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Difference in CD22 molecules in human B cells and basophils.

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Abstract *Objective*. CD22 is believed to be restricted to normal and neoplastic B cells. Human basophils were found to express CD22 molecules. Among the antibodies against CD22, Leu14, which recognized the ligand binding domain reacted to basophils, and B3 and 4KB128, which recognized the amino terminus side and carboxy terminus side of the ligand binding epitope, respectively, did not. To clarify the difference of CD22 antigenicity in human B cells and basophils, we investigated RNA sequence and structures of CD22 molecules. *Materials and Methods*. Purified B cells and basophils were obtained from normal human volunteers by using a MACS magnetic cell sorting system and anti-CD19 and anti-FcεR1 antibodies, respectively. RT-PCR and sequencing of CD22 mRNA were performed in the exons 3 to 8. Western blotting analysis of CD22 was also performed. *Results*. The sequence of CD22 mRNA extracted from the basophils was the same as that of B cells in exons 3 to 8 (epitopes recognized by Leu14, B3 and 4KB128 were translated from exons 4 and 5). Reduced CD22 peptide extracted from the basophils reacted to Leu14 as well as B3 and 4KB128, and the molecular size of the reduced and non-reduced products was 130 kDa as expected. *Conclusion*. Disulfide bonds and the resulting 3D conformation of the CD22 molecules may have important roles in the difference of antigenicity of CD22β in B cells (CD22β1) and basophils (CD22β2). The difference in molecular structure surrounding the ligand-binding domain of CD22 may imply a specialization of the conformational forms of CD22 according to the ligand isoforms.

Key words: CD22, basophils, human, hematopoiesis, disulfide bond

Introduction

The sialoadhesin family of sialic acid binding proteins, which is one of the structurally related groups of the immunoglobulin (Ig) superfamily, includes carbohydrate recognition molecules, i.e., sialoadhesin (Sn), CD22, CD33, myelin-associated glycoprotein (MAG) and other members [1]. The human CD22 gene is located on chromosome 19q13.1 [2]. The predominant form of CD22 in humans (CD22β) [3] contains 7 Ig-like domains (IgLD1 to 7, mapped on exons IV to X of CD22 gene) in the extracellular region, a transmembrane region (exon XI), and an intracytoplasmic region (exons XII to XIV) containing 6 tyrosines, 4 of which are in SH2-binding YxxL motifs [4, 5]. The minor form of CD22 (CD22α) [6], a product of alternative splicing by exon skipping [2], contains 5 IgLD (IgLD1, 2, and 5 to 7) in the extracellular region. The amino terminus of the intracytoplasmic form of CD22 is capped by a signal peptide coded by exon III. The expected ligands for CD22 are CD45 isoforms [7]. CD22 is expected to be restricted to B cells [8-10]. CD22 is detected in the cytoplasm early in B cell development, and appears on the cell surface simultaneously with surface IgD. Cytoplasmic detection of CD22 is, therefore, one of the most important markers for diagnosing B-lineage acute lymphoblastic leukemia [11, 12].

We have observed the expression of CD22 in peripheral blood basophils in patients with basophilia accompanied by chronic myelogeneous leukemia and myelodysplastic syndrome with myelofibrosis using an anti-CD22 antibody, Leu14. However, normal human blood basophils did not react to an anti-CD22 antibody BL-B3C4 [13]. Han et al. recently reported that

human blood basophils react to some clones of monoclonal antibodies raised against human CD22 [14]. We then investigated the character and differences in human CD22 molecules expressed in B cells and basophils.

