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SF20/IL-25, a Novel Bone Marrow Stroma-Derived Growth Factor That Binds to Mouse Thymic Shared Antigen-1 and Supports Lymphoid Cell Proliferation

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Using a forward genetic approach and phenotype-based complementation screening to search for factors that stimulate cell proliferation, we have isolated a novel secreted bone marrow stroma-derived growth factor, which we termed SF20/IL-25. This protein signals cells to proliferate via its receptor, which we have identified as mouse thymic shared Ag-1 (TSA-1). Enforced expression of TSA-1 in IL-3-dependent Ba/F3 cells that do not express endogenous TSA-1 rendered cells to proliferate in a dose-dependent manner when stimulated with SF20/IL-25. FDCP2, a factor-dependent hemopoietic cell line that expresses endogenous TSA-1, could also be stimulated to proliferate with SF20/IL-25. Binding of SF20 to TSA-1 was blocked by anti-TSA-1 Ab and SF20-induced proliferation of TSA-1-expressing cells was inhibited by anti-TSA-1. In vitro assay revealed that SF20/IL-25 has no detectable myelopoietic activity but supports proliferation of cells in the lymphoid lineage.

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Materials and Methods

**Cytokines and cell lines**

Recombinant murine (m)IL-3 was purchased from Upstate Biotechnology (Lake Placid, NY) and rmIL-2 was from R&D Systems (Minneapolis, MN). Anti-FLAG BioM2 Ab was purchased from Sigma-Aldrich (St. Louis, MO). Anti-TSA-1 monoclonal Ab (MTS35) was purchased from BD PharMingen (San Diego, CA). A retrovirus packaging cell line, Plat-E (12), was maintained in DMEM containing 10% (v/v) FCS and selection reagents (8 μg/ml blasticidin and 0.8 μg/ml puromycin; Sigma-Aldrich). The cells were transfected into DMEM/10% FCS without selection reagents 2 days before transfection. A murine pro-B cell line, BaF3, was cultured in RPMI 1640 medium containing 10% FCS in the presence of 1 ng/ml IL-3. A murine factor-dependent cell line, FDCP2, and mast cell line, MC9, were cultured in RPMI 1640 medium containing 10% FCS and 10 ng/ml IL-3. A murine IL-2-dependent T cell line, CTLL-2, was cultured in RPMI 1640 medium containing 10% FCS and 5 ng/ml IL-2. The mouse bone marrow-derived stromal cell lines, ST2, STO, and PA6, and spleen-derived stroma CF-1 were cultured in DMEM/F-12 medium containing 10% FCS. The mouse bone marrow-derived stromal cell lines MS5 and MS10 were cultured in αMEM (Life Technologies, Rockville, MD) containing 10% FCS. COS7 cells were maintained in DMEM/10% FCS.

**Expression cloning of SF20**

Ba/F3 mutagenesis, establishment of ST2 stroma-dependent mutants, and preparation of cDNA library from ST2 cells were performed as previously described (11). Production of retrovirus stocks from the cDNA library and infection of MS10 cells, a bone marrow stroma that does not support proliferation of SB2-33 mutants, were essentially the same as previously reported. To search for the factor that stimulates proliferation of SB2-33 cells, 12,000 independent clones from the ST2 cell cDNA library were screened using subdivided pools (120 clones per pool). After first screening, one pool (no. 6) was identified to support proliferation of SB2-33 clones. This pool contained 120 independent clones and was further subdivided...
into smaller subpools and rescreened. Finally, one clone (6-13-19) was obtained to support the growth of SB2-33 cells.

The nucleotide and deduced amino acid sequence of SF20 has been deposited in GenBank under accession number AY038184.

Identification of human genomic gene for SF20/IL-25

To identify the human counterpart of murine SF20, a homology-based search using the protein query-translated database (TBLASTN) National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) program was performed with the amino acid sequence of murine SF20/IL-25 as a query against the human expressed sequence tag (EST) subset of the GenBank database. The result was a positive match with a human EST clone (GenBank accession number BE387585). The genomic sequence of human SF20/IL-25 was identified by using the EST sequence of BE387585 as a query against the human high-throughput genomic sequence subset of the GenBank database. The identified bacterial artificial chromosome clones (GenBank accession numbers AC005594 and AC005539) derived from human chromosome 19p13.3 encoded lip to lip overlapping partial sequences of SF20/IL-25.

