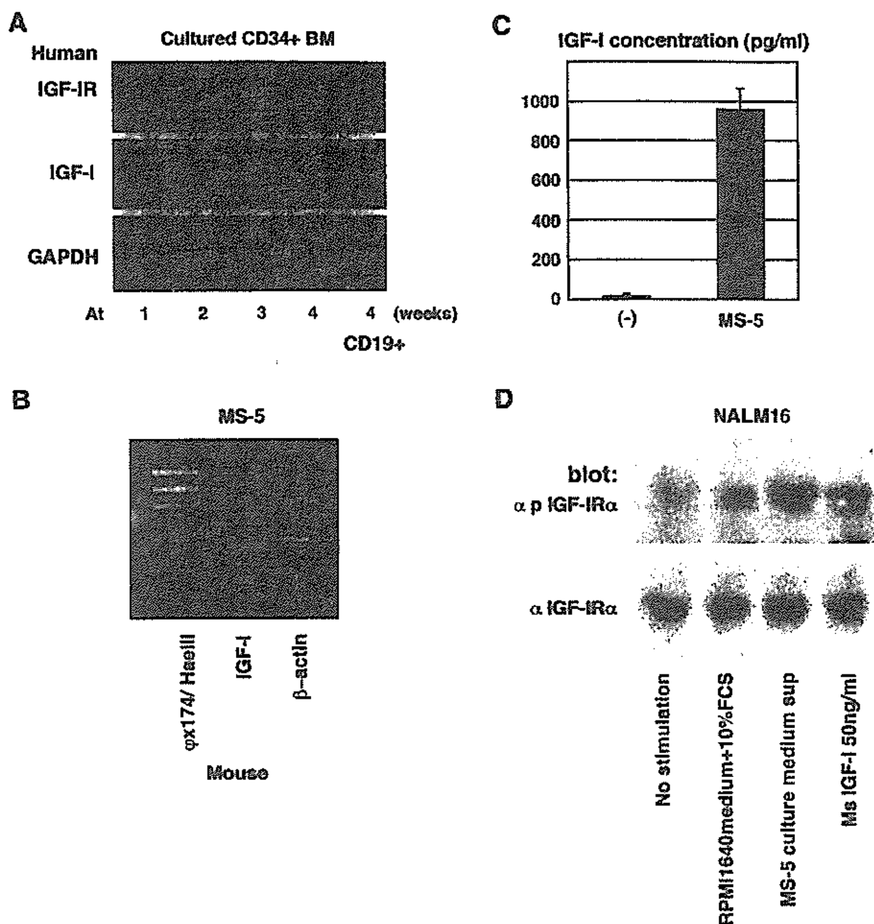


**Figure 2.** Expression of insulin-like growth factors (IGFs) and IGF receptors on cultured human bone marrow (BM) CD34$^+$ cells and murine stromal MS-5 cells. (**A**) Human BM CD34$^+$ cells were cocultured with MS-5 cells for 1, 2, 3, or 4 weeks, as shown in Figure 1. At the end of the culture periods, the floating fraction of the cultured human BM cells was collected and expression of human IGF-I receptor (IGF-IR) and IGF-I was examined using reverse transcriptase polymerase chain reaction (RT-PCR). As an internal control, expression of human glyceraldehyde phosphate dehydrogenase was also examined. CD19$^+$ cells were sorted from 4-week cultured human BM CD34$^+$ cells and similarly examined (CD19$^+$). (**B**) Expression of mouse IGF-I in MS-5 cells was examined using RT-PCR. As an internal control, expression of mouse β-actin was also examined. The φχ174/*Hae*III molecular weight marker was presented in the left side. (**C**) MS-5 cells were cultured alone for 4 weeks and the culture supernatant was collected. Subsequent concentration of mouse IGF-I secreted by MS-5 cells in culture supernatant was determined by enzyme-linked immunosorbent assay. As a negative control, freshly prepared medium containing 10% fetal calf serum (FCS) was also examined, and no significant crossreaction was observed. (**D**) Biological effect of mouse IGF-I secreted by MS-5 cells on human hematopoietic cells was examined using NALM-16 cells that express IGF-IR. Cells at logarithmic growth were stimulated for 5 minutes with either freshly prepared culture medium containing 10% FCS, MS-5 culture supernatant, or recombinant mouse IGF-I (final concentration at 50 ng/mL) and examined by immunoblotting using antiphosphospecific human IGF-IR Ab that only recognize the activated form of IGF-IR. As a control, anti-entire IGF-IR Ab was also used.

human hematopoietic cells. Therefore, the data suggest a possibility that MS-5 cells secrete excess amounts of IGF-I and thus exogenous addition of recombinant IGF-I revealed no effect on pro–B-cell development.

