B Lymphocyte Precursors and Myeloid Progenitors Survive in Diffusion Chamber Cultures but B Cell Differentiation Requires Close Association With Stromal Cells

By Philip C. Kierney and Kenneth Dorshkind

The aim of this study was to investigate the relative contribution of direct contact with stromal cells to stromal cell-derived soluble mediators to the differentiation of B lymphocytes and cells from other hematopoietic lineages. This was investigated by making a comparison between hematopoietic cells grown in direct contact with stroma to those in diffusion chambers (DCs) placed over purified populations of stroma. The source of stromal cells was adherent layers from myeloid or lymphoid long-term bone marrow cultures that had been treated with mycophenolic acid, an antibiotic that depletes hemopoietic cells from the cultures but retains a functional stroma. The cells seeded into the chambers were fresh marrow cells that had been passed through two consecutive nylon wool columns to deplete cell populations capable of forming an adherent cell layer in vitro. DCs were placed in wells in which the adherent stroma, growing under myeloid or lymphoid conditions, was present. The results indicate that progenitors of granulocytes and macrophages survived and differentiated in DCs under myeloid culture conditions, as the number of cells and absolute number of CFU-GM increased over that present in the reseed population. These levels, however, were markedly less than in parallel cultures in which the cells were seeded directly onto stroma. Hematopoiesis in DCs placed over hemopoietically active stroma was not optimal, suggesting that factors were used by those hemopoietic cells closest to the stroma. A B lymphocyte precursor survived in DCs under myeloid but not lymphoid conditions, and its differentiation into B lymphocytes was dependent on close association with stromal cells; B lymphopoiesis initiated when cells from DCs grown under myeloid conditions were harvested from the chambers and seeded directly onto stroma initiated and maintained under lymphoid bone marrow culture conditions. B lymphopoiesis did not initiate if the DC from the myeloid conditions was left intact and placed directly over a lymphoid stromal cell layer in lymphoid conditions.

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The results indicate that B cell precursors, as well as myeloid progenitors and their differentiated progeny, can survive in DCs but that hemopoiesis is not as optimal as when direct stromal cell contact is permitted. The data further indicate that the differentiation of the MBMC B cell precursor into B lymphocytes only occurs when direct stromal cell contact is permitted.

MATERIALS AND METHODS

Animals. Six- to 8-week-old male or female BALB/cAn mice were produced in the vivarium of the Division of Biomedical Sciences, University of California, Riverside, from the breeding stock obtained from Life Sciences Laboratories, St. Petersburg, FL.

Preparation of cell suspensions. Mice were killed by cervical dislocation, and femurs were removed and placed in α-minimal essential medium (α-MEM; GIBCO, Grand Island, NY). Single cell suspensions of bone marrow were obtained by flushing the plug from bones with 3 mL medium. Cell viability was determined by eosin dye exclusion, and all cell counts were performed in a hemocytometer.

Generation of stromal cell cultures and stromal cell lines. Stromal cell cultures were generated by establishing long-term bone marrow cultures under myeloid or lymphoid conditions and subsequently treating these with the antibiotic mycophenolic acid, MPA, (GIBCO). As previously described in detail, this procedure results in the depletion of hemopoietic cells while retaining a viable, functional adherent layer of stromal cells. Purified stromal cells initiated under myeloid conditions were established by plating 4 mL of bone marrow cell suspension at 10^6 cells/mL in α-MEM supplemented with 20% horse serum (GIBCO) and 10^{-7} mol/L hydrocortisone succinate (Upjohn, Kalamazoo, MI) in each 9.5-cm² well of a six-well plate (Costar, Cambridge, MA). Cultures were placed in a 33°C incubator and wrapped in Saran Wrap to prevent drying of cultures. Cultures were fed after 1 week, and by 2 weeks a confluent, adherent layer of stromal cells was established. Purified stromal cells initiated under lymphoid conditions were similarly established as above except that the medium consisted of RPMI 1640 supplemented with 5% fetal calf serum (FCS) (GIBCO), and 5 × 10^{-5} mol/L 2-mercaptoethanol (2-ME), and all incubations were carried out in a 5% CO₂ and air incubator at 37°C. An aliquot of 10^9 cells/mL in a-MEM supplemented with 5% FCS and 1 mg/mL sodium azide. Cells from these dishes were harvested, washed once, and placed in a new a-MEM supplemented with 5% FCS and 1 mg/mL sodium azide. Approximately 7% of the initial cell input remained following passage through the second nylon wool column. To confirm the efficacy of the separation procedure, aliquots of 5 × 10^8 of cells passed twice through nylon wool were cultured alone in tissue culture wells and observed throughout the course of the experiment for growth of stromal or hemopoietic populations.

