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Down-modulation of B cell signal transduction by ligation of mucins to CD22

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ABSTRACT

Epithelial cancer cells secrete mucins carrying carbohydrate antigens such as a sialyl-Tn antigen into cancer tissues and/or the bloodstream, in which mucins may interact with CD22 (Siglec-2). Mucins isolated from colon cancer cells and bovine submaxillary mucins bound to CD22 cDNA transfectants and a human B cell line, Daudi cell, and the binding of soluble recombinant CD22 to the mucins was confirmed by means of a plate assay. The binding specificity was demonstrated by the fact that the mucins bound to the recombinant CD22 with an intact ectodomain but not to that with a mutated ectodomain. Daudi cells were stimulated with anti-IgM $F(ab')_2$ in the presence or absence of mucins. Ligation of mucins to CD22 decreased the phosphorylation of CD22 and SHP-1 recruitment, and the phosphorylation of ERK-1/2 prominently. The in vivo effect of mucins on splenic B cells in the tumor-bearing state was investigated using mucin-producing (TA3-Ha) and non-producing (TA3-St) mammary adenocarcinoma-bearing mice. When fluorescence-labeled epiglycanins were administered to normal mice, a portion of them was taken up by the spleen and became associated with splenic B cells. We found that splenic B cells were reduced in TA3-Ha-bearing mice but not in TA3-St-bearing ones. These results suggest that in the tumor-bearing state a portion of the mucins in the bloodstream was taken up by the spleen and ligated to CD22 expressed on splenic B cells, which may have led to down-regulation of signal transduction.

Upon malignant transformation, many epithelial cells produce mucins in abnormal amounts and/or with abnormal glycosylation patterns [1]. Mucins produced by cancer cells are found in the sera of cancer patients and are used as tumor markers. There have been many reports that patients with high amounts of mucins in their bloodstreams exhibit a lower 5-year survival rate [2]. However, little is known regarding the biological significance of mucins. Mucins easily come into contact with various circulating cells in the bloodstream in cancer patients and/or with the infiltrated cells in cancer tissues. A human colon cancer cell line, LS 180, produces MUC2 mucin [3], which has a high number of tandem repeats and carries various carbohydrate antigens including a sialyl-Tn antigen.

CD22 (Siglec-2) is a lectin recognizing a α2,6 sialic acid-linked glycoconjugate and is composed of seven Ig domains. The cytoplasmic domain of CD22 contains the immunoreceptor-tyrosine based inhibitory motif (ITIM). After B cell receptor (BCR) ligation, the tyrosines are rapidly phosphorylated, followed by recruitment and activation of SHP-1, which inhibits several signaling pathways [4].

It is generally agreed that CD22 is masked by endogenous *cis* ligands [5,6]. However, several reports have suggested that CD22 is unmasked on some subsets of resting B cells or becomes unmasked on a portion of activated B cells following BCR ligation [6,7]. Collins et al. also demonstrated that although *cis* ligands mask the binding of sialoside probes, CD22 is still able to interact with glycoprotein ligands expressed on B or T cells [8].

Thus, it seems likely that CD22 binds preferentially to glycoproteins, which are structurally well accessible and carry high levels of appropriately linked sialic acids. CD22 exhibits high specificity for sialoside ligands containing the sequence $Sia_{\alpha 2\text{-}6}Gal[NAc]$ [9,10]. The $Sia_{\alpha 2\text{-}6}Gal[NAc]$ sequence is present in both the *N*- and *O*-glycan carbohydrate groups of glycoproteins. Therefore, mucins seem to be one of the types of ligands with high affinity due to their high valency.

In the present study, we demonstrated that mucins secreted from colon cancer cells could down-modulate BCR-mediated signal transduction through ligation of mucins to CD22 and that splenic B cells were reduced in mucin-producing mammary adenocarcinoma-bearing mice.

Materials and methods

Cell lines and materials. A human B cell line, Daudi, a colorectal cancer cell line, LS 180, and CHO-K1 and Cos-7 cells were obtained

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from the American Type Culture Collection. The Daudi and LS 180 cells were cultured in RPMI 1640 and Eagle's MEM supplemented with 10% FCS, respectively. The CHO-K1 and Cos-7 cells were cultured in Opti-MEM medium (Invitrogen, Carlsbad, CA) supplemented with 2% FCS. LS 180 mucin and epiglycanin were purified from the conditioned medium of LS 180 cells and the ascites fluid of TA3-Ha-bearing mice, respectively, as described previously [11]. Bovine submaxillary mucin (BSM) was obtained from Roche Diagnostics (Basel, Switzerland). Ovine submaxillary mucin (OSM) was prepared as described previously [12]. An anti-Tn antigen mAb, MLS 128, was prepared as described previously [13]. Alexa Fluor 488-labeled MLS 128 and epiglycanin were prepared using an Alexa Fluor 488 protein labeling kit (Invitrogen). A/J mice (8-wk-old) were purchased from SLC (Shizuoka, Japan). The Kyoto Sangyo University Committee on Animal Welfare approved all animal protocols used in this study. The two murine mammary adenocarcinoma sublines (TA3-Ha and TA3-St) were kindly provided by Dr. J. Hilkens (The Netherlands Cancer Institute).