Materials and methods

Cells and cell sorting

Heparinized peripheral blood (PB) was obtained from normal healthy male adult volunteers after informed consent. Appropriate informed consent according to the institution's guidelines was obtained from the donors. Low-density cells (LDC) separated from 7ml of PB using density centrifugation were used for direct analysis of CD22 expression by flow cytometry. LDCs from 100ml of PB were used for purification of B cells and basophils by using monoclonal antibodies and a MACS™ magnetic cell sorting system (Miltenyi Biotec, Bergisch Gladbach, Germany) [13], and utilized for analysis of mRNA, surface and cytoplasmic CD22 expression by flow cytometry, and Western blotting. In brief, LDCs were washed once with Working Buffer (WB). The cell pellets were suspended in 1ml of WB, 50 μl of mouse myeloma proteins IgG1, IgG2a and IgM and incubated for 10 min at room temperature (RT) to block nonspecific binding of monoclonal antibodies. Then, 50 μl of anti-FcεR1 antibody (0.1 mg/ml, CRA1, IgG2b, Kyokuto Pharmaceutical, Ibaraki, Japan) and 50 μl of anti-CD19-MicroBeads™ (Miltenyi) were added, followed by incubation for an additional 25 min at RT, and one wash with WB. The cells were resuspended in 3ml of WB, applied to a MACS™ LS^+ separation column (Miltenyi), and sorted in the magnetic field of the Vario MACS™ separator (Miltenyi). The positive and negative fractions were utilized for purification of B cells and basophils, respectively. The CD19-positive B cells in the positive fraction were further purified twice using MS^+ separation columns in the magnetic field. Contaminated B cells in the negative fraction were further removed using a LD depletion column. The CD19-negative cells were suspended in 1ml of WB and 100 μl of biotinated goat anti-mouse IgG2b (Southern Biotechnology, Birmingham, AL), incubated for 25 min at RT, and washed once with WB. The cells were resuspended in 1ml of WB and 50 μl of Streptavidine-MicroBeads™ (Miltenyi), incubated for 20 min at RT, and washed once. The cells were resuspended in 3ml of WB, applied to a MACS™ LS^+ separation column, and the CRA1-positive fraction (basophils) was further purified twice using MS^+ separation columns.

Flow cytometry

Anti-CD3 (Leu4), anti-CD19 (Leu12), anti-CD22 (Leu14) and control IgG1 were purchased from Becton Dickinson (San Jose, CA). 4KB128 (anti-CD22, IgG1) was purchased from Dako (Glostrup, Denmark). 5.8HK (anti-CD22, IgG2b) was purchased from Exalpha (Boston, MA). B3 (anti-CD22, IgG2b) was purchased from Coulter (Hialeah, FL). D2 (anti-histamine, Hist, IgG1) was purchased from Biogenesis (Poole, England). BMK-13 (anti-major basic protein, MBP, IgG1) and SF25.5 (anti-eosinophil peroxidase, EP, IgG2a) were purchased from Nichirei (Tokyo, Japan). Bly3 (anti-CD19, IgM) was purchased from YLEM (Rome, Italy). Mouse myeloma proteins IgG1 (MOPC21), IgG2a (UPC10), IgG2b (MOPC141) and IgM (ABPC22) were purchased from Sigma (St. Louis, MO) and used as controls of monoclonal antibodies by an indirect staining method, and/or used to block nonspecific binding of monoclonal antibodies by a direct staining method. FITC-conjugated goat anti-mouse IgG1, IgG2a, PE-conjugated goat anti-mouse IgG1 and IgM were purchase from Southern Biotechnology, and used as 2^{nd} antibodies. 97A6 (anti-CD203c, a human basophil-related antibody, IgG1) was a kind gift from Dr. Hans-Joerg Buhring [15].

The purity of the sorted B cells and basophils was estimated by 2-color direct surface staining of CD3/CD19, indirect 2-color cytoplasmic staining of EP/MBP and EP/Hist or surface staining of CD203c/CD19 [13]. The sorted basophils were pre-incubated with MOPC141 for 10 min at RT to block the biotinated 2^{nd} antibody used for purification of the cells before staining. Fix&Perm™ cell permeabilization kits (Caltag Laboratories, Burlingame, CA) were used for cytoplasmic staining. For indirect cytoplasmic staining, the cells were fixed with reagent A (fixation medium) for 15 min at RT, washed with PBS, incubated with reagent B (permeabilization medium), anti-EP and anti-MBP or anti-Hist for 15 min at RT, washed with PBS, incubated with reagent B and FITC- and/or PE-conjugated 2^{nd} antibodies for 15 min at RT, washed, and then analyzed with a flow cytometer. For surface CD22 staining, the sorted cells were incubated with a PE-conjugated anti-CD22 for 30 min at

4°C, washed and analyzed. For cytoplasmic CD22 staining, the sorted cells were incubated with an unconjugated anti-CD22 for 30 min at 4°C to block surface CD22 antigens, washed, fixed with reagent A, washed, incubated with reagent B and PE-conjugated anti-CD22 of the same clone, washed, and then analyzed. Because unconjugated Leu14 was not available, Leu14-FITC was used to block surface CD22, Leu14-PE was used to stain cytoplasmic CD22, and then suitable compensation between FITC and PE was utilized for measuring the cytoplasmic fluorescence. For estimating the CD19-/CD22+ population, LDCs from normal human peripheral blood were directly stained with Leu12-FITC and PE-conjugated anti-CD22 in 5 male donors (age 30 to 34). To confirm the CD19-/Leu14+ cells were basophils, LDCs were directly stained with Leu14-FITC, CD203c-PE and Leu12-Per/CP in 3 male donors (age 29, 38 and 44). An acquisition gate was set at an enlarged lymphocyte area in a light scatter dot plot to include lymphocytes and basophils as reported previously [13]. Cell fluorescence was analyzed with a FACScan™ Flow Cytometer and CELLQuest™ software (B.D.). Five to fifty thousands events were acquired and analyzed.

