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Immunology

BST-1, a surface molecule of bone marrow stromal cell lines that facilitates pre-B-cell growth

(CD38/rheumatoid arthritis)

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ABSTRACT **Bone marrow stromal cells are essential for B-lymphocyte development. However, how stromal cells regulate B lymphopoiesis is not clear. In this paper, we report the molecular cloning of a stromal cell line-derived glycosylphosphatidylinositol-anchored molecule, BST-1, that facilitates pre-B-cell growth. The deduced amino acid sequence of BST-1 exhibited 33% identity with CD38. BST-1 was expressed in a wide range of tissues and in umbilical vein endothelial cells, whereas it was scarcely expressed in a variety of hematopoietic cell lines. The gene for BST-1 was assigned to chromosome 14q32.3, where immunoglobulin heavy-chain genes are clustered. BST-1 expression was enhanced in rheumatoid arthritis patient-derived bone marrow stromal cell lines that were previously shown to have an enhanced ability to support the growth of a pre-B-cell line as compared with stromal cell lines derived from healthy donors.**

The growth and development of B-lineage cells are regulated by close associations between hematopoietic cells and the bone marrow (BM) microenvironment (1–4). BM-derived stromal cells not only produce soluble factors but also express surface molecules that are key molecules acting on B-lineage cells. For example, interleukin 7 (IL-7) is a stromal cell-derived factor that stimulates B-lineage cell growth (5). Stem-cell factor (SCF) is also involved in B lymphopoiesis, since it shows B-cell growth-stimulatory activity in synergy with IL-7 (6–8). In addition to these factors, other adhesion molecules on stromal cells are crucial for B lymphopoiesis. CD44 and VCAM-1 are involved in *in vitro* interaction between stromal cells and lymphocytes (9, 10). Surrogate immunoglobulin light-chain complexes on B-cell progenitors that can transduce activating signals are essential for normal B lymphopoiesis (11), suggesting the presence of ligand for the surrogate light-chain complexes in the BM microenvironments. Further, it has been suspected that yet unknown stromal cell molecules may be involved in B-lineage cell growth and development (12, 13). We have found that BM stromal cell lines derived from rheumatoid arthritis (RA) patients may provide such unidentified cell surface molecules (14).

To identify these unknown molecules, we prepared monoclonal antibodies (mAbs), RF3 and SG2, against RA-derived BM stromal cell lines and cloned a cell surface molecule, designated as BM stromal cell antigen 1 (BST-1), that facilitates pre-B-cell growth.§

MATERIALS AND METHODS

Cell Lines. All BM stromal cell lines and synovial cell lines were previously established by transfecting the plasmid pACT-SVT (a gift from Y. Kaneda, Osaka University), which encodes the simian virus 40 large tumor (T) antigen into cultured BM stromal cells or synovial cells derived from RA and multiple myeloma (MM) patients and healthy donors (14, 15). Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords by collagenase treatment. The cells were cultured in medium T199 supplemented with 20% fetal bovine serum and endothelial mitogen (50 μg/ml; Funakoshi, Tokyo) on 0.1% gelatin-coated dishes.

mAb Generation. BALB/c mice were immunized with a representative clone of a RA-derived BM stromal cell line, RASV5-5 (14), or a RA-derived synovial cell line, SynSV6-14. Spleen cells from immunized mice were fused with the murine plasmacytoma cell line XAg653 as described (16).

cDNA Cloning. Poly(A)$^+$ RNA was isolated from the RA-derived synovial cell line SynSV1-4 by a FastTrack mRNA-isolation kit (Invitrogen). Double-stranded cDNA was synthesized with a TimeSaver cDNA synthesis kit (Pharmacia), ligated with *Bst*XI adapters, and cloned into the expression vector pEF-BOS, kindly provided by S. Nagata (Osaka Bioscience Institute) (17). Plasmid DNAs were transfected into 293T cells (kindly provided by T. Fujita, Rockefeller University, New York) by calcium phosphate precipitation. A positive pool was identified by the ability to confer reactivity for RF3 as assessed by flow cytofluorometry on a FACScan (Becton Dickinson). The DNA sequence was determined by the dideoxy method on an A.L.F. DNA sequencer (Pharmacia).

Preparation of Soluble Form of BST-1. pΔ63-BOS, an expression plasmid encoding a predicted soluble form of BST-1, was prepared by introducing a termination codon at Thr^{298} in the insert cDNA of p63-BOS. The culture supernatants of 293T cells transfected with pΔ63-BOS DNA were used for experiments.

