Colony Growth of Human Hematopoietic Progenitor Cells in the Absence of Serum Is Supported by a Proteinase Inhibitor Identified as Antileukoproteinase

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Summary

Serum contains many growth factors and nutrients that stimulate colony formation of hematopoietic progenitor cells (HPC) in semisolid cultures. In the absence of serum, no proliferation of HPCs could be obtained in semisolid medium cultures of partially purified bone marrow cells in the presence of multiple hematopoietic growth factors, insulin, cholesterol, and purified clinical-grade human albumin. This appeared to be due to a suppressive activity induced by monocyte- and T lymphocyte-depleted accessory cells on CD34+ HPCs. Serum-free conditioned medium from the bladder carcinoma cell line 5637 could replace serum to support the growth of HPCs in these cultures. After gel filtration and reverse-phase high-performance liquid chromatography of 5637 supernatants, this activity could be attributed to a 15-kD protein that was further identified by NH2-terminal sequence analysis as the serine proteinase inhibitor antileukoproteinase (ALP). The growth-supportive activity from the 5637 conditioned medium and the (partially) purified fractions could be completely neutralized by a polyclonal rabbit IgG antibody against human ALP (huALP). Similar supportive effects on the growth of HPC could be obtained in the presence of recombinant huALP. We demonstrated that the COOH-terminal domain of ALP containing the proteinase inhibitory activity was responsible for this effect. α-1 proteinase inhibitor was capable of similar support in vitro of HPC growth. These results illustrate that proteinase inhibitors play an important role in the in vitro growth of hematopoietic cells by the neutralization of proteinases produced by bone marrow accessory cells. This may be of particular relevance for in vitro expansion of human hematopoietic stem cells in serum-free media.

Characterization of hematopoiesis in humans is dependent on the in vitro analysis of hematopoietic progenitor cells (HPC)1. HPCs can be studied using semisolid medium cultures, and their potential for proliferation and differentiation can be characterized by their ability to form colonies of hematopoietic cells in these cultures. The growth and differentiation of HPC in colony assays depend on the presence of hematopoietic growth factors (HGF) in the cultures (1). These HGF may be added to the culture medium or produced by accessory cells or HPC as paracrine or autocrine growth factors, respectively. In addition, factors that are present in serum, including insulin, cholesterol, or albumin, are critical for the in vitro growth of HPC (2–7). BSA, frequently used as a source of albumin, is usually not >95–99% pure because of its strong protein-binding properties (8, 9). Since in serum-free colony assays, BSA is often used at relatively high concentrations, residual undefined proteins may significantly contribute to the growth-supportive potential of BSA. Pilot studies in our laboratory indicated that in contrast to these BSA preparations, highly purified concentrates of clinical-grade human albumin did not support the proliferation of HPC from

1Abbreviations used in this paper: α-1 PI, α-1 proteinase inhibitor; ALP, antileukoproteinase; bFGF, basic fibroblast growth factor; BFU-E, burst-forming unit erythroid colony; CM, conditioned medium; CPG, controlled pore glass; EGF, epidermal growth factor; EPO, erythropoietin; GCSF, granulocyte colony stimulating factor; GM, granulocyte macrophage; HGF, hematopoietic growth factor; HPC, hematopoietic progenitor cell; HSA, human serum albumin; huALP, human antileukoproteinase; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; rALP, recombinant human antileukoproteinase; RP-HPLC, reverse-phase HPLC; SCF, stem cell factor; TFA, trifluoroacetic acid.

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monocyte- and T lymphocyte-depleted mononuclear bone marrow cells in semisolid medium cultures in the presence of HGF, cholesterol, and insulin. In this study, we analyzed the mechanisms of this finding and showed that this was due to a suppressive effect of monocyte- and T lymphocyte-depleted bone marrow accessory cells on CD34+ purified HPC. We investigated which serum factor, in addition to human albumin, insulin, and cholesterol, was responsible for supporting the in vitro proliferation of bone marrow-derived HPC. We found that a 15-kD protein present in conditioned medium (CM) from the bladder carcinoma cell line 5637 could replace serum. This protein was identified as the serine proteinase inhibitor antileukoproteinase (ALP).