Cloning of human SF20/IL-25

To isolate the cDNA for human SF20, nested PCR primers were designed around the putative start codon on AC005594 and the putative stop codon on AC005539. PCR was then performed on Marathon cDNA from human testis library (Clontech Laboratories, Palo Alto, CA). The 5′ sense primers were 5′-GACCTATGGCAAGCGGCCCCGC3′ and nested 5′-ATGTCGAGGAGCCAGGAGACACCG-3′. The 3′ antisense primer was 5′-TCTGGCATCGCCACAGGAACACCG-3′, and nested 5′-CAGGGCTGCTGGTCACAGCTCAGTGCGCG-3′. The 5′- and 3′-ends of the cDNA were isolated by using RACE according to the manufacturer’s recommendations using Advantage polymerase (Clontech). The PCR products were cloned into a TA vector (Invitrogen, San Diego, CA), and inserts of three positive bacterial transformants were sequenced. All three clones contained identical sequences corresponding to the human SF20 gene. Finally, the full-length cDNA of the human SF20 gene was amplified using specific primers. The sense primer was 5′-CATGTCGAGGAGCCAGGAGACACCG-3′, and the antisense was 5′-TCTGGCATCGCCACAGGAACACCG-3′. The cDNA sequence was allowed for the amplification of a 975-nt fragment, including a 519-bp open reading frame that encodes the mature human SF20/IL-25 protein.

Expression cloning of mouse TSA-1

A cDNA library was prepared from the mutant clone SB2-33 using retroviral vector according to the procedure previously described (13). Twelve thousand independent clones were screened using subpooled pools (120 clones per pool). MiniPrep DNA was prepared from each pool and transfected to packaging cell line Plat-E to obtain library-derived retroviruses. Parental Ba/F3 cells (1 × 105) were infected with 0.5 ml of library-derived retroviruses for each pool in the presence of polybrene (10 µg/ml) for 6 h. Thereafter, 0.5 ml of IL-3-containing medium was added and cells were incubated for 2 days. The library-transduced Ba/F3 cells were harvested, washed once with medium, and cultured on SF20-expressing MS10 stroma. After 2 wk of coculture, Ba/F3 cells transfected by two pools (nos. 35 and 63) were identified to proliferate on SF20-expressing MS10 stroma. These two pools were further subdivided into smaller subpools and rescreened. Finally, four single clones were identified to support strong proliferation of Ba/F3 cells. These four clones contained the same cDNA insert corresponding to mouse TSA-1.

The nucleotide and deduced amino acid sequence of mouse TSA-1 has been deposited in GenBank under accession number K4128-1; Clontech, an in-house stroma cell panel, and in blood cell types using primers 5′-GTTGTGCTGATGCTTTCCTCGAG-3′ (forward) and 3′-CAGAGAGGAGAGGAGAG-3′ (reverse). The PCR was run for a total of 30 cycles (20 s at 94°C and 1 min at 70°C for 5 cycles, and 20 s at 94°C and 1 min at 68°C for 25 cycles) using Advantage polymerase (Clontech).

Expression analyses of human SF20 were performed in human blood fractions MTC panel (K1428-1; Clontech), immune system MTC panel (K1426-1; Clontech), and tumor MTC panel (K1422-1; Clontech) using the primers 5′-ATGTCGAGGAGCCAGGAGACACCG-3′ (forward) and 5′-CAGGGCTGCTGGTCACAGCTCAGTGCGCG-3′ (reverse) with similar conditions as described above.

The expression of TSA-1 in Ba/F3 and FDCP2 cells was analyzed by performing RT-PCR using the primers 5′-GACGAGAGGAGAGGAG-3′ (forward) and 5′-CAAGATTCGGCGCTGACTCTG-3′ (reverse).

