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Developmental Abnormalities in Mice Transgenic for Bovine Oncostatin M

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Oncostatin M belongs to the subfamily of hematopoietin cytokines that binds a receptor complex containing gp130. To date, only the human form of oncostatin M has been identified, and its evolutionary conservation is unresolved. We have isolated a bovine gene whose open reading frame encodes a precursor protein that is 58% identical to human oncostatin M. A comparison of the bovine and human amino acid sequences predicts significant similarity, including the four-α-helical-bundle structure and the placement of disulfide bridges. As with the human protein, bovine oncostatin M binds specific receptors on human H2981 cells and inhibits the proliferation of human A375 tumor cells and mouse M1 leukemia cells. To identify activities regulated in vivo, we injected bovine oncostatin M fusion genes containing various tissue-specific promoters into mouse embryos. The frequencies of transgenic mice were reduced significantly, suggesting that overexpression of the bovine cytokine is detrimental to normal mouse development. In addition to deaths associated with expression in neurons and keratinized epithelia, bovine oncostatin M caused abnormalities in bone growth and spermatogenesis, stimulated fibrosis surrounding islets in the pancreas, and disrupted normal lymphoid tissue development. This work establishes the existence of a nonprimate oncostatin M gene and provides the first demonstration that this cytokine can function in a pleiotropic manner in vivo. Information regarding bovine oncostatin M may help characterize the structure and function of this cytokine in other vertebrate species.

Oncostatin M (OSM) was originally purified as a human protein that could inhibit the proliferation of cells derived from solid tumors (49). Further experimentation suggested that OSM behaved as a cytokine because it is produced by hematopoietic cells and regulates a variety of processes, including the expression of lymphokines such as interleukin-6 (IL-6), granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor (7, 8). Examination of the human OSM (hOSM) protein sequence led to its classification within the hematopoietin superfamily of cytokines and suggested that OSM shares a close relationship with leukemia inhibitory factor (LIF) (4, 10, 39). hOSM and LIF appear to have evolved from a common ancestral gene. Both proteins adopt a four-α-helical-bundle structure, in part as a result of the arrangement of apolar residues within the amphipathic helices, as well as the location of intramolecular disulfide bonds. The primary amino acid sequences are approximately 30% identical and show similarities in regions thought to be important for receptor binding. The genes encoding these cytokines have two introns placed at identical locations within the open reading frames. Moreover, the OSM and LIF genes are tightly linked and appear within 20 kb of each other on human chromosome 22 (23, 40).

One hallmark of OSM is that it binds a receptor complex that contains gp130, a signal-transducing subunit present in the receptors for LIF, IL-11, IL-6, and ciliary neurotropic factor (CNTF) (25, 50). The receptors for these cytokines contain a specific ligand-binding subunit (α receptor) but share common β receptor components. The α receptors for LIF (LIFRα) (20) and IL-11 (21) are transmembrane proteins that are structurally related to their β subunit, gp130. The α receptors for IL-6 (1) and CNTF (15) are cell surface proteins. The β subunit for the IL-6 receptor is a gp130 homodimer, and the β receptor for CNTF is a heterodimer of gp130 and LIFRα. LIFRα functions here as an affinity-enhancing subunit for CNTF, not a ligand-binding protein, and in this context is referred to as LIFRβ (14). Surprisingly, the α receptor for OSM is gp130 itself (25, 44). The direct binding of OSM with gp130 is insufficient for signal transduction, and a β subunit is also required. Interestingly, two different receptor complexes appear to mediate OSM activity; one of these involves gp130 and LIFRβ (19). OSM binds to the gp130-LIFRβ complex with a lower affinity than it binds to LIF, but it is still functional as judged by its induction of mouse M1 cell differentiation (9). The other OSM receptor complex, not recognized by LIF, contains gp130 and a newly discovered affinity-enhancing subunit, OSMRβ (32a). The two classes of OSM receptor have been measured on a variety of human cell lines (47). A375 melanoma and H2981 lung carcinoma cells are two lines that bind OSM with high affinity but lack LIF receptors (19). OSM effectively prevents the replication of these tumor cells.

Although the redundancy in cellular functions controlled by this subfamily of hematopoietin cytokines can now be explained by ligand structure and shared receptor subunits, the role that OSM plays in regulating development and homeostasis remains unclear. Disruption of the mouse LIF gene has helped describe its involvement in embryo implantation (45), regulation of hematopoietic stem cells (17), and activation of neuropeptide gene expression (36). The mouse OSM gene, however, has not been reported, and the OSM-specific receptor has not been demonstrated in mice. Because the evolutionary conservation of OSM remains uncertain, we set out to identify OSM-related genes from other species. This report describes the cloning of a gene that we have identified as bovine OSM (bOSM). The protein encoded by this gene is 58% identical to hOSM and shares many of the same features as the human protein. Experiments suggest that bOSM protein

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produced in transfected COS cells can bind both classes of OSM receptor and stimulate responses in vitro. Using tissue-specific promoters to direct transgene expression in mice, we learned that bOSM has a profound and often lethal effect on mouse development and stimulates phenotypes associated with gp130-based receptors.

MATERIALS AND METHODS

Cloning and expression of bOSM *in vitro*. We screened 10^5 plaques of a bovine genomic library (Stratagene) with the ^{32}P-labelled *Bgl*II-*Xho*I fragment of hOSM cDNA (29). Nitrocellulose filters were hybridized overnight at 42°C in hybridization buffer containing 50% formamide and washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) at 65°C and then in 0.5× SSC–0.1% SDS at 65°C. Purified λ phage DNA was subjected to Southern hybridization analysis, and a 4.7-kb *Eco*RI fragment was subcloned into Bluescript II SK$^+$ (Stratagene). The open reading frame and exon/intron boundaries of the bOSM gene were sequenced from both strands by the dideoxy-chain termination method. The bOSM gene was expressed in cells by using a 3.2-kb *Sst*I-*Eag*I fragment. This DNA begins 12 bp upstream of the initiator Met and continues 340 bp past the termination codon. An AT-rich sequence thought to mediate rapid turnover of various cytokine mRNAs (41) lies downstream of the distal *Eag*I site and was not included in this genomic fragment. The 3.2-kb bOSM DNA was treated with Klenow fragment and cloned by blunt-end ligation into pπH3 (2), which contains the cytomegalovirus promoter and simian virus 40 origin of replication. Cloning of the human OSM gene in pπH3 and its expression in COS cells have been described previously (29). COS cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and transfected with DEAE-dextran sulfate. After 48 h, cultures were fed serum-free medium, and this conditioned medium was collected 24 h later. For Western immunoblot analysis, conditioned medium was dialyzed against 1 M acetic acid and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto nitrocellulose and incubated with rabbit antisera raised against a bOSM peptide (RTAGQVLRGWGERQGRSRRC). The bOSM-antibody complex was detected with alkaline phosphatase-conjugated protein A.

