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Molecular Cloning and Chromosomal Mapping of a Bone Marrow Stromal Cell Surface Gene, BST2, That May Be Involved in Pre-B-Cell Growth

JUN ISHIKAWA,*,[1] TSUNEYASU KAISHO,*,[1] HITOSHI TOMIZAWA,* BYUNG OK LEE,* YOSHIKO KOBUNE,* JOHJI INAZAWA,† KENJI ORITANI,* MOTOYUKI ITOH,* TAKAHIRO OCHI,‡ KATSUHIKO ISHIHARA,* AND TOSHIO HIRANO*,[2]

*Division of Molecular Oncology, Biomedical Research Center and ‡Environmental Medicine, Osaka University Medical School, Suita City, Osaka 565, Japan; and †Department of Hygiene, Hematology and Oncology Unit, Kyoto Prefectural University of Medicine, Kyoto City, Kyoto 602, Japan

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Bone marrow stromal cells regulate B-cell growth and development through their surface molecules and cytokines. In this study, we generated a mAb, RS38, that recognized a novel human membrane protein, BST-2, expressed on bone marrow stromal cell lines and synovial cell lines. We cloned a cDNA encoding BST-2 from a rheumatoid arthritis-derived synovial cell line. BST-2 is a 30- to 36-kDa type II transmembrane protein, consisting of 180 amino acids. The BST-2 gene (HGMW-approved symbol BST2) is located on chromosome 19p13.2. BST-2 is expressed not only on certain bone marrow stromal cell lines but also on various normal tissues, although its expression pattern is different from that of another bone marrow stromal cell surface molecule, BST-1. BST-2 surface expression on fibroblast cell lines facilitated the stromal cell-dependent growth of a murine bone marrow-derived pre-B-cell line, DW34. The results suggest that BST-2 may be involved in pre-B-cell growth.

INTRODUCTION

B lymphopoiesis occurs from hematopoietic stem cells in the bone marrow (BM) (reviewed in Whitlock *et al.*, 1985; Kincade *et al.*, 1989). This process is characterized by successive rearrangement of the immunoglobulin (Ig) loci, expression of surface Ig, and the change of growth requirement. The growth of pre-pro-B cells is dependent on contact with stromal cells but not on IL-7. During the transition from pro-B to pre-B cells, the growth dependency on stromal cells decreases and that on IL-7 increases. Subsequently, pre-B cells differentiate to B cells whose growth is independent of IL-7 (Hayashi *et al.*, 1990; Hardy *et al.*, 1991). BM stromal cells play critical roles in B lymphopoiesis by producing a variety of cytokines such as IL-6 (Hirayama *et al.*, 1992; Gimble *et al.*, 1989), IL-7 (Namen *et al.*, 1988; McNiece *et al.*, 1991; Funk *et al.*, 1993; Sudo *et al.*, 1993) and stem cell factor (Witte, 1990; Flanagan *et al.*, 1991; Martin *et al.*, 1990; Rolink *et al.*, 1991). In addition to these, there are several positive or negative regulators of B-cell formation: positive regulators include pre-B-cell growth stimulating factor (PBSF)/stromal cell-derived factor-1α (SDF-1α), pre-B-cell colony-enhancing factor (PBEF), insulin-like growth factor-I (IGF-I), and bone marrow stromal cell antigen 1 (BST-1), while sex hormones can serve as negative regulators (Nagasawa *et al.*, 1994; Tashiro *et al.*, 1993; Samal *et al.*, 1994; Landreth *et al.*, 1992; Kaisho *et al.*, 1994; Medina *et al.*, 1993; Kincade, 1994). Direct interactions between B lineage cells and BM stromal cells also are critical for early B-cell development (Dorshkind, 1990; Kierney and Dorshkind, 1987). For example, anti-CD44 mAb inhibits lymphoid cell generation in murine long-term BM cultures (Miyake *et al.*, 1990). Furthermore, VCAM-1 is involved in the *in vitro* binding between murine BM stromal cells and B precursor cell lines (Miyake *et al.*, 1991). However, as-yet unidentified stromal cell-derived molecules are involved in B lineage cell growth and development in both murine (Cumano *et al.*, 1990; Gunji *et al.*, 1991; Palacios and Samaridis, 1992) and human (Wolf *et al.*, 1991; Kaisho *et al.*, 1992; Moreau *et al.*, 1993) culture systems.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic polyarthritis, synovial cell proliferation, hypergammaglobulinemia, and polyclonal B-cell activation. Several cytokines that stimulate lymphocyte activation are overproduced in the synovium and may be related to the pathogenesis of RA

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[1] The first two authors contributed equally to this work.

[2] To whom correspondence should be addressed at the Division of Molecular Oncology, Biomedical Research Center, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan. Telephone: 81-6-879-3880. Fax: 81-6-879-3889.

