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Demonstration of the interaction between the CD23 molecule and the galactose residue of glycoproteins

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1. Summary

The CD23 molecule is a low-affinity receptor for IgE and has a marked homology in amino acid sequence with C-type animal lectins, including asialoglycoprotein receptor. We tested whether the CD23 antigen can indeed interact with the sugar chain of glycoproteins. Detergent extract of the membrane component from Epstein-Barr Virus (EBV)-transformed human B-cell line, L-KT9 cells, was incubated with asialofetuin (ASF)-coupled Sepharose, and bound proteins were effectively eluted by 0.3 M lactose or galactose which were among the competitive sugars tested. In this eluate, the CD23 molecule was detected by an immunoblotting technique. Because fetuin has both an N- and O-type sugar chain on the molecule, we tested which type of sugar chain can interact with CD23. The CD23 molecule interacted with asialocasein having a sugar chain with the Gal-GalNAc structure with asialobovine submaxillary mucin having the GalNAc structure, and also with ASF; however, it faintly interacted with ASF after removal of the O-type sugar chain by β -elimination. These results showed that the CD23 molecule can, indeed, interact with the galactose residue, especially with the Gal-GalNAc rather than the Gal-GlcNAc structure of the terminal sugar chain of glycoproteins.

Key words: CD23; Low-affinity Fc ϵ receptor II; Lectin activity; Galactose binding

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Abbreviations: ASF, asialofetuin; ASF-Sepharose, asialofetuin-coupled-Sepharose.

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2. Introduction

The CD23 molecule or Fc ϵ RII is a low-affinity receptor for IgE [1–3] and is a 46 kDa glycoprotein of 321 amino acid residues with an inverse membrane orientation: the N terminus is located in the cytoplasmic portion and the C terminus is exposed at the cell exterior [4]. The CD23 molecule showed a marked homology with a large family of calcium-dependent (C-type) animal lectins, including human and rat asialoglycoprotein receptors, chicken hepatic lectin, and rat mannose-binding proteins [4,5]. The homologous domain of CD23 to C-type lectins spans from cysteine 163 to cysteine 282. The IgE-binding region of CD23 corresponds almost exactly to this domain [6], but the IgE molecule interacts with CD23 by the peptide moiety but not by the sugar chain [7]. No clear evidence has been reported that the lectin domain of CD23 really interacts with carbohydrates, although one report suggested it. Richards and Katz [8] reported that CD23 bound differentially to immobilized monosaccharides (galactose, mannose and N-acetylgalactosamine) in a calcium-dependent manner. In this paper we investigated whether solubilized CD23 molecules from L-KT9 cells bound specifically to glycoproteins the sugar chain of which has a galactose in the terminal, especially with the Gal-GalNAc structure and were eluted by 0.3 M lactose. Thus, this paper directly demonstrates the lectin activity of the CD23 molecule.

3. Materials and Methods

3.1. Cells, antibodies and chemicals

An EBV-transformed human B lymphoma cell line, L-KT9, was a kind gift from Dr. A. Wakisaka (Department of Pathology, Hokkaido University

Questa fotocopia viene fornita
a scopo di studio e per uso
strettamente personale

School of Medicine) and was cultured in RPMI medium supplemented with heat-inactivated 10% fetal calf serum (FCS). Biotin-conjugated mouse monoclonal anti-CD23 antibody (BU-38, IgG1) was obtained from The Binding Site Limited (Birmingham, UK) and magnetic bead-coupled anti-CD23 antibody was obtained from Advanced Magnetic (Cambridge, MA). The following chemicals were obtained commercially: peroxidase-conjugated streptavidin (Zymed Laboratories, San Francisco, CA); silver reagent (Ag-STAIN DAI-ICHI, Dai-ichi, Tokyo, Japan); ECL western blotting detection system (Enhanced Chemiluminescence, Amersham UK); type-III fetuin and type-I bovine submaxillary mucin (Sigma, St. Louis, MO); and casein (Merck). Asialofetuin, asialocasein, and asialobovine submaxillary mucin were prepared according to Spiro [9]. To remove the O-type sugar chain from fetuin, β -elimination was done under the following conditions: 10 mg of ASF/ml of 0.1 N NaOH–0.8 M NaBH₄ at 40°C for 6 h. These glycoproteins were coupled to Sepharose in this laboratory according to Cuatrecasas and Anfinsen [10]. Sepharose was acetylated in dry pyridine and acetic anhydride (3:2) at room temperature for 1 day and then washed with dry toluene.

