macrophages (reviewed in Ref. 19). Thus, multiple cell types may promote B/Mf growth in vivo by secreting M-CSF.

Importantly, although splenic fibroblasts do synthesize mRNA for another CSF, GM-CSF (data not shown), neutralizing Abs specific for M-CSF completely blocked B/Mf outgrowth (Fig. 4, A and B). This suggests that GM-CSF does not act in a redundant fashion to elicit normal splenic B/Mf cells. Further indication that GM-CSF is not the B/Mf-inducing factor came from incubation of B lymphocytes in 50% supernatant from line 1 tumor cells transfected with a GM-CSF expression vector, which did not result in the outgrowth of B/Mf cells (M. A. Borrello and R. Phipps, data not shown). This is in contrast to the work of Katoh et al. (2), where GM-CSF in conjunction with stromal cells was required for macrophage differentiation from a pre-B cell line in long-term bone marrow culture. However, rGM-CSF did act as a proliferative stimulus for already established B/Mf cells (data not shown). Perhaps the two CSFs cooperate, such that splenic fibroblast-secreted M-CSF is an initiation factor for B/Mf cell differentiation from B lymphocyte precursors and GM-CSF is a growth factor, sustaining their continued proliferation in vitro.

The inflammatory cytokine IFN-γ had a strong inhibitory effect on the outgrowth of B/Mf cells. Con A-stimulated spleen cell-conditioned medium displayed IFN-γ mRNA (Fig. 2) and is known to contain high levels of IFN-γ protein (20). Although M-CSF mRNA is also detectable in splenocytes treated with Con A, it appears that IFN-γ overrides any positive signaling to prevent B/Mf differentiation. Likewise, the dominant negative regulatory effect of IFN-γ was shown by its addition to otherwise permissive culture supernatants (splenic fibroblast, line 1) that blocked B/Mf outgrowth (Fig. 2). IFN-γ can also act at a later stage, following
B/M differentiation, to abrogate their \(^{3}H\)thymidine incorporation in response to SF CM (data not shown). Although few to no adherent cells are present in IFN-\(\gamma\)-treated B cell cultures in permissive conditioned media (Fig. 2) or RM-CSF (Fig. 3D), it is possible that the conversion from B lymphocyte to B/M\(\phi\) cell has progressed to an intermediate phase. Information regarding this intermediate could be obtained by adding IFN-\(\gamma\) at later time points (days 1–4) following culture initiation to determine whether this inhibitory cytokine must be present at the onset of B/M\(\phi\) differentiation to abrogate their appearance, or if multiple phases of B/M\(\phi\) cell development are IFN-\(\gamma\)-sensitive. An intriguing finding would be that IFN-\(\gamma\) prevents c-fms expression on developing B/M\(\phi\) cells, especially in light of data showing that this cytokine prevents c-fms expression on developing macrophages (24). C-fms expression in bone marrow-derived macrophages in response to M-CSF (21).

Cell-mediated and humoral immunity are promoted by differing and highly controlled mechanisms that have been the recent subject of intense investigation. The cytokines IFN-\(\gamma\) and IL-4 reciprocally regulate whether a cell-mediated (Th1) or a humoral (Th2) response occurs after infection. In the mouse, IFN-\(\gamma\) promotes a Th1 response to the exclusion of Th2 effectors, and IL-4 precludes Th1 development (reviewed in Ref. 22). Conventional macrophages are stimulated by IFN-\(\gamma\) to express activation and costimulatory molecules, act as APCs, and promote a Th1 response. IL-4, in contrast, has proven to be inhibitory to many aspects of macrophage function, especially intracellular killing and expression of activation Ags (23). Initial evidence that B/M\(\phi\) cells participate in immune responses independent of IFN-\(\gamma\) was their constitutive expression of class II MHC, CD40, and B7-2 (5). The fact that IFN-\(\gamma\) is highly inhibitory to B/M\(\phi\) proliferation and prevents their differentiation from B lymphocytes further supports the hypothesis that B/M\(\phi\) cells may be a Th2-type APC involved in humoral immunity. The notion that B/M\(\phi\) cells can present Ag in the absence of IFN-\(\gamma\) priming is underscored by Bretz et al.’s (24) finding that ras-transformed B/M\(\phi\) cells presented Ag comparably with peritoneal exudate macrophages without prior activation. Whether IL-4 or other Th2 cytokines have a stimulatory effect on B/M\(\phi\) cells, as they do for B cells, such as inducing class II MHC hyperexpression, remains to be investigated.

Expression of the M-CSF receptor, c-fms, is considered to be restricted to the macrophage lineage in mice. Borzillo et al. (4) showed that forced expression of human c-fms in mouse pre-B lymphoma cells was sufficient to induce macrophage differentiation. Furthermore, Tanaka et al. (25) used c-fms to characterize intermediates in the spontaneous differentiation of the pre-B lymphoma 70Z/3 to macrophage-like cells. Their development progressed from nonadherent c-fms mRNA negative pre-B cells to an adherent c-fms intermediate, to adherent macrophage-like, c-fms+ cells. In the present study, c-fms mRNA was undetectable by RT-PCR in purified B lymphocytes (Fig. 6A), but is detected on transition cells by day 3 after culture initiation (Fig. 6B). Interestingly, M-CSF receptor expression has been detected on the surface of normal and malignant human B lymphocytes (26) and hairy cells of hairy cell leukemia patients, which display B cell and monocytoid features (27). In these studies, the level of c-fms gene product on human B lymphocytes was lower than that of normal monocytes, suggesting that the lack of detection of c-fms in mouse B lymphocytes may be due to sensitivity issues, such as expression by a very small subset of the total B cell population, or to species-specific differences. The identification of a B lymphocyte subset expressing c-fms would suggest their ability to differentiate into B/M\(\phi\) cells in the presence of M-CSF and would thus shed light on the B/M\(\phi\) precursor. Further studies of the constitutive expression of c-fms in normal mouse B lymphocytes and the impact of induced expression on development of macrophage characteristics are necessary to address these issues.

There are at least two reports of human B cell lines capable of differentiating to macrophage-like cells in vitro (28) or displaying phosphoprotein patterns resembling cells able to differentiate into macrophages (29). Additionally, there are a number of malignant and nonmalignant diseases where biphenotypic cells have been observed in bone marrow and peripheral sites (30, 31, 32). Patients bearing neoplasms with simultaneous B cell and monocytoid characteristics display a low remission rate and poor prognosis (30). Future work will examine the existence of B/M\(\phi\) cells in humans to identify a normal counterpart to these neoplastic biphenotypic cells. The knowledge of the B/M\(\phi\) growth factor (M-CSF) and inhibitory factor (IFN-\(\gamma\)) may facilitate the identification of these cells in vitro and, ultimately, in understanding and controlling multiple human biphenotypic malignancies.

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References