Northern Blot Analysis. Poly(A)$^+$ RNAs were isolated from SynSV6-14, RASV5-5, and NFSV1-1 cell lines. Poly(A)$^+$ RNA (1 μg per lane) was electrophoresed through a 1% agarose/formaldehyde gel and transferred to a nylon membrane. Human multiple tissue Northern blots were purchased from Clontech. A 948-bp *Pst* I–*Pst* I fragment was labeled with [α-^{32}P]dCTP by a multiprime labeling kit (Amersham).

Phosphatidylinositol-Specific Phopholipase C Treatment of Cells. Cells were washed with phosphate-buffered saline and incubated at 37°C for 1–2 hr with gentle shaking in phosphate-buffered saline containing 1% fetal bovine serum and phosphatidylinositol-specific phospholipase C (2 units/ml; Funakoshi).

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Abbreviations: BM, bone marrow; RA, rheumatoid arthritis; mAb, monoclonal antibody; MM, multiple myeloma; GPI, glycosylphosphatidylinositol; HUVEC, human umbilical vein endothelial cell; IL, interleukin.

§The sequence presented in this paper has been deposited in the GenBank data base (accession no. D21878).

Flow Cytofluorometry. A variety of cell lines or cells were stained with RF3 and analyzed by FACScan as described (14). Fluorescein isothiocyanate labeled goat anti-mouse immunoglobulin was used as second antibody.

Fluorescence *in Situ* Hybridization of the Human BST-1 Gene. Fluorescence *in situ* hybridization was performed (18, 19) with a 1.4-kb BST-1 cDNA probe labeled with biotin-16-dUTP (Boehringer Mannheim) by nick-translation.

Establishment of BALB 3T3 Transfectants Expressing Human BST-1. Twenty micrograms of *Sca* I-cut p63-BOS and 2 μg of *Bam*HI-cut pSV2neo (a gift from T. Taniguchi, Osaka University) were transfected into a mouse fibroblast cell line, BALB 3T3, by a GenePulser (Bio-Rad). Two days later, the neomycin analogue G418 (Sigma) was added to the medium at 2 mg/ml and resistant clones were isolated.

***In Vitro* Analysis of DW34 Pre-B Cells by Coculture with Stromal Cells.** BST-1-positive BALB 3T3 transfectants were examined for their ability to support the growth of a mouse pre-B-cell line, DW34 (20) (kindly provided by S.-I. Nishikawa, Kyoto University). Stromal cell lines were plated at 10^5 cells per well on 24-well plates and irradiated at 30 Gy after 24 hr. Two thousand DW34 cells per well were inoculated onto the irradiated cell monolayers and cultured for 4 days. DW34 cells were harvested from each well and the viable cells were counted by trypan blue dye exclusion. The number of recovered cells was <2000 per well without stromal cells.

RESULTS

Establishment of mAbs RF3 and SG2. Since RA-derived cell lines RASV5-5 and SynSV6-14 had a high ability to support the growth of a pre-B-cell line, DW34 (ref. 14 and data not shown), we first prepared murine mAbs against these cell lines to identify the stromal cell surface molecules involved in pre-B-cell growth. We selected two mAbs, RF3 (IgG2a) and SG2 (IgG2a), that were reactive with RASV5-5 and SynSV6-14, but not with a healthy donor-derived nonsupportive cell line, NFSV1-1 (data not shown). RF3 inhibited the reactivity of SG2 for RASV5-5 and vice versa, suggesting that these two antibodies recognize closely located epitopes on the same molecule (data not shown). The molecule recognized by RF3 and SG2 was termed BST-1.

Molecular Cloning of BST-1. To characterize the molecular nature of BST-1, we cloned the cDNA, p63-BOS, by expression cloning. This clone conferred on transfected 293T cells reactivity with both RF3 and SG2 (Fig. 1 *A* and *B*), confirming that the two mAbs recognize the same molecule. Further, the reactivity with RF3 was inhibited by culture supernatants of 293T cells transfected with pΔ63-BOS (Fig. 1*C*), which encodes a soluble form of BST-1, suggesting that the insert cDNA of p63-BOS encodes BST-1 itself, but not the molecule that induced the expression of BST-1. Northern blot analysis of mRNA from SynSV6-14, RASV5-5, and NFSV1-1 cells revealed a major band of ≈1.4 kb (Fig. 1*D*), a size roughly equivalent to that of the cloned cDNA. The insert cDNA of p63-BOS contains a single open reading frame (Fig. 2). In this frame, the initiator ATG is followed by 317 codons before the termination triplet TAA. Since hydrophobic amino acids are abundant in residues 1–28, this region appears to be a typical signal peptide, suggesting that the mature protein probably consists of 290 amino acids.

