Demonstration of a Second Ligand for the Low Affinity Receptor for Immunoglobulin E (CD23) Using Recombinant CD23 Reconstituted into Fluorescent Liposomes

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Summary

Recombinant full-length human CD23 has been incorporated into fluorescent liposomes to demonstrate the existence of a ligand for CD23 that is different from the previously known ligand, immunoglobulin E (IgE). The novel ligand for CD23 is expressed on subsets of normal T cells and B cells as well as on some myeloma cell lines. The interaction of full-length CD23 with its ligand is specifically inhibited by anti-CD23 monoclonal antibodies and by IgE, and it is Ca^{2+} dependent. Moreover, tunicamycin treatment of a CD23-binding cell line, RPMI 8226, significantly reduced the binding of CD23 incorporated into fluorescent liposomes, and a sugar, fucose-1-phosphate, was found to inhibit CD23-liposome binding to RPMI 8226 cells, suggesting the contribution of sugar structures on the CD23 ligand. In addition, CD23-transfected COS cells were shown to form specific conjugates with the cell line RPMI 8226. These data demonstrate that CD23 interacts with a ligand, which is different from IgE, and that CD23 can be considered as a new surface adhesion molecule involved in cell-cell interactions.

CD23 is a type II transmembrane protein expressed on a wide variety of hemopoietic cell types (1–3). CD23 exists in two forms, *a* and *b*, differing within their intracytoplasmic domains. The *a* form is expressed exclusively on B lymphocytes, whereas the *b* form is expressed on both B cells and other cell types (4).

Studies using anti-CD23 antibodies and soluble CD23 fragments have implicated this molecule in the regulation of IgE synthesis (5, 6). Membrane CD23 has been shown to mediate IgE-dependent antigen focusing (7).

An increasing number of reports have demonstrated that CD23 also plays a role in non-IgE-related activities such as B cell growth (8, 9), germinal center B cell survival (10), prothymocyte maturation (11), myeloid precursor proliferation (12), and antigen presentation (13). This latter observation, together with the earlier finding that CD23 was spatially associated with the MHC class II molecule HLA-DR on the B cell surface (14), suggested that CD23 may be involved in T cell–B cell interactions. Indeed, we recently found that certain anti-CD23 antibodies blocked T cell–B cell conjugate formation in vitro (J. Shields, J.-P. Aubry, D. Estoppey, and J.-Y. Bonnefoy, manuscript submitted for publication).

These findings led us to investigate if CD23 interacts with a membrane structure that is different from IgE, the only known CD23 ligand (15, 16). Using recombinant CD23 incorporated into fluorescent liposomes, the present study demonstrates the existence of a membrane-bound ligand for CD23.

Materials and Methods

Cell Lines

The B cell lines Daudi, RPMI 8226, U266-B1, and IM-9, Jijoye; the T cell lines Molt-4 and Hut 78; the promyelomonocytic cell line HL-60; the histiocytic lymphoma cell line U-937; the chronic myelogenous leukemia K-562; the astrocytoma cell line U-373; and the epidermoid carcinoma Hep-2 were all obtained from the American Type Culture Collection (ATCC), Rockville, MD. The T cell line Jurkat was provided by Dr. A. Bernard (Hôpital de l'Archet, Nice, France). The B cell lymphoma HFB1 was obtained from Dr. R. Callard (University of London, London, UK) and was transfected with CD23 full-length cDNA (a gift of Dr. H. Kikutani, Osaka University, Osaka, Japan) giving HFB1 2/3 (17). COS-CD23 transfectants were obtained by subcloning the CD23 cDNA into the pCDM8 expression vector (Invitrogen, San Diego, CA) and transfecting the resulting expression vector into COS M6 cells (a gift of Dr. I. Stamenkovic, Massachusetts General Hospital, Boston, MA) using DEAE dextran as previously described (18). The Burkitt lymphoma cell line BL2 was obtained from Dr. G. Lenoir (International Agency for Research on Cancer, Lyon, France). The myeloma cell line U266 was obtained from Dr. J. P.

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Revillard (INSERM U80, Lyon, France). The EBV-transformed lymphoblastoid line RPMI 8866 was obtained from Dr. K. Ishizaka (Johns Hopkins University School of Medicine, Baltimore, MD). Cell lines were cultured in RPMI 1640 (Seromed, Berlin, Germany) supplemented with 2 mM glutamine and 10% heat-inactivated FCS (Flow Laboratories, Irvine, Scotland). All cell lines were mycoplasma free. Tonsil mononuclear cells were separated into T and B cell subpopulations (referred to as E^+ and E^-, respectively) by rosetting with AET-treated sheep red blood cells. The germinal center B cells (GCC) were enriched according to the method of Liu et al. (19) with the added modifications of Graber et al. (20). Human umbilical vein endothelial cells (HUVEC) were prepared as previously reported (21). For tunicamycin treatment, B cell lines IM-9 and RPMI 8226 were cultured 48 h in complete medium containing 0.8 μg/ml of tunicamycin (Boehringer Mannheim, Mannheim, Germany). The binding of CD23 liposomes and control liposomes was compared on tumicamycin-treated and non-treated cells by flow cytometric analysis.

mAbs

Anti-CD23 mAbs were kindly provided by P. Beverley (22) (LA1, IgM), by Dr. B. Sugden (23) (EBVCS 1, IgG1), and by Dr. J. Gordon (MHM6, IgG1). The anti-CD23 mAb 25 (16) (IOB8, IgG1) was purchased from Immunotech (Luminy, France). The isotype-matched controls, IgG1, and IgM were purchased from Becton Dickinson & Co. (Mountain View, CA). Sheep FITC-conjugated $(Fab')_2$ antibodies to mouse IgG and IgM were obtained from Grub (Vienna, Austria). Human IgE myeloma protein (The Binding Site Ltd., Birmingham, UK) was purified as previously described (24). The polyclonal antibody Rb55 was obtained by immunization of a rabbit with a CD23 fragment (amino acid position 150–321 of the CD23 sequence) expressed in *Escherichia coli*.