Binding of mucins to Daudi cells. Daudi cells (1×10^6 cells) were treated with anti-CD22 mAb, or biotinylated BSM or asialo BSM (10 μg/ml) at 4 °C for 1 h. After washing with PBS containing 0.5% BSA, the cells were incubated with anti-mouse IgG-FITC or streptavidin-FITC (DAKO, Glostrup, Denmark) at 4 °C for 1 h. After washing as described above, the cells were analyzed with a FACSort (Becton Dickinson, San Jose, CA).

Preparation of FLAG-tagged soluble and membrane bound CD22 cDNA constructs and transfectants. The cDNA of human CD22 was generated from total RNA prepared from Daudi cells stimulated with anti-IgM $F(ab')_2$ (AbD Serotec, Oxford, UK) by RT-PCR using a pair of primers, 5′-AAC AGG CTT GCA CCC AGA-3′ and 5′-TCT GCT CGA GCC CAT CCA GTG T-3′, and then was ligated into pUC 18 (pUC 18-WT CD22). cDNA encoding a mutant with Arg 120 changed to Ala (R120A CD22 cDNA) was generated using a Site-directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Soluble 3 × FLAG-tagged CD22 {sCD22–FLAG(3)} was obtained as follows. cDNA encoding the N-terminal seven Ig domains of CD22 and the corresponding cDNA encoding the mutant with Arg 120 changed to Ala were obtained by PCR using a pair of primers, 5′-TCG AAT TCA AGT AAA TGG GTT TTT GAG CAC-3′ and 5′-ACA GCC AGT CGA CTG CCG AT-3′, and pUC 18-CD22 (WT CD22 cDNA) or pUC 18-mutated CD22 (R120A CD22 cDNA) as a template, respectively, and then subcloned into pFLAG CMV 14 (Sigma, St. Louis, MS). The resulting constructs were transfected into CHO-K1 cells using Fugene 6 transfection reagent (Roche Diagnostics), and then 3 × FLAG-tagged soluble CD22 {WT sCD22–FLAG(3)} and mutated CD22 {R120A sCD22–FLAG(3)} were purified from the conditioned media of the stable transfectants by affinity chromatography on anti-FLAG M2-agarose gels (Sigma). Membrane bound FLAG-tagged CD22 and a mutant were obtained as follows. The WT and R120A CD22 cDNAs were digested with SalI and EcoRI, and then subcloned into pFLAG CMV-5 (Sigma). The resulting constructs were transfected into Cos-7 cells, and the binding of mucins to the transfectants was examined at 48 h after the transfection. Anti-CD22 mAb (BD Biosciences, San Diego, CA) and anti-FLAG M2 mAb (Sigma) were used to confirm the expression of these constructs. The transfectants were incubated with biotinylated LS 180 mucin (10 μg/ml) at 4 °C for 1 h, washed with PBS, and then fixed with 4% paraformaldehyde. After blocking with 5% BSA, the cells were incubated with anti-FLAG mAb, and then the bound mucin and antibodies were detected with goat anti-mouse IgG-Alexa Fluor 488 and streptavidin-Alexa Fluor 594 (Invitrogen), respectively.

Solid phase binding assay. BSM, OSM and LS 180 mucin were coated onto Universal Bind 96-well plates (Corning, Corning, NY). Serially diluted WT sCD22–FLAG(3) and R120A sCD22–FLAG(3) (0.16–10.0 μg/ml) were added to the plates, followed by incubation for 1 h, and then washing with 50 mM sodium phosphate, pH 7.5, 0.2 M NaCl, and 0.05% Tween 20. Bound sCD22–FLAG(3) was quantitated with peroxidase conjugated anti-FLAG M2 mAb (Sigma) and a TMB peroxidase substrate system (Nacalai Tesque, Kyoto, Japan).