The PCR was run for a total of 30 cycles (20 s at 94°C and 1 min at 70°C for 5 cycles, and 20 s at 94°C and 1 min at 68°C for 25 cycles) using Advantage polymerase (Clontech).

Expression of SF20/IL-25 in COS7 cells

COS7 cells (2 × 105 cells/well) were seeded in six-well plates a day before transfection and transfected with a C-terminally FLAG-tagged construct of SF20 cDNA in a mammalian expression vector, pME185, which is driven by SV40 promoter. Transfection was performed using the Lipofectamine method (Life Technologies). After 48 h of culture, supernatant was collected and immunoprecipitated with anti-FLAG BioM2 affinity gel (Sigma-Aldrich) overnight at 4°C. The immunoprecipitate was collected by centrifugation at 5000 rpm for 5 min and washed with immunoprecipitation buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.2 mM NaVO4, 0.2 mM PMSF, and 0.5% Nonidet P-40) three times. The bound proteins were eluted by electrophoresis sample buffer, applied to SDS-PAGE, and electroblotted onto polyvinylidene difluoride membranes (Millipore, Bedford, MA). The blots were probed with anti-FLAG M2 Ab, incubated with HRP conjugate complex, and subsequently developed with ECL Western blotting detection reagents. The bands corresponding to the expected protein and exposed to ECL Hyperfilm were used for signal detection.

Cell proliferation assay

The activity of recombinant SF20/IL-25 was assayed using a two-fold serial dilution of the protein across a 96-well plate. The starting concentration of SF20 was 40 ng/ml while IL-2 and IL-3 started at 20 ng/ml. Background was measured by adding cells to assay medium alone. Cells (1 × 105/well)
were cultured for 72 h and cell proliferation was determined using a standard cell proliferation assay kit (Cell Titer 96 Assay) by Promega (Madison, WI).

**Generation of TSA-1-expressing Ba/F3 stable transformants**

A BstXI fragment of mouse TSA-1 was inserted into a retroviral vector pMX-neo (16). Ba/F3 cells were infected with virus supernatant derived from pMX-neo-TSA-1-transfected packaging cells (Plat-E) for 4 h and were cultured for another 2 days. Cells were then cultured in selection medium (RPMI 1640/10% FCS medium containing 1 ng/ml IL-3 and 1.2 μg/ml G418) for 4 wk to obtain TSA-1-Ba/F3 stable transformants. The expression of TSA-1 in Ba/F3 cells was confirmed by RT-PCR. TSA-1-expressing Ba/F3 cells were harvested, washed twice with factor-free medium, and assayed for proliferative response to SF20/IL-25 as described above.

**Mouse cell proliferation assay**

Bone marrow cells and splenocytes were obtained from C57BL/6 mice using standard methods. T cells (CD3), B cells (CD19), monocytes (CD14), and granulocytes (CD16) were isolated from splenocytes of C57BL/6 mice using CD-specific Dynabeads (CD19; Dynal Biotech, Great Neck, NY) or Abs (CD3, CD14, and CD16; BD PharMingen) followed by CD-specific DETACHaBEADS or Dynabeads M-450 sheep anti-mouse IgG (Dynal Biotech) in accordance with the manufacturer’s recommendation. Cells (1 × 10⁶/well) were cultured in the presence of either SF20 (starting at 40 ng/ml) or IL-2

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**A**

- **FIGURE 2.** Murine SF20/IL-25 cDNA.
  - a, The cDNA and predicted amino acid sequence of SF20 clone 6-13-19. Signal peptide cleavage is shown by the arrow occurring after Ala24.
  - b, Multiple sequence alignment of mouse and human SF20 proteins. The SwissProt/TrEMBL accession numbers are given after the species designation. Potential O-glycosylation sites (S, T) and predicted tyrosine kinase (R-x-x-E-x-x-Y) and protein kinase phosphorylation (S-x-R) sites are indicated.