Indeed, when anti-mouse IGF-I Ab, which neutralizes the effect of IGF-I, was added to the culture, development of CD19$^+$ B cells was significantly reduced (Fig. 3A and B). As shown in Figure 3B, the initial addition of 5 μg/mL polyclonal goat anti-mouse IGF-I Ab was sufficient to induce a significant reduction in pro–B-cell development. When hamster anti-mouse IGF-I mAb was used, however, additional Abs were required to produce a remarkable reduction in subsequent pro–B-cell production (Fig. 3A). In both cases, anti-mouse IGF-I Abs not only reduced the total cell number of cultured CD34$^+$ BM cells, but also remarkably diminished the percentage of CD19$^+$ B cells out of the total number of cells in the culture. Therefore, reduction in CD19$^+$ B cells is not merely the result of an overall cell reduction. Moreover, anti-mouse IGF-I Ab-induced reduction in CD19$^+$ B-cell development was cancelled by the addition of recombinant human IGF-I (Fig. 3D). The data suggests that IGF-I significantly participates in pro–B-cell development. Evidence that anti-human IGF-IR Ab and IGF-IR kinase inhibitor, both of which can block the effect of IGF-I, reduce pro–B-cell development, further supports this notion (Fig. 3B and C).

Of note, RT-PCR analysis also showed a time-dependent expression of IGF-I in a total cell fraction, but not sorted $CD19^+$ cell fraction, of the cultured human BM $CD34^+$ cells during the culture period (Fig. 2A). Beside $CD19^+$ cell fraction, $CD33^+$ myelomonocytic cells were present in the culture (data not shown). Although $CD33^+$ cell population was decreasing with the culture time, they matured with time. Thus, time-dependent expression of IGF-I is most likely due to this cell fraction.

