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# SRCL/CL-P1 Recognizes GalNAc and a Carcinoma-Associated Antigen, Tn Antigen

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**SRCL /CL-P1 was recently identified as a scavenger receptor with a C-type lectin domain, which was expressed in vascular endothelial cells and could bind to Gram-positive and Gram-negative bacteria, yeast and oxidized LDL. We found that SRCL was expressed in some but not all nurse-like cells examined. Furthermore, to characterize the C-type lectin domain of SRCL, the secreted form of the C-type lectin domain (LEC-AP) of SRCL, which was fused to the signal sequence of IgG and alkaline phosphatase, was expressed in 293/EBNA-1 cells and the culture medium was used for the *in vitro* binding assay. LEC-AP specifically bound to GalNAc-conjugated gel in a $Ca^{2+}$-dependent manner, and this binding was inhibited by free GalNAc, L-, D-fucose, D-galactose, lactose, and especially T antigen and Tn antigen. Furthermore, we examined whether or not SRCL could take up saccharide-conjugated particles. 293/EBNA-1 cells stably expressing SRCL were found to take up GalNAc but not mannose-conjugated particles on confocal microscopy. The binding of GalNAc-conjugated particles to these cells was quantitatively measured by comparing the x-means of individual cell populations. An approximately 2.1-fold increase in immunofluorescence intensity was observed for the SRCL transfectants compared to control vector transfectants. Our results provide a basis for understanding the scavenger function of SRCL as to carbohydrate-containing ligands.**

**Key words: C-type lectin, GalNAc, nurse-like cells, SRCL, Tn-antigen**

Abbreviations: BSA, bovine serum albumin; BP, biotinylated-polyacrylamide probe; CS-A, chondroitin sulfate A; FACS, fluorescence-activated cell sorting; FITC, fluorescein isothiocyanate; $IC_{50}$, 50% inhibition concentration; PCR, polymerase chain reaction; 293E, 293/EBNA-1 cells; SRCL, scavenger receptor with C-type lectin.

Nurse cells were first recognized in the thymus, and to express MHC class II, to interact with T cells and to stimulate the proliferation of T cells (*1–3*). We previously identified and characterized nurse-like cells (NLC) from various human tissue sites from their ability to make T cells crawl beneath them (pseudoemperipolesis) and to express the MHC class II molecule (*4*, *5*). NLC are thought to be responsible for initiating T cell-mediated immune responses to antigens in extrathymic organs (*4*).

SRCL was recently identified as a novel scavenger-like receptor with a C-type lectin domain (*6*). SRCL is composed of five domains, an extracellular C-type lectin domain at the C-terminus, a collagen-like domain, a coiled-coil domain, a transmembrane domain and a short N-terminal cytoplasmic domain. SRCL can bind to Gram-positive and Gram-negative bacteria when expressed in Chinese hamster ovary cells (*6*). Recently, the same receptor (CL-P1) was cloned by another group, and found to be expressed in umbilical vein and arterial endothelial cells (*7*). Moreover, when expressed in cells, SRCL/CL-P1 bound to and endocytosed yeasts and heavily oxidized LDL. These ligands are thought to bind to the collagen domain of SRCL due to the sequence similarity to other members of the scavenger receptor family. Therefore, it is possible that SRCL has a function in the process of atherogenesis as well as in protection against bacterial and yeast pathogens.

The C-type lectin domain of SRCL, another putative ligand binding domain, remained to be characterized. The $Ca^{2+}$-dependent (C-type) animal lectins comprise a family of proteins that contain at least one carbohydrate-recognition domain (CRD). The majority of C-type lectins bind to either D-mannose, D-glucose, and related sugars (Man-type ligands), or D-galactose, GalNAc and its derivatives (Gal-type ligands), and comprise a large family of recognition molecules especially in the immune system (*8–14*). For example, macrophages could recognize malignant tumor cells through Tn antigens, the universal carcinoma markers and autoantigens, through macrophage lectin (*9*). Moreover, a recently identified C-type lectin, DC-SIGN, is thought to participate in the interaction between dendritic cells and T cells for antigen presentation (*14*). SRCL has one C-type lectin domain that contains the carbohydrate recognition motif of QPD (Gal-type) (*6*) and exhibits sequence homology to several Gal-type C-type lectins, especially human macrophage lectin (*9*) and hepatic asialoglycoprotein (*15*). Thus, it is important to study the C-type lectin expressed in immunocom-

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petent cells including nurse-like cells and vascular endothelial cells for understanding the immune system.