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**B**

- mouse_{SF20}
- mouse_{BAA23155}
- human_{SF20}
- human_{O75526}
- human_{O75527}
- human_{Q9MF69}
- human_{AAM3639}
- mouse_{SF20}
- mouse_{BAA23155}
- human_{SF20}
- human_{O75526}
- human_{O75527}
- human_{Q9MF69}
- human_{AAM3639}
SF20 was identified based on its ability to support proliferation of a mutant clone, SB2-33, which is dependent on a stroma cell line, ST2, and which was established from chemically mutagenized Ba/F3 cells. To search for SF20, we screened an ST2 stroma cell cDNA library for clones that supported the proliferation of SB2-33 cells. During screening, cDNAs from ST2 stroma cell cDNA library were transduced into a nonsupportive stroma cell line, MS10, and clones that supported the proliferation of SB2-33 cells were selected. After three rounds of screening, we obtained one clone (6-13-19) that gave a phenotype identical to the phenotype observed from SB2-33 cells grown in ST2 stroma. The mutant SB2-33 cells attached and proliferated on the MS10 cell layer transduced with clone 6-13-19 (Fig. 1a) but not on cells transduced with empty vector (Fig. 1b) or on cells infected with a green fluorescent protein construct (Fig. 1c). Parental Ba/F3 cells did not proliferate on MS10 cells transduced with the positive clone (Fig. 1d), which suggests that stroma-dependent proliferation of SB2-33 cells was specific and due to clone 6-13-19.

The SF20 cDNA (clone 6-13-19) contains a single open reading frame of 166 amino acids including a predicted 24-aa signal peptide (Fig. 2a). The mature 142-aa protein does not contain N-glycosylation sites. An in-frame stop codon is located in the short 5’ untranslated region of the cDNA.

Standard protein-protein BLAST (Blastp) and position-specific iterated BLAST (PSI-BLAST) searches with SF20 against the “nr” database (all nonredundant GenBank coding sequence translations plus Brookhaven Protein Data Bank plus SWISS-PROT Protein Knowledgebase plus Georgetown Protein Information Resource plus Protein Research Foundation protein/peptide database) produced only high homology hits, with E-values ranging from 4e-47.
to 4e-77, to hypothetical proteins and fragments of human and mouse (Fig. 2b). No related proteins of lower homology have been found. Both proteins contain two cysteine residues and two potential O-glycosylation sites. The mature proteins encoded by human and mouse SF20 are 91.5% identical. Further homology and topology analyses using Predict Protein (17) revealed a 24-residue region (S-x(10)-S-x(4)-E-x(4)-[FY]-x-C) between the two Cys residues that is similar to framework 3 of IgV domain and conserved among Igκ and Igλ chains, LY-9 Ag. SF20 has been predicted as globular protein, with the region between the two Cys residues in β strand conformation.

The mouse SF20/IL-25 gene is located in the H2 complex region of mouse chromosome 17 (37.0 cM) between C3 (complement 3) and Ir5 (immune response gene 5) (18). Neighboring genes include Rip1, Lama1, Fem1aa, Ir5, Rfx2, and Khsrp. The human SF20 has been mapped to chromosome 19p13.3, which is a paralogous region of 9q34 and the HLA class III region on chromosome 6 (19). Neighboring genes of SF20 on human chromosome 19p13.3 are TIP47, FEM1A, ICBP90, HSPC142, and BST-2 (Ensembl Release 080, http://www.ensembl.org).

Gene expression of SF20/IL-25
RT-PCR analysis revealed that the mouse gene is strongly expressed in the testis, spleen, and heart with moderate expression in the lung and liver (Fig. 3a). No expression was detected in the skeletal muscle, brain, and kidney. In mouse stroma cell panel (Fig. 3b), the gene is expressed in MS5, ST2, and CF-1 cells but not in the other three cell lines (MS10, ST0, and PA6) tested.