Analysis of signal transduction. Daudi cells (2×10^6 cells) were incubated at 37 °C for 10 min in the presence of mucins, and then stimulated with anti-IgM $F(ab')_2$ at 37 °C for 3 min. The cells were lysed in 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl, 1% NP-40, 5 mM EDTA, 100 mM NaF, 1 mM Na_3VO_4, 1 mM PMSF, leupeptin (10 μg/ml), and pepstatin A (10 μg/ml). The lysate was centrifuged at 12,000g for 30 min and from the supernatant, CD22 was immunoprecipitated with anti-CD22 mAb. The immunoprecipitate and co-immunoprecipitated SHP-1 were subjected to SDS–PAGE followed by Western blotting. The PVDF membranes were incubated with anti-CD22 Ab (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phosphotyrosine mAb 4G10 (Upstate Biotechnology, Lake Placid, NY), or anti-SHP-1 Ab (Santa Cruz Biotechnology). For detection of the phosphorylated ERK-1/2, Daudi cells (2×10^6 cells) were incubated at 37 °C for 10 min in the presence or absence of mucins, and then stimulated with anti-IgM $F(ab')_2$ at 37 °C for 5 min. Each cell lysate was subjected to SDS–PAGE, followed by transfer to a PVDF membrane. The membrane was incubated with anti-phosphorylated ERK-1/2 mAb or anti-ERK-1/2 mAb (Cell Signaling Technology, Beverly,

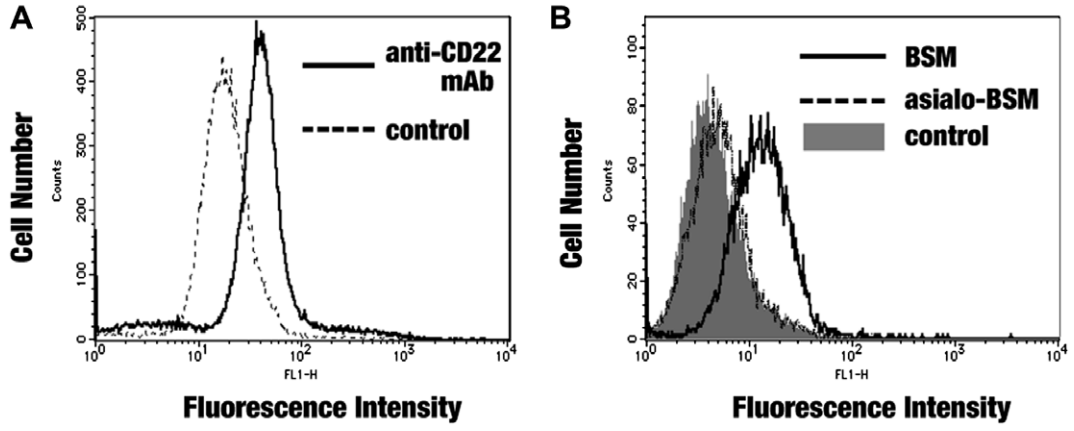

Fig. 1. Binding of CD22 to BSM. (A) Expression of CD22 on Daudi cells. Daudi cells were stained by successive incubation with anti-CD22 mAb and FITC-anti-mouse IgG (solid line). In a control experiment, Daudi cells were treated similarly without the anti-CD22 mAb (dotted line). (B) Binding of BSM to Daudi cells. Daudi cells were incubated with biotinylated BSM (solid line) or biotinylated asialo BSM (dotted line). Bound BSM was detected with FITC-streptavidin.

MA). After washing with PBS containing 0.1% Tween 20, the membranes were incubated with anti-mouse IgG-HRP or anti-rabbit IgG-HRP (Invitrogen), followed by detection with an ECL system (GE Healthcare, Piscataway, NJ).

Immunocytochemical staining of murine mammary adenocarcinoma cells. TA3-Ha or TA3-St cells were fixed in 4% paraformaldehyde for 15 min. After blocking with 5% BSA in PBS, the cells were stained with Alexa Fluor 488-conjugated MLS 128 mAb. Nuclei were stained with DAPI and slides were mounted using a SlowFade Anti-Fade kit (Invitrogen).

Flow cytometric analysis and immunochemical observation of the spleens of tumor-bearing mice. Splenocytes were prepared from TA3-Ha or TA3-St-bearing mice on day 8 after inoculation of tumor cells (5×10^5 cells). After removal of erythrocytes, the leukocytes were incubated with anti-B220-FITC (BD Biosciences) and then analyzed with a FACSort. The spleens of the tumor-bearing mice as described above were resected and fixed with 4% paraformaldehyde in PBS. Cryostat sections of 10 μm were prepared and then treated with anti-CD16/32 Ab (BD Biosciences) to block Fc receptors. CD22 was detected by successive incubation with biotinylated anti-mouse CD22 mAb and Alexa Fluor 488-labeled streptavidin (Invitrogen).

Intravenous injection of epiglycanin. Alexa Fluor 488-labeled epiglycanin (200 μg) was injected into the tail vain. After 20 min, the mice were perfused with 4% paraformaldehyde through the heart and then their spleens were resected. Sections were treated with anti-CD16/32 Ab, and CD22 was detected by successive incubation with biotinylated anti-mouse CD22 mAb and Alexa Fluor 594-labeled streptavidin (Invitrogen).