Recombinant Full-Length CD23 Purification

The 45-kD CD23 *a* form was obtained from Sf9 cell cultures infected with the recombinant baculovirus clone B1 as already described (9). 10^{10} cells were harvested 3 d postinfection by gentle centrifugation of 1,000 *g* for 15 min. The pelleted cells were resuspended in 50 ml of buffer A: 10 mM Tris-HCl, 1 mM TLCK, 1 mM benzamidine, 1 mM PMSF, 10 mM iodoacetamide, pH 7.8, and broken by two passages through an Amicon French pressure cell (Kontron, Basel, Switzerland). The suspension was sonicated, diluted twofold in buffer B: 0.1 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.5 M sucrose, added to buffer A, and centrifuged at 22,500 *g* for 30 min. The pellet was resuspended in buffer C: 1 mM $CaCl_2$, 1 mM $MgCl_2$ in PBS_{240} (240 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.2 mM TLCK, 10 mM iodoacetamide, pH 7.1), mixed with an equal volume of absolute ethanol at −20°C, agitated, and incubated 30 min at room temperature. The ethanol precipitate was then centrifuged 10 min at 3,000 *g*. The membranes were resuspended in 25 ml of buffer C, ethanol precipitated a second time, and redissolved in buffer D: 1% Triton X-100 in PBS_{240}. The detergent extract was incubated overnight at 4°C and centrifuged at 150,000 *g* for 60 min. The solubilized material was immunopurified on a mAb 25-Affigel 10 column (Bio-Rad Laboratories A.G., Glattbrugg, Switzerland) with 3 mg mAb 25 coupled per ml resin at 5 ml/h with constant recycling for 24 h. The immunoaffinity resin was washed with 200 ml buffer D followed by a 200 ml of buffer E: 0.1% Triton X-100 in PBS_{240}. In the third wash (200 ml), Triton X-100 detergent in buffer E was exchanged by 50 mM octyl-β-glucopyranoside (OGP)[1] and 45-kD CD23 was eluted with 50 ml PBS (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.1), 50 mM OGP, 3 M NH_4SCN, pH 6.5. The protein was desalted on a Superdex-200 XK16/60 gel filtration column (Pharmacia, Uppsala, Sweden) run in PBS, 50 mM OGP.

Densitometric scanning of silver-stained SDS-PAGE and Western blotting analysis of the proteins transferred onto a nitrocellulose membrane were used throughout the procedure to follow the purification as already described (20), except that the membrane was immunostained with the polyclonal antibody Rb55.

Liposome Preparation

10 μmol of the synthetic phospholipids POPC (Avanti Polarlipids Inc., Alabaster, AL) in $CHCl_3$ were mixed with 50 nmol of the fluorescent label DiO_{18} (Molecular Probes Inc., Eugene, OR) in EtOH. The solvents were evaporated under a nitrogen stream and the lipids were further dried in a vacuum centrifuge for 30 min. The dried lipids were then completely solubilized in 50 μl of 800 mM OGP in PBS. The detergent concentration was lowered to 200 mM with 150 μl of PBS and 0.2 nmol of the protein (CD23 or glycophorin A [Calbiochem, Lucerne, Switzerland] as control) was added in 800 μl of 50 mM OGP in PBS, at 4°C. The mixture was incubated 1 h at 4°C, filtered through a sterile 0.2 μm Acrodisc 13 (Gelman Sciences, Ann Arbor, MI), and extensively dialyzed in a Spectra/Por membrane (Spectrum Medical Industries, Inc., Los Angeles, CA) (molecular mass cut-off, 12–14 × 10^3 kD; dry thickness, 0.0011 in) against several changes of 140 mM NaCl, 20 mM Hepes, pH 7, for 48 h.

Control liposomes were prepared in the same way except that there was no protein added. Fluorescent liposomes were then ready for flow cytometric studies.

Flow Cytometric Analysis

Liposome Binding Assays. Cells (10^5) were resuspended in 50 μl of the liposome suspension diluted 10 times (or 40 times in the experiments with tunicamycin) in 0.5% BSA, 0.1% azide, 2 mM $CaCl_2$, 140 mM NaCl, 20 mM Hepes, pH 7, and incubated for 2 h at 4°C. Cells were washed twice before analysis.

Inhibition with sugars: glucose, galactose, mannose, cellobiose, lactose, mannobiose, *N, N'*-diacetylchitobiose, glucose-1-phosphate, and fucose-1-phosphate (all from Sigma Chemical Co., St. Louis, MO) have been tested between 0.02 and 50 mM in the buffer mentioned above, for their ability to prevent CD23-liposomes from binding to RPMI 8226 for 2 h at 4°C.