In the present study, we found that SRCL was expressed in some nurse-like cells, and that its C-type lectin domain could bind specifically to several carbohydrates including GalNAc, T and Tn antigen in a Ca2+-dependent manner. Furthermore, 293/EBNA-1 cells overexpressing SRCL were shown to take up GalNAc-conjugated particles.

## MATERIALS AND METHODS

*Reagents*—The following were obtained commercially from the sources shown: Carbohydrate-conjugated Sepharoses (GalNAc-, mannose-, galactose-, and fucose-sepharose) from E-Y Laboratories, mannose- and GalNAc-BP from Seikagaku Kogyo, and GalNAc, D-galactose, D -glucose, D -fucose, L-fucose, D-fructose, D -ribose, D-xylose, L -xylose, D-arabinose, L -arabinose, lactose, melibiose, sucrose, raffinose, mannan, fucoidan, heparin, dextran, and LPS from SIGMA. T antigen (Thomsen-Friedenreich antigen, Galβ1–3GalNAcα-benzene), Tn antigen (*N*-acetylgalactosamine α1-*O*-serine), and chondroitin sulfate A were purchased from CALBIOCHEM. Galactan (gum Arabic) and arabinogalactan were from ALDRICH. methyl-β-D-galactopyranoside were from Lancaster.

*Cell Culture and Transfection*—Nurse-like cell lines, HS729T (ATCC.HTB153), FHs173WE (ATCC.HTB158), HSN27E (*4*), SK-LMS-1 (ATCC.HTB88), RA189SM (*16*), and Hs67 (ATCC.HTB163), and non-nurse cell lines, Hs913T (ATCC.HTB152), SK-HEP-1 (ATCC.HTB52), A549 (ATCC.CCL185), SIHA (ATCC.HTB35), Hs683 (ATCC.HTB138), MC/CAR (ATCC.CRL8083), Jurkat (ATCC.TIB152), HL60 (ATCC.CCL240), and IMR-32 (ATCC.CCL127), and 293/EBNA-1 (Invitrogen, San Diego, USA) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Stably transfected 293/EBNA-1 cells expressing full-length SRCL, secreted alkaline phosphatase (SEAP) and LEC-AP chimeras were generated by transfection in 6-cm dishes with ~1 μg of plasmid DNA using Lipofectamine-plus reagent (Gibco BRL, Rockville, MD, USA). Cells expressing transfected receptors were selected in 200 μg/ml Hygromycin (Sigma) for 1 week and maintained in 100 μg/ml Hygromycin.

*Expression Vector Construction*—The SRCL expression vector was constructed in pDREF (*17*). The full-length cDNA of SRCL was cloned into the pCR2.1 vector by PCR from SRCL-cDNA using primers, 414F 5′-GTCGACACGGTCACCATGAAAGAC-3′ and 414R 5′-GCGGCCGCCCCATCACAGTCCGTTAT-3′.

After digestion with restriction enzymes, *Sal*I and *Not*I, the resulting SRCL insert was ligated into the *Sal*I–*Not*I site of pDREF, generating the plasmid "pDREF-SRCL".

For construction of the LEC-AP expression vector, the C-type lectin domain of SRCL (corresponding to 584 to 742 aa) was amplified by PCR using primers, F10Bam 5′-GGATCCGGCCCATCAGGAGCGGTG-3′ and R10Xba 5′-TCTAGATAATGCAGATGACAGTAC-3′.

After digestion of the resultant PCR product with *Bam*HI and *Xba*I, the cDNA was subcloned into the pDisplay vector (Invitrogen) for fusion to the signal sequence of IgG. Subsequently, chimeric constructs containing the signal sequence were subcloned into pDREF-AP, which was the same vector as pDREF-SEAP used in Ref. *18* and has no signal sequence, generating plasmid "pDREF-LEC-AP." Construction of the secreted-type AP expression vector (pDREF-SEAP) was carried out as shown below. Briefly, SEAP was cloned from the pSEAP2-Enhancer vector (Clontech) into pCR2.1 (Stratagene) by PCR using primers, Xba-AP primer, (5′-CGCTCTAGAAGCTCCGGAATCATCCCAGTTGAGGAGGAGAAC-3′) and AP(HIS)6-NOT primer (5′-CGCGCGGCCGCTCAGTGATGGTGATGGTGATGACCCGGGTGCGCGGCGTCGGT-3′), to generate a HIS-tag and a 3′-*Not*I restriction enzyme site. This SEAP contains no signal sequence. The PCR product was digested with *Bam*HI and *Not*I, and then inserted into the *Bgl*II–*Not*I sites of the pDisplay vector to generate the chimeric SEAP fused to IgG signal sequence. Subsequently, the chimeric SEAP containing the IgG signal sequence was subcloned into pDREF to generate expression vector "pDREF-SEAP."