Materials and Methods

Cell Preparations. After informed consent, bone marrow samples were obtained from healthy donors. The cells were centrifuged on Ficoll Isopaque (density 1.077 g/cm², 20 min, 1,000 g), and mononuclear cells were depleted of monocytes and T lymphocytes as described previously (10). These enriched bone marrow samples were cryopreserved and stored in liquid nitrogen. Immediately before use, the cells were thawed rapidly in a water bath of 37°C, washed once in cold (0°C) RPMI-1640 medium (HyClone Labs., Logan, UT) supplemented with 20% (vol/vol) fetal bovine serum (Boehringer, Mannheim, Germany), and twice with cold RPMI-1640 medium supplemented with 0.1% (wt/vol) clinical grade human serum albumin (HSA; Central Laboratory of the Bloodtransfusion Services, Amsterdam, The Netherlands). The cells were resuspended in IMDM (HyClone Labs., Logan, UT) supplemented with 0.1% (wt/vol) HSA. In some experiments, highly purified CD34+ cells were isolated from the monocyte- and T lymphocyte-depleted bone marrow cells. The CD34+ cells were either selected after staining with FITC-conjugated CD34-specific mAbs (HPCA-2; Becton Dickinson; Mountain View, CA) and sorted with a FACStar® PLUS (Becton Dickinson) or isolated after incubation with magnetic Dynabeads M-450 (Dynal Inc., Lake Success, NY) coated with a primary antibody specific for the CD34 Ag. After magnetizing the CD34+ cells, the nonbinding population was harvested as the CD34+ accessory cells. The CD34+ cells were recovered after incubation with a sheep polyclonal antibody anti-CD34 Fab IgG (Detachable CD34; Dynal Inc.), resulting in >80% pure CD34+ cells.

Cytokines and Antibodies. All cytokines used in this study were recombinant human proteins and were used at plateau concentrations to induce optimal colony growth. The cytokines and final concentrations used were: stem cell factor (SCF) (50 ng/ml), granulocyte-colony stimulating factor (G-CSF) (10 ng/ml), basic fibroblast growth factor (bFGF) (10 ng/ml), and epidermal growth factor (EGF) (10 ng/ml) provided by Amgen (Thousand Oaks, CA); IL-3 (50 ng/ml), IL-11 (10 ng/ml), monocyte-CSF (1,000 U/ml), and GM-CSF (10 ng/ml) were provided by Genetics Institute (Cambridge, MA); IL-1α (10 ng/ml), IL-5 (10 ng/ml), and IL-12 (10 ng/ml) were gifts from Hoffman La Roche (Nutley, NJ); IL-4 (500 U/ml) and IL-7 (10 ng/ml) were gifts from Immunex Corporation (Seattle, WA); IL-6 (1 ng/ml) and IL-8 (10 ng/ml) were gifts from Sandoz (Basel, Switzerland); platelet-derived growth factor (PDGF; 30 ng/ml) was obtained from Gibco BRL (Gaithersburg, MD); insulin-like growth factors (IGF-I, IGF-II, 250 ng/ml) were obtained from Genzyme Corporation (Cambridge, MA); and erythropoietin (EPO) (1 IU/ml) was a gift from Cilag (Herentals, Belgium). IL-9 (1,000 U/ml) was provided by Ludwig Institute for Cancer (Brussels, Belgium). IL-10 (10 ng/ml) was obtained from Schering-Plough Research Institute (Kenilworth, NJ), and IL-2 (300 U/ml) was a gift from Roussel Uclaf (Paris, France). Recombinant human antileukoproteinase (rALP) was kindly donated by Dr. G. Steffens (Gürenenthal, Aachen, Germany) and were used at equivalent molar concentrations of rALP, α-1 proteinase inhibitor (α-1 PI), used at a final concentration of 0.2 to 20 μg/ml, was obtained from Cutter Biologic (Hartford, CT). A neutralizing anti-human antileukoproteinase (huALP) antibody was prepared in rabbits. The antibody has been found to have a high titer and specificity for huALP (11).

Clonogenic Assay. Semisolid medium cultures were performed in sixfold in flat-bottomed 96-well microtiterplates (Greiner, Alphen a/d Rijn, The Netherlands) in aliquots of 0.1 ml per well, containing 105 monocyte- and T lymphocyte-depleted mononuclear bone marrow cells or 150 purified CD34+ cells per well. The culture medium consisted of IMDM, supplemented with 0.6% (wt/vol) human albumin, insulin, and cholesterol. In this study, we analyzed the mechanisms of this finding and showed that this was due to a suppressive effect of monocyte- and T lymphocyte-depleted bone marrow accessory cells. We found that a 15-kD protein present in conditioned medium (CM) from the bladder carcinoma cell line 5637 could replace serum. This protein was identified as the serine proteinase inhibitor antileukoproteinase (ALP).