DC cultures. DCs were seeded with bone marrow cells that had been passed through nylon wool and placed in the wells of the cluster dishes. The chambers, suspended in each well, consisted of a low protein binding, non-tissue culture-treated nucleopore membrane with 0.4 µm pore size (Costar). The distance between the bottom of the DC membrane and the bottom of the tissue culture well is 1 mm, thus minimizing the chance that stromal cell processes could have made contact with cells in the DC through pores in the DC membrane. Cells (10^7 and passed twice through nylon wool) were seeded into the chambers, which were then placed in the tissue culture wells with preestablished stromal layers. The cultures contained a total volume of 4.1 mL, with 1.5 mL contained within the DC and an additional 2.6 mL in the well below. Cultures were fed by first removing 0.75 mL from the chamber. This chamber was then removed from the well with sterile forceps, and one-half the volume was removed from the underlying well and replaced with fresh medium. The DC was then placed back in the well, and 0.75 mL of additional fresh medium was added to it.

Cultures under MBMC conditions were maintained at 33°C in α-MEM supplemented with 20% horse serum (GIBCO) and 10^{-7} mol/L hydrocortisone succinate (Upjohn). The cluster dishes were wrapped in Saran Wrap to prevent dessication because the 33°C 5% CO₂ and air incubator was not humidified. Cultures under lymphoid conditions were maintained at 37°C in a humidified 5% CO₂ and air incubator in RPMI 1640 supplemented with 5% FCS (GIBCO) and 5 × 10^{-5} mol/L 2-ME.

Transfer of cultures from myeloid to lymphoid conditions. In some experiments, cultures were transferred from myeloid to lymphoid growth conditions. For those cells grown in DCs under myeloid conditions, cells were harvested, washed once, and placed in a new dish and passed through nylon wool. Alternatively, aliquots of cells from the DCs were seeded directly onto stroma in BMDC conditions. Those myeloid cultures in which nylon-wool-passed cells had been seeded directly onto stroma were transferred by pooling the nonadherent cells with the loosely adherent ones obtained by vigorously washing the wells by repeated pipetting. After one wash, these cells were then reseded onto adherent stromal cell layers established under lymphoid conditions.

Antibody labeling of cells and fluorescence microscopy. Surface IgM-bearing cells were identified with an affinity-purified, fluorescein-conjugated, goat anti-mouse IgM antibody (Southern Biotechnology, Birmingham, AL). This reagent was diluted 1:5 in α-MEM supplemented with 5% FCS and 1 mg/mL sodium azide. Aliquots of 1 to 2 × 10^6 cells harvested from cultures or fresh bone marrow were suspended in 100 µL diluted antiserum and incubated for 45 minutes on ice. Cells were then washed twice at 4°C in α-MEM without serum and resuspended in 0.2 mL α-MEM. To this was added an equivalent volume of 2% paraformaldehyde in phosphate-buffered saline (PBS). Cells were stored at 4°C until cell counts were performed on wet-mount preparations using a Leitz Laborlux microscope equipped for epifluorescence. At least 500 cells were counted per sample. Those cells that expressed the 14.8 surface antigen were identified by incubating 1 to 2 × 10^6 cells with 100 µL rat anti-mouse hybridoma supernatant (American-type culture collection, Rockville, MD) for 30 minutes at 4°C. Following two washes, cells were resuspended in 50 µL 1:5 dilution of an affinity-
purified goat anti-rat Ig (Southern Biotechnology). No cells in fresh marrow or cultures stained with this reagent alone. After a 30-minute incubation at 4°C, cells were washed twice as above and resuspended in paraformaldehyde. Fresh bone marrow controls were included in all experiments and, on the average, 9% of cells in fresh marrow were surface IgM positive and 20% expressed the 14.8 antigen.