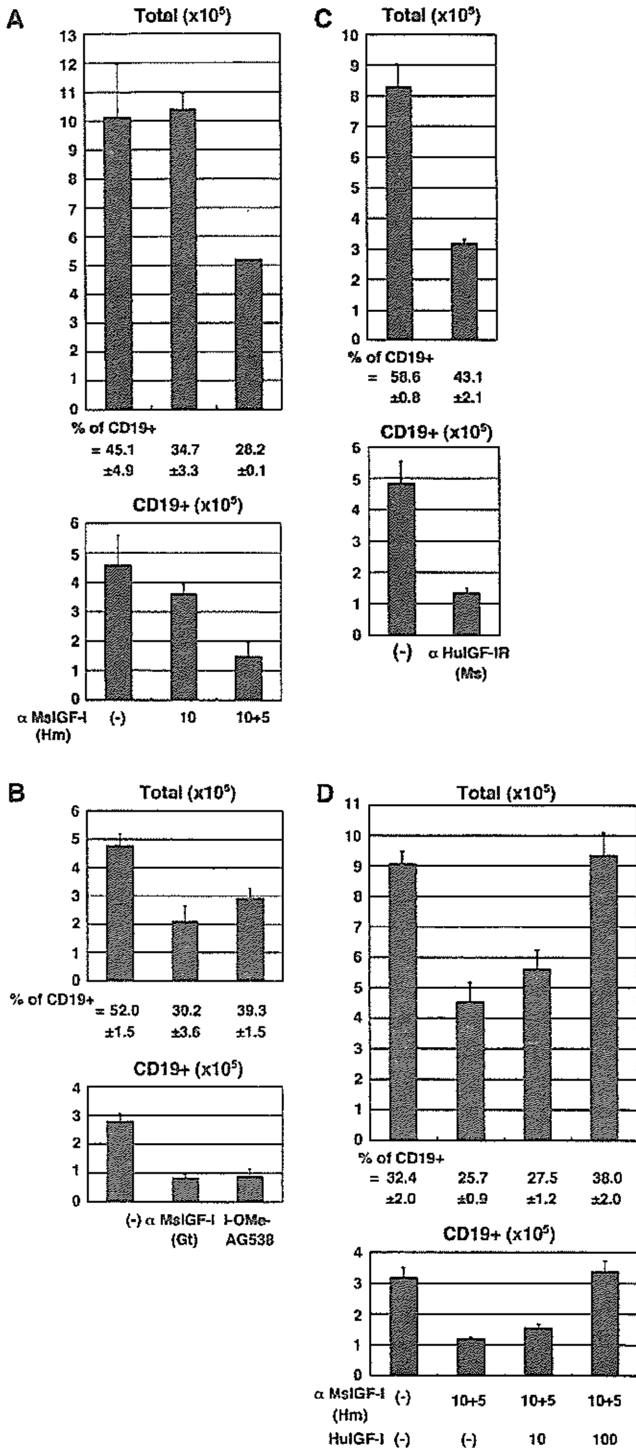


Figure 3. Effect of insulin growth factor-I (IGF-I) inhibition on human pro–B-cell development. Human bone marrow (BM) $CD34^+$ cells were co-cultured with MS-5 for 4 weeks in the presence or absence of hamster anti-mouse IGF-I monoclonal antibody (mAb) [(A) α MsIGF-I (Hm), either initial administration of 10 μg/mL alone (10) or a combination of an initial administration of 10 μg/mL and an additional administration of 5 μg/mL after 2 weeks of culture (10 + 5)], goat polyclonal anti-mouse IGF-I Ab [(B) α MsIGF-1 (Gt), 5 μg/ml)], IGF-IR kinase inhibitor I-OMe-AG538 [(B) 5 μM], mouse anti-human IGF-IR mAb [(C) α HuIGF-IR (Ms), 5 μg/mL], and a combination of α MsIGF-1 (Hm) and human IGF-I (HuIGF-1, 10, and 100 ng/mL) (D). Subsequent $CD19^+$ cell number was estimated using flow cytometry and is shown. Total cell number of cultured $CD34^+$ cells and the percentage of $CD19^+$ cells are also presented.

*Effect of recombinant human IGFBPs on human pro–B-cell development*

To assess the effect of IGFBPs on human pro–B-cell development, we challenged the present culture system with recombinant human IGFBPs. As shown in Figure 4A, of the six IGFBP members, only IGFBP-3 produced a subsequent reduction in the number of pro-B cells; the other IGFBPs did not affect human pro–B-cell development. As shown in Figure 4B, the inhibitory effect of IGFBP-3 on pro–B-cell differentiation was dose-dependent. Furthermore, IGFBP-3–mediated inhibition of $CD19^+$ cell development was cancelled by the addition of recombinant human IGF-I (Fig. 4C).

Because none of the IGFBPs other than IGFBP-3 affected pro–B-cell development from $CD34^+$ BM cells co-cultured with MS-5, we tested a synergistic effect of two or more IGFBPs; no significant synergism was observed and other IGFBPs failed to enhance the effect of IGFBP3 (data not shown). Therefore, we next examined the effect of neutralization of the IGFBP function using specific Abs.

First we tested expression of IGFBPs in MS-5 cells. As shown in Figure 5A and B, RT-PCR experiments revealed that MS-5 transcribe mRNA of IGFBP-4, 5, and 6, but not 1, 2, and 3, while immunoblotting experiments using commercially available Abs detected only IGFBP-6 protein expression. Coincident with the results of immunoblotting, anti-IGFBP-2, 3, and 5 Abs did not affect MS-5–induced pro–B-cell development from $CD34^+$ BM cells (Fig. 5C). Interestingly, however, when anti-mouse IGFBP-6 Ab was added to the culture system, pro–B-cell development was significantly reduced (Fig. 5C). In addition, as shown in Figure 5D, the anti-mouse IGFBP-6 Ab–induced inhibition of pro–B-cell development was completely canceled by the addition of human IGFBP-6. In light of this data, we concluded that the IGFBP-6 produced by the MS-5 cells is essential for pro–B-cell development from $CD34^+$ BM cells.