OSM receptor-binding assay. A 7-ml portion of conditioned medium containing recombinant bOSM (equivalent to 630 ng of hOSM as determined by growth inhibition assays) was lyophilized and resuspended in 1 ml of binding buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.4], 5% bovine serum albumin BSA, and 0.2% sodium azide in RPMI 1640) that included 500 pM [^{125}I]hOSM. This cocktail was diluted in 2-fold increments with binding buffer containing [^{125}I]hOSM to a final dilution of 128-fold. H2981 lung carcinoma cells (22) were cultured (75,000 cells per well) in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum for 24 h. The cells were washed once with binding buffer and then incubated in diluted conditioned medium (200 μl) for 1.5 h at 20°C on a rotary shaker. The cells were washed three times with 500 μl of binding buffer, solubilized in 200 μl of 1 M NaOH at 60°C for 30 min, and then assayed for bound radioactivity with a Packard Cobra Autogamma counter. Each datum point represents the average and standard deviation from triplicate determinations.

Growth inhibition assays. Recombinant bOSM was assayed by previously described methods for its ability to inhibit the proliferation of A375 melanoma cells (28, 39). Briefly, 3.5×10^5 cells were seeded per well in a 96-well microtiter plate containing Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. After 2 h, the medium was replaced with 200 μl containing dilutions of conditioned medium with bOSM protein. At 72 h after plating, cultures were pulsed for 8 h with 100 μl of fresh medium containing ^{125}I-deoxyuridine (0.1 μCi). Cells were then lysed with 1 M NaOH, and the amount of radioisotope incorporated into DNA was determined. Mouse M1 myeloid leukemia cells (2.5×10^4 per well) were incubated for 72 h in RPMI 1640 medium containing 10% fetal bovine serum and in various dilutions of conditioned medium containing bOSM protein. The cells were pulsed for 6 h with 50 μl of medium containing 0.1 μCi of [^{3}H]thymidine and were then harvested, and the counts per minute incorporated into DNA was determined. Each datum point represents the mean of triplicate determinations.

Transgenic mouse analysis. All transgenes contained the 3.2-kb bOSM genomic fragment (see above). Expression vectors and their regulatory sequences were as follows: MtbOSM, which contained the 0.7-kb mouse metallothionein promoter and the 3′ polyadenylation signal sequence (0.63 bp) from the human growth hormone gene (27); RIβbOSM, a 3.5-kb promoter fragment of the RIβ subunit of cyclic AMP (cAMP)-dependent protein kinase (38) and the 3′ intron and polyadenylation signal sequence (0.5 kb) from the mouse protamine-1 (mP1) gene (33); LckbOSM, the 3.1-kb proximal promoter of the *lck* gene (13) and the 0.5-kb mP1 DNA; K14-bOSM, 2.7 kb of the mouse keratin-14 gene promoter (48) and the 0.63-kb human growth hormone DNA; and InsbOSM, the 0.6-kb promoter and noncoding exon 1 of the rat insulin-1 gene (26) and the 0.63-kb human growth hormone DNA. Transgenes were separated from plasmid sequences following restriction enzyme digestion, agarose gel electrophoresis, and elution into TAE (0.04 M Tris-acetate [pH 8.4], 0.001 M EDTA) buffer. The DNA was resuspended (10 μg/ml) in low TE (10 mM Tris [pH 7.5], 0.25 mM EDTA) and filtered through a prerinsed 0.22 μm-pore-size acrodisk (Gelman Sciences). Fertilized oocytes from (C3H × C57BL/6)F_1 mice were used for pronuclear injections. Embryos were cultured overnight and then transferred into the oviducts of pseudopregnant C57BL/6 females. Transgenic mice were identified following hybridization of tail DNA with radiolabeled DNA probes. Tissues prepared for necropsy were fixed in 4% paraformaldehyde and embedded in paraffin. Sectioned material was stained with hematoxylin and eosin and then analyzed microscopically.

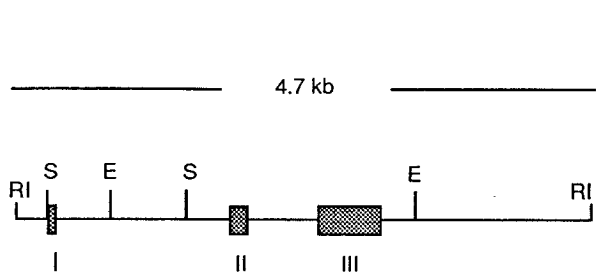
RESULTS

Structure of a bOSM gene and its encoded protein. A lambda phage library containing bovine genomic DNA was screened with the hOSM cDNA under low-stringency conditions. One positive clone was analyzed by Southern hybridization and subjected to DNA sequence analysis. Figure 1 shows a partial restriction map of a 4.7-kb *Eco*RI fragment encoding a protein related to hOSM. The open reading frame for this gene is contained on three exons and is 73% identical to that of hOSM. Introns I and II are 1,418 and 572 nucleotides in length, respectively.

Figure 2 presents a model of the hOSM protein and the alignment of the bOSM amino acid sequence with the human and simian proteins (10). The exon/intron borders dividing the open reading frames of all three genes are identical. The bovine and human precursor proteins are 58% identical. Significant similarities can be observed in various regions, including the signal peptide and the carboxyl extension which is removed from hOSM by proteolysis (24). The hOSM protein is thought to contain four amphipathic helices with a repeated pattern of apolar residues occurring in the *i* and *i*+3 positions of a 7-residue heptad (4, 39). Many of the apolar residues within the four helices of bOSM are identical with those in hOSM, and many of the substitutions represent conservative changes. The four cysteine residues involved in disulfide bonding are also identical, and two regions thought to be important for receptor binding, the AB loop and the D1 region, are strongly conserved. One notable exception in the D1 region is a G-to-N change. This G residue is prominent in helical turns and is conserved in LIF, CNTF, and granulocyte colony-stimulating factor (4). Another change that potentially alters the helical bundle relationship in the bovine protein is a 14-amino-acid deletion that includes the loop region following helix B and the beginning of helix C. This divergence in sequence may not be critical for function, since helices B and C are thought to lie opposite the receptor-binding domain.

bOSM is similar to hOSM in binding cell receptors and regulating cell proliferation *in vitro*. To determine whether the bOSM gene encodes a biologically active protein, we tested its ability to bind receptor and function on cultured cells. A 3.2-kb portion of the bOSM gene with its open reading frame and two introns was inserted into pπH3 (2), an expression plasmid containing the cytomegalovirus promoter and the simian virus 40 origin of replication. COS cells were transfected and incubated in serum-free medium. Supernatants were then assayed for bOSM protein by Western blot analysis with a rabbit antiserum raised against a bOSM peptide. A protein with the expected size of about 30 kDa was detected in bOSM-transfected cells but not in mock-transfected cultures (data not shown).