*Northern Blotting Analysis*—Total RNA (15 μg/lane) was separated on 1.0% agarose gels containing formaldehyde. After transfer to a Hybond-$N^+$ membrane (Amersham Pharmacia Biotech), hybridization was carried out as previously described by Hieshima *et al*. (*18*). A probe was prepared by PCR amplification using primers F1 (5′-TGGAAGAACTTCACAGAC-3′) and R1 (5′-ATGGCCATGACCCCAGTT-3′).

*Saccharide-BP Uptake Study*—HEK293/EBNA-1 cells (293E cells) stably expressing SRCL or ML (human macrophage lectin) grown for 2 days on Lab Tek Chamber Slides (Nunc) precoated with 1 μg/ml Fibronectin overnight, were incubated for 1 h at 37°C in buffer (D-MEM,10% FCS containing 2 mM $CaCl_2$) with biotinylated saccharide-polyacrylamide polymer (GalNAc-BP and Man-BP) at the concentration of 50 μg/ml. After three washes in the same buffer, the cells were fixed for 10 min at room temperature in 4% formaldehyde in PBS. The cells were permeabilized in PBS containing 1% BSA and 0.1% Triton X-100. After three washes with the same buffer, the cells were incubated with FITC-conjugated avidin (Amersham) for 1 h at room temperature. After three washes with PBS 1% BSA, the cells on slides were mounted with a mounting solution (Slow Fade, Molecular Probes) and then examined under a confocal microscope. For flow cytometric analysis, cells were detached from the culture dish with ice-cold PBS containing 2 mM EDTA before fixation. After fixation, the cells were permeabilized with 0.1% Triton X-100 in PBS, incubated with FITC-conjugated avidin for 1 h before a wash in PBS/1% BSA, and then analyzed with FACScan.

*Production of the LEC-AP Fusion Protein*—293/EBNA-1 cells were transfected with expression vector pDREF-LEC-AP. This vector, PDREF, could be episomally maintained in the cytoplasm of 293/EBNA-1 cells, and it is very easy to isolate stable transfectants of a mixed population. The transfected cells were maintained for a few weeks in D-MEM with 10% FCS containing Hygromycin B (100 μg/ml), and the culture supernatant was collected. Alkaline phosphatase activity in the supernatant was measured by means of a chemiluminescent assay using a Great EscAPe detection kit (Clontech).

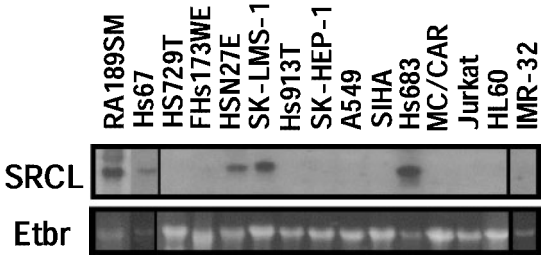


Fig. 1. **Northern blotting analysis of human SRCL expression in human cell lines.** Aliquots of total RNA, 15 μg, from human cells were electrophoresed through 1% agarose gels, transferred to Hybond-$N^+$ membranes, and then blotted with $^{32}$P-labeled probes. Ethidium bromide staining of the gels demonstrated equal loading of the samples. Total RNA was purified from nurse-like cell lines HS729T, FHs173WE, HSN27E, SK-LMS-1, RA189SM, and Hs67, and non-nurse cell lines Hs913T, SK-HEP-1, A549, SIHA, Hs683, MC/CAR, Jurkat, HL60, and IMR32.