3.2. Solubilization of cell membrane and reaction with glycoprotein-coupled Sepharose

The membrane component from L-KT9 cells was detergent extracted according to Kijimoto-Ochiai et al. [11]. The 70 000 $\times$ g supernatant was incubated with glycoprotein-coupled Sepharose at 4°C for 2–6 h and washed stepwise with (1) 0.1% NP-40–PBS, (2) 0.1% NP-40–PBS–0.5 M NaCl, and (3) 10 mM Tris-HCl buffer, and then eluted with 0.3 M lactose–10 mM EDTA in PBS. The eluate was dialyzed against PBS, subjected to SDS-PAGE and analyzed by immunoblotting as follows.

3.3. SDS-PAGE and immunoblotting

The aliquots of the eluate from ASF-Sepharose were applied to mini slab gels of SDS-PAGE according to the method of Laemmli [12] under a non-reducing condition (without 2-mercaptoethanol) in order to maintain the intramolecular S-S bridge. The remaining eluate was further immunopurified with anti-CD23-conjugated magnetic beads. The beads were washed with 0.1% NP-40–PBS–0.5 M NaCl and 10 mM Tris-HCl buffer, boiled in SDS sample buffer [12] without 2-mercaptoethanol, and subjected to SDS-PAGE. The SDS-PAGE gels were

stained by silver reagent. For immunoblotting, the proteins on a gel were electrotransferred to a nitrocellulose membrane. The membrane was incubated in 10 mM Tris-HCl buffer (pH 7.5)–0.05% Tween–0.15 M NaCl with 2.5% skimmed milk and then with biotin-conjugated anti-CD23 antibody followed by incubation with horseradish peroxidase-conjugated streptavidin, and detected by ECL western blotting detection system.

4. Results

4.1. Binding of CD23 molecules to ASF-Sepharose

If the CD23 molecules can interact with galactose residues in the presence of Ca²⁺, CD23 will bind to ASF-Sepharose and will be eluted specifically by competitive sugars or by removing Ca²⁺ with EDTA. L-KT9 cells were solubilized with NP-40 and the 70 000 $\times$ g supernatant was incubated with ASF-Sepharose. Bound proteins were eluted with 0.3 M lactose as a competitive sugar in the presence of 10 mM EDTA–PBS to remove Ca²⁺ (Fig. 1). The eluted fraction was analyzed by SDS-PAGE and subsequently subjected to silver staining or immunoblotting analysis. Several components were detected by silver staining (Fig. 1, lane 1, marked by dots) in the eluate from ASF-Sepharose. After immunopurification of the eluate with anti-CD23-conjugated magnetic beads, a component of about 46 kDa was enriched as indicated (lane 2, $\triangleleft$). Only this 46 kDa component was specifically detected by the anti-CD23 immunoblotting technique (lane 5). In addition, this 46 kDa band was not detected by anti-CD23 immunoblotting when the eluate from ASF-Sepharose was immunopurified by isotype-matched antibody-conjugated magnetic beads (data not shown). This 46 kDa component was not derived from anti-CD23-conjugated magnetic beads but really from L-KT9 cell lysate, because this band was not detected in the eluate of anti-CD23-conjugated magnetic beads with immunostaining (lane 6). Thus, the 46 kDa molecule detected in lane 5 is most probably the complete form of the CD23 molecule the molecular weight of which has been reported as 46 000 [13]. These results show that the CD23 molecule can bind to ASF-Sepharose.

4.2. Elution from ASF-Sepharose

To examine a specific carbohydrate involved in the interaction of CD23 and asialofetuin, a cell lysate