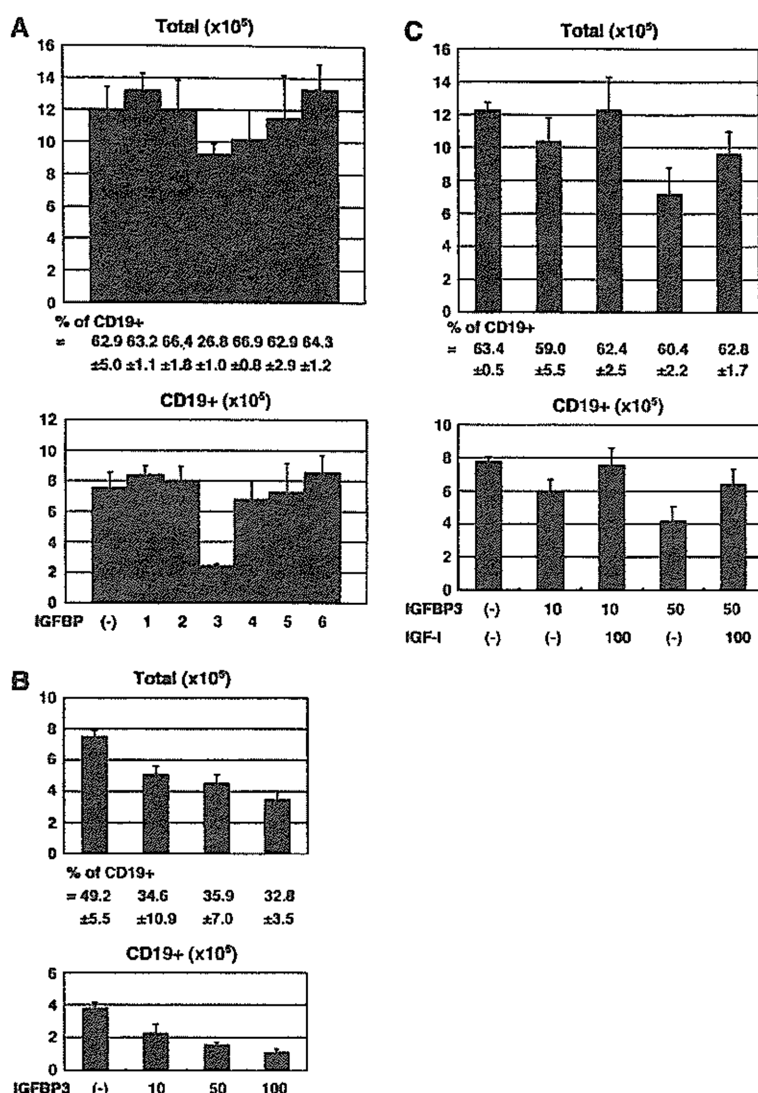


**Figure 4.** Effect of insulin-like growth factor (IGF)-binding proteins on human pro–B-cell development. (A) Human bone marrow (BM) $CD34^+$ cells were cocultured with MS-5 cells with or without 100 ng/mL each recombinant human IGF-binding proteins (IGFBP 1–6) for 4 weeks. The subsequent total cell number of cultured $CD34^+$ cells and the percentage and cell number of $CD19^+$ cells are shown. (B) Human BM $CD34^+$ cells were cocultured with MS-5 cells with or without different doses (ng/mL) of recombinant human IGFBP-3, as indicated, for 4 weeks and examined using the protocol described in (A). (C) Human BM $CD34^+$ cells were cocultured with MS-5 cells with or without indicated combinations of recombinant human IGFBP-3 (ng/mL) and human IGF-I (ng/mL) and examined using the protocol described in (A).

## Discussion

In the present study, when BM $CD34^+$ cells were cultured in the presence of the murine stromal cell line MS-5, pro-B cells, but not pre-B cells, were efficiently induced. MS-5 is well known to be capable of supporting B-cell development [14–17]. However, several different groups have reported this cell line to have distinct effects on induction of B cells. Coincident with our observation, Berardi et al. [14] showed that when human HPSCs derived from umbilical CB were cultured in the presence of MS-5, $CD10^+$, $CD19^+$, and cytoplasmic $\mu^-$ pro-B cells were generated [14]. In contrast, Nishihara et al. [15] and Hirose et al. [16] reported the induction of pre-B cells from CB $CD34^+$ cells after coculturing with MS-5 in the presence of exogenous granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) [14–16]. Moreover, Ohkawara et al. [17] reported that surface $IgM^+$ mature B cells could be produced from CB $CD34^+$ cells using the same culture condition. We tested the effect of the exogenous addition of G-CSF and SCF in our culture system, but failed to observe any signs of pre–B-cell differentiation (data not shown). Although a precise reason for such a difference is unavailable, it may be possible that different stocks of MS-5 exhibit distinct effects on B-cell differentiation. A comparison of the distinct effects of different MS-5 stocks on B-cell differentiation may provide useful and interesting information.