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(Hirano *et al.*, 1988; Hirano, 1992; Feldmann *et al.*, 1992). Synovial cells also express adhesion molecules that bind lymphocytes. For example, ICAM-1 (Hale *et al.*, 1989) and VCAM-1 (Morales-Ducret *et al.*, 1992) are expressed in the blood vessels and on the intimal lining of RA synovial tissue. Furthermore, endothelial cell surface molecules, which mediate the binding of lymphocytes, recently have been identified on the endothelial venules in inflamed synovium (Salmi and Jalkanen, 1992; Airas *et al.*, 1993). We previously showed that BM stromal cell lines derived from RA and multiple myeloma (MM) patients have an enhanced ability to support the stromal cell-dependent growth of a murine BM-derived pre-B-cell line, DW34 (Kaisho *et al.*, 1992). RA-derived synovial cell lines also support DW34 growth. This enhanced ability of supporting pre-B-cell growth is mediated by close interaction through unknown stromal cell surface molecules distinct from ICAM-1, VCAM-1, CD44, NCAM, or ELAM-1 (Kaisho *et al.*, 1992). Furthermore, we cloned a BM stromal cell surface molecule, BST-1, that is overexpressed in RA-derived stromal cell lines and is involved in pre-B-cell growth (Kaisho *et al.*, 1994). However, certain stromal cell lines express low levels of BST-1, although they support pre-B-cell growth. This suggests the presence of other molecules, distinct from BST-1, that are involved in pre-B-cell growth. In this study, we identified a novel type II membrane protein, designated bone marrow stromal antigen 2 (BST-2), that may be involved in pre-B-cell growth.

MATERIALS AND METHODS

Cell lines. We obtained synovial tissues from RA patients during a surgical operation after obtaining informed consent. The tissues were sheared, treated with collagenase for 30 min at 37°C, and trypsinized. Thereafter, the cell suspensions were cultured in DMEM supplemented with 10% FCS and antibiotics for 1 or 2 weeks. After the nonadherent cells were removed with extensive washing, the adherent cells were used as synovial cells. We established two synovial cell lines, SynSV6-14 and SynSV6-8, by expressing SV40 large T antigen in synovial cells as described previously (Kaisho *et al.*, 1992). The stromal cell lines RASV5-5 and RASV10-6 were established from the BM of a patient with RA, NFSV1-1 and NFSV4-3 were established from the BM of a healthy donor, and MMSV3-3 was established from the BM of a patient with MM (Kaisho *et al.*, 1992). A murine BM-derived pre-B-cell line, DW34, the growth of which is dependent on stromal cells, was kindly provided by Dr. S.-I. Nishikawa (Kyoto University, Kyoto, Japan) (Nishikawa *et al.*, 1988).

Hybridoma generation. BALB/c mice were immunized intraperitoneally with SynSV6-14 cells every 2 weeks. Three days after the final immunization, the spleen cells were fused with the murine plasmacytoma cell line XAg653 by PEG 5000. The hybridoma supernatants were screened for reactivity against SynSV6-14, but not NFSV1-1 cells by flow cytometry. A mAb, RS38 (IgM k), was established by this screening procedure. Another mAb, RS38-E (IgG_1 k), which reacted with BST-2, was generated by immunizing BALB/c mice with BALB 3T3 transfectants expressing human BST-2 (as described below).

Flow cytometric analysis. To analyze the specificity of BST-2 surface expression, cells or cell lines of different origins were stained with RS38 and FITC-labeled goat anti-mouse Ig and then analyzed on a FACScan (Becton Dickinson & Co., Mountain View, CA) as described previously (Kaisho *et al.*, 1992).

cDNA cloning. The cDNA library from SynSV6-8 was prepared as described previously (Kaisho *et al.*, 1994). A mammalian expression vector, pEF-BOS (Mizushima and Nagata, 1990), was kindly provided by Dr. S. Nagata (Osaka Bioscience Institute, Osaka, Japan). Plasmid DNA pools were transfected into 293T cells and screened for the ability to confer reactivity with RS38 by flow cytometry. DNA sequencing was carried out by the dideoxy chain termination method using an ALF DNA sequencer (Pharmacia, Uppsala, Sweden). Sequencing data were analyzed by GeneWorks 2.2.1 (IntelliGenetics, Inc. Mountain View, CA). The DNA sequence data have been deposited with DDBJ/EMBL/GenBank under Accession No. D28137.