*Binding Assay for the C-Type Lectin Domain of SRCL*—For the binding and competition experiment on the C-type lectin domain of SRCL, 20 μl (bed volume) of either GalNAc-, fucose, Gal-, or mannose-conjugated gel was incubated and rotated for 1 h at room temperature with culture medium containing expressed LEC-AP or SEAP in the absence or presence of EDTA, monosaccharide or oligosaccharide in 400 μl of D-MEM containing 10% FCS, with 40 mM HEPES (pH7.4) and 2 mM $Ca^{2+}$. For binding inhibition by EDTA, $Ca^{2+}$-depleted buffer was used. After incubation, the gel was spun down in a centrifuge, and washed with the same buffer three times and then incubated for 30 min at 65°C to inactivate native AP. AP activity bound to the gel was measured using a Great EscAPe detection kit and a chemiluminescence detector (Lumat). Data were expressed in relative light units and AP activity in the absence of inhibitor was expressed as 100%.

## RESULTS

*Identification of the SRCL cDNA in Nurse-Like Cells*—We used the subtraction cloning method to isolate cDNA preferentially expressed in a human nurse-like cell. The isolated cDNA was identical to the recently reported human SRCL type I/CL-P1 (*6*, *7*). We have identified several cell lines as nurse-like cells from their pseudoemperipolesis activity (Suzuki, R., unpublished observation). SRCL expression was examined in NLCs and non-nurse cell lines by Northern blotting. NLC lines HS729T, FHs173WE, HSN27E, SK-LMS-1, RA189SM, and Hs67, and non-nurse cell lines Hs913T, SK-HEP-1, A549, SIHA, Hs683, MC/CAR, Jurkat, HL60, and IMR-32 were examined (Fig. 1). RA189SM cells are the primary cultured cells isolated from synovial tissues of patients with rheumatoid arthritis. These cells could present antigens to T cells and stimulate T cell proliferation (*16*). SRCL was expressed in FHs173WE (faint but detectable), HSN27E, Hs683, SK-LMS-1, RA189SM, and Hs67. It is possible that SRCL is expressed in nurse-like cells.

*Construction of a C-Type Lectin Domain-AP Fusion Protein*—The alkaline phosphatase (AP) activity allowed quantitative determination of specific binding. We constructed secreted-type SRCL-AP fusion proteins as described under "MATERIALS AND METHODS." The C-type lectin domain of SRCL (584–742) was fused to the signal sequence of IgG and AP (18–506) in frame in its N- and C- terminus (LEC-AP) (Fig. 2). The chimeric protein was expressed in 293/EBNA-1 cells and the culture medum was used for the *in vitro* binding assay of saccharide-conjugated gels. The SEAP was produced in the same manner as LEC-AP (Fig. 2). For the binding of SEAP to the gel, the same amount of alkaline-phosphatase (AP) activity as that of LEC-AP was used. LEC-AP bound to Gal-, Fuc-, and GalNAc-conjugated gel with 4.0-, 17.8-, 43.7-, and 239.6-fold increases compared to the SEAP control, respectively (Fig. 3A, LEC-AP, black bar). As shown by the results, SRCL could most strongly bind to GalNAc-gel, so we used the GalNAc-gel in subsequent experiments.

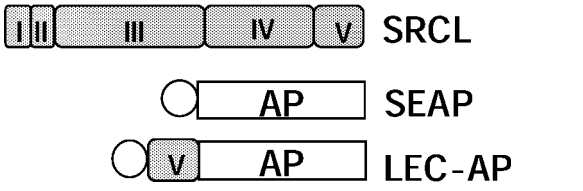


Fig. 2. **Schematic representation of the domain structures of SRCL, AP, and LEC-AP chimera proteins.** I: cytoplasmic. II: transmembrane. III: coiled-coil. IV: collagen. V: C-type lectin: AP: alkaline phosphatase. The circles represent the signal sequence of IgG. Secreted- alkaline phosphatase corresponding to the amino acid sequence (18–506) was fused to either the lectin domain of SRCL which corresponds to the amino acid sequence (584–742) for construction of LEC-AP, or IgG for SEAP

The binding of LEC-AP to GalNAc-gel was inhibited in the presence of 5 mM EDTA, indicating that the interaction was $Ca^{2+}$-dependent (Fig. 3B). The carbohydrate-binding specificity of LEC-AP was examined as to competitive inhibition using various monosaccharides at the concentration of 100 mM (Fig. 3B). Among the free monosaccharides tested, GalNAc, D-galactose, methyl-D-Gal, D- and L-fucose were found to be the most efficient competitors. Then, increasing amounts of competitors including GalNAc, L-fucose, and D-galactose were added to the binding assay mixture, and the $IC_{50}$ values of GalNAc, L-fucose, and D-galactose were determined to be 6, 12, and 16 mM, respectively (Fig. 3C). Then, several disaccharides and trisaccharides, such as β-lactose, melibiose, sucrose and raffinose, were found to inhibit the binding. In particular, β-lactose effectively competed for the binding of LEC-AP to GalNAc-gel. The $IC_{50}$ value of β-lactose was about 0.5 mM (Fig. 4A).

