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Molecular cloning and structure of a pre-B-cell growth-stimulating factor

(stromal cell/B lymphopoiesis/intercrine family/stem cell factor/interleukin 7)

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ABSTRACT **Generation and proliferation of early B-cell progenitors have been known to require stromal cell-derived molecules. A stromal cell line, PA6, was found to produce a soluble mediator, which was distinct from interleukin 7 (IL-7) and stem cell factor and supported the proliferation of a stromal cell-dependent pre-B-cell clone, DW34. A cDNA clone encoding this DW34 growth-stimulating factor was isolated by expression cloning. The nucleotide sequence contained a single substantial open reading frame of 267 nucleotides encoding an 89-amino acid polypeptide. The amino acid sequence of this cytokine, designated pre-B-cell growth-stimulating factor (PBSF), revealed that it is a member of intercrine α subfamily. Recombinant PBSF stimulated the proliferation of DW34 cells for itself and, furthermore, synergistically augmented the growth of DW34 as well as bone marrow B-cell progenitors in the presence of IL-7.**

It is well known that stromal cells play an important role in bone marrow B lymphopoiesis. Whitlock and Witte (1) have developed an *in vitro* long-term B lymphopoiesis system that maintains B-cell progenitors on bone marrow-derived heterogenous stromal cells. In addition, several stromal cell lines that can support long-term B lymphopoiesis have been established (2–7). These cell lines have been used to study the environmental components required for B lymphopoiesis. For example, interleukin 7 (IL-7) (8) was cloned from a stromal cell line and shown to play a key role in B lymphopoiesis (9). However, it appears that IL-7 alone is not sufficient to support B lymphopoiesis in the bone marrow (10–12). Hayashi *et al.* (10) suggested that IL-7 and unidentified molecules produced by stromal cell line PA6 (13) were required for B lymphopoiesis. They showed that B-cell development proceeded through three sequential stages in terms of the growth signal requirement. At the first stage, the cells required PA6 alone for proliferation and differentiated into the second stage, where cells required both PA6 and IL-7 for growth. Then some cells at the second stage acquired the ability to proliferate in response to IL-7 alone. Rolink *et al.* (11) showed that PA6 potentiated the proliferative effect of IL-7 on early pre-B-cell clones that developed to mature B cells. However, these PA6-derived molecules involved in B lymphopoiesis have not yet been identified. One candidate stromal cell-derived molecule is stem cell factor (SCF) (14–16), since it synergizes with IL-7 in stimulating the proliferation of B-cell progenitors (17). However, recent studies showed that B lymphopoiesis was not significantly affected by injection of the antagonistic anti-SCF receptor monoclonal antibody (mAb) ACK2, although myeloid and erythroid hemopoiesis was severely inhibited (18). Therefore, it appears that there are unidentified molecules that compensate the function of neutralized SCF. In this paper, we report a cDNA clone encoding a pre-B-cell growth-stimulating factor (PBSF) that promotes the growth of B-cell progenitors; we show that PBSF is a member of the intercrine family.‡

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MATERIALS AND METHODS

Cells and Cell Culture. Stromal cell lines [ST2 (5) and PA6] and a pre-B-cell clone [DW34 (19)] were kindly provided by S.-I. Nishikawa (Kyoto University Faculty of Medicine). Stromal cell lines (ST2 and PA6) were maintained in RPMI 1640 medium supplemented with 50 μM 2-mercaptoethanol and 10% fetal calf serum. DW34 cells were maintained on the ST2 layer in RPMI 1640 medium supplemented with 50 μM 2-mercaptoethanol and 5% fetal calf serum.

Antibodies and Cytokines. ACK2 mAb was kindly provided by S.-I. Nishikawa. Mouse anti-IL-7 antibody was purchased from Genzyme. Rat anti-mouse IL-5 mAb H7 and mouse IL-5 were kindly provided by K. Takatsu (Tokyo University Institute of Medical Science). Crude IL-7 was obtained from the culture supernatant of COS cells, which were transfected with the mouse IL-7 cDNA clone pSRα mIL-7 (9). Human colony-stimulating factor type 1 (CSF-1), mouse granulocyte/macrophage (GM)-CSF and human G-CSF were kindly provided by S. Nagata (Osaka Bioscience Institute). All antibodies and factors were used at saturating concentrations in biological studies.