SF20/IL-25 is a 20-kDa secreted growth factor
The presence of a signal peptide suggested that SF20 would be secreted when expressed in mammalian cells. Indeed, when the supernatant of COS7 cells transfected with FLAG-tagged SF20 expression construct was analyzed by immunoprecipitation and Western blotting using anti-FLAG Ab, it was found that SF20 was secreted (Fig. 4a, lane 1). The predicted mature protein encoded by SF20 consists of 142 amino acids with a calculated molecular size (Mr) of ~16 kDa. The FLAG-tagged protein in the Western blot was detected at the 20 kDa position, possibly due to the presence of O-linked glycosylation sites. Two potential O-linked glycosylation sites are predicted (Fig. 2b) (20). Recombinant SF20 purified using an anti-FLAG affinity column to homogeneity confirmed the position of this protein (Fig. 4a, lane 2).

Next, we examined the activity of the purified recombinant protein by culturing the mutant clone SB2-33 cells. When stimulated with SF20, SB2-33 cells proliferate in a dose-dependent manner except CF-1, which is spleen-derived stroma, all other stroma cell lines used were derived from bone marrow.

Expression of human SF20 on some normal and cancer human cell lines showed that the human gene is expressed in lung, breast, and colon carcinomas but not in their normal counterparts (Fig. 3c). Interestingly, in normal human blood cells, SF20 was detected in resting mononuclear cells and in resting CD8+ and CD19+ cells but not in resting CD4+ and CD14+ cells. In the activated cell panel, it is only expressed in activated CD8+ T cells (Fig. 3d).

Enforced expression of TSA-1-induced cell proliferation.

a, Expression of TSA-1 and SF20 in parent Ba/F3 cells and mutant clone SB2-33 cells. RT-PCR was performed using TSA-1 and SF20 specific primers as described in Materials and Methods.
b, Enforced expression of TSA-1 in Ba/F3 cells transduced with TSA-1 retrovirus expression construct. Stable transformants of Ba/F3 cells expressing TSA-1 were generated and total RNA was prepared and used for RT-PCR. c, Proliferative response of TSA-1-expressing Ba/F3 cells cultured in the presence of SF20 (○), IL-3 (□), and anti-TSA-1 mAb (10 µg/ml) then SF20 (●). The starting concentration of IL-3 and SF20 are 20 and 40 ng/ml, respectively. Cell proliferation was measured using a Cell Titer Proliferation Assay kit by Promega. Values are means of triplicates in three independent experiments.
similar to that of rIL-3 with half-maximal stimulation at 4 ng/ml (Fig. 4b).

Identification of mouse TSA-1

It is well established that growth factors elicit their biological effects by binding to receptors expressed on the surface of responsive cells. Thus, to determine the mechanism by which SF20 rendered SB2-33 cells to proliferate, we searched for the SF20 receptor. cDNA clones derived from SB2-33 cell cDNA library were transduced into parent Ba/F3 cells and the library-transduced Ba/F3 cells were cultured in SF20-expressing MS10 stroma. Finally, four clones were identified which when transduced into parent Ba/F3 cells rendered Ba/F3 cells to proliferate strongly on SF20-expressing MS10 stroma. The phenotype of Ba/F3 cells transduced with the positive clones is identical to the proliferative phenotype of SB2-33 cells grown in SF20-expressing stroma as shown in Fig. 1a. These four clones contained the same cDNA insert corresponding to mouse TSA-1.

TSA-1 is a known protein belonging to the Ly-6 family (1, 7). Members of this family share a Ly-6 Ag/urokinase-type plasminogen activator receptor-like domain (Protein Families Database of Alignments and Hidden Markov Models (HMMs) (Wellcome Trust Sanger Institute, Hinxton, U.K.) accession no. 00021); comprising 10 highly conserved cysteine residues important for forming the intrachain disulfide bonds, and an asparagine residue (Asn-79), which is the putative GPI linkage site. The Ly-6 Ag/uPAR-like domain is found in the Ly-6, glycolipid-anchored urokinase-type plasminogen activator receptor family, and the family of secreted, single-domain snake venom α-neurotoxins (21). Moreover, some other important membrane proteins, such as epidermal growth factor receptor, low-density lipoprotein receptor, and insulin receptor, share some homology within limited regions to TSA-1. The overall identity of TSA-1/uPAR-like domain members (CD59, CD87, LY-6E, LY-6A, LY-6C, LY-6D, LY-6A/2/LY-6E.1, LY-6F.1) is 18–27%. In the human genome, human TSA-1, also called RIG-E (14) and 9804 (15), shares 61% similarity. CD59, a GPI-anchored glycoprotein involved in host-mediated complement attack, shares 26% similarity to TSA-1 (22).