Production of soluble recombinant BST-2–immunoglobulin fusion protein. At first, an expression plasmid encoding the control chimeric protein that has the BST-1 secretory signal sequence and human IgG_1 Fc region was constructed. Briefly, a BST-1 *Xba*I fragment, derived from p63-BOS (Kaisho *et al.*, 1994), was subcloned into pUC19. The resulting plasmid was cut with *Pst*I and *Hin*dIII and used as a vector arm. The following oligonucleotides were annealed and inserted into the *Pst*I and *Hin*dIII sites: 5′-GCTTCTGCTTCT-ACTGTTGCTGCTGGCGGCGGGCGGGGCGGATCCA-3′ and 3′-ACGTCGAAGACGAAGATGACAACGACGACCGCCGCCCGCC-CCGCCTAGGTTCGA-5′. The newly generated *Bam*HI site is indicated by underlines. The *Eco*RV and *Bam*HI fragment, derived from the resulting plasmid, was subcloned into the *Eco*RV and *Bam*HI sites of p63Ig-BOS. This p63Ig-BOS was an expression plasmid encoding a chimeric protein with the BST-1 extracellular domain and a human IgG_1 Fc region. The cDNA encoding human IgG_1 Fc was kindly provided by Dr. B. Seed (Massachusetts General Hospital, Boston, MA). The resulting plasmid, p63EBIg-BOS, encoded a chimeric protein with the BST-1 secretory signal sequence and a human IgG_1 Fc region. The DNA fragment encoding a putative extracellular region of BST-2 between an asparagine residue at position 49 and a serine residue at position 162 was amplified by PCR with the oligonucleotides 5′-TCGAAGATCTTAACAGCGAGGCCTGCCGG-3′ and 5′-TCGCGGATCCGAGCTGGAGTCCTGGG-3′. The resultant DNA fragment was cut with *Bgl*II and *Bam*HI and subcloned into the *Bam*HI site of p63EBIg-BOS to yield pRS38Ig-BOS, which expresses a chimeric protein consisting of the BST-1 secretory signal peptide, the putative extracellular region of BST-2, and a human IgG_1 Fc region. 293T cells were transfected with p63EBIg-BOS or pRS38Ig-BOS using a standard calcium phosphate method as described previously (Kaisho *et al.*, 1994). Three days later, the supernatants were collected.

Fluorescence in situ hybridization (FISH) of the human BST2 gene. FISH was carried out as previously reported (Inazawa *et al.*, 1993). Metaphase chromosomes were prepared by the thymidine synchronization, bromodeoxyuridine release technique for chromosome banding. A 0.9-kb BST-2 cDNA was labeled with biotin-16–dUTP (Boehringer Mannheim, Mannheim, Germany) by nick-translation and hybridized to metaphase chromosomes. The hybridization signals were detected with FITC–avidin (Boehringer) and biotinylated anti-avidin (Vector) as described previously (Pinkel *et al.*, 1986).

Northern blot hybridization. $Poly(A)^+$ RNAs were isolated from SynSV6-14, RASV5-5, and NFSV1-1 cells using a FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). One microgram of $poly(A)^+$ RNA per lane was electrophoresed through a 1.0% agarose formaldehyde gel and transferred to a GeneScreen Plus membrane (NEN Research Products, Boston, MA). Human multiple tissue Northern blots were purchased from Clontech (Palo Alto, CA). Hybridization was performed at 65°C in the presence of 0.5 mmol/liter sodium phosphate, 1 mmol/liter EDTA, 7% SDS, and 1% bovine serum albumin (Kaisho *et al.*, 1994). The blots were washed at 55°C (0.1× SSC, 0.1% SDS). For the BST-2 probe, a 318-bp *Hin*dIII–*Hin*dIII fragment of pRS38-BOS was used. CHOB (Nakajima and Wall, 1991) was used as an internal control. The probes were labeled with [α-^{32}P]dCTP using a multiprime labeling kit (Amersham, Tokyo, Japan).

Immunoprecipitation. The cells were surface labeled with a sulfo-NHS-biotin (Pierce Chemical Co., Rockford, IL). Briefly, the cells

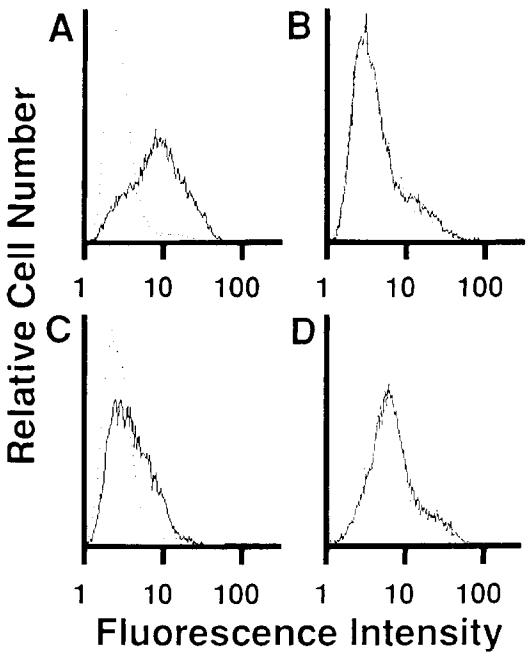

FIG. 1. BST-2 expression on RA synovial and BM stromal cell lines. SynSV6-14 (**A**), NFSV1-1 (**B**), MMSV3-3 (**C**) and RASV5-5 (**D**) cells were incubated with (solid lines) or without (dotted lines) RS38 followed by FITC-goat anti-mouse Ig.