Bone Marrow Culture with Stromal Cells. Stromal cell-dependent lymphopoiesis cultures from bone marrow cells were performed under culture conditions similar to those described by Whitlock and Witte (1). Prior to coculturing, stromal cells were grown to confluence in 3.5-cm dishes (no. 25000; Corning). Femoral bone marrow plugs from 3-wk-old BALB/c mice were extruded and suspended in RPMI 1640 medium. After washing, cells were placed into 6.0-cm dishes and incubated in RPMI 1640 medium with 5% fetal calf serum for 40 min. To prevent direct contact between stromal cells and bone marrow cells, an inner chamber with a 0.45-μm membrane filter (Millicell CM; Millipore) was placed on a prepared monolayer of PA6 cells within a 3.5-cm-diameter dish. Nonadherent cells were harvested and 3×10^5 bone marrow cells were placed in an inner chamber and cultured in RPMI 1640 medium with 5% fetal calf serum (lot no. 1115741; HyClone) and 50 μM 2-mercaptoethanol in the presence of 1% crude IL-7 with or without ACK2 (15 μg/ml). These dishes were incubated at 37°C in 5% CO_2/95% air. Half of the medium in the dishes was replaced with fresh medium at day 4 of culture. After 7 days of culture, nonadherent cells

Abbreviations: IL, interleukin; SCF, stem cell factor; CSF, colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; MIP, macrophage inflammatory protein; mAb, monoclonal antibody; PBSF, pre-B-cell growth stimulating factor.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. D21072).

were harvested, counted with trypan blue dye exclusion, and analyzed by flow cytometry.

Bone Marrow Culture Without Stromal Cells. Femoral bone marrow plugs from 3-wk-old BALB/c mice were extruded and suspended in RPMI 1640 medium. After washing, cells were placed into 6.0-cm-diameter dishes and incubated in RPMI 1640 medium with 5% fetal calf serum (lot no. 1115741; HyClone) for 40 min. Nonadherent cells were harvested and 360 μl of the cell suspension (4.5×10^5 cells) was mixed with 240 μl of conditioned medium from COS-7 cells transfected with the pME18S vector alone (COS-7-CM), COS-7-CM plus 3% crude IL-7, pS3H7-CM, or pS3H7-CM plus 3% crude IL-7 in each well of a 24-well cell culture plate (no. MS-8024R; Sumitomo Bakelite, Tokyo) and incubated at 37°C in 5% CO_2/95% air. At day 4 of culture, cells were harvested, counted with trypan blue dye exclusion, and analyzed by flow cytometry. One hundred microliters of the cell suspension was placed into each well of a 96-well plate (no. MS-8096R; Sumitomo Bakelite, Tokyo) and pulsed with 0.5 μCi of [^{3}H]thymidine per well (1 Ci = 37 GBq). After 6 hr of incubation, the cells were harvested with an automated harvester onto glass fiber filters and the radioactivity was determined by liquid scintillation counting.

Bioassay with DW34 Cells. Conditioned medium from PA6 (PA6-CM) was generated in the following manner. When PA6 cells reached confluence, the medium was replaced and incubated for 4 days. The supernatant was harvested and centrifuged. For screening the cDNA library, COS-7 cells were transfected with a cDNA library, and the culture supernatants were harvested at 72 hr and assayed for the growth-stimulating activities of DW34 cells. DW34 cells were harvested, washed, and incubated at a concentration of 10^4 cells per well in RPMI 1640 medium with 5% fetal calf serum and 50 μM 2-mercaptoethanol in a 96-well plate for 30 hr at 37°C in the presence of conditioned medium. [^{3}H]Thymidine uptake was measured as described above.

Molecular Cloning and RNA Analysis. Poly(A)$^+$ RNA was prepared from PA6 cells by standard methods. A cDNA expression library was prepared from 5 μg of poly(A)$^+$ RNA. Size-selected cDNAs were ligated via *Bst*XI linkers into the COS-7 expression vector pME18S and transformed into *Escherichia coli* strain DH5α to generate a library of $\approx 5 \times 10^5$ ampicillin-resistant colonies. Transformants comprising $\approx$50 clones were added to each well of 96-well microtiter plates (no. 25850; Corning). Plasmid DNA was isolated in each well and used to transfect COS-7 cells by the DEAE-dextran-mediated transfection method. Culture supernatants from COS-7 cells were harvested 72 hr after transfection and analyzed by bioassay with DW34 cells (see above). One positive pool was identified from $\approx 10^4$ pools and subdivided to isolate a single positive plasmid. The insert of this cDNA was sequenced by the dideoxynucleotide method.