Amino Acid Homology of BST-1 with CD38 and ADP-Ribosyl Cyclase. The nucleotide sequence of the insert cDNA was compared with other DNAs in the GenBank (release 76) and EMBL (release 34) data bases by GENEWORKS 2.2.1 (IntelliGenetics); no significant similarity with any known DNA sequences was found. However, comparison of the deduced amino acid sequence of BST-1 with the Swiss-Prot (release 25) protein data base revealed that BST-1 showed 33% amino acid identity with human lymphocyte antigen CD38 (Fig. 3) (21, 22), which exhibited a 29% identity with *Aplysia* ADP-ribosyl cyclase (23). BST-1 itself showed less but significant (26%) identity with the cyclase. The positions of nine cysteine residues in the extracellular region of BST-1 were completely conserved among CD38 and the cyclase, suggesting that these molecules may be distantly related.

BST-1 Is a Glycosyl-Phosphatidylinositol (GPI)-Anchored Membrane Protein. In addition to a signal peptide, BST-1 contains a short C-terminal hydrophobic region without an intracytoplasmic region, suggesting that BST-1 is a GPI-anchored membrane protein. Treatment with phosphatidylinositol-specific phospholipase C decreased the BST-1 surface expression of a BALB 3T3 cell line transfected with p63-BOS by 49% but did not decrease that of a type I membrane protein, R25 antigen (16). The same treatment decreased the surface expression of another GPI-anchored molecule, Thy-1, on EL4 mouse lymphoma cells by 73%. Further, the surface expression of BST-1 on RASV5-5 decreased by 47% after treatment with phospholipase C, but CD29 (VLAβ1) did not. These results suggest that BST-1 may be anchored to the cell membrane by a GPI linkage.

Distribution of BST-1 on Human Cell Lines and Tissues. Surface expression of BST-1 on a variety of cell lines and cells was investigated by flow cytofluorometry (Table 1). BST-1 expression was observed on unstimulated HUVECs. IL-1, which induced the expression of the adhesion molecules VCAM-1 and ELAM-1 (refs. 24 and 25; data not shown), had no significant effect on BST-1 expression of HUVECs. In general, hematopoietic cell lines tested had little expression of BST-1, although Jurkat and U-937 showed significant expression. Tissue distribution of BST-1 was

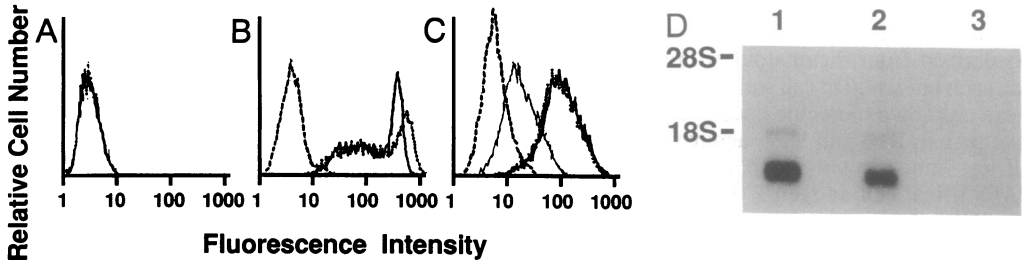

FIG. 1. (*A* and *B*) Insert cDNA of p63-BOS encodes BST-1. Transformed human embryonic kidney cells (293T cell line) were transfected with pEF-BOS (*A*) or p63-BOS (*B*) and incubated with RF3 (solid lines) or SG2 (dotted lines) followed by fluorescein-labeled goat anti-mouse immunoglobulin. In *A*, all flow cytofluorometry profiles were almost identical. (*C*) Inhibition of RF3 reactivity of RASV5-5 by the presence of a soluble form of BST-1. RASV5-5 cells were incubated with RF3 (2 μg/ml) as first antibody in the absence (thick solid line) or presence (thin solid line) of 293T cell supernatants containing soluble BST-1 or in the presence of control supernatant (dotted line). The control supernatant did not affect the flow cytofluorimetry profile of cells incubated with RF3 alone. In *A–C*, control data of cells stained with second antibody alone are represented as dashed lines. (*D*) BST-1 mRNA expression in RA synovium-derived cell line SynSV6-14 (lane 1), RA BM stromal cell line RASV5-5 (lane 2), and healthy donor BM stromal cell line NFSV1-1 (lane 3). One microgram of poly(A)$^+$ RNA was fractionated per lane in an agarose/formaldehyde gel. A *Pst* I–*Pst* I fragment derived from p63-BOS was used as probe. Positions of 28S and 18S rRNAs are indicated.