Results and discussion

Binding of mucins to CD22

Many studies have suggested potential functions for CD22 ligation of endogenous ligands in regulation of the B cell function. However, the physiological importance of CD22-mediated ligand binding and adhesion in vivo has not been elucidated clearly. First, we examined the binding of mucins to CD22.

The expression of CD22 on Daudi cells was confirmed by FACS analysis (Fig. 1A). As shown in Fig. 1B, BSM but not asialo BSM bound to Daudi cells. To further demonstrate the binding of mucins to CD22, FLAG-tagged wild type CD22 and mutated CD22 were added to microtiter plates coated with OSM, BSM or LS 180 mucin. As shown in Fig. 2A, all the mucins examined bound to FLAG-tagged wild type CD22. As expected, the level of binding of OSM was the highest among these mucins, because most carbohydrate chains of OSM are composed of a sialyl-Tn antigen, which is known to bind to CD22 [9]. This binding specificity was confirmed by the fact that the mucins bound to the CD22 fusion protein with an intact ectodomain but not to that with a mutated ectodomain (Fig. 2B).

To demonstrate that cell surface CD22 could bind to mucins, two types of cDNAs encoding membrane-bound CD22 were transfected into Cos-7 cells, as described under Materials and methods. As shown in Fig. 2C, mucins could bind to Cos-7 cells expressing the wild type CD22 but not to ones expressing the mutated CD22. These results indicate that the Arg 120 residue is essential for the binding, like in the cases of other glycoconjugates [14,15]. In addition, the mucins bound to Daudi cells and CD22 cDNA transfectants without sialidase treatment, indicating that mucins favorably compete with *cis* ligands for occupancy on CD22 binding. These results are consistent with the report that CD22 is redistributed to the sites of cell contact on its interaction in *trans* with glycoprotein ligands on opposing cells [8,16].

Inhibition of signal transduction through BCR on ligation of mucins to CD22

Next, we investigated the effect of mucins on signal transduction through the B cell receptor. Daudi cells were incubated with various amounts of mucins at 37 °C for 10 min and then stimulated with anti-IgM $F(ab')_2$ at 37 °C for 3 min. CD22 was immunoprecipitated with anti-CD22 mAb from each lysate and the immunoprecipitate was subjected to SDS–PAGE followed by Western blotting. Engagement of the BCR with anti-IgM $F(ab')_2$ induced rapid tyro-