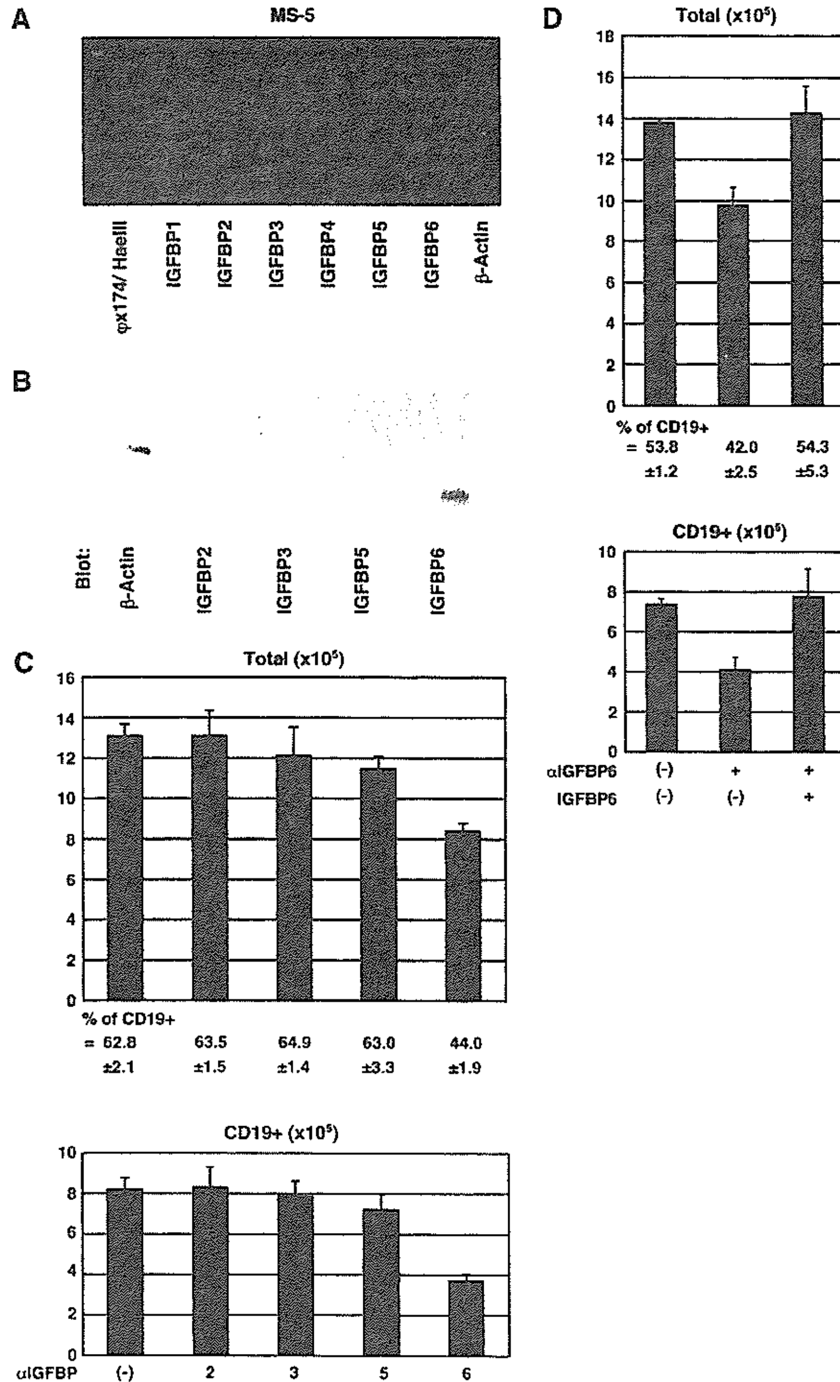


**Figure 5.** Effect of antibodies against insulin-like growth factor (IGF)-binding proteins on human pro–B-cell development. (A) Gene expression of mouse IGF-binding proteins (IGFBP) 1–6 on MS-5 cells was examined using reverse transcriptase polymerase chain reaction. As an internal control, expression of mouse β-actin was also examined. The φχ174/*Hae*III molecular weight marker was presented in the left side. (**B**) Immunoblot analysis with goat anti-mouse IGFBP-2, 3 5, and IGFBP-6 antibodies (αIGFBP2-6, respectively) on MS-5 cell lysates was performed. (C) Human bone marrow (BM) $CD34^+$ cells were cocultured with MS-5 cells for 4 weeks with or without 10 μg/mL each goat antibodies against mouse IGF-binding proteins (αIGFBP), as indicated. Subsequent cell number of $CD19^+$ cells was examined using flow cytometry and is shown. Total cell number of cultured $CD34^+$ cells and percentage of $CD19^+$ cells are also presented. (D) Human BM $CD34^+$ cells were cocultured with MS-5 cells for 4 weeks with or without the indicated combination of αIGFBP6 (5 μg/mL) and recombinant human IGFBP-6 (100 ng/mL). Subsequent total cell number of cultured $CD34^+$ cells and percentage and cell number of $CD19^+$ cells was examined using the protocol described in (C).