were washed three times and resuspended in labeling buffer (0.15 *M* NaCl, 0.1 *M* Hepes, pH 8.0) with 0.1 mg/ml sulfo-NHS-biotin. After incubation at room temperature with gentle shaking for 40 min, the cells were washed three times with cold RPMI medium and solubilized in a lysis buffer containing 50 m*M* Tris–HCl, pH 7.0, 150 m*M* NaCl, 1% NP-40, phenylmethylsulfonyl fluoride (1 m*M*), aprotinin (1 U/ml), and leupeptin (1 mg/ml). Following centrifugation, the lysates were precleared with BALB/c mouse serum-conjugated protein G–Sepharose (Pharmacia) and then immunoprecipitated with RS38-E mAb (10 μg/sample). The bound proteins were released by boiling for 5 min in Laemmli's SDS sample buffer containing 5% 2-ME, electrophoresed by SDS–PAGE, and blotted onto a PVDF membrane (Millipore, Bedford, MA). Nonspecific binding sites on the membrane were blocked using PBS containing 0.1% Tween-20 (PBS-T), 5% skim milk, and 0.1% NaN_3. The blocked membranes were washed three times with PBS-T and incubated for 1 h with horseradish peroxidase-conjugated streptavidin (Amersham). Biotinylated proteins on the membrane were visualized with the ECL Western blot detection system (Amersham).

In vitro analysis of DW34 cell growth. BALB 3T3 transfectants expressing human BST-2 were established as follows. Ten micrograms of *Sph*I-cut pRS38-BOS and 1 μg of *Bam*HI-cut pSV2-neo were cotransfected into a murine fibroblast cell line BALB 3T3 using a GenePulser (Bio-Rad Laboratories, Tokyo, Japan). Electroporation was performed at 250 μF and 0.25 kV. G418-resistant clones were isolated after 2 to 3 weeks. BST-2-positive or -negative clones were identified by flow cytometry with RS38. The ability of these transfectants to support DW34 cell growth was evaluated as described previously (Kaisho *et al.*, 1994). Briefly, monolayers of BALB 3T3 transfectants were prepared on 24-well plates and irradiated at 30 Gy after 24 h. Two thousand DW34 cells per well were added onto the monolayers and cultured for 4 days. The numbers of DW34 cells were counted using the trypan blue dye exclusion assay. The number of recovered cells was less than 400 per well when the stromal cells were not present.

RESULTS

Production of a mAb against BST-2 and its expression in BM stromal and RA synovial cell lines. To identify the human stromal cell surface molecules involved in pre-B-cell growth, we first generated murine mAbs against a RA synovial cell line, SynSV6-14, since it could strongly support the stromal cell-dependent growth of DW34. Murine hybridomas were screened for reactivity with SynSV6-14, but not NFSV1-1 cells, because NFSV1-1 cells had little supporting ability (Kaisho *et al.*, 1992). One mAb, RS38 (IgM, k), was identified, and the molecule recognized by RS38 was designated BST-2. Flow cytometric analysis showed that SynSV6-14 cells and a MM BM-derived stromal cell line, MMSV3-3 (Figs. 1A and 1C), were positive for the surface expression of BST-2, whereas NFSV1-1 cells and a RA BM-derived stromal cell line, RASV5-5, were negative (Figs. 1B and 1D).

BST-2 is a novel type II transmembrane protein. To characterize the molecular nature of BST-2, we performed an expression cloning to isolate its cDNA (Fig. 2). Pools of several thousand plasmid DNAs were transfected into 293T cells and screened for reactivity with RS38 by flow cytometric analysis. A positive pool was identified and broken down into smaller pools until a single cDNA clone, pRS38-BOS, was isolated. RS38 reacted against 293T cells transfected with pRS38-BOS, but did not react against control 293T cells (Figs. 3A and 3B). DNA sequence analysis of the pRS38-BOS cDNA showed that the 996-bp insert contained a 9-bp 5′ untranslated region, an open reading frame of 540 bp, and a 447-bp 3′ untranslated region, encoding a protein of 180 amino acids (Fig. 4). A hydropathy plot

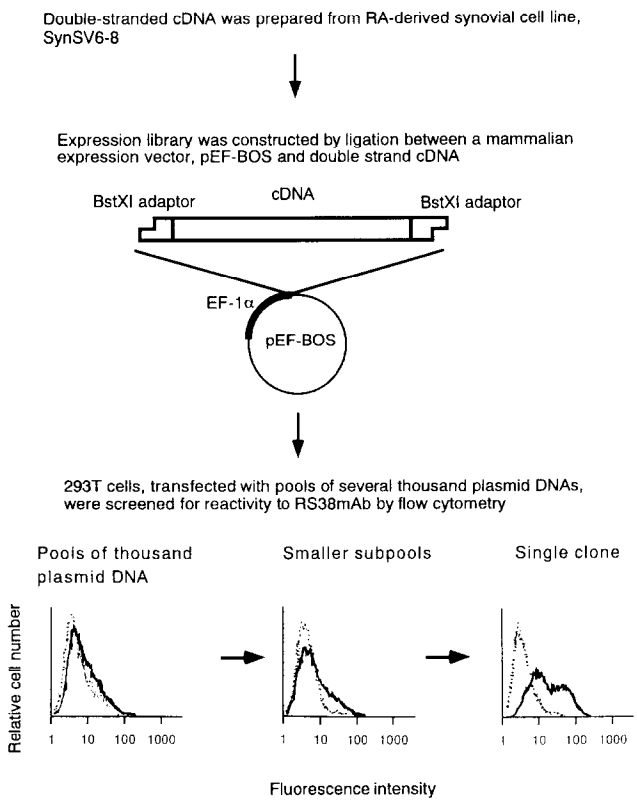

FIG. 2. The cloning strategy for isolating the BST-2 cDNA.