To test whether bOSM competes with hOSM for receptor binding, we incubated human H2981 lung carcinoma cells with [^{125}I]hOSM and various dilutions of protein prepared from either bOSM-transfected or mock-transfected COS cell supernatants. Ninety minutes later cultures were rinsed, lysed, and measured for [^{125}I]hOSM binding. As shown in Fig. 3, medium containing the bOSM protein prevented 85% of hOSM from



B

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                                                                  Sst I
                                                          ....aggccggagctcggtcacccagc

ATG GGG GCA CAG CGT ATG CAG AGA ACA CTG CTC A-- gtaagtactt....cgtccccag -GT
 M   G   A   Q   R   M   Q   R   T   L   L   S       Intron I (1418 bp)

CTG GTC CTC AGA CTT CTG CTC CTG TGC ACA GTG GCC ACG GGC AAG TGC TCG GGC AAG
 L   V   L   R   L   L   L   L   C   T   V   A   T   G   K   C   S   G   K

TAC CAC GAG CTT CTT CTG CAG CTC CAG CGC CAG GCG GAC CTC ATG CAG GAC CCC AGC
 Y   H   E   L   L   L   Q   L   Q   R   Q   A   D   L   M   Q   D   P   S

ACG CTC CTG GAT CCC TAT gtgagtgcc....ctctccccag ATC CAC CTC CAA GGC CTA CAC
 T   L   L   D   P   Y       Intron II (572 bp)         I   H   L   Q   G   L   H

AGT CCC GTA CTG CAG GAA CAC TGC AGG GAG CGC CCG GGG GAC TTT CCC AGC GAG GAT
 S   P   V   L   Q   E   H   C   R   E   R   P   G   D   F   P   S   E   D

GCC CTG TGG AGG CTC AGC AGG CAG GAC TTC CTG CAG ACC CTC AAC ACC ACA CTG GGC
 A   L   W   R   L   S   R   Q   D   F   L   Q   T   L   N   T   T   L   G

CTC ATC CTT CGC ATG CTG AGT GCC CTG CAG CAG GAC CTC CCG GAA GCA GCC CAC CAA
 L   I   L   R   M   L   S   A   L   Q   Q   D   L   P   E   A   A   H   Q

CAG GCG GAG ATG AAT GTC CGC GGG TTC GGG AAC AAC ATC CAC TGC ATG GCC CAG CTG
 Q   A   E   M   N   V   R   G   F   G   N   N   I   H   C   M   A   Q   L

CTG CGT GGC TCC TCG GAC CCA AAG GCT GCT GAA CCC ACC CAG CCG GGC CCA GGG CCC
 L   R   G   S   S   D   P   K   A   A   E   P   T   Q   P   G   P   G   P

ACC CCG CTG CCA CCC ACC CCG CCT TCC AGT ACC TTC CAG CGC AAG CTC AGG AAC TGT
 T   P   L   P   P   T   P   P   S   S   T   F   Q   R   K   L   R   N   C

GGG TTC CTG CGA GGC TAC CAC CGC TTC ATG CGC ACA GCC GGG CAG GTC CTC CGG GGC
 G   F   L   R   G   Y   H   R   F   M   R   T   A   G   Q   V   L   R   G

TGG GGG GAG AGA CAG GGC CGC AGC CGC AGA CAC AGC CCC TGC CGG GCC CTG AAG AGG
 W   G   E   R   Q   G   R   S   R   R   H   S   P   C   R   A   L   K   R

GGG GCC CGC AGG ACA CAG CCC TTC CCG GAG ATC AGG AGA CTC GCG CCC AGG GGC CAG
 G   A   R   R   T   Q   P   F   P   E   I   R   R   L   A   P   R   G   Q

CCG CCC GGT AGC CTT TGG GGT GCC CCT GCC AGG TGA aaggtcagcaggtggcatgcaggacgg
 P   P   G   S   L   W   G   A   P   A   R   *

caggccgctgccacccatctcctctctgctccccacatctccgaagcgcactttaatgggtgtgagccgcgcagc

cccctctagctcccccaaggctgggctgcaggggtatggtcaggctgtggagtgctcccagccctgggttcctcg

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FIG. 1. bOSM gene. (A) Partial restriction endonuclease map of bovine genomic DNA (4.7 kb). RI, *Eco*RI; S, *Sst*I; E, *Eag*I. (B) Open reading frame of the bOSM gene. This DNA sequence includes the exon/intron borders and the flanking regions that are included in the bOSM expression vectors.

binding to cells, a level similar to that obtained with excess unlabeled hOSM (25). Supernatants from control COS cells had little effect on hOSM binding. This result suggests that bOSM can recognize the OSM-specific receptor on human cells, since H2981 cells do not express LIFRβ or bind LIF (19).

A375 cells, derived from a human melanoma, become round and stop growing in the presence of nanomolar levels of hOSM (22). This inhibition occurs through the OSM-specific receptor (19). Mouse M1 leukemia cells also stop dividing in the presence of hOSM and differentiate into a quiescent macrophage-like cell. This effect occurs through the receptor complex that contains gp130 and LIFRβ (9). To test the functional activity of bOSM on these two cell types, supernatants from bOSM-transfected COS cells and control transfected cells were di-