Table 1

| Name of gene | Primer sequence forward reverse | Product size (bp) |
|---|---|---|
| human IGF-I | 5′-ACAGGTATCGTGGATGAGTG-3′<br>5′-GTAACTCGTGCAGAGCAAAG-3′ | 263 |
| human IGF-IR | 5′-ATGTGCTGGCAGTATAACCC-3′<br>5′-ACAGCCTTGGATGAACGATG-3′ | 929 |
| mouse IGF-I | 5′-ATGCTCTTCAGTTCGTGTGT-3′<br>5′-CTTCTCCTTTGCAGCTTCGT-3′ | 271 |
| mouse IGFBP-1 | 5′-AGATTAGCTGCAGCCCAAC -3′<br>5′-TGTTCTAGGCAGCATCACTCT-3′ | 535 |
| mouse IGFBP-2 | 5′-ATCCCCAACTGTGACAAGCA-3′<br>5′-CCTCTCTAACAGAAGCAAGGGA-3′ | 407 |
| mouse IGFBP-3 | 5′-TCCAAGTTCCATCCACTCCA-3′<br>5′-GAGGCAATGTACGTCGTCTT-3′ | 372 |
| mouse IGFBP-4 | 5′-AATTAGAGATCGGAGCAAGA-3′<br>5′-TGGGAATTCCTATTGCTACA-3′ | 598 |
| mouse IGFBP-5 | 5′-ATGAGACAGGAATCCGAACA-3′<br>5′-TCAACGTTACTGCTGTCGAA-3′ | 269 |
| mouse IGFBP-6 | 5′-TGCTAATGCTGTTGTTCGCT-3′<br>5′-TGAGTGCTTCCTTGACCATC-3′ | 652 |
| human GAPDH | 5′-CCACCCATGGCAAATTCCATGGCA-3′<br>5′-TCTAGACGGCAGGTCAGGTCCACC-3′ | 598 |
| mouse Actin | 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′<br>5′-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3′ | 661 |

It was previously reported that earlier stages of B-cell development depend on contact with the stromal cells [15,17]. Consistently, culture supernatant of MS-5 cells failed to support pro–B-cell development from $CD34^+$ BM cells (data not shown), suggesting that cell-to-cell interaction between $CD34^+$ BM cells and MS-5 cells is essential for pro–B-cell development in our culture system. In contrast, however, we detected pro-B cells not only in adherent cell fraction, but also in floating cell fraction, while pro-B cells were more enriched in adherent cell fraction. Although contact with stromal cells should be necessary for pro-B cell development from $CD34^+$ cells, it may not always be necessary for further differentiation and/or proliferation of pro-B cells. Therefore, a portion of pro-B cells will dissociate from stromal cells and move to floating cell fraction.

As demonstrated in the present study, the MS-5 cells produced IGF-I, and elimination of IGF-I function resulted in the failure of pro–B-cell development. Previous reports have revealed that IGF-I is essential for differentiation of pro-B to pre-B cell [6,18] and expansion of B-cell population [7,8]. Therefore, our data extends previous observations and indicates that IGF-I likely plays an important role in induction of pro-B cells from HPSCs.

As we presented in Figure 2A, IGF-I is possibly secreted by a non–B-cell fraction of the cultured $CD34^+$ cells. IGF-I may also be present in the supplemented FCS. Therefore, in addition to the IGF-I secreted by MS-5 cells, IGF-I produced by a non–B-cell fraction of the cultured $CD34^+$ cells as well as that provided by supplemented FCS can also affect to pro–B-cell development. However, as we presented in Figure 2D, the biological assay has indicated that the contribution of IGF-I that may be present in the supplemented FCS can be excluded. Furthermore, because the Abs used to neutralize IGF-I activity in this study have higher specificity for mouse IGF-I than human IGF-I, it is most likely that the majority of IGF-I–mediated effects in our culture system are due to mouse IGF-I secreted by MS-5 cells.

Because IGF-I is thought to play an integrating role in hematopoiesis, it seems reasonable to consider that IGFBPs may also contribute to regulation of hematopoiesis. Evidence that IGFBPs are produced by stromal cells in the BM, yolk sac, and liver, where hematopoiesis occurs [19,20], strongly supports this notion. Indeed, a recent report by Liu et al. [21] suggested that IGFBP-3 may block differentiation of HPSCs and be capable of promoting proliferation of primitive $CD34^+CD38^-$ hematopoietic cells, contributing to expansion of the HPSC pool [21]. In this study, we further demonstrated that addition of exogenous IGFBP-3 inhibited the effect of IGF-I on pro–B-cell induction from $CD34^+$ BM cells. Although MS-5 cells do not express IGFBP-3, it has been reported that BM stromal cells produce IGFBP-3 upon stimulation with several factors, such as vitamin D3 and transforming growth factor (TGF)-β1 [22,23]. Therefore, it is conceivable that IGFBP-3 could be secreted by BM stromal cells and involved in the regulation of early hematopoiesis, including B-cell development, depending on conditions in the hematopoietic microenvironment.