TSA-1 maps to mouse chromosome 15 (41.7 cM) (18). Alterations in this region have been associated with lymphoid malignancies. The protooncogenes, c-sis and c-myc, which are involved in cell growth, are also encoded in this region. The corresponding localization on human LY6E is in chromosome 8q24.3 (14). Neighboring genes of human LY6E on chromosome 8q24.3 include LY6H, GPAA1, GRINA, TNFRSF1IB, and RAD21 (Ensemble Release 080, http://www.ensemble.org/).

To confirm that mouse TSA-1 is the receptor of SF20, we generated stable transformants of Ba/F3 cells expressing TSA-1. Parent Ba/F3 cells do not express endogenous TSA-1 and SF20 (Fig. 5a). The expression of TSA-1 in TSA-1-transformed Ba/F3 cells was confirmed by RT-PCR (Fig. 5b). Ba/F3 cells expressing TSA-1 responded to SF20 (Fig. 5c,d) and proliferated in a dose-dependent manner similar to that of rIL-3 (Fig. 5c, □). When TSA-1-transformed Ba/F3 cells were cultured in the presence of

**FIGURE 6.** SF20 stimulation and binding assay. a, Expression of TSA-1 and SF20 in mouse factor-dependent cell lines. Total RNA was prepared from cells and used for RT-PCR analysis using TSA-1- and SF20-specific primers. b, Proliferative response of FDCP2, MC9, and CTLL-2 cells cultured in the presence of SF20. Cells (1 × 10^4/well) were grown in 96-well round-bottom plates containing two-fold serial dilution of mouse IL-3 and IL-2 (both starting at 20 ng/ml) and SF20 (starting at 40 ng/ml). To test the effect of anti-TSA-1 on SF20-induced proliferation of FDCP2 cells, FDCP2 cells were first treated with anti-TSA-1 mAb for 1 h before addition of SF20. Cells were grown for 72 h and cell proliferation was measured as described above. Values are means of triplicates in three independent experiments. c, Binding assay of SF20 to TSA-1. SF20 binds to TSA-1 in FDCP2 cells and in TSA-1-transformed Ba/F3 cells but not to MC9 and CTLL-2 cells. This binding was inhibited by anti-TSA-1. Control, cells plus secondary Ab (PE-conjugated anti-mouse IgG) only; SF20, 1 µg/ml; anti-TSA-1, 10 µg/ml. Control cells with no added Ab gave the same result as the control above (data not shown).
anti-TSA-1 mAb (Fig. 5c, ●), proliferation of TSA-1-transformed Ba/F3 cells upon SF20 stimulation was inhibited, indicating that the engagement of SF20 to TSA-1 was blocked with anti-TSA-1.

Next, we investigated whether SF20 could stimulate other factor-dependent hematopoietic cell lines. FDCP2 is a mouse factor-dependent hematopoietic cell line that requires IL-3 to proliferate. MC9 is a mouse IL-3-dependent mast cell line and CTLL-2 is a mouse IL-2-dependent T cell line. FDCP2 strongly expressed the endogenous TSA-1 receptor while MC9 and CTLL-2 did not express TSA-1 (Fig. 6a). All cell lines did not express endogenous SF20. When these cells were stimulated with SF20, only FDCP2 responded and proliferated (Fig. 6b). In the absence of TSA-1, MC9 and CTLL-2 could not be induced to proliferate. To confirm that the response of FDCP2 to SF20 was due to the expression of TSA-1, we challenged FDCP2 cells with anti-TSA-1 mAb and found that proliferation of FDCP2 cells in the presence of anti-TSA-1 was inhibited (Fig. 6b). This result confirmed that TSA-1 acts as a receptor of SF20 in inducing FDCP2 cells to proliferate.