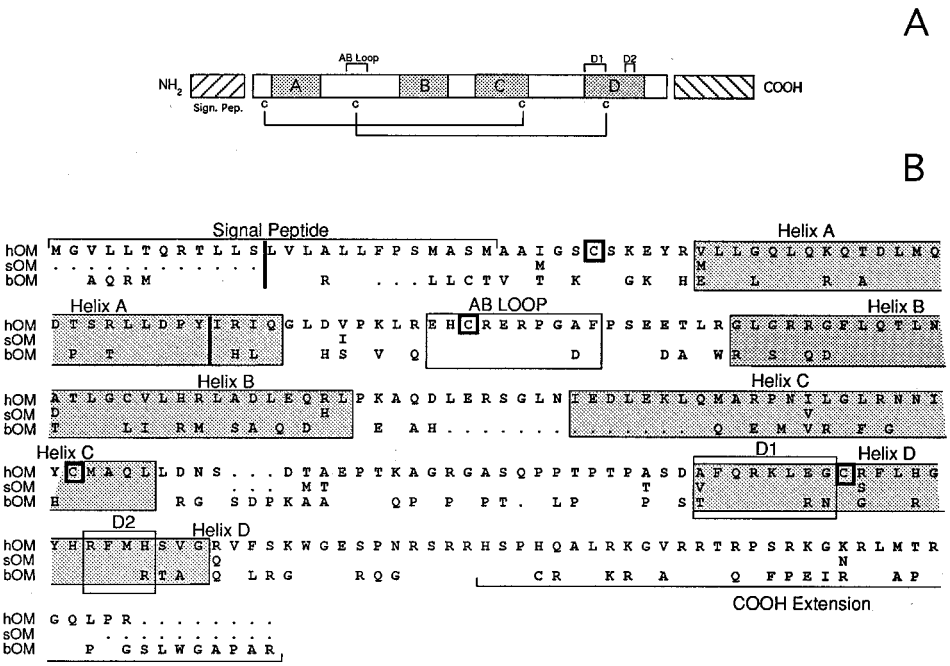

FIG. 2. (A) Structural domains of hOSM protein. Shaded areas indicate the locations of the four amphipathic helices. The hatched regions represent portions of the precursor protein that are processed by proteolysis. The cysteine residues and intramolecular disulfide bonds are indicated. The AB loop and the D1 and D2 regions have been implicated in receptor binding (4). (B) Best-fit alignment of the hOSM (29), simian OSM (10), and bOSM precursor proteins. Differences in amino acid sequence relative to the human sequence are indicated. Deletions in primary sequence are indicated by a dot. The two heavy lines indicate the location of the intron/exon boundaries in the human, simian, and bovine genes. Cysteine residues involved in disulfide bonding are shown in black boxes.

luted in culture medium containing 10% serum. A375 cells (Fig. 4) and M1 cells (Fig. 5) were incubated in the presence of various dilutions for 3 days. As indicated, DNA synthesis was inhibited in both cell lines in a dose-dependent manner. Control experiments with dilutions of supernatants from mock-transfected cells had no effect on cell proliferation. These results, in combination with the receptor competition assay described above, strongly suggest that the gene isolated from the bovine genomic DNA library encodes a biologically active protein that can interact with both mouse and human gp130-based receptors.

Targeted expression of bOSM in transgenic mice. hOSM regulates a range of biological responses in cultured cells, but very little is known about its effects in vivo. Accordingly, a series of experiments designed to target bOSM expression in transgenic mice was undertaken. Table 1 lists the various tissue-specific promoters used to regulate the bOSM gene and the frequency of transgenic mice obtained with these and other nonrelated transgenes. The initial test for identifying organ systems that may respond to bOSM utilized the metallothionein promoter, which directs transcription in numerous tissues (6). The frequency of bOSM transgenic mice obtained with this promoter was significantly reduced in comparison with the frequency obtained when using the osteoglycin (5) or epithelin (35) genes, suggesting that expression of bOSM in multiple organs is deleterious during mouse development. This argument is strengthed by the phenotypes displayed by the lone transgenic animal (TG 943) that was mosaic for MtbOSM; the transgene was intact as determined by Southern analysis, but the mouse contained less than one copy of the transgene per cell (data not shown). This mouse displayed a chronic tremor and had grossly enlarged hind limbs. Efforts to breed this male animal failed. Following necropsy, it was determined that spermatogenesis was defective in this mouse; no mature sperm were detected in the ducts of the epididymus, nor were developing spermatocytes apparent in the seminiferous tubules. Spermatogonia which line the periphery of the tubule were absent (data not shown). The enlarged hind legs of TG 943 resulted from excessive growth of bone in the femur and phalanges. As shown in Fig. 6 the marrow cavities were filled with immature bone and new periosteal bone deposition. Closer examination revealed that the growth plate of the femur was disrupted and disorganized. The haversian system was present but minimal, and very little reorganization, if any, occurred within this new bone.

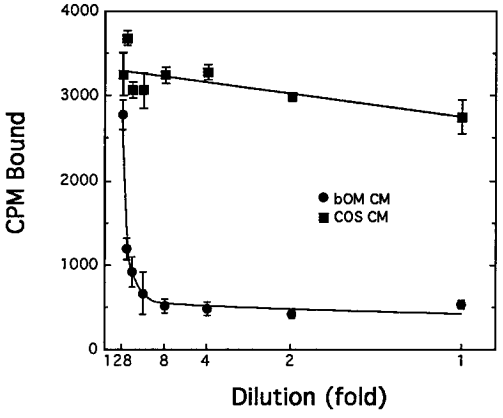

FIG. 3. Inhibition of [^{125}I]hOSM binding to H2981 lung carcinoma cells by bOSM protein. Conditioned medium from COS cells transfected with a bOSM expression vector was serially diluted in binding buffer and incubated with cells for 1.5 h at 20°C. The cells were then rinsed and assayed for [^{125}I]hOSM binding (see Materials and Methods).

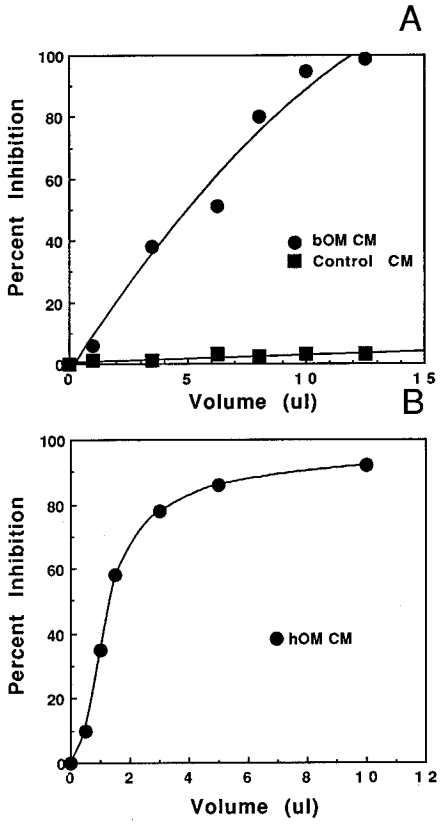

FIG. 4. Inhibition of human A375 melanoma cell proliferation by bOSM. (A) The indicated volumes of conditioned medium (CM) from COS cells transfected with either pπH3bOSM or pπH3M were diluted to a final volume of 200 μl with fresh culture medium and incubated with cells for 72 h. Cultures were pulsed with ^{125}I-deoxyuridine for 8 h and then assayed for radionucleotide incorporation into DNA. (B) A similar experiment performed with conditioned medium isolated from COS cells transfected with the hOSM plasmid pπH3hOSM (29).