Amplification and sequencing of the CD22 cDNA

RT-PCR and the sequencing were performed as reported previously [16]. In brief, total RNA of the sorted B cells and basophils was isolated by Trizol, and 5 μg was then utilized for the cDNA synthesis. The cDNA was synthesized from the total RNA of the cells with random primers and murine Moloney leukemia virus reverse transcriptase in a final volume of 20 μl. The CD22 cDNAs were amplified with AmpliTaq™ polymerase (Toyobo, Osaka, Japan). Each primer was used to determine the sequences around the ligand binding domain (exons III to VIII, Table 1 and Fig. 3) from 1 μl of the cDNA according to the following amplification profile: 35 cycles at 94°C for 60 seconds, 58°C for 90 seconds, and 73°C for 120 seconds. The amplified products were separated on 3% agarose gels and stained with ethidium bromide. Some of the PCR products were purified with microcon-100 (Millipore, Bedford, MA). The purified PCR products were sequenced with a TaqDye™ Deoxy terminator cycle sequencing kit (PE Applied Biosystems, Foster City, CA) and a DNA sequencer (model 310, PE Applied Biosystems).

Glycopeptidase treatment of intact cells

LDCs obtained from 2 normal volunteers were used to observe the antigenicity alteration of CD22 epitopes. One to 1.5 x 10^6 LDCs in 1ml of gelatin-veronal buffer (GVB^{2+}, pH 7.8) were incubated in the absence or presence of 50 units of recombinant neuraminidase (New England Biolabs, Beverly, MA) or 5 and 50 units of recombinant glycopeptidase F (Wako, Osaka, Japan) in a 37°C water bath for 30 min [17]. Deglycosilation was terminated by the addition of 10 ml cold WB, and the cells were pelleted. The cells were resuspended in 0.1 ml WB, with 20 μl of MOPC21, UPC10, MOPC141 and ABPC22 added, divided into 5 tubes, stained simultaneously with Leu12-FITC and PE-cinjugated anti-CD22 clones, and analyzed using a flow cytometer.

Western blotting of the CD22

Western blotting analysis of CD22 was utilized to estimate the role of disulfide bonds in the molecular structure and the antigenicity of CD22 epitopes. SDS-PAGE and Western blotting were performed as reported previously [18]. About 2 x 10^6 purified B cells or basophils sorted from normal human blood were solubilized by 100-400 μl of RIPA-A buffer containing 10 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml leupeptin. The cell lysate was vortexed for 3 min and then centrifuged at 4°C for 10 min at 16,000g. The aliquots of the cell lysate were combined with Laemmli's reducing or non-reducing buffer [19] and boiled for 3 min at 100°C. The samples were kept at –70°C until use.

The samples were separated by SDS-PAGE (5.0% gels) and analyzed by immunoblotting. Western blotting was performed using a modification of the method of Beardsley [20]. The blots were electrophoretically transferred to PVDF blotting membranes. Nonspecific binding was blocked with 5% low-fat milk powder in Tris-buffered saline (TBS). After extensive washing with TBS, the immunoblots were incubated overnight with anti-CD22 clones at appropriate concentrations in the blocking buffer at 4°C, then washed a further 3 times with TBS-T (0.05% Triton-X100 in TBS). Antibody binding was detected using horseradish-peroxidase-conjugated sheep anti-mouse IgG (Amersham, Little Chalfont, UK) and visualized

with ECL chemiluminescence reaction reagents and ECL-hyperfilm (Amersham).