Conjugate Formation. COS cells or RPMI 8226 and Molt-4 were labeled with BCECF-AM at 10 ng/ml and Indo-1-AM at 1 μg/ml, respectively (both from Calbiochem), for 35 min at 37°C, washed three times, and resuspended at 5 × 10^5/ml. Equal volumes of COS cells and RPMI 8226 or Molt-4 cells (0.5 ml at 5 × 10^5/ml) were mixed, centrifuged at 120 *g* for 5 min at room temperature, then incubated at 37°C in a waterbath for 10 min. Cells were gently resuspended by inversion and analyzed on a FACStar Plus® (Becton Dickinson & Co., Erembodeggen, Belgium). Parameter settings and compensation were set on the instrument using a single population of labeled cells before conjugate analysis. Conjugates were defined as those events that registered a positive signal for both Indo-1 and BCECF. A minimum of 10,000 events were analyzed for each set of conjugates.

[1] *Abbreviations used in this paper:* MFI, mean fluorescence intensity; OGP, octyl-β-glucopyranoside.

For antibody inhibition studies, saturating concentrations of antibody were added to COS cells for 30 min at 4°C, followed by one wash before conjugates were formed as described above.

Results

Recombinant CD23 Incorporated into Fluorescent Liposomes Bind to Some T Cells and B Cells. To search for a ligand for CD23 that is different from IgE, recombinant full-length CD23 was incorporated into fluorescent liposomes. The recombinant membrane form of CD23 was expressed in insect cells using the baculovirus expression system (9), and purified to homogeneity by immunoaffinity chromatography, resulting in a preparation that was 95% pure (Fig. 1 *A*) as assessed by coomassie blue–stained SDS-PAGE and Western blotting analysis (Fig. 1 *B*).

This material, or glycophorin as control, was then reconstituted into artificial membranes, and the presence and accessibility of the protein was demonstrated by double fluorescence analysis or dot-blot immunostaining using the respective antibody (data not shown). Light scattering experiments were performed to analyze the vesicles' size distribution, which was found homogenous and devoid of aggregates. The mean diameter of CD23 liposomes and liposomes containing no protein was ~150 nm (data not shown).

Flow cytometric analyses of different cell types were performed after incubation of the cells with the CD23-incorporated fluorescent liposomes or control liposomes (Table 1). As predicted from some of the reported biological activities of soluble CD23 in vitro, germinal center B cells as well as a subpopulation of T cells bound to CD23 liposomes. Interestingly, two myeloma cell lines, U266-B1 and RPMI 8226, were the most positive cell lines. Variations in the intensity of the staining (related to the mean fluorescence intensity [MFI]) obtained with CD23 liposomes were observed, according to the growth phase of the cells (data not shown).

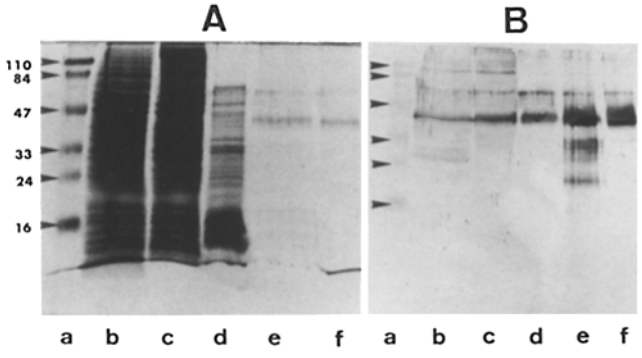


Figure 1. SDS-PAGE and Western blotting analysis of full-length CD23 expressed in insect cells. Aliquots from different steps of the purification were subjected to electrophoresis on a 15% (wt/vol) polyacrylamide gel and stained with coomassie blue (*A*) or transferred onto nitrocellulose membrane and immunostained with Rb55 (*B*). Lanes *a*, prestained molecular mass standards (kD) (Bio-Rad Laboratories); lanes *b*, extract of recombinant Sf9 cells; lanes *c*, membranes after ethanol precipitation; lanes *d*, detergent extract of membranes; lanes *e*, mAb 25 immunoaffinity elution; lanes *f*, Superdex-200 final pool.

The RPMI 8226 cell line was mainly used for further studies. EBV-transformed B cell lines were either slightly positive (RPMI 8866, IM-9) or negative (Daudi). Cell lines of the macrophage type (U-937 and HL-60) were negative. In all cases, negligible binding was observed with liposomes that did not contain protein compared with cells alone. The cell lines that bound most CD23 liposomes, RPMI 8226 and U266-B1, did not bind the glycophorin-containing liposomes. Taken together, these data strongly suggest the existence of a ligand for CD23 on B cell and T cell subsets. Further studies using anti-CD23 antibodies support this conclusion.

The Binding of CD23 to Its Ligand Is Specifically Inhibited by Anti-CD23 mAbs and Is Ca^{2+} Dependent. To show the specificity of the interaction of CD23 with its ligand, antibodies to CD23 were tested for their capacity to inhibit the binding of CD23 liposomes to the RPMI 8226 cell line. As shown in Fig. 2, all the anti-CD23 antibodies tested were able to decrease the binding of the CD23 liposomes. mAb 25, EBVCS 1, and LA1 mAbs, although directed against three different epitopes (17), all inhibited the interaction of CD23 with its ligand. Isotype-matched controls of IgG1 and IgM (data not shown) had no effect. Inhibition was also observed with IgE, the other known ligand for CD23. EBVCS 1, which does not inhibit binding of IgE to CD23 (17), was the weakest inhibitor of CD23-liposome binding.