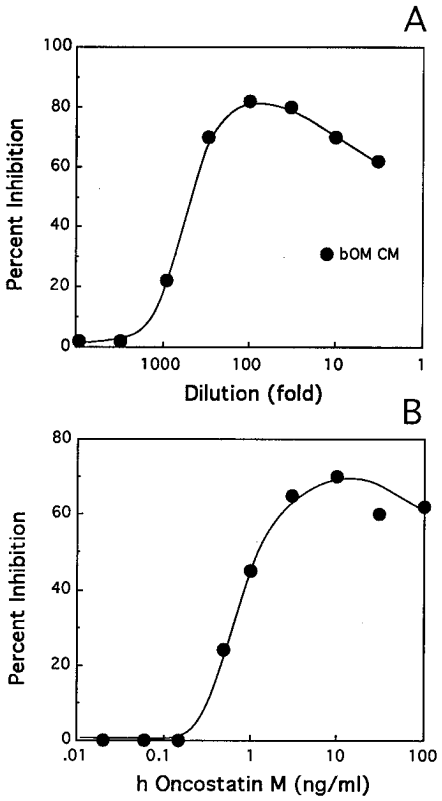

FIG. 5. Inhibition of mouse M1 leukemia cell proliferation by bOSM. (A) The indicated volumes of conditioned medium (CM) from bOSM-transfected COS cells were diluted to a final volume of 200 μl with fresh culture medium and incubated with cells in microtiter plates. After 72 h, cultures were pulsed with [^{3}H]thymidine for 6 h and then assayed for radionucleotide incorporation into DNA. (B) Replicate conditions with pure recombinant hOSM protein.

Because OSM is a potent mitogen for AIDS-related Kaposi's sarcoma cells (32), we tried, using the keratin-14 (K14) promoter, to derive a mouse that expressed bOSM in the basal layer of stratified epithelium (48). The frequency of transgenic mice obtained with this promoter and an unrelated gene, βH3 (43), was nearly 20%. As a result of severe keratinosis, the frequency of transgenic mice expressing mouse amphiregulin, a ligand for the epidermal growth factor receptor (34), was reduced to approximately 10% (33a). However, no transgenic animals were recovered when K14-bOSM was used, suggesting that expression of bOSM within developing skin is lethal.

As with LIF and CNTF, OSM induces neuropeptide gene expression in neuroblastoma cells (37). To test the potential effects of bOSM on neurons in vivo, we used the promoter for the RIβ subunit gene of cAMP-dependent protein kinase, which directs neuron-specific gene expression within the brain, spinal cord, and peripheral ganglia (38). The transgenic bOSM frequency (7.3%) was approximately three times lower than normal. Nearly 50% of the RIβbOSM animals (7 of 16) died at birth. Five animals, all runted in comparison with littermates, experienced severe tremors with ataxia. These animals displayed a progressive weakness, initiating with the hind limbs, and died within 6 to 8 weeks. The remaining four animals showed no phenotype and no transgene expression. These results suggest that expression of bOSM in neurons was detrimental, but the inability to establish a viable mouse line with this promoter precluded the analysis of bOSM action in nerve.

The frequency of bOSM transgenic mice obtained with the rat insulin-1 promoter (26) was also reduced significantly. A line of animals was established with this construct that showed consistent bOSM mRNA expression in pancreatic islet cells (data not shown). These mice demonstrated massive stromal cell proliferation around islets and blood vessels (Fig. 7). This fibrosis was accompanied by acinar atrophy and edema. Most islets appeared normal within the stroma, although some were surrounded by large cuffs of lymphocytes. Hyperglycemia was not observed in these mice, suggesting that islet function was not compromised. Possibly because of an accumulation of bOSM in serum, we found that the number of megakaryocytes in the marrow cavities in numerous InsbOSM animals was increased more than 10-fold (Fig. 8). This result is consistent with the finding that OSM stimulates megakaryocyte colony formation in vitro and platelet production in vivo (48a).

Finally, we tested whether bOSM could influence T-cell development by targeting its expression to the thymus by using the proximal *lck* promoter (13). Animals within the LckbOSM lineage acquired a lethal autoimmune phenotype and significant changes in lymphoid tissue development (Fig. 9). For instance, the thymus architecture was disrupted and the demarcation of cortical and medullary regions was lost. The major cell type normally found within the cortex, $CD4^+$ $CD8^+$ thymocytes, was dramatically underrepresented, and these regions were supplanted with follicles containing B lymphocytes. In addition to the presence of splenomegaly, the nodes became enlarged because of a lymphoproliferation of mostly $CD4^+$

TABLE 1. Targeted expression of bOSM in transgenic mice

Promoter	Tissue	Gene	No. of pups born	No. of transgenic pups	% of transgenic pups
Mt-1	Ubiquitous	Osteoglycin	51	12	23.5
		Epithelin	41	8	18.1
		bOSM	116	1	0.8
Keratin-14	Basal-cell	H3	93	13	17.3
	Epithelia	Amphiregulin	75	9	9.6
		bOSM	136	0	0
RIβ	Neurons	RIβ-LacZ	50	10	20.0
		bOSM	219	16	7.3
Insulin-1	β cells	NGF[a]	47	8	17.0
		bOSM	73	3	4.1
Lck	Thymocytes	bOSM	55	2	3.6

[a] NGF, nerve growth factor.

$CD8^+$ "thymocytes." Thus, the normal separation of primary (thymus) and secondary (node) lymphoid tissues is lost in these animals, and this may contribute to the autoimmune phenotype.

DISCUSSION

We have described the isolation of a gene that encodes a bOSM protein. This gene shares many features with the hOSM and simian OSM genes, including the relative lengths of the three exons and two introns and the locations of the exon/intron boundaries within the open reading frame. The coding regions of the bovine and human genes are 73% identical, and some sequence similarity within the introns of these genes has been observed. Further evidence that the bOSM gene is the human counterpart and is not a member of a small gene family is provided by Southern blot analysis, in which a single band was detected following hybridization of [^{32}P]bOSM probe with bovine DNA (data not shown).