Production of CM. The human bladder carcinoma cell line 5637 (12, 13) was used to produce a serum-free CM. Confluent adherent cells were washed three times with RPMI-1640 and incubated for 24 h with RPMI-1640 serum-free medium, supplemented with 0.6% (wt/vol) human albumin, insulin, and cholesterol, was responsible for supporting the in vitro proliferation of bone marrow-derived HPC. We found that a 15-kD protein present in conditioned medium (CM) from the bladder carcinoma cell line 5637 could replace serum. This protein was identified as the serine proteinase inhibitor antileukoproteinase (ALP).
by elution with 18% ethylene glycol buffer in 1.55 M NaCl, 8
PBS containing 10% (wt/vol) polyethylene glycol (PEG 20,000) glycine/HC1, pH 3.5. The protein fraction was eluted by care-
chymotrypsinogen (Mr 25,000), and lysozyme (Mr 14,300) were
teins, a gel filtration was performed on Ultrogel ACA 54 (Phar-
growth of purified CD34 + cells in the presence of multiple HGF (G-CSF
of HPC were supplemented with Tween 20 (0.01% vol/vol),
macia, Uppsala, Sweden) by using a 2.6 × 100-cm column and a
concentrated on a centricon-3 filter (Amicon Inc., Beverly, MA),
centrifuged (15 rain, 500 g, 20°C) irradiated (50 Gy), and frozen
through [L-12), separately or in various combinations, or
Replacement of AB serum by HSA (0.6% wt/vol), cho-
sterol (20 μg/ml), and insulin (10 μg/ml) resulted in only
plated (background colony growth). In contrast,
cultures of purified CD34 + cells, HPC growth in the
presence of 10% AB serum yielded 16 ± 1 CFU-GM and
4 ± 1 BFU-E per 103 cells plated, whereas in the absence
of AB serum, 9 ± 2 CFU-GM and 6 ± 1 BFU-E were
Table 1. Effect of 5637 CM on Colony Growth of Monocyte- and
T Lymphocyte-depleted Bone Marrow Mononuclear Cells in the
Absence of Serum
| Condition       | CFU-GM | BFU-E |
|-----------------|--------|-------|
| Serum-free medium| 4 ± 1  | 0     |
| 5637 CM (vol/vol) | 28 ± 3 | 17 ± 2 |
| AB serum (vol/vol) | 18 ± 3 | 13 ± 3 |
|                 | 9 ± 2  | 7 ± 2  |
|                 | 21 ± 2 | 20 ± 2 |

All cultures were performed in the presence of optimal concentrations of HGF (GM-CSF 10 ng/ml, G-CSF 10 ng/ml, IL-3 50 ng/ml, SCF 50 ng/ml, and EPO 1 IU/ml).
Fractionation of HPC growth-promoting activity from CPG-purified 5637 CM by gel filtration (Ultrigel ACA 54). The shaded area represents the protein concentration per fraction. The growth supportive activity for CFU-GM (x-x) and BFU-E (□-□) colonies from the monocyte- and T-lymphocyte depleted bone marrow mononuclear cells was analyzed in the presence of multiple HGF in the absence of serum (n = 4). Colony growth supported by each column fraction at a final concentration of 0.3% was expressed as percentage of growth of control cultures in the presence of 10% AB serum. The absolute numbers of colonies in the control culture were 17 ± 4 CFU-GM and 20 ± 4 BFU-E (mean ± SE per 10^3 cells plated). Protein concentrations were determined by the Coomassie blue binding assay. Molecular mass markers: BSA, (M, 67,000), ovalbumin (OV, M, 45,000), chymotrypsinogen (CHYM, M, 25,000), and lysozyme (LYS, M, 14,300).

Figure 3. Purification of HPC growth-promoting activity by RP-HPLC. In the absence of serum, each RP-HPLC fraction (400 μl) was analyzed for the supportive activity for CFU-GM, (x-x) and BFU-E (□-□) growth from monocyte- and T-lymphocyte-depleted bone marrow mononuclear cells in the presence of HGF (n = 3). Colony growth supported by each fraction at a final concentration of 0.3% was expressed as percentage of growth of control cultures, obtained in the presence of 10% (vol/vol) AB serum. Absolute numbers of colonies in the control culture were 12 ± 3 CFU-GM and 16 ± 4 BFU-E (mean ± SE per 10^3 cells plated). Fraction 32 contained the highest level of supportive activity for both the myeloid and erythroid colony growth, 75 ± 12% and 125 ± 20% (mean percentage of growth ± SE), respectively.