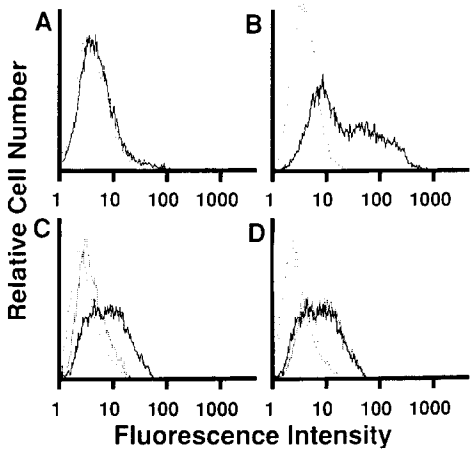

FIG. 3. The plasmid pRS38-BOS confers reactivity with RS38. (**A** and **B**) 293T cells transfected with pEF-BOS (**A**) or pRS38-BOS (**B**) were incubated with RS38 (solid lines) followed by FITC-goat anti-mouse Ig. (**C** and **D**) SynSV6-14 cells were stained with RS38 (solid lines). The fine dotted lines represent the data of the cells that were stained with RS38 following preincubation with culture supernatants of 293T cells transfected with pRS38Ig-BOS (**C**) or p63EBIg-BOS (**D**). The broken lines (**A–D**) represent the control data of cells stained with FITC-goat anti-mouse Ig alone.

of the deduced amino acid sequence showed a hydrophobic region of 28 amino acids preceded by a hydrophilic region at the amino terminus. This is a feature typical of a type II transmembrane protein. The basic amino acid residues at positions 18, 19, and 21 most probably serve as a membrane anchoring region. To prepare a secretory form of BST-2, we constructed pRS38Ig-BOS, which encodes a chimeric protein consisting of the BST-2 molecule, which was deleted of its putative amino-terminal cytoplasmic and transmembrane regions, the secretory signal sequence of BST-1, and the human IgG_1 Fc region. The culture supernatants of 293T cells transfected with pRS38Ig-BOS but not p63EBIg-BOS inhibited the reactivity of RS38 with SynSV6-14 cells (Figs. 3C and 3D). This result showed that pRS38-BOS encodes BST-2 itself but not the molecule that induces the expression of BST-2 in 293T cells. Furthermore, the result confirmed that BST-2 is a type II transmembrane protein, the amino terminus of which is in the cytoplasm. The putative extracellular domain contains two potential N-linked glycosylation sites (Fig. 4). A database search for both the DNA and the predicted amino acid sequences did not show any significant homology with known sequences (GenBank release 81, EMBL release 37, and SWISS-PROT release 28 database).

Chromosomal location of the BST2 gene. To determine the chromosomal location of the BST2 gene, we performed FISH analysis. Of a total of 100 metaphase cells examined, 6 cells exhibited twin-spot signals on both homologous chromosomes 19p13.2, and the other 21 cells had twin-spot signals on one chromosome 19p13.2 and a single spot on another 19p13.2. Such specific accumulation of the signals was not detected on any other chromosome. These results showed that the BST2 gene is located on chromosome 19p13.2 (Fig. 5).

BST-2 is a 30- to 36-kDa membrane protein. Biochemical analysis of BST-2 was performed by immunoprecipitation with RS38-E. RS38-E precipitated a molecule of 30–36 kDa from SynSV6-14 and CL-4 cells under reducing conditions (Fig. 6). The presence of a broad band suggested that BST-2 is glycosylated. BALB 3T3 cells transfected with pRS38-BOS, but not the parent BALB 3T3 cells, expressed a polypeptide of 30–36 kDa that is recognized by RS38-E. These results provided additional evidence that pRS38-BOS encoded BST-2 itself.

The expression of the BST2 gene. The expression of the BST2 gene was examined by Northern blot analysis. A single ~1.0-kb transcript was observed in SynSV6-14, but not in RASV5-5 or NFSV1-1 cells (Fig. 7A), consistent with the surface expression of BST-2 (Fig. 1). The size of the transcript was equivalent to that of the cloned cDNA. Multiple tissue Northern blots

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                                                    GTGGAATTC    9

ATG GCA TCT ACT TCG TAT GAC TAT TGC AGA GTG CCC ATG   48
Met Ala Ser Thr Ser Tyr Asp Tyr Cys Arg Val Pro Met   13

GAA GAC GGG GAT AAG CGC TGT AAG CTT CTG CTG GGG ATA   87
Glu Asp Gly Asp Lys Arg Cys Lys Leu Leu Leu Gly Ile   26

GGA ATT CTG GTG CTC CTG ATC ATC GTG ATT CTG GGG GTG  126
Gly Ile Leu Val Leu Leu Ile Ile Val Ile Leu Gly Val   39