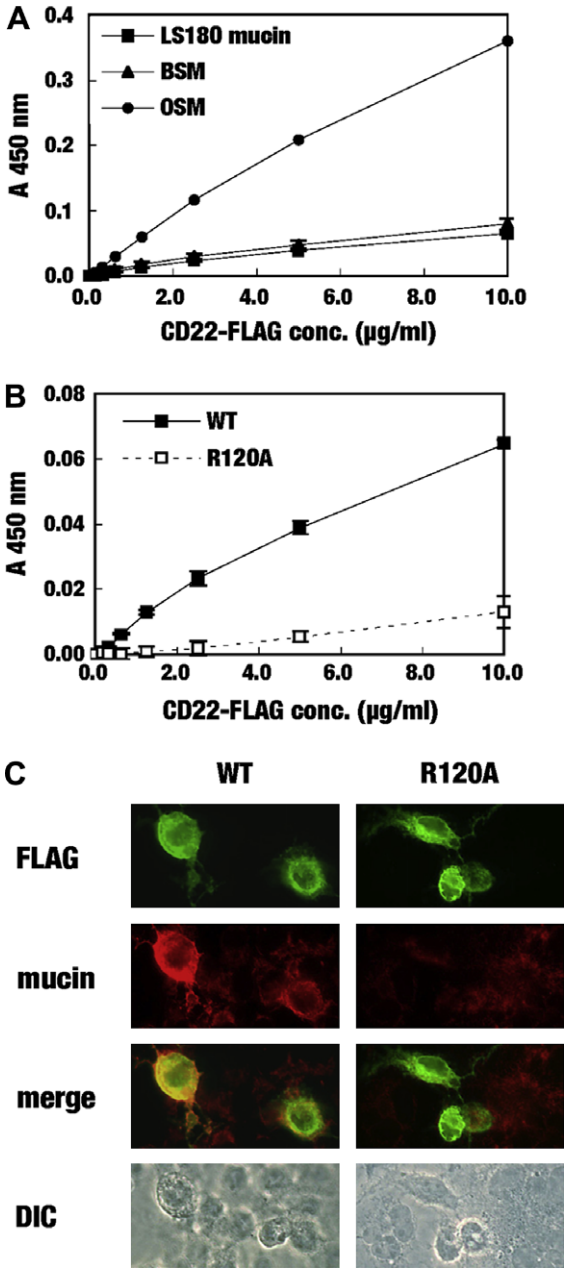

Fig. 2. Binding of recombinant CD22 to mucins. (A) WT sCD22–FLAG(3) was added to plates coated with OSM (circles), BSM (triangles), or LS 180 mucin (squares), and its binding was examined as described under Materials and methods. Data are means ± SD ($n = 3$). (B) Plates coated with LS 180 mucin were incubated with WT sCD22-FLAG(3) (closed squares) or R120A sCD22-FLAG(3) (open squares), and bound WT sCD22-FLAG(3) or R120A sCD22-FLAG(3) was detected as described under Materials and methods. (C) Cos-7 cells expressing FLAG-tagged recombinant CD22 were incubated with anti-FLAG mAb or biotinylated LS 180 mucins. Bound antibodies or mucin were detected as described under Materials and methods.