The primary amino acid sequence of the bovine precursor protein is 58% identical to that of hOSM. A comparison suggests that the two precursor proteins share similar signal peptides, as well as a carboxyl extension that in hOSM is subject to proteolysis (24). As with the human protein, bOSM is thought to contain four amphipathic helices and a pattern of apolar residues arranged in a 7-residue heptad. Many of the substitutions observed within the helices of these two proteins rep-

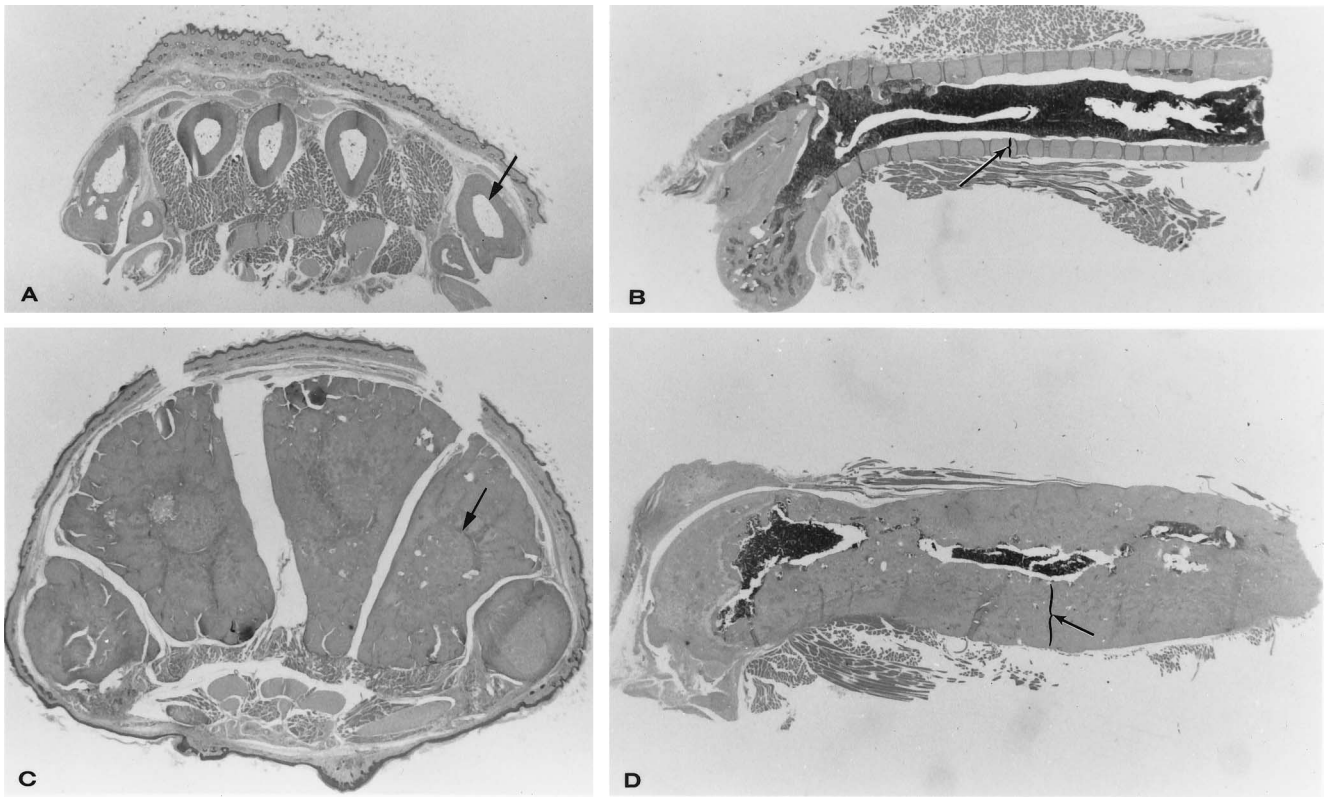

FIG. 6. Excessive bone growth in a MtbOSM transgenic mouse. (A and B) Cross section through the hind foot and femur of a normal mouse. The arrows point to the central space in a metatarsal bone (A) and the compact bone layer in the femur (B). (C and D) Comparable regions of a bOSM transgenic mouse have filled in with new bone and have increased in overall bone size.