As demonstrated in the present article, while all six members of the IGFBP family possess the ability to bind with IGF, only IGFBP-3 inhibited the effect of IGF-I on

pro–B-cell development. Because all other IGFBPs failed to exert synergism with the IGFBP-3 in an inhibitory effect on pro–B-cell development in our experiment (data not shown), IGFBPs may compete with each other for binding with IGF-I, and IGFBP-3 should have the highest binding affinity. Furthermore, our data indicated that IGFBP-6 is required for pro–B-cell development, and neutralization of IGFBP-6 resulted in a marked reduction in the subsequent pro–B-cell number. Therefore, it is suggested that each IGFBP can have a distinct effect on the IGF action in regulation of hematopoiesis.

Interestingly, recent reports have indicated that IGFBPs have an intrinsic ability to affect cells directly [24–29]. For example, IGFBP-2 has been shown to be mitogenic in uterine endometrial and osteosarcoma cells in the absence of IGFs [30]. IGFBP-3 has been reported to induce a reduction in cell growth, DNA synthesis inhibition, and apoptosis in specific cells in an IGF-independent manner [31–34]. Furthermore, IGFBPs have been reported to have specific cell-surface receptors; IGFBP-2 and IGFBP-3 directly bind to target cells through $\alpha5\beta1$ integrin and TGF-$\beta$ type-V receptors, respectively, thereby inducing intracellular signals [35,36]. Therefore, IGFBPs might not only inhibit the effects of IGF-I during hematopoiesis, but also have intrinsic and direct bioactivities that directly affect hematopoietic cells independently of IGF-I function.

In conclusion, IGF-I and IGFBPs appear to play important roles in early B-lymphopoiesis. Although details of their molecular functions remain uncertain, IGFBPs can possibly affect hematopoietic cells in both IGF-dependent and IGF-independent manners. The present observations should contribute to a better understanding of the functional roles of IGF-I and IGFBPs in regulation of B-lymphopoiesis.

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## References

1. Clark R. The somatogenic hormones and insulin-like growth factor-1: stimulators of lymphopoiesis and immune function. Endocr Rev. 1997; 18:157–179.
2. Baxter RC. Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. Am J Physiol. 2000;278: E967–E976.
3. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev. 1997;18:801–831.
4. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev. 2002;23:824–854.
5. Kurtz A, Matter R, Eckardt KU, Zapf J. Erythropoiesis, serum erythropoietin, and serum IGF-I in rats during accelerated growth. Acta Endocrinol (Copenh). 1990;122:323–328.
6. Landreth KS, Narayanan R, Dorshkind K. Insulin-like growth factor-I regulates pro-B cell differentiation. Blood. 1992;80:1207–1212.
7. Gibson LF, Piktel D, Landreth KS. Insulin-like growth factor-1 potentiates expansion of interleukin-7-dependent pro-B cells. Blood. 1993; 82:3005–3011.
8. Jardieu P, Clark R, Mortensen D, Dorshkind K. In vivo administration of insulin-like growth factor-I stimulates primary B lymphopoiesis and enhances lymphocyte recovery after bone marrow transplantation. J Immunol. 1994;152:4320–4327.
9. Melchers F, Karasuyama H, Haasner D, et al. The surrogate light chain in B-cell development. Immunol Today. 1993;14:60–68.
10. Kiyokawa N, Kokai Y, Ishimoto K, Fujita H, Fujimoto J, Hata J. Characterization of the common acute lymphoblastic leukemia antigen (CD10) as an activation molecule on mature human B cells. Clin Exp Immunol. 1990;79:322–327.
11. Takenouchi H, Kiyokawa N, Taguchi T, et al. Shiga toxin binding to globotriaosyl ceramide induces intracellular signals that mediate cytoskeleton remodeling in human renal carcinoma-derived cells. J Cell Sci. 2004;117:3911–3922.
12. Saito M, Kiyokawa N, Taguchi T, et al. Granulocyte colony-stimulating factor directly affects human monocytes and modulates cytokine secretion. Exp Hematol. 2002;30:1115–1123.
13. Kiyokawa N, Lee EK, Karunagaran D, Lin S-Y, Hung M-C. Mitosis-specific negative regulation of epidermal growth factor receptor, triggered by a decrease in ligand binding and dimerization, can be overcome by overexpression of receptor. J Biol Chem. 1997;272: 18656–18665.
14. Berardi AC, Meffre E, Pflumio F, et al. Individual CD34+ CD38lowCD19-CD10- progenitor cells from human cord blood generate B lymphocytes and granulocytes. Blood. 1997;89:3554–3564.
15. Nishihara M, Wada Y, Ogami K, et al. A combination of stem cell factor and granulocyte colony-stimulating factor enhances the growth of human progenitor B cells supported by murine stromal cell line MS-5. Eur J Immunol. 1998;28:855–864.
16. Hirose Y, Kiyoi H, Itoh K, Kato K, Saito H, Naoe T. B-cell precursors differentiated from cord blood CD34+ cells are more immature than those derived from granulocyte colony-stimulating factor-mobilized peripheral blood CD34+ cells. Immunology. 2001;104:410–417.
17. Ohkawara JI, Ikebuchi K, Fujihara M, et al. Culture system for extensive production of CD19+IgM+ cells by human cord blood CD34+ progenitors. Leukemia. 1998;12:764–771.
18. Funk PE, Kincade PW, Witte PL. Native associations of early hematopoietic stem cells and stromal cells isolated in bone marrow cell aggregates. Blood. 1994;83:361–369.
19. Clawson TF, Lee WH, Yoder MC. Differential expression of insulin-like growth factor binding proteins in murine hematopoietic stromal cell lines. Mol Cell Endocrinol. 1996;120:59–66.
20. Abboud SL, Bethel CR, Aron DC. Secretion of insulinlike growth factor I and insulinlike growth factor-binding proteins by murine bone marrow stromal cells. J Clin Invest. 1991;88:470–475.
21. Liu LQ, Sposato M, Liu HY, et al. Functional cloning of IGFBP-3 from human microvascular endothelial cells reveals its novel role in promoting proliferation of primitive CD34+CD38- hematopoietic cells in vitro. Oncol Res. 2003;13:359–371.