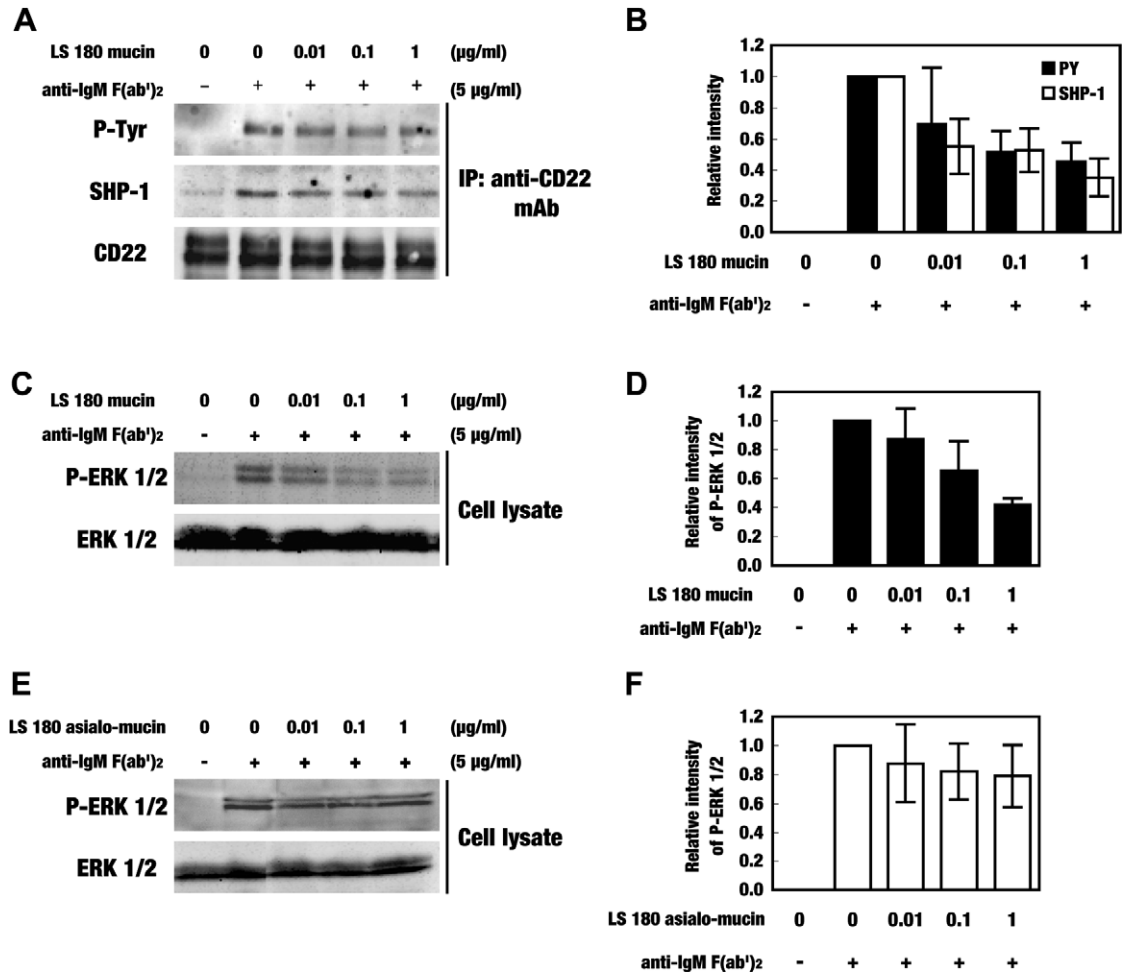

Fig. 3. BCR-mediated signal transduction in the presence of mucins. (A) Daudi cells (1×10^6 cells) were incubated with mucins at 37 °C for 10 min, and then stimulated with anti-IgM F(ab′)$_2$ at 37 °C for 3 min. CD22 and SHP-1 prepared from the cell lysate were subjected to SDS–PAGE and Western blotting, and then phosphorylated tyrosines, SHP-1 and CD22 were detected as described under Materials and methods. (B) The intensities of the bands in (A) were determined with Image J software and normalized as to the levels detected in experiments performed in the presence of anti-IgM F(ab′)$_2$ and in the absence of LS 180 mucin (mean ± SD, $n = 3$). (C) Daudi cells were stimulated as described above except that the incubation time was 5 min. The cell lysate was directly subjected to SDS–PAGE, followed by Western blotting. Phosphorylated ERK-1/2 and ERK-1/2 were detected as described under Materials and methods. (D) The relative amounts of phosphorylated ERK-1/2 in (C) were determined and normalized as described for (B). (E) Daudi cells were incubated with asialomucin instead of a mucin and then the same procedure as in (C) was performed. (F) The relative amounts of phosphorylated ERK-1/2 in (E) were determined and normalized as in (B).

sylphosphorylation of CD22 and recruitment of SHP-1, as described previously [17]. Ligation of mucins to CD22 reduced the tyrosylphosphorylation and recruitment of SHP-1 as shown in Fig. 3A and B. We also detected phosphorylated ERK-1/2 with a specific mAb. It should be noted that pre-incubation with mucins prevented the phosphorylation of ERK-1/2 prominently (Fig. 3C and D). The effect was dose-dependent and asialomucin had no effect (Fig. 3E and F). When mucins were added to the cells without BCR stimulation, tyrosylphosphorylation of CD22, recruitment of SHP-1 and phosphorylation of ERK-1/2 were not affected (data not shown). The effect of CD22 engagement on signal transduction through BCR has been investigated using an anti-CD22 antibody [18]. It is generally agreed that sequestration of CD22 away from the BCR lowers the activation threshold, which results in activation of the MAP kinase pathway [19]. Yu et al. demonstrated that a synthetic glycan ligand of CD22 blocks translocation of CD22, but not that of BCR to lipid rafts in a mouse B cell line, BAL17 [20]. Since mucins possess an extremely larger number of binding sites for CD22 compared to its antibody, mucins may have some different effects on signal transduction through the BCR. Mucin ligation decreased the phosphorylation of CD22 and SHP-1 recruitment, and unexpectedly reduced the phosphorylation of ERK-1/2 in a dose-dependent manner. Lanoue et al. reported an interesting study in which B cell activation by an antigen expressed on cancer cells was found to be depressed if the target co-expressed α2-6 sialoglycoproteins [21], suggesting that B cell signaling is inhibited by *trans* ligands on such antigen-presenting cells. In this case, it is also considered that translocation of the BCR to the lipid rafts at the sites of cell contact may be hindered, resulting in down-modulated signal transduction. Studies on this issue are underway.

Reduction of splenic B cells in mice bearing mucin-producing tumor cells

To investigate the biological effect of circulating mucins in the tumor-bearing state, we used mouse mammary adenocarcinoma cell lines TA3-Ha and TA3-St. They are a closely matched pair that can be exploited to compare the effects of mucins on B cells in vivo, because TA3-Ha cells produce a mucin named epiglycanin, but a subline of them, TA3-St, does not. Expression of epiglycanin in TA3-Ha cells but not in TA3-St cells was confirmed immunochemically using anti-Tn mAb (MLS 128) (Fig. 4A). The binding of murine CD22 to epiglycanin was also confirmed by means of a plate assay (data not shown). Epiglycanin is known to be shed from