CCC TTG ATT ATC TTC ACC ATC AAG GCC AAC AGC GAG GCC  165
Pro Leu Ile Ile Phe Thr Ile Lys Ala Asn Ser Glu Ala   52

TGC CGG GAC GGC CTT CGG GCA GTG ATG GAG TGT CGC AAT  204
Cys Arg Asp Gly Leu Arg Ala Val Met Glu Cys Arg Asn   65
                                                  *
GTC ACC CAT CTC CTG CAA CAA GAG CTG ACC GAG GCC CAG  243
Val Thr His Leu Leu Gln Gln Glu Leu Thr Glu Ala Gln   78

AAG GGC TTT CAG GAT GTG GAG GCC CAG GCC GCC ACC TGC  282
Lys Gly Phe Gln Asp Val Glu Ala Gln Ala Ala Thr Cys   91

AAC CAC ACT GTG ATG GCC CTA ATG GCT TCC CTG GAT GCA  321
Asn His Thr Val Met Ala Leu Met Ala Ser Leu Asp Ala  104
 *
GAG AAG GCC CAA GGA CAA AAG AAA GTG GAG GAG CTT GAG  360
Glu Lys Ala Gln Gly Gln Lys Lys Val Glu Glu Leu Glu  117

GGA GAG ATC ACT ACA TTA AAC CAT AAG CTT CAG GAC GCG  399
Gly Glu ILe Thr Thr Leu Asn His Lys Leu Gln Asp Ala  130

TCT GCA GAG GTG GAG CGA CTG AGA AGA GAA AAC CAG GTC  438
Ser Ala Glu Val Glu Arg Leu Arg Arg Glu Asn Gln Val  143

TTA AGC GTG AGA ATC GCG GAC AAG AAG TAC TAC CCC AGC  477
Leu Ser Val Arg ILe Ala Asp Lys Lys Tyr Tyr Pro Ser  156

TCC CAG GAC TCC AGC TCC GCT GCG GCG CCC CAG CTG CTG  516
Ser Gln Asp Ser Ser Ser Ala Ala Ala Pro Gln Leu Leu  169

ATT GTG CTG CTG GGC CTC AGC GCT CTG CTG CAG TGAGATC  556
ILe Val Leu Leu Gly Leu Ser Ala Leu Leu Gln ...      180

CCAGGAAGCTGGCACATCTTGGAAGGTCCGTCCTGCTCGGCTTTTCGCTTG 606

AACATTCCCTTGATCTCATCAGTTCTGAGCGGGTCATGGGGCAACACGGTT 657

AGCGGGGAGAGCACGGGGTAGCCGGAGAAGGGCCTCTGGAGCAGGTCTGGA 708

GGGGCCATGGGGCAGTCCTGGGTGTGGGGACACAGTCGGGTTGACCCAGGG 759

CTGTCTCCCTCCAGAGCCTCCCTCCGGACAATGAGTCCCCCCTCTTGTCTC 810

CCACCCTGAGATTGGGCATGGGGTGCGGTGTGGGGGGCATGTGCTGCCTGT 861

TGTTATGGGTTTTTTTTGCGGGGGGGGTTGCTTTTTTCTGGGGTCTTTGAG 912

CTCCAAAAAATAAACACTTCCTTTGAGGGAGAGCAAAAAAAAAAAAAAAAA 963

AAAAAAAAAAAAAAAAAAAAGAATTCCACCACA                    996
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FIG. 4. Nucleotide and predicted amino acid sequence of human BST-2 cDNA. The putative transmembrane region is underlined. Two potential sites of N-linked glycosylation are indicated by asterisks. This sequence data have been deposited with DDBJ/EMBL/GenBank under Accession No. D28137.

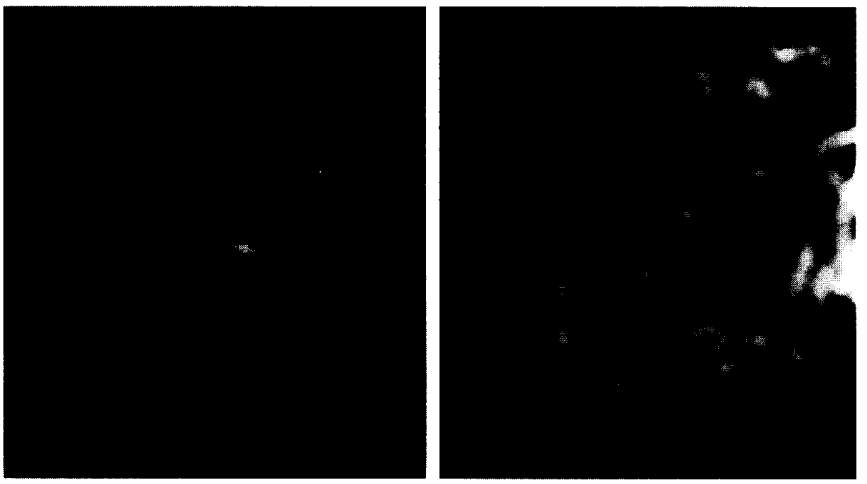

FIG. 5. Chromosomal assignment of human BST2. (**Left**) Metaphase chromosomes stained with propidium iodide showing the twin-spot signals on the short arm of chromosome 19 (arrow). (**Right**) The G-banding pattern of the same chromosomes was delineated through a UV2-A filter (Nikon), indicating that the BST2 gene is located on the region of chromosome 19p13.2.

with a BST-2 probe (Fig. 7B) showed that the BST-2 transcript is expressed abundantly in liver, lung, placenta, and heart. To a lesser extent, it is expressed in pancreas, kidney, skeletal muscle, and brain.