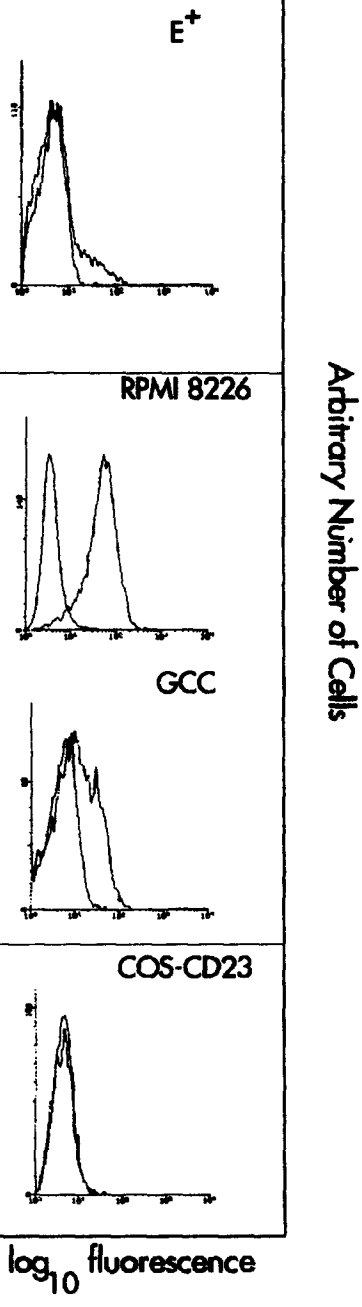
Since CD23 is a member of Ca^{2+}-dependent lectin family, the effect of Ca^{2+} on CD23-liposome binding was investigated (Fig. 3). The binding of CD23 liposomes was Ca^{2+} concentration dependent with a maximum binding of CD23 liposomes being reached at 2 mM of Ca^{2+}. In contrast, the binding of protein-free liposomes was independent of calcium up to a concentration of 5 mM. In addition, CD23-liposome binding to RPMI 8226 was displaced by 10 mM EGTA (85% inhibition). These data demonstrate that the interaction of CD23 with its ligand is specific and depends on the presence of Ca^{2+}.

The Binding of CD23 to Its Ligand Is Decreased by Tunicamycin Treatment and Fucose-1-phosphate. To determine the potential role of oligosaccharides in the interaction with CD23, the effect of tunicamycin, an inhibitor of N-glycosylation, was investigated using two ligand-bearing cell lines. Tunicamycin treatment of RPMI 8226 and IM-9 significantly decreased the binding of CD23 liposomes, down to the control level in the case of IM-9, as shown in Fig. 4. A panel of sugars were then tested for their capacity to inhibit CD23-liposome binding to RPMI 8226 cells. Fucose-1-phosphate was able to prevent CD23-liposome binding to RPMI 8226 cells, as shown in Fig. 5, *B* and *D*. The inhibition by fucose-1-phosphate was concentration dependent with a maximum reached at 12.5 mM (not shown). Glucose-1-phosphate (Fig. 5, *C* and *D*) glucose, galactose, mannose, lactose, *N*, *N'*-diacetylchitobiose, cellobiose, and mannobiose had no effect. These data suggest a role for a glycan structure containing, or related to, fucose-1-phosphate type, in the interaction with CD23.

CD23-transfected COS Cells form Conjugates with the CD23 Ligand–positive Cell Line, RPMI 8226. To confirm the ob-

Table 1. *Binding of Recombinant CD23-incorporated Fluorescent Liposomes to a Panel of Human Cell Lines*

Origin	Cells*	CD23‡ liposomes	Control‡ liposomes	FACS¶ profiles
Promyelocytic leukemia	HL-60	-	-	
Histiocytic lymphoma	U-937	-	-	
Chronic myelogenous leukemia	K-562	-	-	
T cells				
Leukemia	Molt-4	-	-	
Lymphoma	Hut 78	-	-	
	Jurkat	-	-	
Tonsil	E+	+	-	
B cells				
myeloma	RPMI 8226	++	-	
	U266-B1	++	-	
	U266	-	-	
EBV-LCL	RPMI 8866	+	-	
	IM-9	+	-	
	Daudi	-	-	
Burkitt lymphoma	BL2	-	-	
	Jijoye	-	-	
Lymphoma	HFB1 WT	+/-	-	
	HFB1 2/3	+/-	-	
Tonsil	GCC	+	-	
Non-lymphoid				
Umbilical vein	HUVECs	-	-	
Astrocytoma	U-373	-	-	
Epidermoid Carcinoma	Hep-2	-	-	
Monkey kidney cells	COS	-	-	
	COS - CD23	-	-	



The different cell lines were incubated with CD23 liposomes or control liposomes for 2 h at 4°C in presence of 2 mM Ca^{2+} (detailed in Materials and Methods). After washes, cells were analyzed by flow cytometry.

* Cell lines are from human origin except the COS cells.

‡ Results were classified as no staining (-), weak staining (+/-), clear staining (+), and strong staining (++).

¶ Representative staining of cell subpopulations (E^+ and GCC), staining of all cells (RPMI 8226), and absence of staining (COS-CD23) are shown.