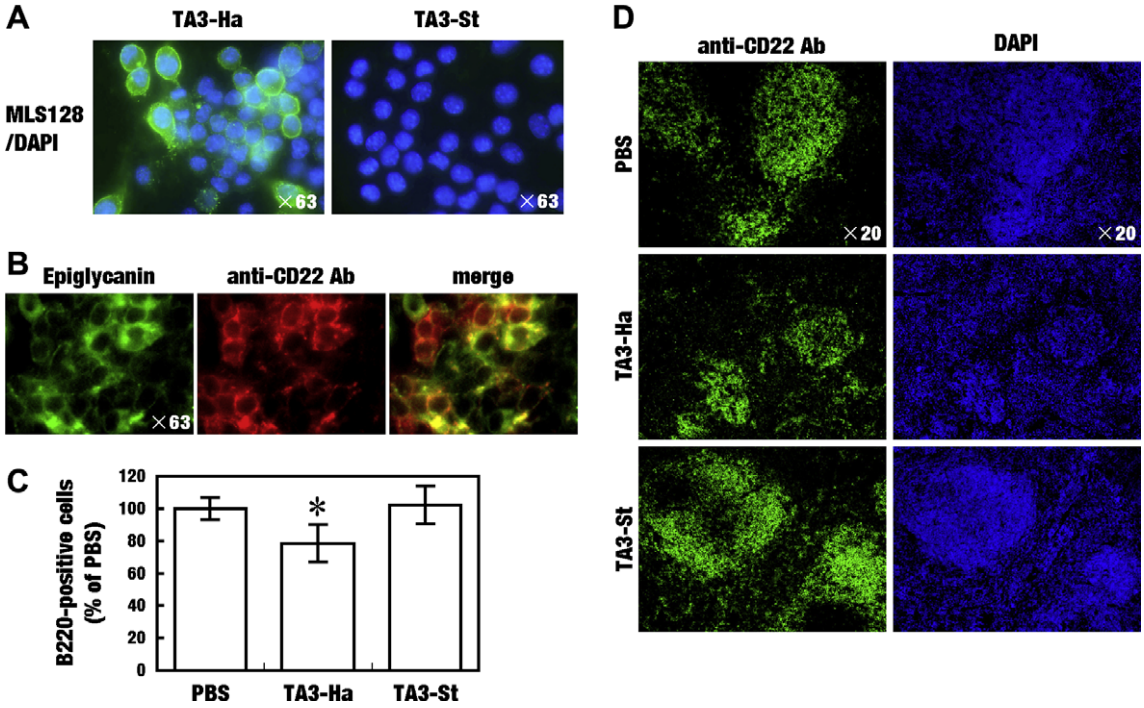

Fig. 4. In vivo effect of mucins in the tumor-bearing state. (A) TA3-Ha or TA3-St cells were stained with Alexa Fluor 488-conjugated MLS 128 mAb. Nuclei were stained with DAPI. (B) Spleens were resected from mice that had been treated with 4% paraformaldehyde 20 min after i.v. injection of Alexa Fluor 488-labeled epiglycanin. Sections were stained as described under Materials and methods. (C) Splenocytes were prepared from TA3-Ha or TA3-St-bearing mice on day 8 after tumor implantation, stained with FITC-conjugated anti-B220 Ab, and then analyzed by flow cytometry. The values in the histograms are percentages relative to that in a control experiment taken as 100% ($n = 6$, each). $^{*}P < 0.01$. (D) Spleen sections obtained from tumor-bearing mice were stained as described under Materials and methods.

the cell surface and can be detected in ascites fluid as well as in the bloodstream of TA3-Ha-bearing mice [22]. When Alexa Fluor 488-labeled epiglycanin was administered intravenously to normal mice, labeled epiglycanin was definitely detected in the spleen and associated with CD22-expressing B cells (Fig. 4B). To examine the effect of mucins in the tumor-bearing state, TA3-Ha or TA3-St cells were injected intraperitoneally into syngeneic strain A mice. On day 8 after tumor inoculation, splenocytes were prepared and B220-positive cells were quantitated by flow cytometry. As shown in Fig. 4C, similar levels of B220-positive cells were present in the splenocytes of TA3-St-bearing mice and control mice, whereas B220-positive cells were clearly reduced in those of TA3-Ha-bearing mice. A reduction of CD22-positive cells was also observed immunohistochemically in the spleens of TA3-Ha-bearing mice but not in those of TA3-St-bearing mice, and control mice (Fig. 4D). Similar impairment of splenic tissues has been observed in CD22 knockout mice and in mice expressing mutated CD22 molecules that lack ligand-binding activity [23,24]. It has also been demonstrated that splenic marginal zone B cells are especially reduced in these mice.

It would be interesting to determine which subpopulations of splenic B cells are reduced and how they are reduced in the tumor-bearing state. A study along these lines is underway.