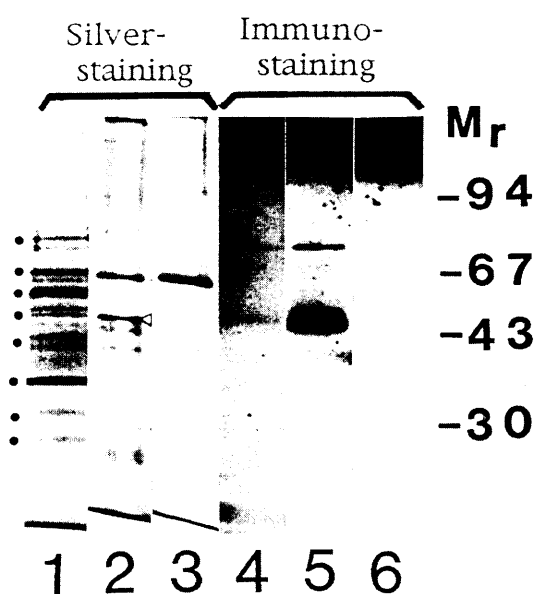


Fig. 1. Detection of CD23 in the bound fraction of ASF-Sepharose. About 2 ml of L-KT9 cell lysate ($70\,000 \times g$ supernatant, 2.5 mg protein/ml) was incubated at 4°C for 6 h with ASF-Sepharose (packed volume about 0.5 ml), washed with 3 types of buffer as described in Materials and Methods and eluted with 0.5 ml of 0.3 M lactose in the presence of 10 mM EDTA. The aliquots of eluate (30 μl) were separated on SDS-PAGE gels (lanes 1 and 4). In addition, the aliquots of eluate (210 μl) were further immunopurified with anti-CD23-coupled beads (25 μl of suspension), and the beads were washed with 3 types of buffer as for Sepharose gel and applied to SDS-PAGE (lanes 2 and 5). The same amounts of anti-CD23-coupled beads (25 μl for each lane) were also subjected to SDS-PAGE (lanes 3 and 6). After SDS-PAGE, the gels were stained by silver staining reagent (lanes 1–3) or the proteins on the gels were transferred to nitrocellulose membranes (lanes 4–6) and stained by biotin-conjugated anti-CD23, avidin-peroxidase and ECL western blotting reagent. The numbers on the right side show the positions of molecular weight markers.

containing CD23 was incubated with ASF-Sepharose and eluted with various carbohydrates. CD23 molecules in the eluates were immunopurified by anti-CD23-conjugated magnetic beads, subjected to SDS-PAGE and blotting. The presence of CD23 antigen on nitrocellulose sheet was then determined by immunostaining (Fig. 2). Lactose and then galactose (0.3 M) could effectively elute the CD23 molecule (Fig. 2, lanes 1 and 2), but N-acetylglucosamine, mannose and glucose eluted the CD23 molecule poorly (Fig. 2, lanes 3–6). EDTA (10 mM) alone also could elute the CD23 molecule effectively (lane 6). After EDTA elution, no CD23 molecule was eluted by 0.3 M lactose (lane 7).

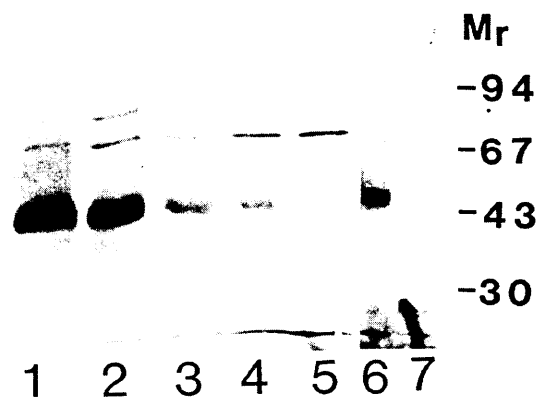


Fig. 2. Effect of various carbohydrates to elute the bound CD23 from ASF-Sepharose. L-KT9 cell lysate was incubated at 4°C for 2 h with ASF-Sepharose gel (lanes 1–6) (approximately 2 mg cell lysate per 0.2 ml of packed volume of Sepharose). The gel was eluted with 0.3 M carbohydrates or 10 mM EDTA after washing as described in Materials and Methods. The eluates were immunopurified by anti-CD23-coupled beads, subjected to SDS-PAGE and immunoblotting as described in Fig. 1. Carbohydrates used for elution were lactose (lane 1), galactose (lane 2), N-acetylglucosamine (lane 3), mannose (lane 4) or glucose (lane 5). ASF-Sepharose gel was also eluted with 10 mM EDTA alone (lane 6) then the gel was further eluted with 0.3 M lactose (lane 7).

4.3. CD23 molecule interacts with O-type sugar chain (Gal-GalNAc structure)

Fetuin has both N- and O-type sugar chains on the molecule. Generally, the N-type sugar chain has a Gal-GlcNAc-structure, while the O-type sugar chain has a Gal-GalNAc or GalNAc structure after removal of sialic acid. In order to know which type of sugar chain can interact with CD23, several glycoproteins having a known carbohydrate structure were coupled to Sepharose and incubated with cell lysate containing CD23. The CD23 molecule was detected by the immunoblotting technique used in Fig. 2. As shown in Fig. 3, the sugar chain of asialocasein having a Gal-GalNAc structure (lane 2) and of asialobovine submaxillary mucin having a GalNAc structure (lane 5) could bind with CD23. ASF (lane 3) also interacted with CD23, while the removal of an O-type sugar chain from ASF reduced the reactivity to CD23 (lane 4). The bound fraction of protein-uncoupled Sepharose gel also showed a faint band (lane 6), probably because Sepharose is a polymer of galactose derivatives. After the OH groups of Sepharose were acetylated, no band was seen (lane 7). These results showed that the CD23 molecule can interact with galactose residue especially with Gal-GalNAc or GalNAc structure rather than Gal-GlcNAc of the