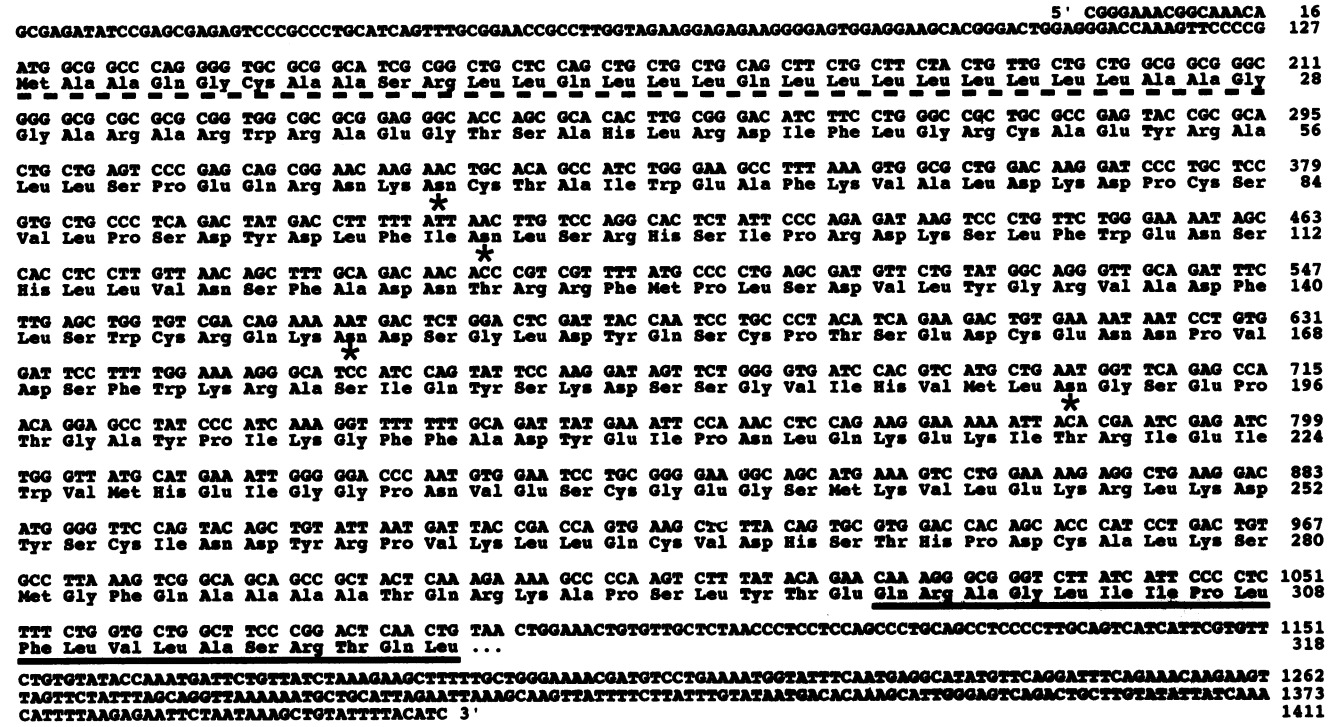

FIG. 2. Nucleotide and deduced amino acid sequence of the open reading frame encoding human BST-1. The putative signal peptide and the C-terminal hydrophobic region are indicated by the dashed and solid lines, respectively. Four potential N-linked glycosylation sites are indicated with asterisks.

examined by Northern blot analysis (Fig. 4). Expression of BST-1 mRNA was detected in various tissues, including placenta, lung, liver, and kidney.