Acknowledgments

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References

[1] Y.S. Kim, J. Gum Jr., I. Brockhausen, Mucin glycoproteins in neoplasia, Glycoconj. J. 13 (1996) 693–707.
[2] M. Miyake, T. Taki, S. Hitomi, S. Hakomori, Correlation of expression of H/Le(y)/Le(b) antigens with survival in patients with carcinoma of the lung, N. Engl. J. Med. 327 (1992) 14–18.
[3] D.J. McCool, J.F. Forstner, G.G. Forstner, Synthesis and secretion of mucin by the human colonic tumour cell line LS180, Biochem. J. 302 (Pt. 1) (1994) 111–118.
[4] T.F. Tedder, J. Tuscano, S. Sato, J.H. Kehrl, CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling, Annu. Rev. Immunol. 15 (1997) 481–504.
[5] E.R. Sjoberg, L.D. Powell, A. Klein, A. Varki, Natural ligands of the B cell adhesion molecule CD22 beta can be masked by 9-*O*-acetylation of sialic acids, J. Cell Biol. 126 (1994) 549–562.
[6] N. Razi, A. Varki, Masking and unmasking of the sialic acid-binding lectin activity of CD22 (Siglec-2) on B lymphocytes, Proc. Natl. Acad. Sci. USA 95 (1998) 7469–7474.
[7] C.P. Danzer, B.E. Collins, O. Blixt, J.C. Paulson, L. Nitschke, Transitional and marginal zone B cells have a high proportion of unmasked CD22: implications for BCR signaling, Int. Immunol. 15 (2003) 1137–1147.
[8] B.E. Collins, O. Blixt, A.R. DeSieno, N. Bovin, J.D. Marth, J.C. Paulson, Masking of CD22 by cis ligands does not prevent redistribution of CD22 to sites of cell contact, Proc. Natl. Acad. Sci. USA 101 (2004) 6104–6109.
[9] E.C. Brinkman-Van der Linden, A. Varki, New aspects of siglec binding specificities, including the significance of fucosylation and of the sialyl-Tn epitope. Sialic acid-binding immunoglobulin superfamily lectins, J. Biol. Chem. 275 (2000) 8625–8632.
[10] A. Varki, T. Angata, Siglecs—the major subfamily of I-type lectins, Glycobiology 16 (2006) 1R–27R.
[11] T. Inaba, H. Sano, Y. Kawahito, T. Hla, K. Akita, M. Toda, I. Yamashina, M. Inoue, H. Nakada, Induction of cyclooxygenase-2 in monocyte/macrophage by mucins secreted from colon cancer cells, Proc. Natl. Acad. Sci. USA 100 (2003) 2736–2741.
[12] G. Tettamanti, W. Pigman, Purification and characterization of bovine and ovine submaxillary mucins, Arch. Biochem. Biophys. 124 (1968) 41–50.
[13] N. Tanaka, H. Nakada, M. Inoue, I. Yamashina, Binding characteristics of an anti-Siaalpha2-6GalNAcalpha-Ser/Thr (sialyl Tn) monoclonal antibody (MLS 132), Eur. J. Biochem. 263 (1999) 27–32.
[14] P.A. van der Merwe, P.R. Crocker, M. Vinson, A.N. Barclay, R. Schauer, S. Kelm, Localization of the putative sialic acid-binding site on the immunoglobulin superfamily cell-surface molecule CD22, J. Biol. Chem. 271 (1996) 9273–9280.
[15] P. Engel, N. Wagner, A.S. Miller, T.F. Tedder, Identification of the ligand-binding domains of CD22, a member of the immunoglobulin superfamily that uniquely binds a sialic acid-dependent ligand, J. Exp. Med. 181 (1995) 1581–1586.
[16] M. Zhang, A. Varki, Cell surface sialic acids do not affect primary CD22 interactions with CD45 and surface IgM nor the rate of constitutive CD22 endocytosis, Glycobiology 14 (2004) 939–949.

[17] J.C. Poe, M. Fujimoto, P.J. Jansen, A.S. Miller, T.F. Tedder, CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux, J. Biol. Chem. 275 (2000) 17420–17427.
[18] R.M. Tooze, G.M. Doody, D.T. Fearon, Counterregulation by the coreceptors CD19 and CD22 of MAP kinase activation by membrane immunoglobulin, Immunity 7 (1997) 59–67.
[19] G.M. Doody, L.B. Justement, C.C. Delibrias, R.J. Matthews, J. Lin, M.L. Thomas, D.T. Fearon, A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP, Science 269 (1995) 242–244.
[20] J. Yu, T. Sawada, T. Adachi, X. Gao, H. Takematsu, Y. Kozutsumi, H. Ishida, M. Kiso, T. Tsubata, Synthetic glycan ligand excludes CD22 from antigen receptor-containing lipid rafts, Biochem. Biophys. Res. Commun. 360 (2007) 759–764.
[21] A. Lanoue, F.D. Batista, M. Stewart, M.S. Neuberger, Interaction of CD22 with alpha2,6-linked sialoglycoconjugates: innate recognition of self to dampen B cell autoreactivity, Eur. J. Immunol. 32 (2002) 348–355.
[22] A.G. Cooper, J.F. Codington, M.C. Brown, In vivo release of glycoprotein I from the Ha subline of TA3 murine tumor into ascites fluid and serum, Proc. Natl. Acad. Sci. USA 71 (1974) 1224–1228.
[23] T. Samardzic, D. Marinkovic, C.P. Danzer, J. Gerlach, L. Nitschke, T. Wirth, Reduction of marginal zone B cells in CD22-deficient mice, Eur. J. Immunol. 32 (2002) 561–567.
[24] J.C. Poe, Y. Fujimoto, M. Hasegawa, K.M. Haas, A.S. Miller, I.G. Sanford, C.B. Bock, M. Fujimoto, T.F. Tedder, CD22 regulates B lymphocyte function in vivo through both ligand-dependent and ligand-independent mechanisms, Nat. Immunol. 5 (2004) 1078–1087.