We next examined the effects of various oligosaccharides on the binding of LEC-AP to GalNAc-gel. Galactan and also arabinogalactan effectively competed for the binding at the concentration of 1 mg/ml (Fig. 4B).

*LEC-AP Recognizes T and Tn Antigens*—We next examined the effects of T and Tn antigens, carcinoma-associated antigens, on the binding of LEC-AP to GalNAc-gel. Both T and Tn antigens specifically inhibited the binding of LEC-AP to GalNAc-gel. The $IC_{50}$ values of T-antigens and Tn-antigens were <3 mM and <1 mM, respectively (Fig. 5).

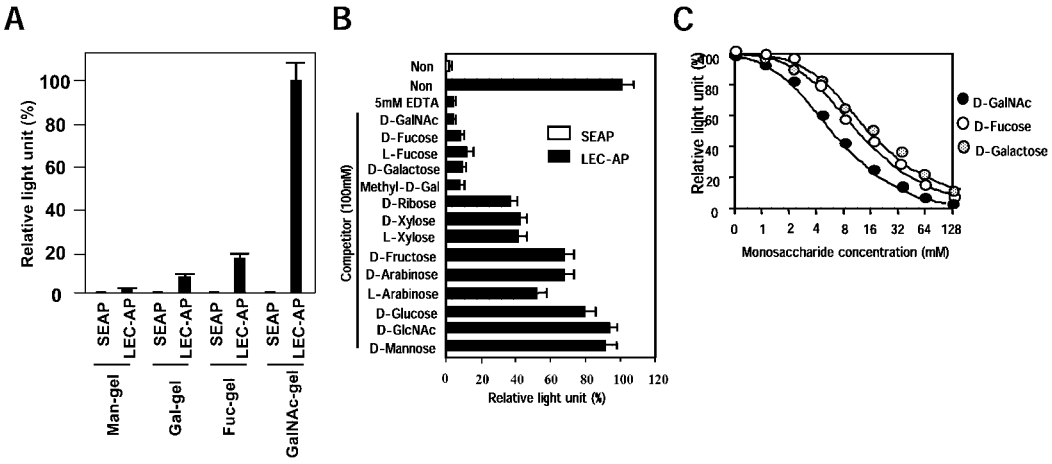


Fig. 3. **In vitro binding and binding competition assaying of LEC-AP chimeras on GalNAc-conjugated gel.** (A) Culture medum containing LEC-AP proteins was applied to 20 μl (bed volume) of mannose-, galactose-, fucose-, or GalNAc-conjugated Sepharose gel at room temperature for 1h. Culture medium containing expressed SEAP was used as a negative control. (B) Culture medum containing LEC-AP proteins was applied to GalNAc-gel in the absence or presence of the indicated concentrations of competitors as described under "MATERIALS AND METHODS." As a negative control, the same level of AP activity as that of LEC-AP was used for in vitro binding. In (A) and (B), data are means ± SD for triplicate determinations and are representative of three experiments. (C) Inhibition of binding of LEC-AP to GalNAc-conjugated gel by free GalNAc, D-fucose, or D-galactose. Briefly, 20 μl (bed volume) of GalNAc-gel was incubated with LEC-AP in the presence of increasing concentrations of free GalNAc, L-fucose, or D-galactose. Data representative of three experiments was shown.