Results

Surface and intracytoplasmic expression of CD22

The surface and cytoplasmic expressions of CD22 epitopes in the sorted B cells and basophils are shown in Fig. 1 and summarized in Table 2. Domain specificity of the anti-CD22 clones is shown in Table 2 as well [21]. The same experiments were carried out in 4 normal male adult volunteers (age, 28 to 30). The percentages of CD3+ cells and CD19+ cells in the sorted B cells and basophils were 0.1 $\pm$ 0.02% and 99.3 $\pm$ 0.19% in the B cell samples, and 0.1 $\pm$ 0.09% and 0.1 $\pm$ 0.07% in the basophil samples, respectively. The MBP-positive and histamine-positive percentages in the sorted basophil samples were 95.6 $\pm$ 3.17% and 84.0 $\pm$ 8.76%, respectively. The surface and cytoplasmic expressions of epitopes recognized by Leu14, 4KB128 and 5.8HK were intensive in the sorted B cells. In contrast, the fluorescence by B3 was high on the surface and low in the cytoplasm in the same samples. The epitope recognized by the B3 clone may have been hidden in part by the capping of signal peptides in the cytoplasm. The fluorescence of the surface epitope recognized by Leu14 (the ligand binding interface between IgLD1 and IgLD2) and 5.8HK was high in the sorted basophils, while that of 4KB128 (IgLD2) and B3 (IgLD1) was very low in the same samples. The fluorescence of cytoplasmic CD22 epitopes was lower than that of surface epitopes in the basophils, and the propensity of the fluorescence difference in the different epitopes was the same. Therefore, it is possible that the antigen structures of CD22 around the ligand binding domain, but not the ligand-binding interface itself, in the basophils are different from those in B lymphocytes in humans.

The antibody against FcεR1 possibly stimulated human basophils, and the surface expression of CD22 may have been modified by the stimulation. We therefore studied the CD22 expression in CD19-negative cells directly in the LDCs. The $Leu14^{+}/CD19^{-}$ cells expressed CD203c (Fig.2-I). The epitopes recognized by clones Leu14 or 5.8HK were fully expressed, and those recognized by 4KB128 or B3 were reduced or absent in CD19-negative cells, thought to be basophils, as shown in Fig.2-II and summarized in Table 2. The data were compatible to the observations in the sorted basophils.

RT-PCR analysis of CD22

In the first instance, we considered whether the alteration of antigenicity of CD22 in human basophils was caused by the lack of epitopes recognized by the 4KB128 and B3 clones by alternative splicing as exon skipping or cryptic splicing. We then performed RT-PCR analysis of CD22 mRNA. The data of the CD22 RT-PCR analysis are shown in Fig. 3. The same experiments were carried out in 5 normal male adult volunteers (age 29 to 32). The percentages of CD3+ cells and CD19+ cells in the sorted B cell samples and basophil samples were 1.7 $\pm$ 2.63% and 97.1 $\pm$ 3.64% in the B cell samples, and 0.1 $\pm$ 0.17% and 0.3 $\pm$ 0.57% in the basophil samples, respectively. The CD203c-positive percentages in the sorted basophil samples were 59.2 $\pm$ 30.45%. The expected sizes of CD22β in lanes 1 to 4 in Fig. 3 were 1,424, 1,025, 907 and 489, and the sizes of CD22α in lanes 1, 2 and 3 were 893, 494 and 376 bp, respectively. The observed sizes of the CD22β bands in lanes 1 to 4 were almost the same in the sorted B cells and basophils, therefore alternative splicing longer than 100bp was not expected in basophil CD22β.

We then analyzed the sequences of the CD22β bands in lanes 1 to 4 of the sorted B cells and basophils. The CD22β PCR products in lanes 1 to 4 (exons III to VIII) were purified from 2 different samples of the 5 normal donors, and sequenced using all the primers in Table 1. The sequences of the purified and amplified mRNA of CD22β in the B cells and basophils were the same as in the previous report [3], and no alternative splicing was observed in either of the samples examined (data not shown).

Deglycosilation of intact cells

We then considered the possible loss of antigenicity of the epitopes in the highly N-glycosilated molecule, CD22, by different sugar moiety in human basophils. The reactivity of LDCs to anti-CD22 clones, Leu14, 4KB128, 5.8HK and B3, was not altered by the treatment using 50 units of neuraminidase or 5 and 50 units of glycopeptidase F, i.e., the flow cytometric analysis showed the same data as the non-treated cells shown in Fig. 2 (data not shown).

Western blotting of CD22 proteins and their reactivity to antibodies

Although it was possible that the treatment of

the LDCs by neuraminidase or glycopeptidase F was insufficient to expose the antigenic epitopes, we then considered the possible different conformation of CD22 molecules in human B cells and basophils, which was significantly affected by disulfide linkages in the molecule just like the relationship between epitope antigenicity and the 3-dimensional conformation of MAG [22]. The same experiment was performed in 2 individuals. The percentages of CD3+ , CD19+ and CD203c+ cells in the sorted B cells and basophils samples from two individuals were as follows: 0.0 and 0.0% CD3+ cells, 0.3 and 0.6% CD19+ cells and 56.9 and 52.8% CD203c+ cells in the basophil samples, and 0.0 and 0.1% CD3+ cells and 99.9 and 99.1% CD19+ cells in the B cell samples. The reduced and non-reduced proteins extracted from the purified B cells and basophils reacted similarly to Leu14 (Fig 4). The non-reduced proteins extracted from the purified basophils reacted weakly to 4KB128 and faintly to B3. The reactivity of the basophil CD22 to 4KB128 and B3 was obviously, but not fully, recovered by reduction of the proteins.