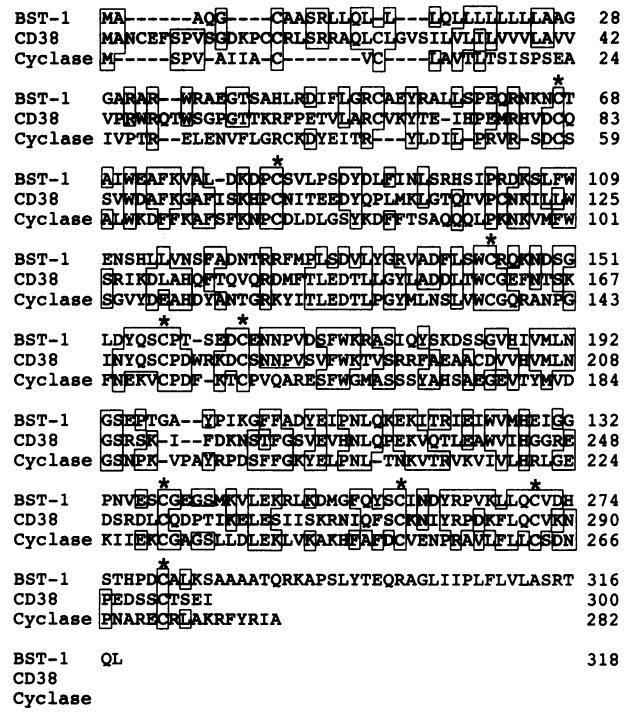

FIG. 3. Homology of BST-1 with human CD38 antigen and *Aplysia* ADP-ribosyl cyclase. The deduced amino acid sequences of the above proteins were aligned by using the new protein alignment program (GENEWORKS 2.2.1) and by inspection. Two or more identical amino acids are boxed. Gaps are indicated as hyphens. Amino acids are numbered at the right of each line. Conserved cysteines are marked by asterisks.

Chromosomal Mapping of the Human BST-1 Gene. Fluorescence *in situ* hybridization showed that the human BST-1 gene was located on chromosome 14 at q32.3 (Fig. 5), where immunoglobulin heavy-chain genes are clustered.

Table 1. Expression of BST-1 in a variety of cells

Name	Origin	MFI	
		Control	RF3
Nalm6	Pre B	2.8	2.8
Daudi	B	5.2	6.8
CL4	B	2.8	2.8
Ramos	B	3.2	3.2
U-266	Myeloma	3.5	3.5
MOLT-4	T	3.8	3.8
Jurkat	T	4.8	9.2
U-937	Histiocyte	2.1	38.0
K562	Erythroblast	3.2	3.2
HL-60	Promyelocyte	4.1	5.9
HUVECs*	Endothelium		
− IL-1		6.2	42.0
+ IL-1		6.2	42.0
Flow6000	Fibroblast	7.5	10.0
FL	Amnion	4.8	4.8
HepG2	Hepatoma	2.8	2.8
T24	Bladder carcinoma	2.7	2.7
SKMG4	Glioblastoma	6.7	6.7
HeLa	Cervical carcinoma	3.2	3.2
RASV5-5	RA BM stroma	6.5	90.0
SynSV6-14	RA synovium	8.7	78.0
NFSV1-1	Normal BM stroma	6.7	8.7

Cells were incubated without (control) or with RF3 and were washed before addition of fluorescein-labeled goat anti-mouse Ig. Results are expressed as mean fluorescence intensity (MFI) of stained cells.

*HUVECs were unstimulated or were stimulated with IL-1 (100 units/ml) for 4 hr.

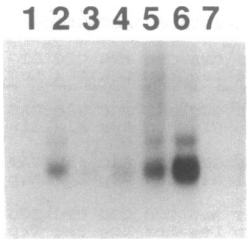

FIG. 4. Expression of BST-1 mRNA in a variety of tissues. A *Pst* I–*Pst* I fragment derived from p63-BOS was used as a probe. Lanes: 1, pancreas; 2, kidney; 3, skeletal muscle; 4, liver; 5, lung; 6, placenta; 7, brain.

Effect of BST-1 on DW34 Cell Growth. To examine whether BST-1 was involved in pre-B-cell growth, we transfected p63-BOS into a murine fibroblast cell line, BALB 3T3, and obtained several stable transfectants expressing human BST-1. These transfectants were cocultured with a stromal cell-dependent mouse pre-B-cell line, DW34 (20). The mean number of DW34 cells recovered from the cocultures [(2.3 ± 0.4) × 10^5, mean ± SEM, n = 6] was about twice that from cocultures with BST-1-negative clones [(1.3 ± 0.3) × 10^5, n = 5; P = 0.0001, unpaired t test where $P < 0.01$ was considered significant].