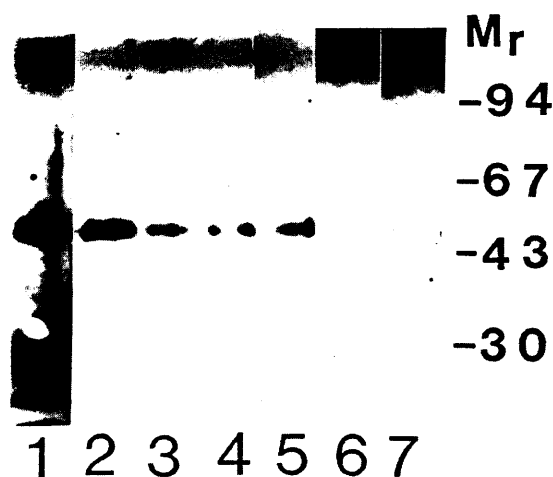


Fig. 3. CD23 mainly interacts with O-type sugar chains. L-KT9 cell lysate was incubated with various glycoprotein-coupled Sepharose (approximately 1 mg protein of the cell lysate per 0.1 ml of packed volume of Sepharose) at 4°C for 2 h and analyzed as in Fig. 2. Lane 1: the cell lysate was directly immunopurified without incubation with Sepharose gel. Lanes 2–5: Sepharose gel coupled with asialocasein (lane 2); ASF (lane 3); ASF- β -eliminated (lane 4); asialobovine submaxillary mucin (lane 5). Lane 6: Sepharose

terminal sugar chain of glycoproteins.

5. Discussion

Recently, many cell adhesion molecules have been reported to have a lectin domain in their molecules. The selectin family including L-, E- and P-selectin are all type-I membrane proteins and containing a 'C-type' lectin domain [14]. In some cases, for example gp90MEL, the lectin activity was demonstrated directly [15,16]. Each C-type lectin domain appears to have a highly restricted carbohydrate binding specificity [14].

With CD23, low-affinity IgE receptor, no clear evidence has been reported that CD23 interacts with carbohydrate. The present paper clearly demonstrates the binding of CD23 with the galactose residue of glycoproteins. In our system, CD23 antigen is not a major galactose-binding protein in L-KT9 cell lysate (Fig. 1, lane 1). A faint band was detected with immunostaining of CD23 molecule in the eluate from ASF-Sepharose (Fig. 1, lane 4). The CD23 molecule was specifically enriched with anti-CD23-conjugated magnetic beads (Fig. 1, lanes 2 and 5). The bands around 60–70 kDa observed in the immunoblots are probably the 'fibronectin-like' molecule reported to have cross-reactivity with the anti-eosinophil Fc ϵ RII

antibody [17] or vimentin reported to have cross-reactivity with BU-38 anti-CD23 antibody [18]. In addition, we clearly demonstrate the specificity of the carbohydrate chain which interacts with CD23 molecules. We showed that the CD23 molecule can interact with Gal-GalNAc rather than Gal-GlcNAc structures. This is also coincident with the results obtained by Richards and Katz [8] that CD23 interacted with galactose and GalNAc rather than GlcNAc in a calcium-dependent manner.

Although our results indicated that CD23 can interact with the galactose residue of asialoglycoprotein, the physiological importance of the interaction remains to be determined. It has been reported that IgE is a natural ligand for CD23. The carbohydrate moiety of IgE, however, is not involved with the binding to CD23, in spite of the fact that the IgE-binding region of Fc ϵ RII corresponds almost exactly to a domain of 123 amino acid residues homologous to the carbohydrate-binding domain of C-type animal lectins [6]. Indeed, it has recently been suggested that lectins may possess biologically important functions other than carbohydrate binding [19,20]. On the other hand, there are some papers supporting the presence of natural ligand for CD23 besides IgE. Recently it has been reported that CD21 is a natural ligand for CD23 [21,22]. It is uncertain, however, whether CD21 interacts with CD23 through the peptide moiety or sugar chain. Furthermore, Flores-Romo et al. [23] showed that human allogeneic mixed lymphocyte responses are blocked by anti-CD23 antibodies recognizing the lectin domain of the CD23 molecule. In this reaction, the B lymphoblastoid cell line and T-enriched population were used and thus the participation of IgE was excluded. There is no evidence, however, that a lectin-carbohydrate interaction is involved in this reaction. The ligand for CD23 in the mixed lymphocyte reaction has not yet been clarified. They assumed that the candidate molecule for the ligand would be on the responding T-cell surface. Our present paper has shown that CD23 can bind to the galactose residue of asialoglycoprotein. Therefore, it must now be shown whether this lectin activity of CD23 has a physiological importance in the interaction of CD23 with such putative receptors.

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