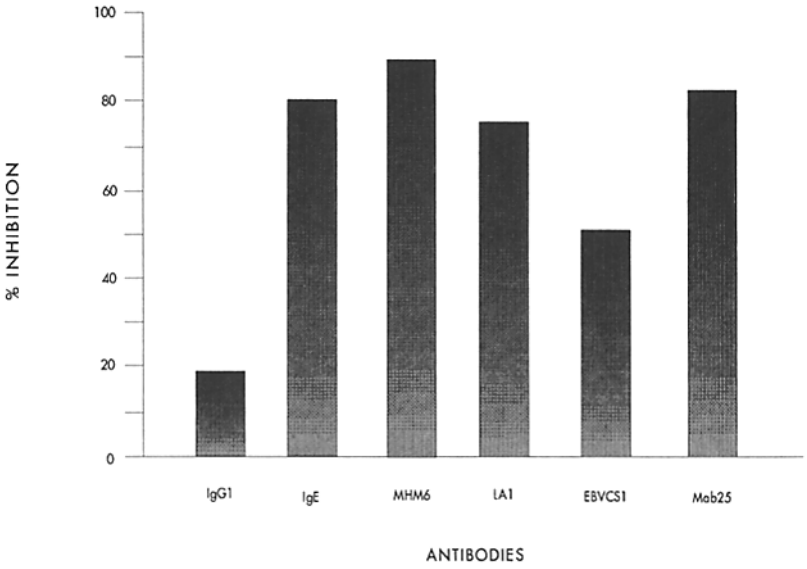


Figure 2. Inhibition of the binding of CD23 liposomes to RPMI 8226 by anti-CD23 mAbs and IgE. RPMI 8226 cells were incubated with fluorescent CD23 liposomes and different anti-CD23 mAbs (30 μg/ml), IgG1 (30 μg/ml) as control, or IgE (200 μg/ml) for 2 h at 4°C. After two washes, cells were analyzed by FACS® as described in Table 1. The results of a representative experiment are presented and are expressed as a percentage inhibition calculated according to the following formula: $100\times [(MFI_{CD23\ liposomes}) - (MFI_{control\ liposomes})] - [MFI_{CD23\ liposomes+mAb}) - (MFI_{control\ liposomes})]/(MFI_{CD23\ liposomes} - MFI_{control\ liposomes}$. The MFI of RPMI 8226 cells in absence of antibody was 47 arbitrary units (AU) with CD23 liposomes and 6 AU with control liposomes.

servation made with artificial membranes in a more natural system, we investigated the cellular interaction between COS cells expressing CD23 transiently and RPMI 8226. CD23-transfected or mock-transfected COS cells were labeled with BCECF and incubated with Indo-1-labeled RPMI 8226 or Indo-1-labeled Molt-4 (which do not bind CD23 liposomes).

As shown in Fig. 6, CD23-transfected COS cells were able to bind to RPMI 8226 (Fig. 6 *A*) but not to Molt-4 (Fig. 6 *C*), confirming the data obtained with fluorescent liposomes. 82% of CD23-positive COS cells were able to form conjugates. The interaction of CD23-COS cells with RPMI 8226 was inhibited by an anti-CD23 mAb (Fig. 6 *B*), whereas, as expected, this antibody had no effect on the interaction between CD23-COS cells and Molt-4 (Fig. 6 *D*). These results demonstrate that recombinant CD23 in cell membranes can interact with a membrane ligand.

Discussion

In this study, the existence of a second ligand for CD23 is demonstrated. First, CD23 reconstituted into fluorescent liposomes was used to show the presence of a binding structure on lymphoid cells by flow cytometric analysis. Second, the demonstration of a ligand for CD23 was not restricted

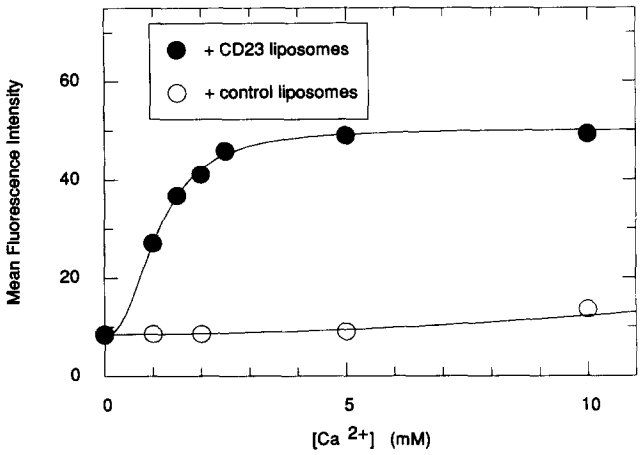


Figure 3. Effect of Ca^{2+} concentration on the binding of CD23 liposomes to RPMI 8226 cells. RPMI 8226 cells were incubated with CD23 liposomes or with control liposomes as described in Table 1 in the presence of increasing concentrations of Ca^{2+}. The MFI was measured by FACS®.

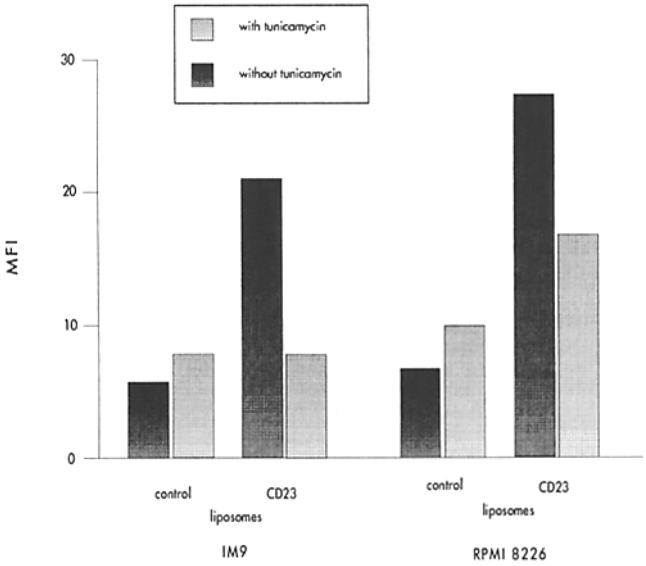


Figure 4. Effect of tunicamycin treatment on the binding of CD23 liposomes to CD23 ligand–positive cell lines. RPMI 8226 and IM-9 cell lines treated with or without tunicamycin for 48 h were stained with suboptimal concentration of CD23 liposomes or control liposomes (dilution, 1/40), as indicated in Table 1. The MFI was measured by FACS®.