**Myeloid colony assay.** Myeloid progenitor cells capable of forming colonies in semisolid medium were assayed by culturing 5 × 10⁶ cells in one cm² plastic Petri dishes containing 1 mL methylcellulose medium.²² The latter contained 0.8% methylcellulose, 30% FCS, 5 × 10⁻³ M 2-ME (Sigma, St Louis), and 10% medium conditioned by WEHI-3B (D') cells. This was prepared by culturing WEHI cells at 10⁶ cells/mL in 40 mL a-MEM supplemented with 1% FCS. Following a 24-hour incubation, supernatants were collected, centrifuged, and stored at 4°C until used. Colonies were enumerated on day 8.

**RESULTS**

**Hemopoietic cell survival in diffusion chamber under MBMC conditions.** To determine how the growth of cells in diffusion chambers under MBMC conditions compared with that usually observed in primary MBMC, 10⁵ bone marrow cells passed twice through nylon wool were seeded in DCs placed over purified stromal cell layers. The nylon wool passage depleted stromal cells that could potentially form a microenvironment within the DC, thus ensuring that survival of hemopoietic populations would be dependent on stromal cells in the underlying well. The source of this stroma was a primary MBMC that has been treated with MPA to deplete hematopoietic cells. This growth was compared with that in DCs placed over hemopoietically active stroma or following direct seeding of the nylon-wool-passed cells on purified stromal cell layers. The data in Table 1 show the results of three such experiments in which triplicate wells were evaluated for each condition.

Hemopoietic cells were consistently recovered following seeding in DCs placed over stromal cell layers obtained by MPA treatment. Three weeks following initiation of the cultures, the number of hematopoietic cells recovered from the chambers had increased three- to sevenfold over the number initially seeded. Morphological examination of these populations showed them to be primarily neutrophilic granulocytes and macrophages, a finding consistent with the fact that 70% to 90% of the cells expressed the MAC-1 surface antigen (data not shown). The data also indicate that progenitors of granulocytes and macrophages survived under DC conditions for the 3-week observation period as the absolute number of CFU-GM had increased up to fivefold over the number in the initial seed population. In no case was the absolute number of CFU-GM present 3 weeks postinitiation less than that present in the reseeded population.

A second aliquot of the same nylon-wool-passed bone marrow cells was seeded directly onto MPA-derived stromal cell layers. As shown in the middle column of Table 1, a higher level of hematopoiesis was observed in these cultures, as the numbers of cells recovered had increased up to 33 times the initial number seeded. This was generally reflected in the number of CFU-GM present; in experiment 2, the numbers of these progenitors had increased to 91 times the numbers in the reseeded population.

A third aliquot of the above cells was seeded into DCs placed over hematopoietically active stroma. These cultures were established by simultaneously seeding 10⁵ of the nylon-wool-passed bone marrow cells into DCs and directly onto the MPA stromal cell layers over which the chambers were placed. The use of reseeded, MPA-treated stroma in this third condition ensured that the stromal cells in all experiments were manipulated in a comparable manner. The number of hematopoietic cells recovered from these DCs 3 weeks postseeding had decreased twofold in one experiment and had doubled in the other two. These conditions were not optimal, however, since the numbers of CFU-GM present were always less than in the initial nylon-wool-passed reseed population.

In all experiments, aliquots of nylon-wool-passed cells were seeded directly into empty wells. No adherent layer formed under these conditions, and hematopoietic cells did not survive past 1 week. Neither did hematopoiesis initiate in DCs placed into wells in which no adherent stromal layer was present. Before they were used, MPA-treated stromal cell cultures were observed for 2 weeks following the last drug treatment to ensure that hematopoiesis did not initiate from drug-resistant precursors.

**Survival of B cell precursors in DCs.** An early B cell

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Growth Conditions of Nylon-Wool-Passed Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postincubation</td>
<td>DC Over MPA Stroma</td>
</tr>
<tr>
<td>Cell No.</td>
<td>1</td>
</tr>
<tr>
<td>(relative change)</td>
<td>over 10⁵ seeded</td>
</tr>
<tr>
<td>2</td>
<td>0.5 × 10⁶ ± 0.3 (15)</td>
</tr>
<tr>
<td>CFU-GM/10⁶</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>210 ± 165</td>
</tr>
<tr>
<td>CFU-GM/10⁶</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>Absolute no.</td>
<td>1</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>2</td>
</tr>
<tr>
<td>(relative change)</td>
<td>over 10⁵ seeded</td>
</tr>
<tr>
<td>3</td>
<td>273 (8)</td>
</tr>
</tbody>
</table>