For Northern blot analysis, 5 μg of poly(A)$^+$ RNA was fractionated on a 10% agarose-formaldehyde gel and blotted onto GeneScreen*Plus* (NEN) as recommended by the manufacturer. The blots were probed with ^{32}P-labeled cDNA probes. After prehybridization, hybridization, and posthybridization washes of filters were performed, a filter was exposed to x-ray film at −70°C.

RESULTS

Stromal Cell-Derived Molecule(s) Distinct from SCF and IL-7 Is Involved in the Stromal Cell-Dependent Proliferation of Bone Marrow B-Cell Progenitors and a Pre-B-Cell Line. A stromal cell line, PA6, has been known to support the proliferation of B-cell progenitors in the presence of IL-7 (9). We found that PA6 could stimulate the proliferation of B-cell progenitors in the presence of IL-7 even in the culture in which PA6 cells and bone marrow cells were separated by a membrane filter. On the other hand, very few viable cells were present 7 days after the culture in the presence of IL-7 alone without PA6 cells (Fig. 1). Anti-SCF receptor mAb ACK2, which can block the function of SCF receptor (18), did not abrogate the B-cell generation in this culture condition. This result suggested the existence of a PA6-derived soluble factor(s) other than SCF and IL-7 that stimulates the proliferation of B-cell progenitors in the presence of IL-7.

This culture system, however, was not suitable for further characterization and molecular cloning of the molecule(s), since it required fresh bone marrow cells and a relatively long culture period. Therefore, we tested whether a stromal cell-dependent B-cell clone, DW34, could be used as responder cells to develop a more simple assay system. A pre-B-cell clone, DW34, was established from Whitlock–Witte-type long-term culture by limiting dilution on a stromal cell line ST2 layer, which could support B lymphopoiesis (19). DW34 cells grown on stromal cell lines such as ST2 and PA6, however, die out without them. Although DW34 cells proliferated in response to IL-7 alone, the proliferative effect of IL-7 on DW34 cells could be potentiated by PA6 cells (data not shown). In addition, a conditioned medium from PA6 was capable of stimulating the proliferation of DW34 cells in the presence of anti-SCF receptor mAb (ACK2), anti-IL-7 mAb, or both (Fig. 2). Anti-IL-7 mAb inhibited the proliferation of DW34 cells induced by PA6-derived conditioned medium to some extent but not completely (Fig. 2). None of the factors tested, including IL-1β, IL-4, IL-5, IL-6, IL-11, CSF-1, GM-CSF, G-CSF, and leukemia inhibitory factor, induced the stimulation of [^{3}H]thymidine uptake by DW34 cells over background levels throughout a tested range of factor concentrations (data not shown). None of the antibodies tested, including anti-IL-4, anti-IL-5 receptor, and anti-IL-6, inhibited the stimulation of the proliferation of DW34 cells (data not shown). In addition, conditioned medium from PA6 did not elicit any response in standard bioassays for IL-3 (data not shown). These results suggested that a PA6-derived soluble factor(s) stimulated the proliferation of pre-B-cell clone DW34 cells. We then attempted to isolate a PA6-derived factor to stimulate the growth of DW34 cells in the following experiments.

Molecular Cloning and Structure of a DW34 Growth-Promoting Factor. An expression cDNA library was prepared from PA6 by using pME18S and then screened for the activity

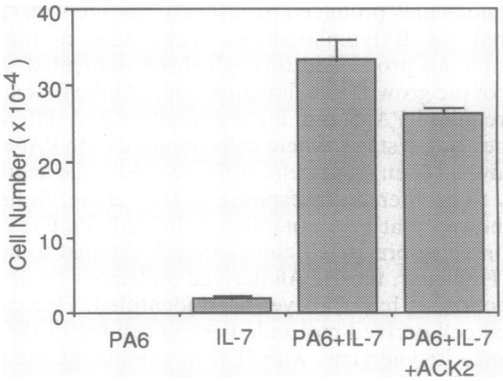

FIG. 1. Generation of B-cell progenitors on PA6 layers. PA6 cells were physically separated from bone marrow cells by the membranes (0.45 μm) and cultured in the presence of IL-7. An inner chamber containing bone marrow cells was placed in a 3.5-cm-diameter dish containing PA6 cells. Cells were cultured in the medium containing IL-7 or IL-7 plus anti-SCF receptor mAb (ACK2). In one experiment, bone marrow cells were cultured without the stromal cells; 7 days later, cells in the inner chambers were harvested and analyzed by flow cytometry. Data are presented as numbers of B220$^+$ cells. Numbers of B220$^+$ cells were obtained by multiplying the harvested total cell numbers by the ratio of cells within the lymphocyte gate.