The observed size of non-reduced protein in the SDS-PAGE was almost 130 kDa, and the size of reduced protein was slightly longer as reported in a previous article [23]. Therefore, the naked glycoprotein itself, but not a complex of CD22 and other molecules, had the different antigenicity to anti-CD22 clones. And therefore, the major CD22 molecules (CD22β) in human have conformationally different forms, i.e., CD22β1 in B cells and CD22β2 in basophils.

Discussion

The expected mechanism of the different antigenicity of CD22 molecules expressed in human B cells and basophils was as follows: 1) hidden epitopes by other membrane components as lipids, sugar chains or combined polypeptides; 2) loss of epitopes by alternative splicing as exon skipping or cryptic splicing; 3) hidden epitopes by different 3D conformation of the peptide itself; 4) and other possible reasons. The reported sizes of unreduced CD22β and CD22α were 130 and 120 kDa, and those of reduced ones were 140 and 130 kDa, respectively [23]. The epitopes recognized by clones Leu14, 4KB128 and B3 are translated from exons 4 and 5. No alternative splicing of major and full length CD22, CD22β, were observed in exons 3 to 8. The antigenicity of CD22 in deglycosilated intact basophils by treatment with glycopeptidase F or neuraminidase was not altered. The antigenicity of intracytoplasmic basophil CD22 was basically the same as that of surface. Therefore, the expected mechanism of masked epitopes by other membrane components does not explain the observations. Moreover, the observed sizes of CD22 in the SDS-PAGE were 130 to 140 kDa. Hence, naked CD22 molecules themselves have the different antigenicity. The difference in the antigenicity of CD22 molecules arises from inside of the molecules but not from outside.

Mouse monoclonal antibodies against human CD22 are raised to react to CD22 expressed on human B cells. Immunological analysis of the surface expressed CD22 in human B lymphocytes and basophils revealed that some clones against CD22 reacted to human basophils, and others did not. A clone recognizing the ligand binding interface between IgLDs 1 and 2, Leu14, reacted to human basophil CD22, while 2 clones which recognize the membrane-distal side and membrane-proximal side of the ligand binding interface, IgLD1 and IgLD2, clones B3 and 4KB128, respectively, did not react with the human basophils. Although the epitope recognized by the clone 5.8HK is not known, the clone may react to the ligand binding interface or an epitope which is located far from the ligand binding domain, IgLDs 3 to 7. Some of the expected ligands for B cell CD22, CD45 isoforms are, therefore, expected to react to basophil CD22 as well. CD22α is an isoform of CD22, which is a product of alternative splicing by exon skipping. CD22β expressed in B cells, CD22β1, is a full-length product of the CD22 gene. We have found a novel conformational form of CD22β expressed on human basophils, CD22β2, which is also a product of the CD22 gene without alternative splicing between exons 3 to 8. Human basophils also expressed CD22α mRNA; therefore there might be another conformational form of CD22, CD22α2. These 4 possible isoforms and conformational forms of CD22 may appropriately choose different isoforms of the ligand according to the types of effector and target cells, or the situation and circumstance of an inflammation.

Sn, CD22, CD33 and MAG have a variable Ig domain (V in Fig. 5) at the amino terminus and 1 to 16 C2 domains [1]. C2 domains have features intermediate between variable (V) and constant (C) Ig domains in containing the cysteine spacing and seven β strands typical of C domains, but sequences which are more