Nylon-wool-passed bone marrow (10⁵) seeded into DCs placed over MPA-purified stroma, directly onto MPA stroma, or in DC over stroma on which 10⁵ hematopoietic cells had been seeded in parallel. Number of CFU-GM/10⁵ in reseed population in experiments 1, 2, and 3 were 125, 83 and 280, respectively. Each experiment represents triplicate cultures per condition. Numbers indicate mean ± SD.
precursor, but not pre-B or mature B cells, is present under MBMC conditions in which direct contact with the stroma is permitted,\textsuperscript{12,13} and these cells can be induced to differentiate into mature B cells following transfer to LBMC conditions.\textsuperscript{28,29} This is demonstrated by the data in Table 2 in which nylon-wool-passed cells were seeded onto stromal cell layers under MBMC conditions (condition 1, Table 2); 3 weeks later, the cells were harvested and replated on LBMC stroma under LBMC conditions. As shown in Table 2, cells that expressed surface IgM were induced by 4 weeks later.

To determine whether the MBMC B cell precursor also survived in DCs under MBMC conditions for 3 weeks, cells from cultures were either transferred to a new DC that was placed over a stromal cell adherent layer established by treating a primary LBMC with MPA (condition 2, Table 2) or harvested from the DCs in MBMC conditions and plated directly onto LBMC stroma (condition 3, Table 2). These cultures were then maintained at 37°C in LBMC medium. Less than 5 \times 10^{6} cells were recovered from DCs after 4 weeks in LBMC conditions, and no IgM-expressing cells were included among this population. This was consistent with morphological observations that demonstrated these cells to be granulocytes and macrophages. It is not clear why these myeloid cells survived under the LBMC conditions, but very low levels of colony-stimulating factors sufficient to allow this could have been secreted by the stromal cells under lymphoid conditions. A markedly different profile was observed in cultures in which cells had been seeded directly on stroma (condition 3). Up to 2 \times 10^{6} cells were recovered from these cultures, and up to 10\% expressed surface IgM. Morphological examination demonstrated virtually all these cells to be lymphoid. These data indicate that a B cell precursor can survive in DCs under MBMC conditions but requires a close association with stromal cells to differentiate into surface IgM-positive B cells.

The adherent layers in LBMC conditions onto which DC cells were seeded were MPA-treated LBMC stroma. This left open the possibility that the surface IgM-positive B cells detected could have arisen from MPA-resistant precursors present in the adherent layer. To ensure that the B cells were indeed derived from the DC cells, replicate experiments were performed in which 10^{6} nylon-wool-passed cells were seeded into DCs over MPA-treated stroma under MBMC conditions. Following a 3-week incubation period, cells were harvested from the chambers and plated onto a confluent layer of S17, a stromal cell line shown to support B cell differentiation,\textsuperscript{30} under LBMC conditions. Three separate experiments were performed, and the mean number of cells (\pm SD) recovered following 4 weeks in LBMC conditions was 2.1 \pm 0.6 \times 10^{6}. Phenotypic analysis demonstrated that 48\% \pm 3.6\% of these expressed the 14.8 antigen and 12.4 \pm 7.3\% were surface IgM positive. These data confirm that a B cell precursor is present in DCs and can differentiate into B cells when provided with the proper conditions.

**Adherent populations of functional stroma do not establish in DCs.** The conclusion that a B cell precursor survives under DC conditions is dependent on confirming that the nylon-wool-passed cells are not in contact with an adherent layer of functional stromal cells. That the nylon-wool-passed cells cultured alone do not form adherent layers and that the DC membranes are designed to prevent cell adherence suggest that this is the case. Nevertheless we considered it critical to examine the DC membrane to exclude the possibility that a functional adherent layer had established under the influence of soluble mediators produced by the stromal cells in the lower chamber.

Following 3 weeks of growth under MBMC conditions, nonadherent cells were removed from the DC growing over MPA-treated stroma in MBMC conditions. The membranes were gently washed and stained with Wright’s stain. Examination of the membranes at 100x under a dissecting microscope indicated that a few foci of cells adherent to the membrane were observed in one-half of the experiments. These appeared to be macrophages, as they presented with a central nucleus and abundant cytoplasm. In view of this result, however, experiments were designed to establish whether populations of cells that could support hematopoiesis did indeed establish on these DC membranes. This was tested by placing the DC, seeded with 10^{6} nylon-wool-passed cells, over a well in which MPA-derived stroma was growing under MBMC conditions. After 3 weeks, the nonadherent cells were removed from the chambers and the membranes were gently washed. The DCs were then placed over a well containing medium only in MBMC conditions and reseeded with 10^{6} nylon-wool-passed cells. If a functional stromal layer had established in the DC, hematopoiesis would have been expected to initiate. Cultures were fed weekly and then examined for cell growth at 3 weeks postseeding, but no cells were recovered at that time. This experiment indicates that functional adherent layers do not establish in DCs under the experimental conditions used and suggests that the B cell precursor and CFU-GM that survive in DCs do so under the influence of soluble mediators secreted by stromal cells in the underlying well.