Figure 2. Fractionation of HPC growth-promoting activity from CPG-purified 5637 CM by gel filtration (Ultrigel ACA 54). The shaded area represents the protein concentration per fraction. The growth supportive activity for CFU-GM (x-x) and BFU-E (□-□) colonies from the monocyte- and T-lymphocyte depleted bone marrow mononuclear cells was analyzed in the presence of multiple HGF in the absence of serum (n = 4). Colony growth supported by each column fraction at a final concentration of 0.3% was expressed as percentage of growth of control cultures in the presence of 10% AB serum. The absolute numbers of colonies in the control culture were 17 ± 4 CFU-GM and 20 ± 4 BFU-E (mean ± SE per 10^3 cells plated). Protein concentrations were determined by the Coomassie blue binding assay. Molecular mass markers: BSA, (M, 67,000), ovalbumin (OV, M, 45,000), chymotrypsinogen (CHYM, M, 25,000), and lysozyme (LYS, M, 14,300).

Characterization of the Supportive Factor from 5637 CM. The protein fraction from the 5637 CM, concentrated by adsorption to and elution from the CPG beads, contained the colony growth supportive factor with a fivefold higher concentration of activity compared to the crude 5637 supernatant (data not shown). After gel filtration of the CPG-eluate into 90 fractions, the major protein peaks were recovered from fractions 30 to 40 (>45 kD). As illustrated in Fig. 2, addition of 0.3% (vol/vol) of these high M protein-containing fractions to the culture medium did not result in an enhancement of colony growth. However, addition of 3–10% (vol/vol) CM, produced by 5637 cells in the absence of serum, could support the colony growth up to levels similar to the growth observed in the presence of 10% AB serum. Replacement of AB serum by 10% (vol/vol) 5637 CM to the culture medium supported 136 ± 12% of CFU-GM growth and 85 ± 4% of BFU-E growth as compared to the control culture. These results show that in the presence of purified human albumin, 5637 cells produce factors that can support the growth of CFU-GM and BFU-E comparable to the effect of AB serum (Table 1).

The HPLC fractions, which contained the highest growth supportive activity, were electrophoretically analyzed for purity by means of SDS-PAGE, and the molecular mass was defined as shown in Fig. 4. The HPLC fractions 30–32 from gel filtration fraction 62 showed a distinct protein band of 15 kD, with the highest concentration in fraction...
SDS-PAGE of ILP-HPLC fractions containing biologically active (Fig. 3) 15-kD protein from 5637-cell CM. Of each fraction, 20 µl was loaded on a polyacrylamide gel under reducing conditions and proteins were silver stained. Molecular mass markers (Bio-Rad, Richmond, CA) were phosphorylase b (M₉ 92,500), BSA (M₉ 66,200), ovalbumin (M₉ 45,000), carbonic anhydrase (M₉ 31,000), soybean trypsin inhibitor (M₉ 21,500), lysozyme (M₉ 14,400), and the low M₉ marker aprotinin (Pierce Chemical Company, Rockford, IL) (M₉ 6,500).

32(f–32). An automated protein sequencer was used to sequence the first 30 NH₂-terminal amino acid residues of the protein, and they revealed complete structural identity with homology to the NH₂-terminal sequence of the serine proteinase inhibitor ALP. ALP consists of a total of 107 residues. When purified natural protein was digested with formic acid, sequence analysis of the internal amino acids 50 to 76 showed again identity to ALP, confirming its complete homology to ALP (Fig. 5). Similarly, the second peak of activity from gel filtration (fractions 68 and 69) was purified by HPLC, yielding again a predominant 15-kD protein (fraction 28), which was also identified as ALP by NH₂-terminal sequence analysis (45 residues) (Fig. 5). As shown in Fig. 6, purified rALP could also support the growth of CFU-GM and BFU-E similar to 5637 CM or the purified 15-kD protein. Polyclonal anti-huALP antibodies could completely neutralize the biologic activity of 5637 CM (10% vol/vol), HPLC fraction-32 (0.1% vol/vol), and rALP (10 ng/ml). However, the support of AB serum (1% vol/vol) on HPC growth could not be neutralized by polyclonal anti-huALP antibodies (Fig. 6). Replacement of serum by the serine proteinase inhibitor α-1 PI (2 µg/ml), present in serum at a concentration high as 25 µM, supported the growth of both CFU-GM and BFU-E progenitors similar to rALP. α-1 PI supported 69 ± 20% of CFU-GM and 137 ± 38% of BFU-E growth as compared to AB serum (n = 3). Replacement of rALP by its domains ALP-D-1 or ALP-D-2, separately or in combination, at molar concentrations equivalent to rALP, showed that the COOH-terminal domain ALP-D-2 contained the active site (Fig. 7).