homologous to V domains [22]. The binding site of the ligands in CD22, CD33 and MAG is arginine of RGD motif in murine MAG and RME motif in human CD22 and CD33 between IgLD1 (V) and IgLD2 (C2). Each of IgLD1 and IgLD2 has 3 cycteines, 2 of them make an intradomain disulfide bond and the rest make interdomain disulfide linkage between IgLD1 and IgLD2 (Fig. 5-A) [24]. Some of the monoclonal antibodies raised against synthesized polypeptide fragments of MAG did not react to native MAG, and the lost accessibility of MAG epitopes to the antibodies recovered in the reduced MAG protein by cutting the disulfide bonds off [24]. CD22β1 and CD22β2 had the same sequence from exons 3 to 8, and therefore from the signal peptide and IgLD1 to IgLD5. The antigenicity of the CD22β2 epitopes against 4KB128 and B3 recovered after reduction of the protein. Therefore, disulfide bonds and the resulting 3 dimensional conformation of CD22 are thought to have an important role in the antigenicity of the epitopes (Fig. 5-A). The mechanism of the possible difference of CD22 conformation is not known. Chaperone proteins in the cytoplasm bind to immature or unfolded polypeptides and ensure correct folding or transport, and therefore they are thought to have an important role in the 3D-conformation of multidomain proteins [25]. Heat-shock proteins (hsp) are an important subset of chaperones, and chaperone proteins consist of the chaperonins (groEL and hsp60), the hsp70 family, the hsp90 family, and other proteins. Although the chaperones utilized for CD22 conformation are not known, the difference of chaperones may rightly explain the mechanism of CD22 conformation in B cells and basophils by choosing different sheets of the protein structure. If so, some of antigens simultaneously expressed in human B cells and basophils may also have conformationally different forms through the same mechanisms of the chaperone machinery.

The receptor of B lymphocyte to an antigen is B cell receptor (BCR). BCR activated by an antigen transmits signals through two heterodimers of CD79a and CD79b analogous to the relationship of T cell receptor and CD3 (Fig 5-C). Activated CD22 in B lymphocytes is expected to down-modulate the activation threshold of BCR through its association with PTP-1C (SHP-1) and other signaling molecules [4, 9]. IgE, the receptor to an allergic antigen, binds to FcεR1 on basophils, and transmits signals through a heterotetramer of the components of FcεR1 (αβγ2) [26]. Therefore, CD22 expressed in basophils may also down-modulate the activation threshold of FcεR1.

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Table 1 Primer sequence of CD22 for RT-PCR.

Primer Name	Sequence
CD22 3-5'	gacacgacaccatgcatctc (exon III)
CD22 4-5'	acctcaatgacagtggtcagc (exon IV)
CD22 5-5'	agaaattcaagagtcccaggaagt (exon V)
CD22 6-5'	cagaatacattcacgctaaacctg (exon VI)
CD22 6-3'	tgcacttgcaggaacacttc (exon VI)
CD22 8-3'	aacactggggttactggaattgta (exon VIII)

Table 2 Expression of epitopes recognized by anti-human CD22 clones in B cells and basophils.

clone	epitope	% of CD19-/CD22+ cells in sLDC (n=5)	rMFI of sCD22-PE (n=4)		rMFI of cCD22-PE (n=4)	
			in B cells	in basophils	in B cells	in basophils
control		0.1 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00
Leu14	IgLD1-2	0.9 ± 0.73	65.9 ± 11.36	17.6 ± 3.20	10.6 ± 5.20	3.5 ± 1.28
4KB128	IgLD2	0.2 ± 0.05	62.0 ± 10.87	2.3 ± 1.01	15.0 ± 6.75	1.9 ± 0.81
5.8HK	?	0.9 ± 0.56	37.5 ± 7.30	15.6 ± 4.33	6.7 ± 3.41	2.4 ± 0.86
B3	IgLD1	0.1 ± 0.05	35.9 ± 7.10	2.5 ± 0.15	2.2 ± 0.93	2.0 ± 0.59

IgLD1 and 2, Ig-like domains 1 and 2, respectively; IgLD1-2, reported to block the binding of the ligand; sLDC, small low-density peripheral blood cells with light scattering properties including lymphocytes and basophils; rMFI, relative mean fluorescence intensity, MFI of CD22 / MFI of isotype control.