**SF20/IL-25 binds to TSA-1 and induces cell proliferation**

To investigate the molecular basis of the proliferative effect of SF20 to TSA-1-expressing cells, we performed an assay to demonstrate that SF20 specifically binds to TSA-1 and this binding was inhibited by anti-TSA-1. Flow cytometric analyses (Fig. 6c) revealed that binding of SF20 was detected on FDCP2 cells that expressed the endogenous TSA-1, and on TSA-1-transformed Ba/F3 cells but not on MC9 and CTLL-2 cells that do not express endogenous TSA-1. When FDCP2 cells and TSA-1-transformed Ba/F3 cells were incubated in the presence of anti-TSA-1, binding of SF20 to TSA-1 was inhibited. Taken together, these results suggest that SF20 binds to TSA-1 and induces proliferation of FDCP2 cells and TSA-1-transformed Ba/F3 cells.

**SF20/IL-25 supports proliferation of mouse lymphoid cells**

To determine whether SF20 could induce proliferation of mouse hemopoietic cells in vitro, we first examined the proliferative activity of SF20 to freshly isolated bone marrow cells and splenocytes. Unfractionated bone marrow cells and splenocytes were cultured for 7 days in the presence of SF20, medium alone, or with IL-3 (bone marrow cells) and IL-2 (splenocytes). Indeed, SF20 could induce proliferation of bone marrow cells, mostly B lymphocytes as judged by anti-CD19 Ab staining (data not shown), comparable to that of IL-3 (Fig. 7a), and splenocytes comparable to that of IL-2 (Fig. 7b).

Next, we investigated the biological activity of SF20 to mouse lineage-specific cells. Expression analysis of TSA-1 in normal murine lineage-specific cells showed expression of the gene in T (CD3) and B cells (CD19) but not on monocytes (CD14) and granulocytes (CD16) (Fig. 8a). Binding of SF20 to TSA-1 was also detectable in T and B cells but not on monocytes and granulocytes (Fig. 8b). In addition, binding of SF20 to TSA-1 was also inhibited by anti-TSA-1. To examine whether SF20 could stimulate proliferation of TSA-1-expressing CD3 and CD19 cells, we cultured mouse CD3, CD19, CD14, and CD16 cells in the presence of SF20. SF20 induced a concentration-dependent proliferation of CD3 (T cells) and CD19 (B cells), similar to that of rIL-2 (Fig. 8c, right panels) but not of CD14 (monocytes) and CD16 (granulocytes) cells (left panel). Moreover, when CD3 and CD19 cells were challenged with anti-TSA-1 Ab, the proliferative effect of SF20 was abolished (right panels). Thus, this result suggests that SF20 was able to support proliferation of mouse lymphoid cells through TSA-1.

**Discussion**

In this report, we have identified SF20, a bone marrow stroma-derived factor, and the subsequent identity of its receptor called mouse TSA-1. SF20 has no distinct homology to...
known cytokines or growth factors. The growth-promoting activity of this protein to hemopoietic cells in the lymphoid lineage suggests that this protein plays an important role in lymphocyte activation and differentiation. Aside from bone marrow and spleen stroma cells, SF20 is expressed in mononuclear cells, resting CD8^{+} and CD19^{+} cells, and activated CD8^{+} cells. SF20 can be differentiated from other growth factors because of its receptor, TSA-1. Expression of TSA-1 is not confined to the T lineage, because B lineage cells in the bone marrow (B220^{+}) (1) and CD19^{+} cells are TSA-1 positive. It was reported that in T lineage cells, the expression of TSA-1 appears on a more pluripotent subset, including prothymocytes (23). As T cell development continues, TSA-1 expression is lost during entry into the CD4^{+}CD8^{+} (double positive) stage and reappears at the end stage of T cell development. Thus, TSA-1 may be functionally important in thymocyte maturation.