Influence of Antileukoproteinase on the Suppressive Effect of Accessory Cells on HPC Growth in the Absence of Serum. Monocyte- and T lymphocyte-depleted bone marrow cells were separated with Dynabeads into CD34⁻ cells and a population containing 80% CD34⁺ cells. Fig. 8 shows the colony growth of these purified CD34⁺ cells in the absence or presence of increasing numbers of CD34⁻ cells. In two separate experiments, it was shown that the inhibited growth of CFU-GM and BFU-E, induced by the CD34⁻ accessory cells in the absence of serum, was completely restored by the addition of rALP (100 ng/ml) or CM (10% vol/vol).

Figure 4. SDS-PAGE of RP-HPLC fractions containing biologically active (Fig. 3) 15-kD protein from 5637-cell CM. Of each fraction, 20 µl was loaded on a polyacrylamide gel under reducing conditions and proteins were silver stained. Molecular mass markers (Bio-Rad, Richmond, CA) were phosphorylase b (M₉ 92,500), BSA (M₉ 66,200), ovalbumin (M₉ 45,000), carbonic anhydrase (M₉ 31,000), soybean trypsin inhibitor (M₉ 21,500), lysozyme (M₉ 14,400), and the low M₉ marker aprotinin (Pierce Chemical Company, Rockford, IL) (M₉ 6,500).

HPLC f32 from gel filtration f62 no digestion

| Fraction (f) | Sequence |
|-------------|----------|
| HPLC f32 from gel filtration f62 no digestion | SGKSFHAGVCPPKSAQCLYKRPPFPQSDM |

HPLC f30+31 from gel filtration f62 HCOOH digestion

HPLC f28 from gel filtration f68+69 no digestion

Antileukoproteinase 1

| Antileukoproteinase 1 | Sequence |
|-----------------------|----------|
| SGKSFHAGVCPPKSAQCLYKRPPFPQSDM | 50 55 60 65 70 75 |
| 1 5 10 15 20 25 30 35 40 45 | PVDTNPTRKKCPVTQQLMLNP |
| 1 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 | 81 85 90 95 100 105 |
| 1 5 10 15 20 25 30 35 40 45 | EMDQGCKRLKCCGMGKSCVSPVX |

50 55 60 65 70 75

Figure 5. Amino acid sequence analysis of HPC growth-supporting fractions (f) purified from 5637-cell CM. Amino acid sequences were determined by Edman degradation with an on-line Applied Biosystems 477A/120A sequencer. Since cysteine residues cannot be detected unless modified, their presence was deducted from the absence of any detectable amino acid signal at such position. ALP sequence data are available from EMBL/GenBank/DDBJ under accession numbers P03973 and P07757.
BFU-E growth by CD34- cells in the absence of serum. Purification of this CM led to the isolation of a protein similar to the numbers of colonies in the presence of serum present in serum at concentrations of 1-2 μg/ml, could for suppression of growth of HPC in the absence of serum. The number of CFU-GM and BFU-E in the control culture was 27 and 28 per 10^3 cells plated, respectively. In the presence of antihuALP antibodies, colony growth supported by 5637 CM, fraction f-32 or rALP, but not by 1% (vol/vol) AB serum, was completely neutralized.