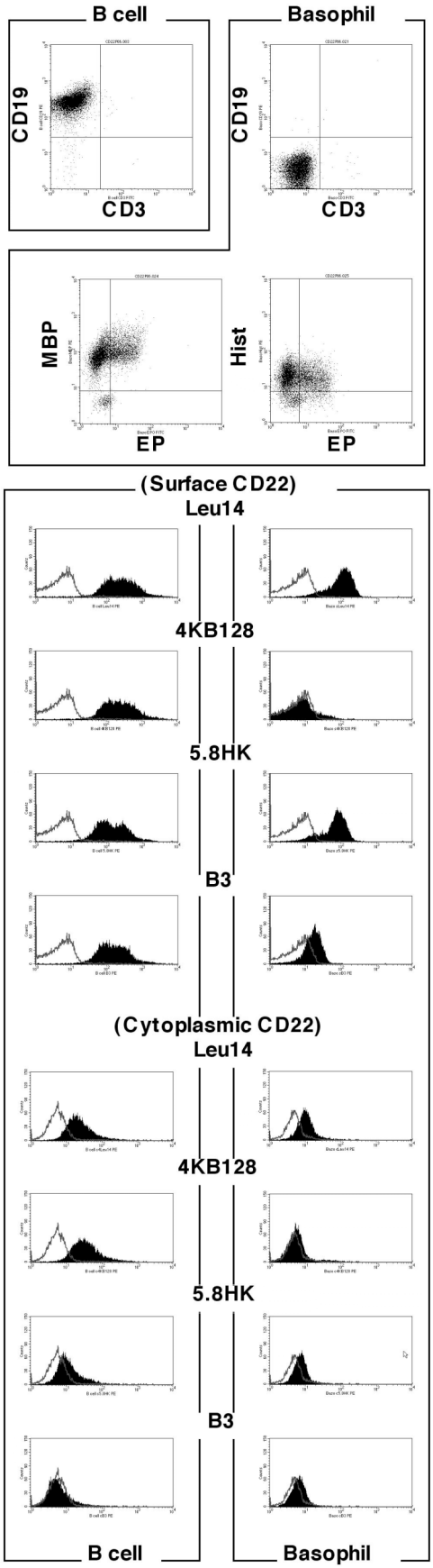

Figure 1. Extracellular and intracytoplasmic expressions of CD22 in the purified B cells and basophils from normal human peripheral blood. The purity of the samples is demonstrated with 2 color dot plots of CD3/CD19, EP/MBP and EP/Hist. CD3, Leu4; CD19, Leu12; EP, eosinophil peroxidase; MBP, major basic protein; Hist, histamine.

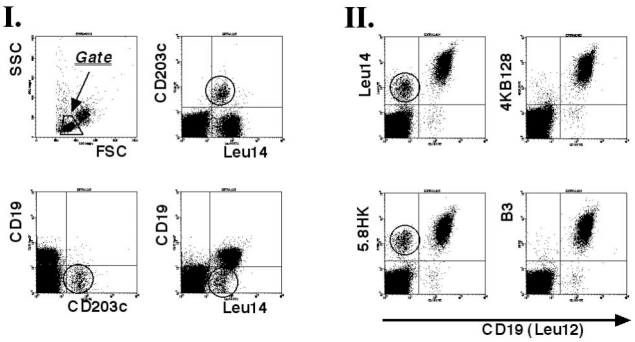

Figure 2. CD22 expression in CD19-positive and CD19-negative cells analyzed directly in low-density cells from normal human peripheral blood. Panels I, 3-color analysis of Leu14-FITC/97A6 (anti-CD203c)-PE/Leu12 (anti-CD19)-Per/CP, and panels II, 2-color analysis of CD19-FITC/CD22-PE.

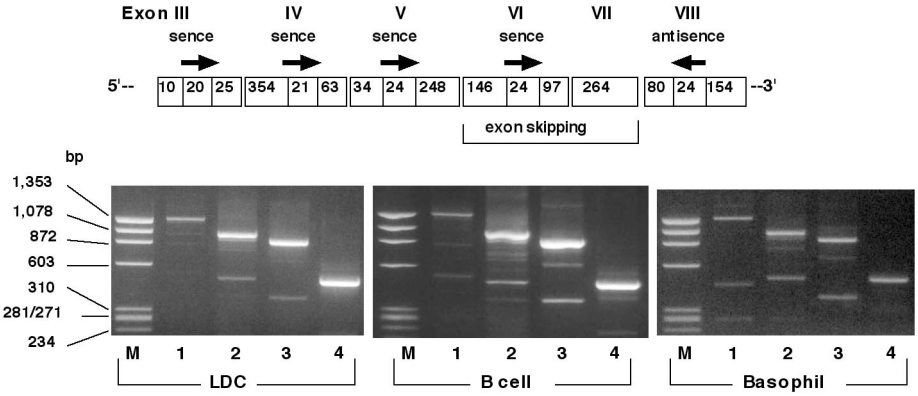

Figure 3. RT-PCR for the CD22 mRNA extracted from low density cells (LDC), purified CD19-positive B cells and purified FcεR1-positive basophils from normal human peripheral blood. Lane M, markers (1,353, 1,078, 872, 603, 310, 281/271 and 234 bp). Lanes 1, 2, 3 and 4, RT-PCR products using primers in exons III, IV, V and VI as sense primers and in exon VIII as an antisense primer in Table 1, respectively. LDC, low-density cells.