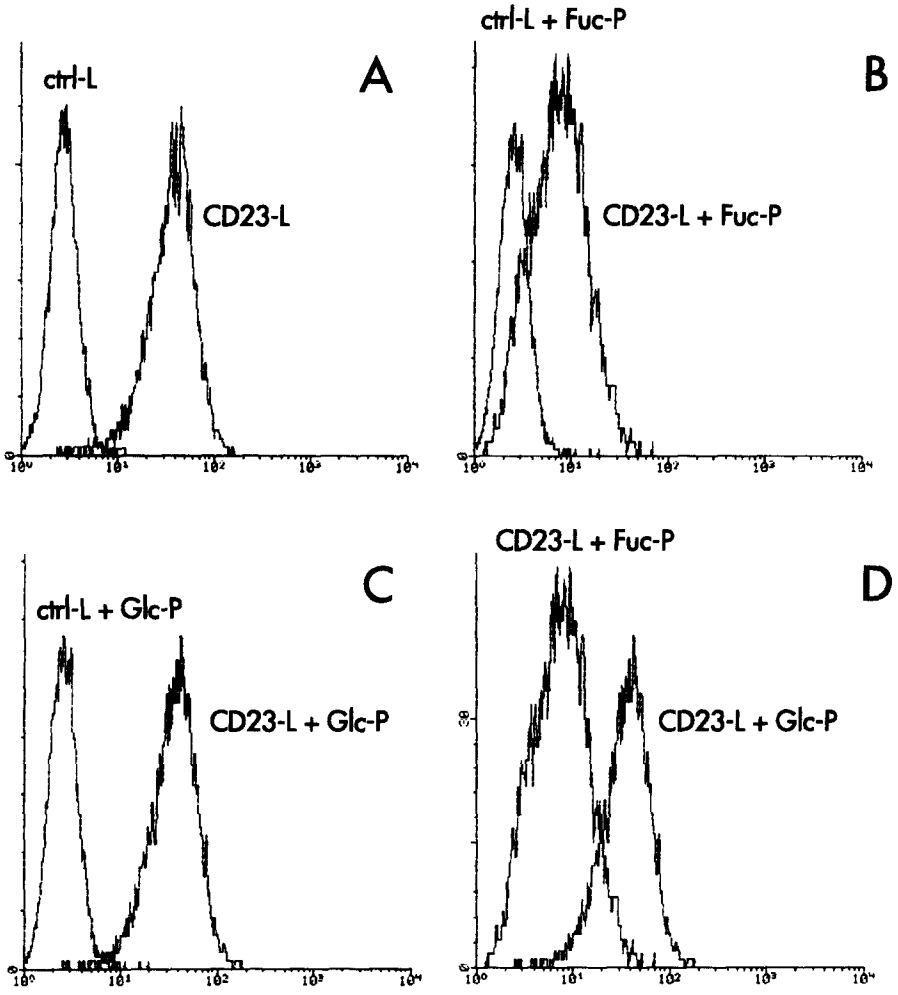


Figure 5. Inhibition of CD23-liposome binding to RPMI 8226 cells by fucose-1-phosphate. RPMI 8226 cells were incubated with CD23 liposomes (CD23-L) or control liposomes (ctrl-L) in the presence of 12.5 mM of fucose-1-phosphate (Fuc-P) or glucose-1-phosphate (Glc-P) for 2 h at 4°C. After washes, cells were analyzed by FACS®. (*A*) RPMI 8226 cells were incubated with CD23 liposomes and control liposomes. *B* is as *A*, but in the presence of fucose-1-phosphate. *C* is as *B*, but in the presence of glucose-1-phosphate. (*D*) RPMI 8226 cells were incubated with CD23 liposomes in the presence of fucose-1-phosphate or glucose-1-phosphate.

to the use of the reconstituted CD23 since recombinant CD23-transfected COS cells were shown to interact specifically with the cell line RPMI 8226 by forming conjugates. Altogether, these two approaches clearly demonstrate the interaction of CD23 with a cell surface ligand.

It is interesting that binding studies using radiolabeled purified recombinant and natural soluble CD23 failed to demonstrate specific binding to any cell types (not shown). Reconstituted full-length CD23 was probably creating a multivalency important for the interaction with its ligand.

Our results on the distribution of the ligand for CD23 are in agreement with the reported biological activities of CD23. RPMI 8866 has been reported to proliferate in response to recombinant CD23 (9), and germinal center B cells have been shown to respond to soluble CD23 with IL-1 by increased survival (10). Moreover, we show here that a subset of T cells also binds the CD23 liposomes. Bertho et al. (25) reported the effect of soluble CD23, when added with IL-1, on the proliferation of CD4-positive T cells. We recently also found that the membrane form of CD23 was involved in conjugate formation between CD4-positive T cells and B cells (Shields et al., manuscript submitted for publication). Our results provide a basis for understanding how CD23 could strongly increase T cell/B cell cooperation since each cell type can express both CD23 and the CD23 ligand.