**Discussion**

In the presence of multiple HGF, insulin, cholesterol, and a highly purified clinical grade source of human albumin, we observed that in vitro HPC present in monocyte- and T lymphocyte-depleted bone marrow failed to proliferate and differentiate into mature colonies of myeloid and erythroid cells. In contrast, purified CD34+ cells proliferated in the absence of human AB serum. Reconstitution experiments showed that accessory cells were responsible for suppression of growth of HPC in the absence of serum and that the addition of serum could restore HPC growth. This study, similar to studies presented by several other investigators (1-7), shows that in the presence of high concentrations of BSA, growth of HPC was observed almost similar to the numbers of colonies in the presence of serum (data not shown). Since BSA is usually <95-99% pure (8, 9), unidentified serum factors bound to albumin may have influenced the results. Serum-free CM from the bladder carcinoma cell line 5637 could replace the need for serum or BSA in the semisolid medium cultures to support the growth of HPC from normal bone marrow. Activity-based purification of this CM led to the isolation of a protein with a molecular mass of 15 kD (SDS-PAGE) that was identified by NH2-R-terminus sequence analysis as ALP, a potent inhibitor of leukocyte elastase. The effect of this purified ALP on HPC growth could also be confirmed by rALP. Both purified natural and recombinant ALP supported 100% of erythroid colony growth and >60% of the myeloid colony growth as compared to the control culture in the presence of 10% AB serum. In addition, we could completely neutralize the biologic activity from 5637-CM and purified natural ALP with a polyclonal IgG anti-huALP, illustrating that ALP was the factor essential for the in vitro HPC growth from monocyte- and T lymphocyte-depleted bone marrow cells in the presence of highly purified human albumin. ALP is a 107-amino acid cationic protein consisting of two domains of similar architecture but with different inhibitory activities (18, 19). The NH2-terminal domain ALP-D-1 that is assumed to bind trypsin could not support growth of HPC in the absence of serum. The COOH-terminal domain ALP-D-2, containing a strong inhibitory activity against chymotrypsin, neutrophil elastase, and trypsin, restored the colony growth, indicating that the proteinase inhibitory activity of ALP was indeed responsible for the support of the HPC growth in vitro.

ALP, also known as secretory leukocyte proteinase inhibitor, is present in a wide variety of mucous secretions and produced by epithelial-like cells (20). This local production of ALP with very high affinity for leukocyte elastase serves as a potential regulatory feedback mechanism to prevent epithelial damage caused by proteolytic proteinases (21). Regulatory effects of ALP in hematopoiesis have not been described previously. Serum contains various serine proteinase inhibitors, of which α1-PI and α2-macroglobulin are present at relatively high concentrations of 1.3 mg/ml and 1.7 mg/ml, respectively (22); ALP has been found to circulate at a concentration of 50 ng/ml (21). When serum was replaced by α1-PI, the main plasma pro-
Figure 8. Inhibition of the suppressive effect of CD34- accessory cells on HPC growth of purified CD34+ cells in the absence of serum by proteinase inhibitors. In the presence of HGF, the CD34+ cells were cultured with increasing numbers of CD34- cells, up to a ratio 1:16 supplemented with IMDM, rALP (100 ng/ml), CM (10% vol/vol), AB serum (10% vol/vol), or α-1 PI (2 μg/ml). The growth of CFU-GM and BFU-E is expressed as percentage of growth of control culture, obtained in the presence of 10% (vol/vol) AB serum in the absence of CD34- cells. The results of two experiments (A and B) are shown.

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The proteinase inhibitor of neutrophil elastase (22), the growth support was comparable to rALP. These results support the evidence that the proteinase inhibitory activity is responsible for the observed effects. The high concentrations of other serine proteinase inhibitors may explain why anti-huALP antibodies did not abolish the stimulatory effect of serum.

Since the bone marrow accessory cells responsible for the suppression of growth of CD34+ purified HPC were depleted of monocytes and T lymphocytes, the myeloid cells appeared to be the mediator cells for this effect. Probably, these myeloid cells secrete proteolytic proteinases like neutrophil elastase that are capable of degrading cytokines, growth factor receptors, or other proteins essential for the proliferation of HPC (23-25). ALP or other proteinase inhibitors present in serum apparently neutralized these proteolytic enzymes. The mechanisms by which ALP supported the proliferation of HPC in vitro may be at the cell membrane level by protecting from degradation cellular receptors or proteins in the culture medium that are essential for HPC growth. In conclusion, we demonstrated that monocyte- and T lymphocyte-depleted accessory bone marrow cells suppressed the in vitro growth of CD34+ HPC in the absence of serum. The serine proteinase inhibitor ALP was capable of restoring the proliferation of HPC in vitro in the presence of multiple HGF, highly purified albumin, insulin, and cholesterol. These results show that proteinase inhibitors are important for optimal in vitro growth of HPC and may be essential for in vitro expansion of human hematopoietic stem cells in serum-free medium.
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