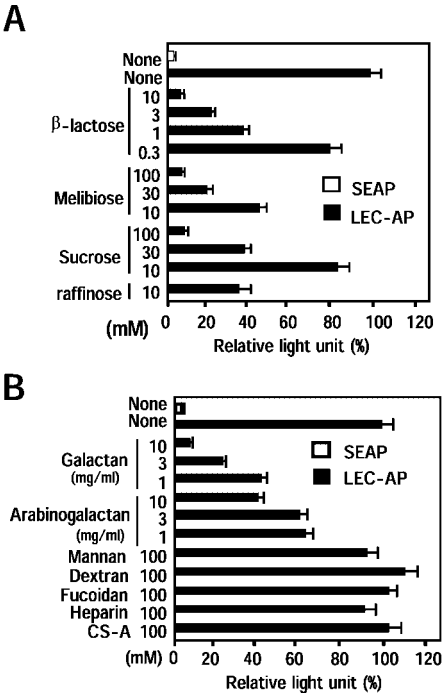


Fig. 4. ***In vitro*** **binding competition assay of LEC-AP to GalNAc-gel by di- and oligosaccharides.** Culture medium containing LEC-AP proteins was applied to GalNAc-gel in the absence or presence of the indicated concentration of di- and trisaccharides (A), or oligosaccharides (B). In (B), the concentrations of galactan and arabinogalactan are expressed in mg/ml, and those of others were expressed in mM. Data are means ± SD for triplicate determination and are representative of three experiments.

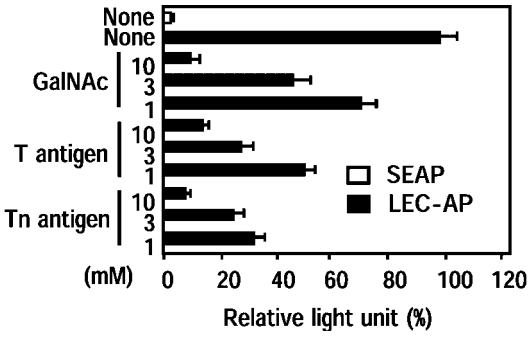


Fig. 5. ***In vitro*** **binding competition assaying of LEC-AP on GalNAc-gel with T and Tn antigens.** Briefly, 20 μl (bed volume) of GalNAc-gel was incubated with LEC-AP in the presence of the indicated concentrations (mM) of free T antigen and Tn antigen, and free GalNAc as a positive control. AP activity bound to the GalNAc-gel was measured. Data are means ± SD for triplicate determinations and are representative of three experiments.

*Carbohydrate-Binding Activity of SRCL*—To determine whether or not SRCL bound to carbohydrate chains in transfected cells, the full-length SRCL cDNA was cloned into the pDREF vector (pDREF-SRCL) and then the expression vector was transfected into 293/EBNA-1 cells. Stable transfectants were assayed for the binding of biotinylated carbohydrate ligands at 37°C. As a positive control, pDREF-ML, a human macrophage lectin (*9*), was used. In SRCL-transfected cells, specific binding and uptake were observed on confocal microscopy only when *N*-acetyl-D-galactosamine-BP (GalNAc-BP) was used as the ligand (Fig. 6A). Mannose-conjugated BP did not show any binding (Fig. 6A). To confirm ligand uptake by SRCL, we observed the cells detached from the bottom of a slide and rounded cells because we could easily detecting the cell interior signals by observing such rounded cells. The GalNAc-BP uptake by SRCL was evident because a series of optical sections revealed dot-like signals in both the cell interior (Fig. 6B, white arrow) and on the cell surface (Fig. 6B, black arrow).