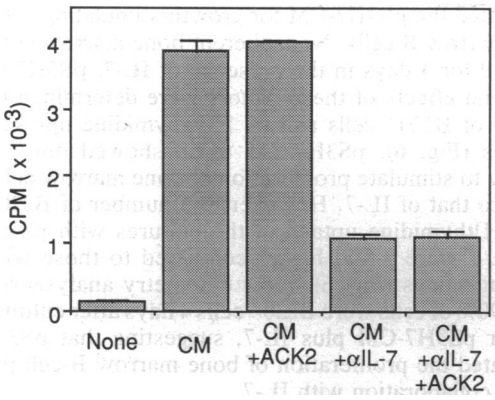

FIG. 2. Effect of conditioned medium from stromal cell line PA6 on proliferation of pre-B-cell clone DW34. The culture medium containing 50% conditioned medium was used to test the ability to stimulate [^{3}H]thymidine uptake (cpm) of DW34 cells in the presence of neutralizing anti-IL-7 mAb (40 μg/ml), ACK2 (20 μg/ml), or both.

to stimulate the growth of DW34 cells after expression in COS-7 cells. Approximately 10^4 pools were screened and the positive pool was subdivided until a single positive clone (clone pS3H7) was identified. The nucleotide sequence of clone pS3H7 was determined and is shown in Fig. 3. The insert is 1776 base pairs long, containing only one substantial open reading frame of 267 base pairs. This cDNA contains 1451 base pairs of 3′ noncoding sequence with multiple copies of the A+U-rich sequence, which was reported to be an important regulatory element for cytokine gene expression (20). The predicted polypeptide encoded by the cDNA insert consists of 89 amino acids. A stretch of 18 hydrophobic amino acids that resembles a conventional protein secretory leader sequence is located adjacent to the putative initiation codon. The amino acid sequence of the mature protein contains four cysteine residues. Studies of their positions suggested that it was a member of the intercrine α subfamily (21), which includes cytokines such as IL-8 and macrophage inflammatory protein 2 (MIP-2). Northern blot analysis of mRNA from a PA6 cell line revealed the existence of two distinct transcripts, 1.8 and 3.6 kb long (Fig. 4). Two species of mRNA observed may be the result of alternative splicing.

When searched for all the reported amino acid sequences, the product of pS3H7 is identical to stromal cell-derived factor 1α (SDF-1α) (22). SDF-1α was recently cloned by Tashiro *et al.* (22) as a signal sequence containing proteins, although its biological function was not known.

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                                                                                (79)
CTCGGTGTCCTCTTGCTGTCCAGCTCTGCAGCCTCCGGCGCGCCCTCCCGCCCACGCCATGGACGCCAAGGTCGTCGCC
                                                                  MetAspAlaLysValValAla
                                                                  ---------------------

GTGCTGGCCCTGGTGCTGGCCGCGCTCTGCATCAGTGACGGTAAACCAGTCAGCCTGAGCTACCGATGCCCCTGCCGGTTC
ValLeuAlaLeuValLeuAlaAlaLeuCysIleSerAspGlyLysProValSerLeuSerTyrArgCysProCysArgPhe
---------------------------------                  *          *
                                                                                (241)
TTCGAGAGCCACATCGCCAGAGCCAACGTCAAGCATCTGAAAATCCTCAACACTCCAAACTGTGCCCTTCAGATTGTTGCA
PheGluSerHisIleAlaArgAlaAsnValLysHisLeuLysIleLeuAsnThrProAsnCysAlaLeuGlnIleValAla
                                                                *