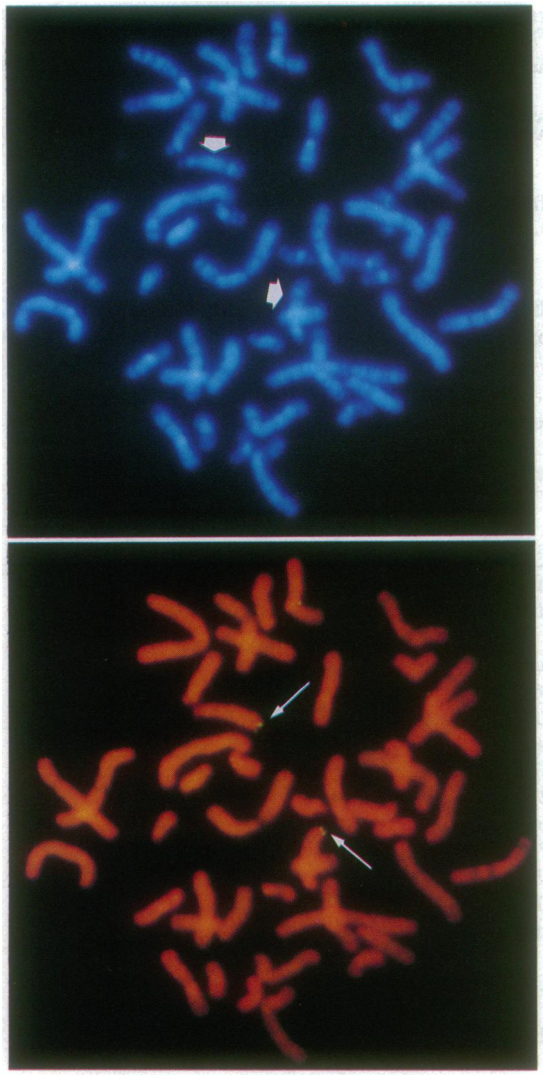

FIG. 5. Localization of the human BST-1 gene by fluorescence *in situ* hybridization. (*Upper*) G-banding pattern of same chromosomes through a UV-2A filter (Nikon). (*Lower*) Propidium iodide-stained metaphase chromosomes (the same chromosomes as in *Upper*) showing the fluorescein signals (arrows) through a B-2A filter (Nikon). Sixty-eight metaphase cells were examined. Of these, 7 cells exhibited double spots on both homologous 14q32.3 chromosome segments and other 23 cells had double spots on one 14q32.3 and a single spot on the other 14q32.3.

Enhanced Expression of BST-1 on RA BM-Derived Stromal Cell Lines. Surface expression of BST-1 on panels of RA, MM, and healthy donor-derived BM stromal cell lines was investigated by flow cytofluorometry (Fig. 6). The results are represented as subtracted mean fluorescence intensity (SMFI) of 13 BM stromal cell lines derived from six RA patients, 11 lines from four MM patients, and 12 lines from four healthy donors. SMFI was calculated by subtracting the mean fluorescence intensity of cell lines stained with second antibody alone. All of the RA-derived lines expressed BST-1, whereas only 7 out of 11 of MM-derived lines and 7 out of 12 of healthy donor-derived lines did. Furthermore, BST-1 expression in the RA-derived clones was significantly higher than in the healthy donor-derived clones (SMFI = 53.1 ± 9.3 vs. 18.5 ± 5.2, P = 0.004). BST-1 expression of the MM-derived clones was not significantly different from that of the healthy donor-derived clones (SMFI = 17.5 ± 4.8 vs. 18.5 ± 5.2, P = 0.884). The data suggest that both the frequency of BST-1-positive cells and the frequency of the cells with an enhanced expression of BST-1 increase in RA-derived BM stromal cell lines.

DISCUSSION

We have cloned a surface molecule, BST-1, from BM stromal cell lines. BST-1 is a GPI-linked membrane protein showing a significant similarity with CD38 and facilitates the stromal cell-dependent growth of a mouse pre-B-cell line.