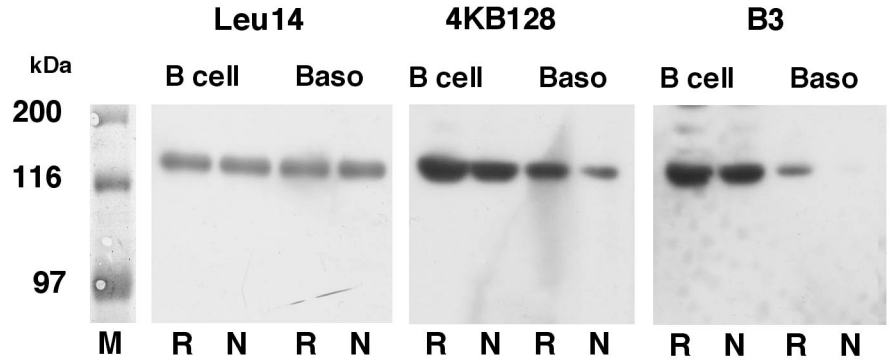

Figure 4. Western blotting of CD22 glycoproteins extracted from purified CD19-positive B cells and purified FcεR1-positive basophils from normal human peripheral blood. Lane M, markers (200, 116 and 97 kDa). Lanes R and N, reduced and non-reduced proteins, respectively.

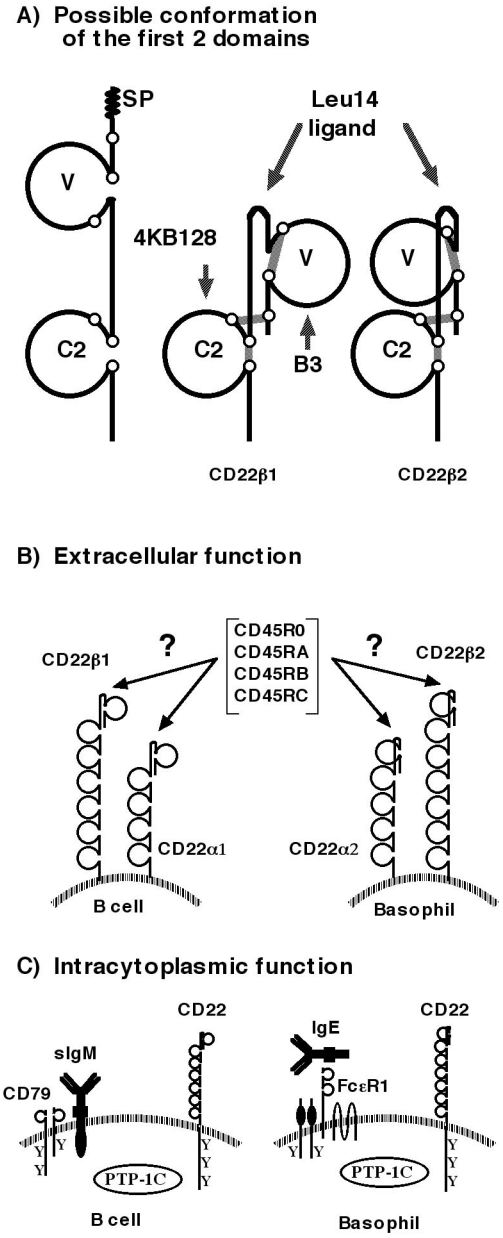

Figure 5. Possible structures of conformational forms of CD22 and future problems in studying CD22 biology. A) The epitope recognized by B3 is masked by signal peptide (SP) in the cytoplasm. The epitopes recognized by B3, Leu14 and 4KB128 are exposed in the full length form of B cell surface CD22 (CD22β1), while those by B3 and 4KB128 are possibly masked by its conformation in basophil surface CD22 (CD22β2). V and C2, Ig like domains 1 and 2, respectively; open circles, cysteines; gray lines, disulfide bonds. B) Analogy of extracellular function of CD22. The expected ligands for B cell CD22 are the isoforms of CD45R in T cells. C) Intracytoplasmic function of CD22. CD22 down-modulates the B cell activation threshold, presumably through its association with PTP-1C (SHP-1) and other signaling molecules. Physical interaction of CD40L, TCR and CD45R in T cells and CD40, HLA-DR and CD22 in B cells co-stimulates the signal of B cell receptor (BCR) by allergen through CD79α/β. Physical interaction of CD40L in basophils and CD40 in B cells also induces IgE production by allergen-activated B cells [27]. Allergen-specific IgE combines with FcεR1 in basophils to react to allergen, and then basophil-involved circuit of allergy reaction is closed.