CGGCTGAAGAACAACAACAGACAAGTGTGCATTGACCCGAAATTAAAGTGGATCCAAGAGTACCTGGAGAAAGCTTTAAAC
ArgLeuLysAsnAsnAsnArgGlnValCysIleAspProLysLeuLysTrpIleGlnGluTyrLeuGluLysAlaLeuAsn
                           *
                                                                                (403)
AAGTAAGCACAACAGCCCAAAGGACTTTCCAGTAGACCCCCGAGGAAGGCTGACATCCGTGGGAGATGCAAGGGCAGTGGT
Lys
GGGGAGGAGGGCCTGAACCCTGGCCAGGATGGCCGGCGGGACAGCACTGACTGGGGTCATGCTAAGGTTTGCCAGCATAAA

GACACTCCGCCATAGCATATGGTACGATATTGCAGCTTATATTCATCCCTGCCCTCGCCCGTGCACAATGGAGCTTTTATA

ACTGGGGTTTTTCTAAGGAATTGTATTACCCTAACCAGTTAGCTTCATCCCCATTCTCCTCATCCTCATCTTCATTTTAAA

AAGCAGTGATTACTTCAAGGGCTGTATTCAGTTTGCTTTGGAGCTTCTCTTTGCCCTGGGGCCTCTGGGCACAGTTATAGA

CGGTGGCTTTGCAGGGAGCCCTAGAGAGAAACCTTCCACCAGAGCAGAGTCCGAGGAACGCTGCAGGGCTTGTCCTGCAGG

GGGCGCTCCTCGACAGATGCCTTGTCCTGAGTCAACACAAGATCCGGCAGAGGGAGGCTCCTTTATCCAGTTCAGTGCCAG

GGTCGGGAAGCTTCCTTTAGAAGTGATCCCTGAAGCTGTGCTCAGAGACCCTTTCCTAGCCGTTCCTGCTCTCTGCTTGCC
                                                                                (1051)
TCCAAACGCATGCTTCATCTGACTTCCGCTTCTCACCTCTGTAGCCTGACGGACCAATGCTGCAATGGAAGGGAGGAGAGT

GATGTGGGGTGCCCCCTCCCTCTCTTCCCTTTGCTTTCCTCTCACTTGGGCCCTTTGTGAGATTTTTCTTTGGCCTCCTGT

AGAATGGAGCCAGACCATCCTGGATAATGTGAGAACATGCCTAGATTTACCCACAAAACACAAGTCTGAGAATTAATCATA

AACGGAAGTTTAAATGAGGATTTGGACTTTGGTAATTGTCCCTGAGTCCTATATATTTCAACAGTGGCTCTATGGGCTCTG

ATCGAATATCAGTGATGAAAATAATAATAATAATAATAACGAATAAGCCAGAATCTTGCCATGAAGCCACAGTGGGGA

TTCTGGGTTCCAATCAGAAATGGAGACAAGATAAAACTTGCATACATTCTTACGATCACAGACGGCCCTGGTGGTTTTTGG

TAACTATTTACAAGGCATTTTTTTACATATATTTTTGTGCACTTTTTATGTTTCTTTGGAAGACAAATGTATTTCAGAATA

TATTTGTAGTCAATTCATATATTTGAAGTGGAGCCATAGTAATGCCAGTAGATATCTCTATGATCTTGAGCTACTGGCAAC
                                                                                (1699)
TTGTAAAGAAATATATATGACATATAAATGTATTGTAGCTTTCCGGTGTCAGCCACGGTGTATTTTTCCACTTGGAATGAA

ATTGTATCAACTGTGACATTATATGCACTAGCAATAAAATGCTAATTGTTTCATGCTGTAAAAAAAAAAAAAAAAAA
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FIG. 3. Nucleotide sequence and deduced amino acid sequence of mouse PBSF cDNA. Predicted amino acid sequence of the single substantial open reading frame is indicated. Hydrophobic core of the predicted signal sequence is underlined with a dashed line. Asterisks represent positions of four cysteines that are conserved among members of the intercrine α subfamily. A+U-rich sequences that are often observed within the 3′ untranslated regions of cytokine mRNAs are underlined.

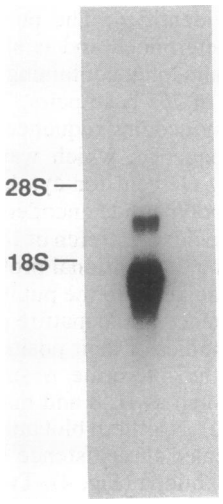

FIG. 4. Northern blot analysis of pS3H7 mRNA. Poly(A)$^+$ RNA (5 μg) from the stromal cell line PA6 was hybridized with a ^{32}P-labeled PBSF cDNA insert.