### Table 2. Survival of B Cell Precursors in DC

<table>
<thead>
<tr>
<th>Condition</th>
<th>Growth Under MBMC Conditions (3 weeks)</th>
<th>Following Switch to</th>
<th>LBMC Conditions (4 weeks)</th>
<th>Cell No. After 4 Weeks in LBMC Conditions</th>
<th>IgM Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stroma</td>
<td>--</td>
<td>Stroma</td>
<td>1 \times 10^{6} \pm 0.1</td>
<td>6.4 \pm 5</td>
</tr>
<tr>
<td>2</td>
<td>DC</td>
<td>--</td>
<td>DC</td>
<td>0.3 \times 10^{6} \pm 0.15</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>DC</td>
<td>--</td>
<td>Stroma</td>
<td>1.4 \times 10^{6} \pm 0.7</td>
<td>11 \pm 0.7</td>
</tr>
</tbody>
</table>

After 3 weeks under MBMC conditions (a-MEM, 20% horse serum, 10^{-4} mol/L steroids, 33°C), either on stroma directly or in DCs over MPA stroma layers, cultures switch to LBMC conditions (RPMI 1640, 5% FCS, 5 \times 10^{-4} mol/L 2-ME, 37°C). Cells were left in contact with stroma, kept in DC, or removed from DC and placed directly on LBMC stroma. All data are based on two separate experiments in which three wells per condition were evaluated.
LBMC stromal cells do not support B cell precursors under DC conditions. The above experiments suggest that MBMC stromal cells produce soluble mediators that allow survival of B cell precursors in DC. Whether LBMC stromal cells can also support this precursor was tested by seeding 10⁵ marrow cells passed twice through nylon wool into DCs placed over LBMC stromal cells in lymphoid conditions or directly onto the stroma. The data in Table 3 indicate that a close association with stromal cells is required for cell survival under LBMC conditions. Following 4 weeks of culture, B lymphopoiesis had not initiated and no hematopoietic cells were recovered from the DCs. Cells that expressed the 14.8 antigen and surface IgM were consistently recovered from parallel cultures in which the nylon-wool-passed cells had been directly seeded onto the stromal cell layer. These data establish that the initiation of B lymphopoiesis in LBMC requires a close association with the stroma and suggest that under LBMC conditions LBMC stromal cells do not secrete a soluble mediator that can support the B cell precursor under DC conditions.

DISCUSSION

The experiments reported herein used an in vitro DC culture system to evaluate the relative contribution of direct contact with stromal cells vs the soluble mediators produced by them in the differentiation of B lymphocytes and other myeloid populations. The findings clearly indicate that, at least for the 3-week period of observation, hematopoiesis can proceed in DCs under MBMC conditions. Progenitors of granulocytes and macrophages were detected, and their numbers had increased over those present in the reseed population. This could indicate that self-renewal of these progenitors had occurred or that they were derived from an earlier cell through differentiation and amplification. The presence of morphologically identifiable granulocytes and macrophages in the chambers also suggests that differentiation of CFU-GM had occurred as well. A B lymphocyte precursor was detected in the DCs, but no conclusion can be drawn as to whether it is part of a stem cell pool or a restricted progenitor cell population. We must assume that the survival of these cell types was dependent on the production of soluble mediators from the underlying stroma; no cells survived in DCs placed in wells in which no stroma was present, and analysis of the DC membrane indicated that functional stroma had not established. A few adherent foci were observed, but these cells did not support hematopoiesis. Their identity has not been established, but they resemble macrophages morphologically.