The observation that some myeloma cell lines bind CD23 liposomes needs further investigation to determine whether this is linked to any biological effect. The future molecular characterization of the CD23-binding structure(s) on various cell types will determine if more than one ligand is involved in the CD23 interaction.

The specificity of the CD23 interaction with a ligand was demonstrated. Liposomes containing an unrelated protein (glycophorin), as well as protein-free liposomes, did not bind to CD23-binding cell lines. Moreover, the binding of CD23 liposomes was specifically inhibited by anti-CD23 mAbs and IgE but not by isotype-matched controls.

The CD23 ligand detected here is different from IgE: (*a*) none of the CD23-binding lymphoid cells, except U266-B1, expressed detectable IgE on their membranes: (*b*) anti-IgE antibodies did not inhibit CD23-liposome binding to U266-B1; and (*c*) the IgE-positive cell line U266 did not bind CD23 liposomes. Moreover, CD23 does not interact with itself since CD23 liposomes did not bind to CD23-transfected COS cells

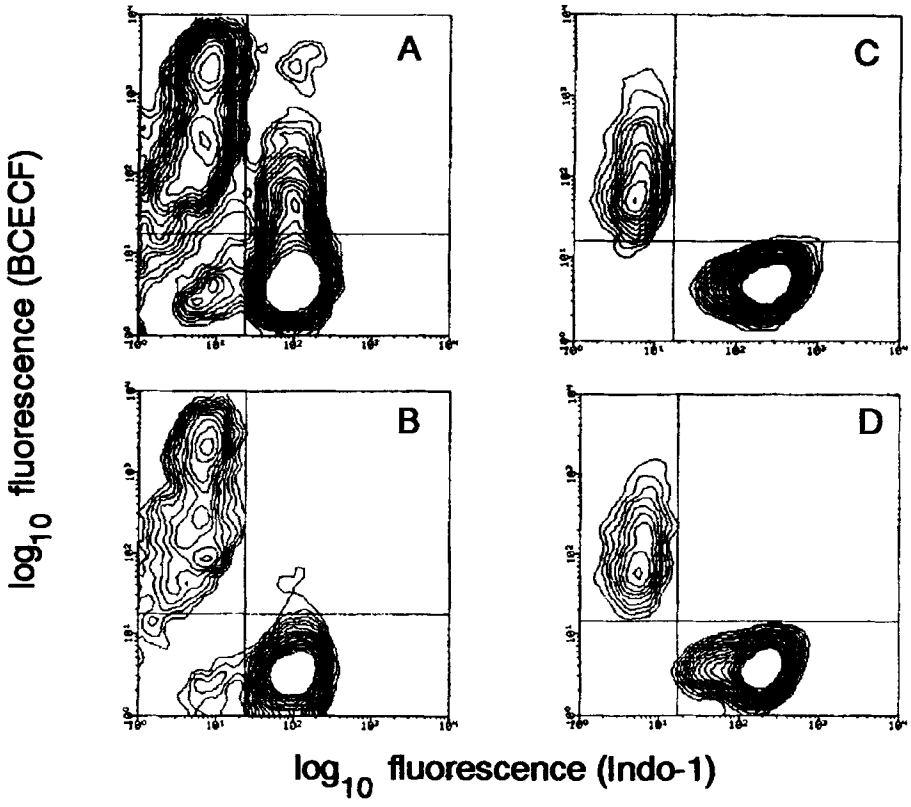


Figure 6. COS cells expressing recombinant membrane CD23 form conjugates with RPMI 8226 cells. CD23-transfected COS cells (28% CD23 positive) and RPMI 8226 or Molt-4 cells were labeled with BCECF-AM and Indo-1-AM, respectively. After mixing and centrifugation, cells were incubated for 10 min at 37°C. Conjugates are positive events for both BCECF and Indo-1 as measured by FACS®. The percentage of conjugates compared with the number of labeled CD23-transfected COS cells is given. (*A*) CD23-COS cells were incubated with control IgG1 mAb and then added to RPMI 8226 (23%). *B* is as *A*, but CD23-COS cells were incubated with mAb 25 anti-CD23 antibody (6%). (*C*) CD23-COS cells were incubated with control IgG1 and then added to MOLT-4 cells (2%). *D* is as *C*, but CD23-COS cells were incubated with mAb 25 anti-CD23 antibody (2%).

and no aggregates were observed in the CD23 liposome preparations as assessed by photon correlation spectroscopy (data not shown).

Our findings suggest that membrane CD23 should be considered as a new adhesion molecule. In fact, CD23 appears to be an additional molecule involved in the interaction between T and B cells as well as in follicular dendritic cell/germinal center cell interactions, since follicular dendritic cells express high amounts of CD23 (1) and germinal center cells bind CD23 liposomes. The existence of a ligand for CD23 on some B cells indicates that in addition, CD23 could mediate homotypic B cell adhesion.