Biological Activities of pS3H7-Transfected COS-7 Cell Conditioned Medium. To confirm the biological activity of the cloned gene product, we prepared a conditioned medium (pS3H7-CM) from the pS3H7-transfected COS-7 cells and tested it for DW34 growth-stimulating activity. pS3H7-CM stimulated [^{3}H]thymidine uptake by DW34 cells ≈9-fold compared to the mock conditioned medium prepared from COS-7 cells transfected with the pME18S vector alone (Fig. 5*A*). Furthermore, pS3H7-CM augmented synergistically the proliferation of DW34 cells induced by IL-7 (Fig. 5*B*). We also tested the pS3H7-CM for growth-stimulating activity of bone marrow B cells. Nonadherent bone marrow cells were cultured for 4 days in the presence of IL-7, pS3H7-CM, or both, and effects of these factors were determined by generation of B220$^+$ cells and by [^{3}H]thymidine uptake in the cultures (Fig. 6). pS3H7-CM alone showed only a weak activity to stimulate proliferation of bone marrow cells compared to that of IL-7. However, the number of B220$^+$ cells and [^{3}H]thymidine uptake in the cultures with pS3H7-CM plus IL-7 were 3-fold higher compared to those with IL-7 alone at 4 days (Fig. 6). Flow cytometry analysis revealed that >90% of cells were B220$^+$ cells 4 days after cultures with IL-7 or pS3H7-CM plus IL-7, suggesting that pS3H7-CM stimulated the proliferation of bone marrow B-cell progenitors in collaboration with IL-7.

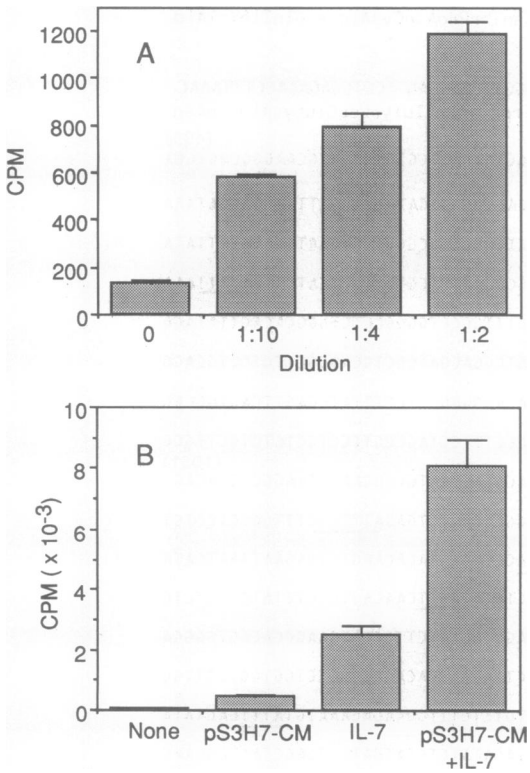

FIG. 5. Effect of conditioned medium from COS-7 cells transfected with pS3H7 (pS3H7-CM) on proliferation of pre-B-cell clone DW34. (*A*) DW34 cells were cultured at the identicated dilutions of pS3H7-CM for 24 hr. (*B*) DW34 cells were cultured in the presence of 50% pS3H7-CM, 1% crude IL-7, or 50% pS3H7-CM plus 1% crude IL-7 for 24 hr. The cells were pulsed with [^{3}H]thymidine for 6 hr and harvested, and the radioactivity was determined.

DISCUSSION

Previous studies have shown that stromal cell-derived unidentified molecules are required for B lymphopoiesis in addition to IL-7 (10–12). The major objective of the present study was to identify those stromal cell-derived molecules that stimulate B lymphopoiesis. The initial studies showed that the culture supernatant of PA6 cells could stimulate proliferation of B-cell progenitors as well as stromal cell-dependent pre-B-cell clone DW34. In this paper, we have cloned cDNA of a factor, PBSF, from PA6 cells and characterized its function. This factor can stimulate the proliferation of bone marrow-derived B progenitor cells in the presence of IL-7 as well as growth of the stromal cell-dependent B-cell clone DW34 cells.