Distribution of BST-2 in human cells and cell lines. The surface expression of BST-2 in a variety of cells and cell lines was analyzed by flow cytometry (Table 1). Hematopoietic cell lines, including certain B and myeloid lineage cell lines, expressed BST-2. Human umbilical vein endothelial cells (HUVEC) were negative for BST-2. IL-1 stimulation did not affect BST-2 expression on HUVEC. Several, but not all, BM stromal cell lines and synovial cell lines expressed BST-2.

Effect of BST-2 on DW34 cell growth. To examine whether BST-2 is involved in pre-B-cell growth, pRS38-BOS was transfected into a murine fibroblast cell line, BALB 3T3, and stable transfectants expressing human BST-2 were established. BST-2-positive transfectants showed enhanced ability to support DW34 cell growth (Table 2). The mean number of DW34 cells recovered from coculture with BST-2-positive BALB 3T3 cells was $2.0 \pm 0.3 \times 10^5$/well, whereas that from coculture with BST-2-negative BALB 3T3 cells was $0.9 \pm 0.4 \times 10^5$.

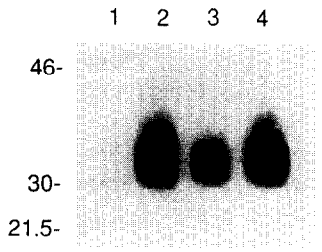

FIG. 6. Immunoprecipitation of BST-2 from BALB 3T3 (lane **1**), a BALB 3T3 transfectant expressing BST-2 (lane **2**), SynSV6-14 (lane **3**), and CL4 (lane **4**) cells. The cells were surface labeled with a sulfo-NHS-biotin and immunoprecipitated with mAb RS38-E. The immunoprecipitates were analyzed by SDS–PAGE, and blots were visualized with the ECL Western blot detection system. Molecular mass standards in kilodaltons are shown to the left.

These results suggest that BST-2 may be involved in stimulating pre-B-cell growth.

DISCUSSION

In this study, we made a mAb, RS38, that recognized a cell surface molecule expressed in synovial and bone marrow stromal cell lines. Furthermore, we cloned the cDNA encoding this molecule, designated BST-2, that was recognized by RS38 mAb. BST-2 is a novel human type II transmembrane protein that facilitated the growth of a murine stromal cell-dependent pre-B-cell line. The results suggested that BST-2 may be one of the stromal cell surface molecules involved in pre-B-cell growth.

We previously showed that bone marrow stromal cell lines derived from patients with RA or MM showed an enhanced ability to support the stromal cell-dependent growth of a murine pre-B-cell line, DW34 (Kaisho *et al.*, 1992). Furthermore, we cloned a novel GPI-anchored

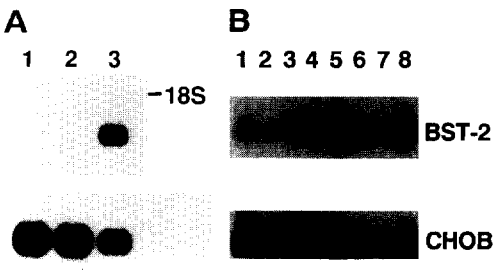

FIG. 7. Northern blot analysis of BST-2 transcripts. (**A**) One microgram of poly(A)$^+$ RNAs isolated from NFSV1-1 (lane **1**), RASV5-5 (lane **2**), and SynSV6-14 (lane **3**) cells was electrophoresed through a 1.0% agarose gel, transferred to a nylon membrane, and hybridized with a BST-2 probe. The same filter was rehybridized with a CHOB probe. The migration position of 18S rRNA is indicated. (**B**) Expression of BST-2 mRNA in a variety of tissues. Lanes: **1,** pancreas; **2,** kidney; **3,** skeletal muscle; **4,** liver; **5,** lung; **6,** placenta; **7,** brain; and **8,** heart.