BST-1 is considered to be one of the cell surface molecules expressed on BM stromal cells that play roles in B lymphopoiesis, although we have not examined whether BST-1 is indeed expressed in the BM microenvironment. (*i*) Enhanced expression of BST-1 was observed in RA-derived BM stromal cell lines that showed an enhanced ability to support the growth of the mouse pre-B-cell line DW34 as compared with those derived from healthy donors. (*ii*) Stable transfectants of BALB 3T3 cells expressing human BST-1 facilitated pre-B-cell growth better than BST-1-negative BALB 3T3 cells. Human BST-1-negative BALB 3T3 transfectants or

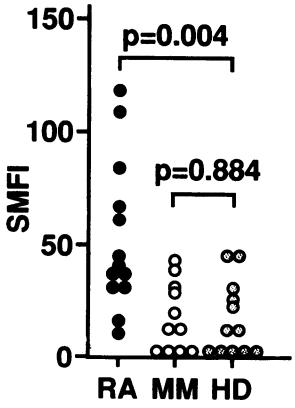

FIG. 6. Surface expression of BST-1 on BM stromal cell lines. BST-1 surface expression was evaluated by flow cytofluorometry with RF3 and fluorescein-labeled goat anti-mouse immunoglobulin as first and second antibodies, respectively. Results are represented as subtracted mean fluorescence intensity (SMFI; see text) on the vertical axis. An unpaired t test was performed to compare the SFMI of stromal cell lines derived from three groups, RA and MM patients and healthy donors (HD). Values of $P < 0.01$ were considered to be significant.

parental BALB 3T3 cells significantly supported DW34 cell growth, suggesting that BST-1 may not be essential for DW34 cell growth. However, it is possible that the mouse BST-1 homologue is expressed on BALB 3T3 cells.

Although BST-1 is a GPI-linked membrane protein, whereas CD38 is a type II integral membrane protein, they showed a similar overall amino acid structure, suggesting that BST-1 may have a function similar to that of CD38. Anti-CD38 induces an increase of intracellular Ca^{2+} and an immunoglobulin-independent activation and proliferation of B cells (26). This activation is suggested to be mediated by the ADP-ribosyl cyclase activity of CD38 that catalyzes the synthesis of cyclic ADP-ribose from nicotinamide adenine dinucleotide (27). Cyclic ADP-ribose is a potent mediator to induce Ca^{2+} release independently of the inositol 1,4,5-trisphosphate receptor (28, 29). However, it is unknown how CD38 plays a role in B-cell activation as a cell surface enzyme.

Human CD38 is expressed on immature lymphocytes and has been suggested to be involved in lymphocyte development (21, 22). On the other hand, BST-1 is expressed on stromal cell lines and umbilical vein endothelial cells and is distributed in a wide range of tissues, suggesting that BST-1 may have a function different from that of CD38. BST-1 may act as a ligand for CD38 through like–like interaction; this possibility should be examined. Ubiquitous expression of BST-1 suggests that BST-1 may have multiple functions in addition to its role in B-cell growth. Further characterization of BST-1-positive cell lineages in normal tissues should be useful in elucidating the physiological role(s) of BST-1.

RA is a systemic autoimmune disease of unknown etiology and primarily affects joint synovial tissues. We previously showed that RA-derived BM stromal cell lines had an enhanced ability to support pre-B cell growth (14). Further, the expression of BST-1 was enhanced in RA-derived BM stromal cell lines compared with healthy donor-derived ones, suggesting the presence of abnormalities in the BM microenvironment in RA patients. This hypothesis may be supported by the fact that BM transplantation resulted in a complete remission of RA or psoriatic arthritis in certain cases (30, 31). Although it remains to be clarified how BST-1 functions *in vivo*, we assume that polyclonal B-cell abnormalities in RA may be, at least in part, attributed to BST-1 overexpression on the stromal cell population. The molecular mechanism(s) that induces such abnormal expression of multiple genes, including those encoding BST-1 and IL-6, may lead to better understanding of the etiology of RA (32, 33).