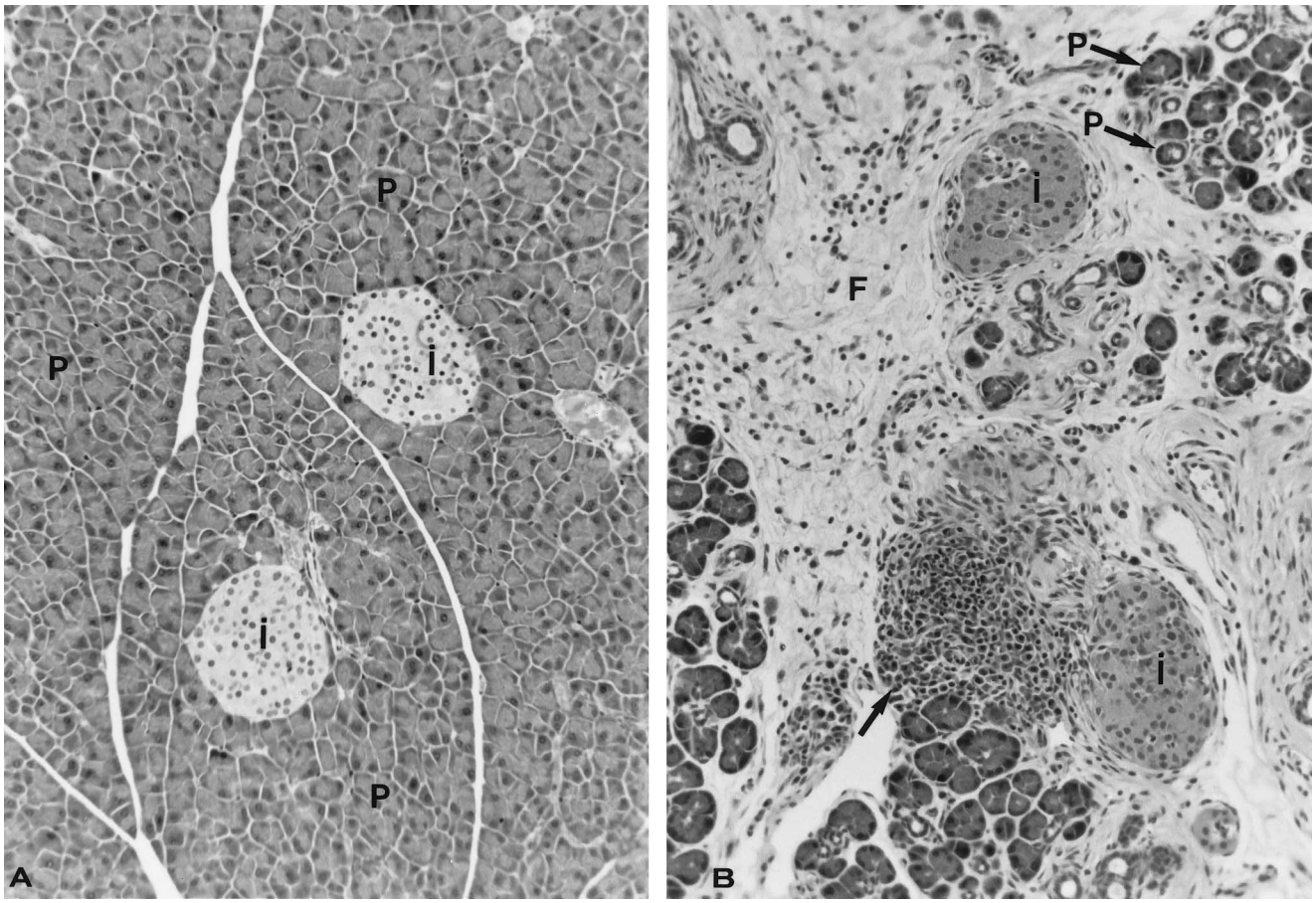

FIG. 7. Pancreas of an InsbOSM transgenic mouse. (A) Pancreatic acinar cells (P) and islets (i) of a normal mouse. (B) The islets of InsbOSM mice become encased in large regions of fibrosis (F), marked by the accumulation of fibroblasts and collagen. Infiltration of leukocytes (arrows) is also common.

resent conservative changes, and the cysteine residues involved in disulfide bonding appear to be identical. One striking difference between the human and bovine proteins is the 14-amino-acid deletion between helices B and C of the bovine protein. Relative to hOSM protein, this modification might introduce considerable change in the alignment of the two helices that are thought to face away from the receptor. Potential differences in shape, however, do not preclude binding of the bOSM protein to the receptors containing mouse gp130 and LIFRβ or human gp130 and presumably OSMRβ, nor do they prevent cellular responses in vitro. For instance, conditioned medium from COS cells transfected with a bOSM expression vector inhibited [^{125}I]hOSM binding to H2981 cells in a dose-dependent manner and blocked the replication of A375 melanoma cells. In addition, supernatants with bOSM protein inhibited mouse M1 cell proliferation in a manner similar to that of pure hOSM.

The demonstration that bOSM can activate both classes of OSM receptor in vitro led to the question how this protein might act in transgenic mice. One conclusion, inferred from the significant drop in frequency of production of transgenic mice, is that overexpression of bOSM prevents development of normal mice. The use of promoters that would theoretically direct bOSM transcription in numerous tissues or in large organs like the nervous system or skin failed to generate viable mice. Also, when more tissue-restricted promoters were used and transgene expression was established, we still observed serious abnormalities. All mice that inherited the InsbOSM transgene acquired interstitial pancreatitis, and all mice in the LckbOSM lineage displayed aberrant thymus function and T-cell development.

The second conclusion from these experiments is that the phenotypes elicited with bOSM have also been observed with other gp130-directed cytokines. For instance, administration of LIF at high doses in vivo caused numerous defects, including extensive bone growth (30). As with InsbOSM animals, transgenic mice that produced LIF or IL-6 in islet β cells acquired pancreatic fibrosis and infiltration of inflammatory cells (3, 12). Abnormalities associated with autoimmunity have been reported in IL-6 (18) and LIF (42) transgenic mice, and, similar to LckbOSM animals, the lymphoid tissues of LIF mice showed interconversions of various cell types (42). Targeting LIF expression with a neuron-specific promoter resulted in neonatal death (32b), and transgenic mice that overexpressed IL-6 in nervous tissue acquired neuropathologies (11). A second experiment designed to test neural responses to bOSM has established that the mRNA encoding vasoactive intestinal polypeptide is induced in the celiac ganglion of both bOSM and LIF transgenic mice (2a). Finally, the increased number of megakaryocytes found in InsbOSM transgenic mice and the increased production of platelets seen in mice treated with recombinant hOSM (48a) have been achieved in vivo with IL-11 (16), LIF (31), and IL-6 (1).