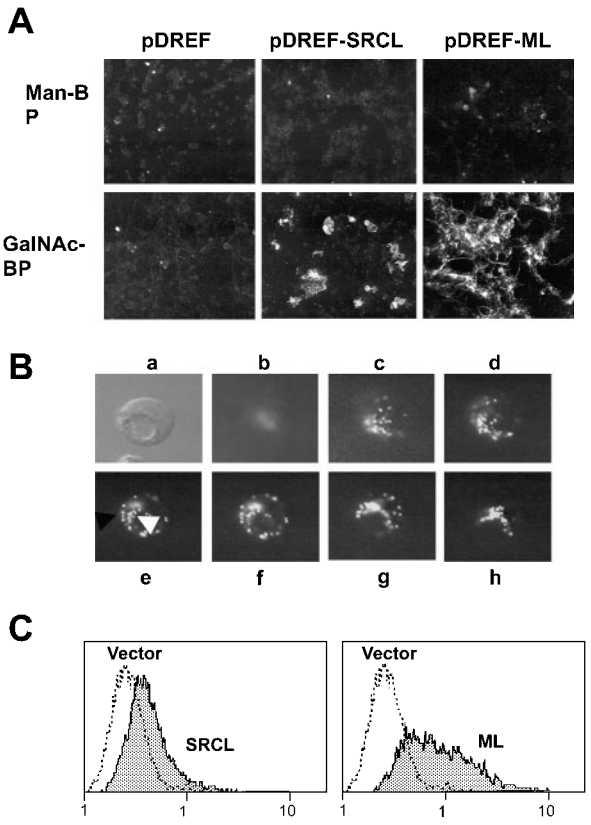


Fig. 6. **The uptake of GalNAc-conjugated polyacrylamide particles by SRCL-transfected cells**. (A) The uptake of GalNAc-BP by 293/EBNA-1 cells expressing SRCL. Cells stably transfected with pDREF-SRCL or control vector pDREF were incubated with Man- or GalNAc-BP (50 μg/ml) for 1 h at 37°C. As a positive control, human macrophage lectin (pDREF-ML) expressed in 293/EBNA-1 cells was used. After washing, the cells were fixed and stained with FITC-avidin and visualized by fluorescence microscopy. (A) Serial section photographs (b–h) showing the uptake of dot-like signals in the cell interior. Cells stably transfected with pDREF-SRCL were incubated with GalNAc-BP (50 μg/ml) for 1 h at 37°C. After washing, the cells were fixed and stained with FITC-avidin, and the rounded cells were visualized by fluorescence microscopy. (a) Phase contrast microscopy. (A) Quantitative measurement of the binding of the GalNAc-BP to SRCL-expressing cells by FACS analysis. Representative data for three experiments are shown. The *x*-means of individual cell populations were calculated and expressed as fold increases compared to in control vector transfectants.

Moreover, we performed quantitative FACS analysis of the binding of GalNAc-BP (Fig. 6C) to SRCL-expressing cells. In this experiment, we detached 293 cells bound to GalNAc-BP by treating the cells with 2 mM EDTA. With this treatment, the GalMAc-BPs bound to the cell surface was thought to be dissociated from the cells. However, all of the bound-BP was not completely dissociated. Thus, it seems likely that the GalNAc-BPs bound to SRCL is internalized into the cells. The binding of GalNAc-BP to cells was quantitatively measured by comparing the x-means of individual cell populations. Approximately 2.1- and 5.4-fold increases in immunofluorescence intensity compared to in control vector transfectants were observed in the SRCL and ML transfectants, respectively. The binding of SRCL to GalNAc-BP ligands was weak than that of ML. We thought that these binding activities were dependent on the gene expression levels, because the expression of SRCL was lower than that of ML. Thus, this type of quantitative analysis was not so important but confirmed that SRCL expressed in 293/EBNA-1 cells specifically binds to and takes up GalNAc-conjugated polyacrylamide particles.

## DISCUSSION

In this study we showed that SRCL is expressed in several nurse-like cells, the C-type lectin domain of SRCL specifically interacts with Gal-type ligands, and T- and Tn antigens, and SRCL-transfected cells specifically internalize GalNAc-conjugated particles. The human SRCL was originally identified as a bacteria binding receptor (*6*). Subsequently, the same receptor (CL-P1) was independently identified by other groups as a receptor of oxidized-LDL (ox-LDL) on vascular endothelial cells (*7*). SRCL/CL-P1 has both scavenger receptor-like and collectin-like structures. The alternative splicing form of SRCL, SRCL type II, which has no C-type lectin domain, was found to be able to bind to bacteria (*6*), so that C-type lectin domain thought to be dispensable for the binding to bacteria. We examined whether or not LEC-AP bound to such microorganisms including *S. aureus*, *Escherichia coli*, and zymosan. However, no specific binding was observed (data not shown). However, LEC-AP could bind to galactan and arabinogalactan with moderate specificity. Although the structures of these materials were complex and not uniform, the similar structural components of these materials were thought to be present in some bacterial cell walls (*19*, *20*), especially in Gram-positive bacteria. Thus, the C-type lectin of SRCL may recognize some bacteria.

The C-type lectin domain of SRCL has been shown to contain the QPD motif, which was expected to bind to D-galactose and GalNAc (*6*). In the present study, SRCL expressed in 293E cells was actually shown to possess binding activity as to GalNAc-conjugated BP. Moreover, in the *in vitro* binding assay, the C-type lectin domain-AP chimera expressed in 293E cells was shown to possess affinity to Gal-type ligands, D-galactose (D-Gal), GalNAc, L-D-fucose, T-antigen, and Tn-antigen. The binding affinity of LEC-AP as expressed as $IC_{50}$, as to several carbohydrates examined in this study was equivalent to that observed for other C-type lectins (*21*, *22*). The binding specificity was very similar to that of human macrophage C-type lectin (HML), which recognizes GalNAc, D-Gal and Tn antigen (*9*, *21*, *23*). Therefore SRCL may play a role as a scavenger receptor for asialoglycoprotein in nurse-like cells as well as vascular endothelial cells.