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1. Cooper, M. D. (1981) *J. Clin. Immunol.* **1**, 81–89.
2. Whitlock, C. A., Denis, K. A., Robertson, D. & Witte, O. N. (1985) *Annu. Rev. Immunol.* **3**, 213–235.
3. Kincade, P. W., Lee, G., Pietrangeli, C. E., Hayashi, S. & Gimble, J. M. (1989) *Annu. Rev. Immunol.* **7**, 111–143.
4. Dorshkind, K. (1990) *Annu. Rev. Immunol.* **8**, 111–137.
5. Namen, A. E., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D. Y., Schmierer, A., Mosley, B., March, C. J., Urdal, D., Gillis, D., Cosman, D. & Goodwin, R. G. (1988) *Nature (London)* **333**, 571–573.
6. Witte, O. N. (1990) *Cell* **63**, 5–6.
7. McNiece, I. K., Langley, K. E. & Zsebo, K. M. (1991) *J. Immunol.* **146**, 3785–3790.
8. Funk, P. E., Varas, A. & Witte, P. L. (1993) *J. Immunol.* **150**, 748–752.
9. Miyake, K., Medina, K., Hayashi, S.-I., Ono, S., Hamaoka, T. & Kincade, P. W. (1990) *J. Exp. Med.* **171**, 477–488.
10. Miyake, K., Medina, K., Ishihara, K., Kimoto, M., Auerbach, R. & Kincade, P. W. (1991) *J. Cell Biol.* **114**, 557–565.
11. Kitamura, D., Kudo, A., Schaal, S., Müller, W., Melchers, F. & Rajewsky, K. (1992) *Cell* **69**, 823–831.
12. Cumano, A., Dorshkind, K., Gillis, S. & Paige, P. J. (1990) *Eur. J. Immunol.* **20**, 2183–2189.
13. Palacios, R. & Samaridis, J. (1992) *Mol. Cell. Biol.* **12**, 518–530.
14. Kaisho, T., Oritani, K., Ishikawa, J., Tanabe, M., Muraoka, O., Ochi, T. & Hirano, T. (1992) *J. Immunol.* **149**, 4088–4095.
15. Tomita, N., Higaki, J., Morishita, R., Kato, K., Mikami, H., Kaneda, Y. & Ogihara, T. (1992) *Biochem. Biophys. Res. Commun.* **186**, 129–134.
16. Kaisho, T., Nagasawa, T., Kishimoto, T. & Kikutani, H. (1992) *J. Immunol.* **148**, 989–995.
17. Mizushima, S. & Nagata, S. (1990) *Nucleic Acids Res.* **18**, 5322.
18. Inazawa, J., Saito, H., Ariyama, T., Abe, T. & Nakamura, Y. (1993) *Genomics* **17**, 153–162.
19. Pinkel, D., Straum, T. & Gray, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2934–2938.
20. Nishikawa, S.-I., Ogawa, M., Nishikawa, S., Kunisada, T. & Kodama, H. (1988) *Eur. J. Immunol.* **18**, 1767–1771.
21. Jackson, D. G. & Bell, J. I. (1990) *J. Immunol.* **144**, 2811–2815.
22. Dörken, B., Möller, P., Pezzutto, A., Schwartz-Albiez, R. & Moldenhauer, G. (1990) in *Leukocyte Typing IV: White Cell Differentiation Antigens*, eds. Knapp, W., Dörken, B., Gilks, W. R., Rieber, E. P., Schmidt, R. E., Stein, H. & von dem Borne, A. E. G. Kr. (Oxford Univ. Press, New York), p. 86.
23. States, D. J., Walseth, T. F. & Lee, H. C. (1992) *Trends Biochem. Sci.* **17**, 495.
24. Osborn, L. (1990) *Cell* **62**, 3–6.
25. Lublin, D. M. (1992) *Curr. Top. Microbiol. Immunol.* **178**, 141–162.
26. Santos-Argumendo, L., Teixeira, C., Preece, G., Kirkham, P. A. & Parkhouse, M. E. (1993) *J. Immunol.* **151**, 3119–3130.
27. Howard, M., Grimaldi, J. C., Bazan, J. F., Lund, F. E., Santos-Argumendo, L., Parkhouse, R. M. E., Walseth, T. F. & Lee, H. C. (1993) *Science* **262**, 1056–1059.
28. Lee, H. C., Walseth, T. F., Bratt, G. T., Hayes, R. N. & Clapper, D. L. (1989) *J. Biol. Chem.* **264**, 1608–1615.
29. Galione, A., Lee, H. C. & Busa, W. B. (1991) *Science* **253**, 1143–1146.
30. Liu Yin, J. A. & Jowitt, S. N. (1992) *Bone Marrow Transplant.* **9**, 31–33.
31. Lowenthal, R. M., Cohen, M. L., Atkinson, K. & Biggs, J. C. (1993) *J. Rheumatol.* **20**, 137–140.
32. Hirano, T., Matsuda, T., Turner, M., Sato, K., Buchan, G., Tang, B., Miyasaka, N., Shimizu, M., Maini, R., Feldmann, M. & Kishimoto, T. (1988) *Eur. J. Immunol.* **18**, 1797–1801.
33. Hirano, T. (1992) *Res. Immunol.* **143**, 759–763.