OSM can be distinguished from the other members of this

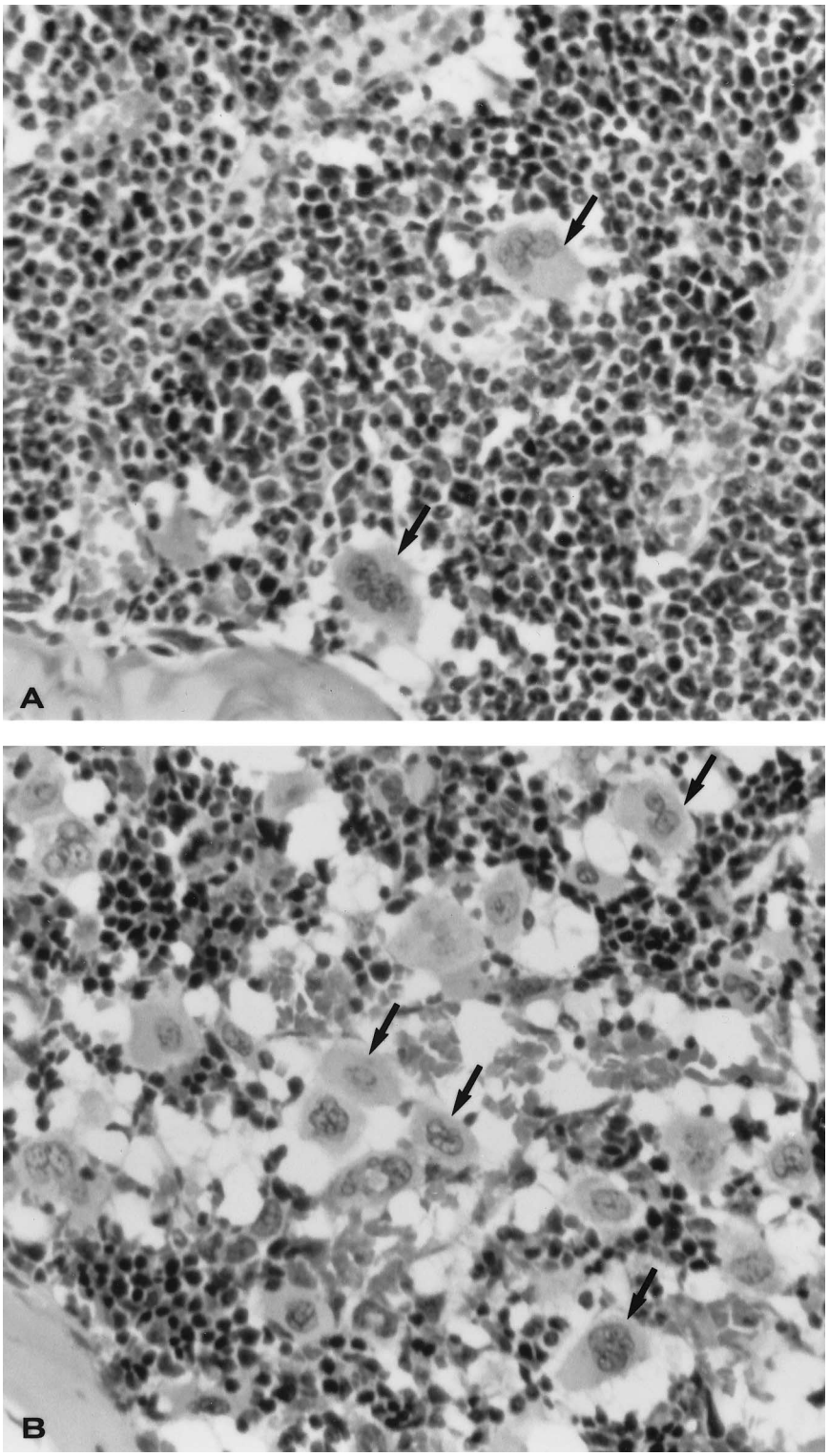

FIG. 8. Increased numbers of megakaryocytes in bOSM transgenic mice. Representative regions through the bone marrow of a control mouse (A) and a transgenic mouse (B). An approximate 10-fold increase in the number of megakaryocytes (arrows) was detected in several individuals of the InsbOSM lineage.

cytokine family in its utilization of gp130 as a receptor-binding α subunit, as well as its association with two different enhancing receptor subunits, LIFRβ and OSMRβ. Given the expression pattern of the common β receptor subunits shared by this group of cytokines, the ability to bind gp130 directly may provide OSM with a broader range of activities than any one family member. This would make OSM a potent developmental cytokine, and its expression by activated T cells and mac-

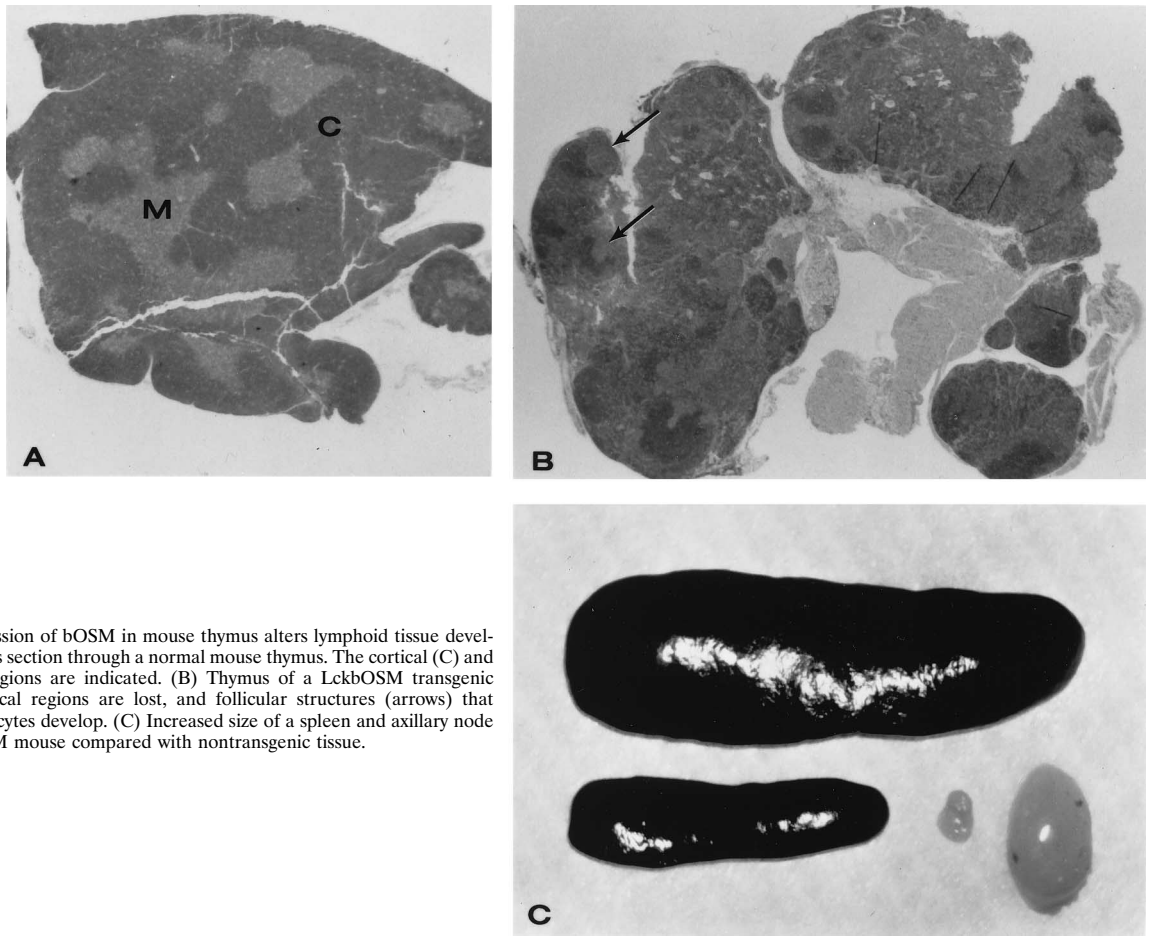

FIG. 9. Expression of bOSM in mouse thymus alters lymphoid tissue development. (A) Cross section through a normal mouse thymus. The cortical (C) and medullary (M) regions are indicated. (B) Thymus of a LckbOSM transgenic mouse. The cortical regions are lost, and follicular structures (arrows) that contain B lymphocytes develop. (C) Increased size of a spleen and axillary node from an LckbOSM mouse compared with nontransgenic tissue.

rophages would provide a versatile stimulus for wound repair. Our results are the first to demonstrate the pleiotrophic nature of OSM in vivo. The specific actions of this cytokine, however, will be better understood once the mouse gene has been identified. The characterization of a bovine protein that is 58% identical to hOSM strongly suggests that this cytokine has evolved in nonprimate mammals. Undoubtedly, the murine homolog has a low sequence similarity to hOSM. Such is the case for the mouse and human IL-6 proteins, which are 42% identical (1). Information regarding bOSM may help characterize this homolog in other species, including mice.

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