Proliferation assay of mouse lineage-specific cells revealed that SF20 supports proliferation of CD3 and CD19 cells but had no observed myelopoietic activity. Binding of SF20 to TSA-1 in TSA-1-expressing FDCP2 cells and CD3 and CD19 cells has been demonstrated, and this binding was inhibited by anti-TSA-1 Ab. In addition, SF20-induced proliferation of FDCP2 cells and mouse CD3 and CD19 cells was also inhibited by anti-TSA-1, indicating that TSA-1 is a putative receptor of SF20.

SF20 may interact with TSA-1 through IgV framework 3-like domain. IgV-like domains are known to facilitate protein-protein interactions. The regulatory mechanism by which SF20 promotes cell proliferation upon binding to TSA-1 is unknown. TSA-1 is a GPI-anchored protein and lacks transmembrane regions for signaling. In contrast to the TCR/CD3 complex, which signals through the plasma membrane via coupling to membrane-associated G proteins and possibly ion channels that induce the production of several intracellular second messengers (24), these molecules are anchored on the outer side of the plasma membrane and are spatially distant from cytoplasmic/intramembranous proteins. Several murine GPI-anchored proteins such as CD48 (25), TAP (26), and a number of human GPI-anchored proteins, such as CD55 (27) and CD59 (22), have been implicated in T cell activation. The finding...
that a large number of GPI-anchored proteins stimulate cells when cross-linked has led to the suggestion that the GPI-anchored molecules must be in an active conformation to initiate intracellular signaling, and that the GPI moiety might be directly involved in cellular activation (3, 4, 27–29). It has been suggested that GPI-anchored proteins transmit signals to the cell interior by interacting with non-receptor-type tyrosine kinases, p56lck and p59fyn (30–32). This interaction could be mediated by an as-yet-undefined membrane protein, which could serve as a linker between these two proteins.

We have noted that TSA-1 is strongly expressed in malignant cells. Expression analysis of SF20 mRNA in normal and cancer cell lines revealed that SF20 is expressed in lung, breast, and colon cancer cell lines, but not in their normal counterparts. Thus, we propose that SF20 may function as an autocrine factor for proliferation of malignant cells.

The bone marrow stromal Ag 2 (BST2) that is implicated in pre-B cell development and expressed on bone marrow stromal line cells and thymoma lymphoid synovial cell lines (33) is located ~300 kb 3′ of SF20. The proximity of two cytokines involved in lymphocyte development may suggest long-distance transcriptional coregulation (34). Given the location of both SF20 and BST2, we predict that more genes with yet unknown functions on the MHC paralogous region will be cytokines involved in stromal thymic cross-talking and lymphocyte development.

Finally, the identification of SF20/IL-25 as a ligand of TSA-1 has added more insights into the functional role of TSA-1 in lymphocyte activation. In T cell, besides the major TCR-CD3 activation pathway, several lines of evidence indicate that T cell activation can also be achieved independently through the interaction of multiple adhesion and activating molecules, such as CD28/CTLA-4, CD152/ICOS, and CD40/CD40L. Thus, SF20/IL-25 may develop a physiological role of CD28/CTLA-4 in the differentiation of T cell in the thymus and T cell in the periphery. The biological activity of SF20/IL-25 in the stimulation of CD3 and CD19 cells indicates that the potential role of SF20/IL-25 in lymphocyte development. As such, the SF20/IL-25 receptor-TSA-1 may find medical application in the treatment of lymphocyte disorders associated with autoimmune diseases, lymphoplasia, or immunodeficiency syndromes.

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Letter of Retraction


In the article above, we isolated a novel secreted bone marrow stroma-derived growth factor, SF20/IL-25, which supports lymphoid cell proliferation via mouse thymic shared Ag-1. In subsequent work, we were unable to reproduce our published findings reported in Figs. 4B, 5C, 6B, and 8C of the article. At this point, we are unable to explain why these prior experiments were flawed, but some minor contaminant in the purified SF20 in the original experiments could have brought the inconsistency. Since the original data is indispensable for demonstration of the physiological role of SF20, the published findings are unsound. Therefore, we would like to inform the scientific community of this error.

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