Myeloid progenitors also survive upon implantation of DC in vivo into the peritoneal cavity of mice, further substantiating the role of soluble mediators in the maintenance of selected hematopoietic populations. The factor-dependent survival of various long-term hematopoietic lines also substantiates this view. Greenberger and colleagues described a permanent progenitor line that was dependent on factor(s) present in WEHI-3-conditioned medium. This line was multipotent as it could generate neutrophils, basophils, mast cells, and erythroid cells. Growth of factor-dependent cell lines was also reported by Dexter et al. and Nagao et al. Seemingly contradictory results from studies of long-term cultures from hamster marrow indicated that neither an adherent layer nor an exogenous factor addition was required for progenitor cell survival in vitro. As noted by those investigators, however, cell survival in that system may have been due to greater sensitivity to factors present in the serum used or the production of mediators by endogenous cell populations.

Additional indirect evidence for the dependence of cells in DCs on soluble mediators was obtained in experiments in which hematopoietic cells were simultaneously seeded into DCs and onto the underlying stroma. Very few hemopoietic cells were recovered from DCs under those conditions, and progenitor cell numbers had declined markedly. That hematopoiesis had established in association with the adherent layer suggests that those hematopoietic cells closest to the stroma had used the factors produced by it, leaving only low amounts available to the cells in DCs. This may indicate that localized hematopoietic microenvironments exist in vivo and that mediators produced by the stromal cells were used by the hematopoietic cells closest to them. Thus, although some mediators could potentially diffuse longer distances, they indeed may function as short-range molecules. This

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4 Weeks Postinitiation</th>
<th>Growth Conditions of Nylon-Wool-Passed Bone Marrow</th>
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<td>Cell No.</td>
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<td>0</td>
</tr>
<tr>
<td>(relative change</td>
<td>2</td>
<td>0</td>
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<tr>
<td>over 10³ seeded)</td>
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<td>0</td>
</tr>
<tr>
<td>% 14.8</td>
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<td>0</td>
</tr>
<tr>
<td>(relative change</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>in absolute no.)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>% IgM</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(relative change</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>in absolute no.)</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Nylon-wool-passed bone marrow (10⁵) seeded into DCs placed over MPA-purified LBMC stroma or directly onto MPA-purified LBMC stroma under LBMC conditions. Assays were performed 4 weeks postseeding. Numbers indicate mean ± SD. Percentage of 14.8+ cells in reseed population in experiments 1 and 2 in the NW reseed population were 10 and 6.5, respectively. Percentage of IgM+ cells in reseed population in experiments 1 and 2 in the NW reseed population were 1 and 0, respectively. Data expressed as mean ± SD. Each well (three per condition) counted individually, but cells were pooled for cell surface staining.
could further explain why it is difficult to detect factors in supernatant medium from ongoing long-term bone marrow cultures, since all that is produced is bound by the hematopoietic populations in the cultures. Although hematopoiesis did proceed in DCs under MBMC conditions, blood cell differentiation is not necessarily independent of close stromal cell associations. Indeed, the results we present argue that such associations are required, since the number of cells and progenitors was always greater in parallel cultures in which cells were seeded onto stroma. The inability of the DC B cell precursor to differentiate into B lymphocytes in DC under LBMC conditions further supports this position. These associations could be mediated by direct contact between hematopoietic and stromal cells. Alternatively, developing blood cells may require interactions with growth factors presented within the context of the hematopoietic microenvironment, such as by their attachment to components of the extracellular matrix. Thus, DCs may permit short-term, low-level hematopoiesis in the absence of optimal conditions. Nevertheless, the DC approach is useful for demonstrating the influence of factors on particular lineages and maturational states.

The demonstration of a B cell precursor in DCs makes this point particularly evident. A B cell precursor was present in DCs in MBMC medium 3 weeks postseeding. That mature B and pre-B cells are not present under these conditions suggests that a factor that acts on B cell precursors exists. Whether this putative mediator promotes survival of these populations or their self-renewal cannot be determined from these experiments. How it might relate to the pre-B cell differentiation factor secreted by stromal cell line S17 is also unknown. The latter agent potentiates the expression of the 14.8 antigen and cytoplasmic μ heavy chains in bone marrow B cell progenitors in a 24-hour short-term assay. Different mediators, whose actions affect distinct B lineage target cells, may exist. Ultimately, identification of the factor that maintains B cell precursors in DC will be dependent on replacing the underlying stroma with a conditioned medium that can be characterized.

ACKNOWLEDGMENT

The expert technical assistance of Anne Johnson and Linda Collins is greatly appreciated.

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PC Kierney and K Dorshkind