It is not clear whether B-cell growth-promoting activities derived from PA6 are entirely attributed to PBSF. Since anti-IL-7 antibody significantly blocked the activity of the PA6 culture supernatant to stimulate the growth of DW34

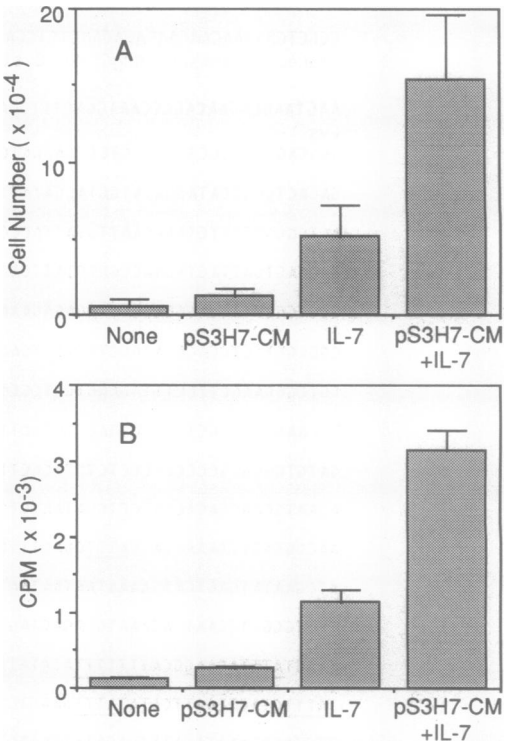

FIG. 6. Effect of pS3H7-CM on proliferation of bone marrow B-cell progenitors. Bone marrow cells were cultured for 4 days in the presence of IL-7, pS3H7-CM, or pS3H7-CM plus IL-7. Cells were harvested and analyzed by flow cytometry or pulsed with [^{3}H]thymidine for the last 6 hr of culture. (*A*) Numbers of B220$^+$ cells. (*B*) Growth activity of the cells determined by [^{3}H]thymidine uptake.

cells, PA6 may produce a small amount of IL-7. These results suggest that the growth of DW34 cells requires both PBSF and IL-7 in the culture supernatant of PA6 cells. Although PBSF stimulated the proliferation of B-cell progenitors in the presence of IL-7, the activity of PBSF is much lower than that induced by PA6 under the conditions in which direct contact between PA6 cells and bone marrow cells was inhibited. It is possible that the optimum concentration of PBSF constantly secreted by PA6 cells adjacent to B cells may be important. Alternatively, the growth-stimulating activity of PBSF may be limited to a small fraction of B-cell subpopulations, since bone marrow B cells are heterogenous in terms of their differentiation stages or other PA6-derived molecules may synergize with PBSF to stimulate the proliferation of B cells.

The number of B cells generated in the cultures in which cells were mixed together allowing direct contact was high compared to those generated under the conditions in which direct contact between PA6 cells and bone marrow cells was inhibited. This suggests that direct contact between stromal cells and B cells is important in addition to stromal cell-derived cytokines such as IL-7, SCF, and PBSF. The development of neutralizing antibodies against recombinant PBSF will further clarify the function and involvement of PBSF in bone marrow B lymphopoiesis.

Recent studies have shown that the growth factor requirement of B-cell progenitors is different depending on differentiation stages as defined in terms of immunoglobulin-gene rearrangement status (10, 12, 23, 24). Therefore, it is important to identify the target cells for PBSF in the course of differentiation. Since DW34 cells have μ chains in the cytoplasm (19), the target cells for PBSF may include the cells at a relatively late stage in the developmental pathway of immature B cells. Various populations of B-cell progenitors from bone marrow sorted by flow cytometry should be tested for the ability to respond to PBSF.

Putative amino acid sequence analysis of PBSF revealed it is a member of the intercrine family. Most of the members in the family have proinflammatory and reparative activities. However, MIP-1 and MIP-2 were reported to enhance colony formation by GM progenitor cells in collaboration with GM-CSF or M-CSF (25). Another study showed that MIP-1α is a negative regulator of pluripotent stem cell proliferation (26). These and our results suggest that some members of the intercrine family play important roles in hemopoiesis. Therefore, it will be interesting to investigate the effect of PBSF on myelopoiesis. At the same time, development of a PBSF gene transgenic mouse will reveal physiological and pathological roles of PBSF.

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