stromal cell molecule, BST-1, that is overexpressed on RA-derived BM stromal cell lines and involved in pre-B-cell growth (Kaisho *et al.,* 1994). We did not find any structural similarity between BST-1 and BST-2: the former is a GPI-anchored protein with 30% homology to CD38, while the latter is a type II membrane molecule without any homology to other known proteins. Both BST-1 and BST-2 are expressed in various tissues and cell lines other than BM stromal cell lines. However, the expression pattern of each molecule was different: HUVEC expressed BST-1 but not BST-2; a MM patient-derived BM stromal cell line, MMSV3-3, that exhibited an enhanced ability to support DW34 growth (Kaisho *et al.,* 1992) expressed BST-2 but not BST-1; and RASV5-5, derived from a RA patient, was BST-1-positive and BST-2-negative. There seem to be three types of stromal cell lines: BST-1^+/BST-2^- (RASV5-5 type), BST-1^-/BST-2^+ (MMSV3-3 type), and BST-1^+/BST-2^+ (RASV10-6, NFSV4-3 type), although more detailed studies are required before such a classification of stromal cells can be confirmed. In addition to the stromal cell lines, several lymphohemopoietic lineage cell lines express BST-2, although BST-1 is only weakly expressed in lymphohemopoietic lineage cell lines (Kaisho *et al.,* 1994). The broad tissue distribution of BST-2 is consistent with the fact that none of the factors and cell surface molecules, such as IL-7, stem cell factor, PBSF/SDF-1α, IGF-I, PBEF, and BST-1, that influence the growth and differentiation of lymphohemopoietic precursors is restricted in its distribution to the BM (Nagasawa *et al.,* 1994; Tashiro *et al.,* 1993; Samal *et al.,* 1994; Landreth *et al.,* 1992; Kaisho *et al.,* 1994; Medina *et al.,* 1993; Kincade, 1994). The BM microenvironment suitable for B lineage differentiation may be determined by a unique combination and concentration of molecules rather than by tissue-restricted factors as discussed by Kincade (1994). The broad tissue distribution of BST-2 also suggested its potential role in other organs like other pre-B-cell stimulating molecules that are expressed in various tissues.

TABLE 1

Expression of BST-2 in Human Cell Lines[a]

Name	Origin	Control	RS38
Nalm6	PreB	2.8	2.8
Daudi	B	6.2	10.0
CL4	B	3.0	20.0
Ramos	B	3.2	3.2
Molt4	T	4.2	4.2
Jurkat	T	4.8	4.8
U937	Histiocyte	2.2	10.0
K562	Erythroblast	3.5	12.0
HL60	Promyelocyte	4.2	10.0
Mo7	Megakaryoblast	2.2	30.0
HUVEC[b]	Endothelium		
	Unstimulated	4.0	4.0
	Stimulated with IL-1	5.8	5.8
FL	Amnion	2.1	15.0
HepG2	Hepatoma	3.0	5.2
T24	Bladder cell carcinoma	2.7	7.2
SKMG4	Glioblastoma	6.0	8.0
HeLa	Cervical cell carcinoma	3.2	7.2
RASV5-5	RA BM stroma	6.5	6.7
RASV10-6	RA BM stroma	5.4	17.7
MMSV3-3	MM BM stroma	3.2	6.8
NFSV1-1	HD BM stroma	9.0	9.2
NFSV4-3	HD BM stroma	4.8	21.7
SynSV6-14	RA synovial cell	5.0	10.3
SynSV6-8	RA synovial cell	3.8	11.0

[a] Cells were incubated with or without RS38 followed by washing and addition of FITC-goat anti-mouse Ig. Results are expressed as mean fluorescence intensity of stained cells.

[b] HUVEC were stimulated with 100 U/ml IL-1 for 4 h (Kaisho *et al.,* 1994).

TABLE 2

DW34 Cell Growth on BALB 3T3 Transfectants[a]

BST-2 negative (n = 5)	BST-2 positive (n = 5)	
0.9 ± 0.4	2.0 ± 0.3	$P = 0.0001$[b]

[a] Results are presented as the mean number of recovered DW34 cells ($\times 10^{-5}$/well) on five independent BST-2-negative BALB 3T3 transfectants and five positive ones ± SE. Experiments were performed in triplicate for each clone.

[b] An unpaired t test was performed to compare them. Values of $P < 0.01$ were considered to be significant.

Synoviocytes express several adhesion molecules that interact with lymphocytes. Synovial cell adhesion molecules, VAP-1 and L-VAP-2, which interact with lymphocytes, have been identified at the protein level (Salmi and Jalkanen, 1992; Airas *et al.,* 1993). VCAM-1 expression is augmented in the synovial lining of RA (Morales-Ducret *et al.,* 1992) and is involved in mediating lymphocyte adhesion and homing (Van Dinther-Janssen *et al.,* 1991). In addition, unidentified molecules are involved in synovial-specific adhesion (Salmi *et al.,* 1992). Furthermore, Moreau *et al.* (1993) have shown that synoviocytes could support B-cell progenitor growth in the presence of IL-7 and suggested that molecules other than CD44, VCAM-1, stem cell factor, and IL-7 are critical for growth support. These interactions between synovial cells and lymphocytes may play a critical role in the synovial inflammation in RA patients. In addition to SynSV6-14 and SynSV6-8 cells (Table 1), several RA patient-derived synovial cell lines expressed BST-2 (data not shown). Therefore, BST-2 may play a role in B-cell activation in the synovium of a patient with RA.

The molecular cloning of a novel type II membrane molecule, BST-2, that is expressed in stromal cell lines and may be involved in pre-B-cell growth should help to facilitate further studies of the BM microenvironment, which is important for B-cell development.

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