Collectins comprise a family of proteins that contain at least two characteristic structures, a collagen-like region and a carbohydrate recognition domain (CRD). There are four groups in this family: mannan-binding protein, surfactant protein A, surfactant protein B and membrane-bound type collectin,SRCL/CL-P1. SRCL could recognizes GalNAc-type carbohydrate, so it is a novel type of collectin.

GalNAc is an about threefold more potent inhibitor than D-Gal, indicating that the *N*-acetamide group at C-2 in the pyranose ring participates in the interaction. L-Arabinose, which has the same configuration as D-Gal or

L-Fuc but lacks the $CH_2OH$ or $CH_3$ of C6, respectively, was inactive at levels up to 10-fold higher than the molar amount of D-Gal used, indicating that the $CH_2OH$ or $CH_3$ of C6 is necessary for the binding. Among the oligosaccharides examined, β-lactose (Galβ1–4Glc) was the best inhibitor, the activity being about 4-, 10-, and 10- fold greater than those of D-Gal, raffinose (Galα1–6Glcβ1–2Dfruf), and melibiose (Galα1–6Glc), respectively. Tn antigen (GalNAcα1-*O*-Ser), which has the α-anomer linkage of GalNAc, also showed strong inhibitory activity. These results indicated that SRCL recognizes both the α- and β-anomers of D-Gal.

It is interesting to note that a lectin with Galβ1–4GlcNAc binding activity on microvascular endothelial cells can contribute to retention and secondary tumor formation of blood-borne tumor cells (*24*). Thus, SRCL may be identical to these lectin-like substances observed in the endothelium and may participate in the retention of tumor cells.

Another point of interest is our finding that the C-type lectin domain of SRCL could bind to T and Tn antigens. T (Galβ1–3GalNAcα-Ser/Thr) and Tn (*N*-acetylgalactosamineα1-*O*-serine), found on mucin-type glycoproteins, has been well documented to be expressed on a variety of human carcinoma cells, and thought to be autoantigens (*25*, *26*) and to participate in metastasis (*27*, *28*). A direct link has been demonstrated between vessel invasion by malignant cells and the cell-surface density of Tn antigens (*29*). These epitopes might contribute to the recognition of malignant cells by immune cells and are thought to stimulate anti-tumor immunity (*25*, *30*). Several proteins have been described that specifically recognize the Tn antigen, including monoclonal antibodies (*31*, *32*), plant lectins (*33*), human macrophage lectin (*9*), and glycosyltransferase (*34*). Among them, human macrophage lectin (ML), which has a C-type lectin domain and is expressed in macrophages, specifically binds to the Tn antigen for the recognition of malignant cells (*9*, *21*). Therefore, it is possible that SRCL has a similar function to that of ML.

The presence of SRCL in immunocompetent cells such as nurse-like cells and vascular endothelial cells suggests its role as a scavenger receptor for antigen presentation. Cytokine-activated human vascular endothelial and skin nurse-like cells play roles in initiating immune responses by interacting with immunocompetent cells *via* their class II MHC molecules (*4*, *35–37*). The RA189SM cells shown in Fig 1, which were primary cultured nurse-like cells isolated from synovial tissues of patients with rheumatoid arthritis, and can maintain and activate T cells *in vitro*, are thought to be involved in the development of rheumatoid arthritis (*16*). As SRCL was expressed in these cells and was suggested to have endocytic activity based on the results in Fig. 6, SRCL may function in the uptake of soluble glycoconjugates released from pathogens and endogenous glycoproteins bearing oligosaccharides having terminal galactose residues including T- and Tn antigens for antigen presentation to T cells by MHC class II molecules, thereby initiate immune responses like DEC-205 (*13*). It is important to examine whether or not SRCL in nurse-like cells and endothelial cells actually functions as a molecular scavenger as to Gal-type ligands. The isolation of antibodies specific to SRCL now in progress will help us to answer these questions.

Our finding that SRCL is expressed in several nurse-like cells, and recognizes T- and Tn antigens reveals a novel function for SRCL. Additional studies on the function of SRCL may provide more insight into the roles of nurse-like cells and endothelial cells.

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