22. Kveiborg M, Flyvbjerg A, Eriksen EF, Kassem M. 1,25-Dihydroxyvitamin D3 stimulates the production of insulin-like growth factor-binding proteins-2, -3 and -4 inhuman bone marrow stromal cells. Eur J Endocrinol. 2001;144:549–557.
23. Kveiborg M, Flyvbjerg A, Eriksen EF, Kassem M. Transforming growth factor-beta1 stimulates the production of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in human bone marrow stromal osteoblast progenitors. J Endocrinol. 2001; 169:549–561.
24. Valentinis B, Bhala A, DeAngelis T, Baserga R, Cohen P. The human insulin-like growth factor (IGF) binding protein-3 inhibits the growth of fibroblasts with a targeted disruption of the IGF-I receptor gene. Mol Endocrinol. 1995;9:361–367.
25. Oh Y, Muller HL, Lamson G, Rosenfeld RG. Insulin-like growth factor (IGF)-independent action of IGF-binding protein-3 in Hs578T human breast cancer cells. Cell surface binding and growth inhibition. J Biol Chem. 1993;268:14964–14971.
26. Oh Y, Muller HL, Pham H, Rosenfeld RG. Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells. J Biol Chem. 1993;268:26045–26048.
27. Rajah R, Valentinis B, Cohen P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism. J Biol Chem. 1997;272: 12181–12188.
28. Conover CA, Bale LK, Durham SK, Powell DR. Insulin-like growth factor (IGF) binding protein-3 potentiation of IGF action is mediated through the phosphatidylinositol-3-kinase pathway and is associated with alteration in protein kinase B/AKT sensitivity. Endocrinology. 2000;141:3098–3103.
29. Rajah R, Lee KW, Cohen P. Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-alpha-induced apoptosis: role of Bcl-2 phosphorylation. Cell Growth Differ. 2002;13:163–171.
30. Slootweg MC, Ohlsson C, Salles JP, de Vries CP, Netelenbos JC. Insulin-like growth factor binding proteins-2 and -3 stimulate growth hormone receptor binding and mitogenesis in rat osteosarcoma cells. Endocrinology. 1995;136:4210–4217.
31. Villaudy J, Delbe J, Blat C, Desauty G, Golde A, Harel L. An IGF binding protein is an inhibitor of FGF stimulation. J Cell Physiol. 1991;149:492–496.
32. Imbenotte J, Liu L, Desauty G, Harel L. Stimulation by TGF beta of chick embryo fibroblasts—inhibition by an IGFBP-3. Exp Cell Res. 1992;199:229–233.
33. Cohen P, Lamson G, Okajima T, Rosenfeld RG. Transfection of the human IGFBP-3 gene into Balb/c fibroblasts: a model for the cellular functions of IGFBPs. Growth Regul. 1993;3:23–26.
34. Bernard L, Babajko S, Binoux M, Ricort JM. The amino-terminal region of insulin-like growth factor binding protein-3, (1-95)IGFBP-3, induces apoptosis of MCF-7 breast carcinoma cells. Biochem Biophys Res Commun. 2002;293:55–60.
35. Schutt BS, Langkamp M, Ranke MB, Elmlinger MW. Intracellular signalling of insulin-like growth factor binding protein-2 [abstract]. Growth Horm IGF Res. 2000;0. A29.
36. Leal SM, Liu Q, Huang SS, Huang JS. The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor. J Biol Chem. 1997;272:20572–20576.