In this context, it is interesting to note that CD23 is a member of a Ca^{2+}-dependent lectin family (26–28). We demonstrate here that the interaction of CD23 with its ligand is Ca^{2+} dependent. Some members of this Ca^{2+}-dependent lectin family have been identified as adhesion molecules, such as GMP-140 (29), MEL-14 (30), ELAM-1 (31), and CD72 (32). This suggests that CD23 recognizes a sugar structure on its ligand. Our finding that tunicamycin treatment of the CD23-binding cells decreases the binding of CD23 liposomes supports this, although we cannot exclude the possibility that tunicamycin affects the intracellular transport and cell surface expression of the ligand. Moreover, fucose-1-phosphate was able to prevent CD23-liposome binding to its ligand on RPMI 8226 cells, suggesting that fucose-1-phosphate, or a fucose-like sugar moiety, is involved on the CD23 ligand in the interaction with CD23. It is interesting to note that the same sugar, fucose-1-phosphate, has been shown to prevent IgE binding to CD23 (33). Inhibition data with fucose-1-phosphate together with our observation that IgE was able to prevent CD23 binding to its new ligand on RPMI 8226 cells suggest that the sites of interaction of CD23 with IgE and its other new ligand are close.

Another feature of human CD23 is the presence of an inverted RGD motif in its sequence. RGD sequences are known to be target sequences for adhesion receptors, and the inverted sequence can also play a role (34). However, the importance of this sequence needs to be demonstrated since a similar sequence is absent in the mouse CD23 homologue (35, 36), and sequences important for biological function would be expected to be conserved.

In conclusion, CD23, a low affinity receptor for IgE, interacts with at least two molecules: one is IgE and the other, as demonstrated here, is a membrane bound ligand. The latter interaction suggests that CD23 can act as an adhesion molecule enhancing cell-cell interaction. The identification of the ligand for CD23 is currently under investigation.

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Note added in proof: We recently identified CD21 as being a ligand for CD23 (37).

References

1. Gordon, J., L. Flores-Romo, J.A. Cairns, M.J. Millsum, P.J. Lane, G.D. Johnson, and I.C.M. MacLennan. 1989. CD23: a multi-functional receptor/lymphokine. *Immunol. Today.* 10:153.
2. Delespesse, G., M. Sarfati, and H. Hofstetter. 1989. Human IgE-binding factors. *Immunol. Today.* 10:159.
3. Conrad, D.H. 1990. FcERII/CD23: the low affinity receptor for IgE. *Annu. Rev. Immunol.* 8:623.
4. Yokota, A., H. Kikutani, T. Tanaka, R. Sato, E.L. Barsumian, M. Suemura, and T. Kishimoto. 1988. Two species of human Fc epsilon receptor II (Fc epsilon RII/CD23): tissue specific and IL-4-specific regulation of gene expression. *Cell.* 55:611.
5. Pène, J., F. Rousset, F. Brière, I Chretien, J.Y. Bonnefoy, H. Spits, T. Yokota, N. Arai, K.I. Arai, J. Banchereau, and J.E. de Vries. 1988. IgE production by normal human lymphocytes is induced by IL-4 and suppressed by alpha-interferon, gamma-interferon and prostaglandin E2. *Proc. Natl. Acad. Sci. USA.* 85:6880.
6. Sarfati, M., E. Rector, K. Wong, M. Rubio-Trujillo, A.H. Sehon, and G. Delespesse. 1984. In vitro synthesis of IgE by human lymphocytes. II. Enhancement of the spontaneous IgE synthesis by IgE-binding factors secreted by RPMI 8866 lymphoblastoid B cells. *Immunology.* 53:197.
7. Kehry, M.R., and L.C. Yamashita. 1989. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. *Proc. Natl. Acad. Sci. USA.* 86:7556.
8. Gordon, J., J.A. Cairns, M.J. Millsum, S. Gillis, and G.R. Guy. 1988. Interleukin-4 and soluble CD23 as progression factors for human B lymphocytes: analysis of their interactions with agonists of the phosphoinositide 'dual pathway' of signalling. *Eur. J. Immunol.* 18:1561.
9. Jansen, K.U., J. Shields, J. Gordon, J. Cairns, P. Graber, and J.Y. Bonnefoy. 1991. Expression of human recombinant CD23 in insect cells. *J. Recept. Res.* 11:507.
10. Liu, Y.J., J.A. Cairns, M.J. Holder, D.S. Abbott, K.U. Jansen, J.Y. Bonnefoy, J. Gordon, and I.C.M. MacLennan. 1991. Recombinant 25 kDa CD23 and interleukin-1 alpha promote the survival of germinal center cells. *Eur. J. Immunol.* 21:1107.
11. Mossalayi, M.D., J.C. Lecron, A.H. Dalloul, M. Sarfati, J.M. Bertho, H. Hofstetter, G. Delespesse, and P. Debré. 1990. Soluble CD23 (FcERII) and interleukin-1 synergistically induce early human thymocyte maturation. *J. Exp. Med.* 171:959.
12. Mossalayi, M.D., M. Arock, J.M. Bertho, C. Blanc, A.H. Dalloul, H. Hofstetter, M. Sarfati, G. Delespesse, and P. Debré. 1990. Proliferation of early human myeloid precursors induced by interleukin-1 and recombinant soluble CD23. *Blood.* 75:1924.
13. Flores-Romo, L., G.D. Johnson, A. Veronesi, A. Ghaderi, D.R. Stanworth, and J. Gordon. 1990. Functional implication for the topographical association between MHC class II and the low affinity IgE receptor: occupancy of CD23 blocks the ability of B cells to present alloantigen to T cells. *Eur. J. Immunol.* 20:2465.
14. Bonnefoy, J.-Y., O. Guillot, H. Spits, D. Blanchard, K. Ishizaka, and J. Banchereau. 1988. The low affinity receptor for IgE (CD23) on B lymphocytes is spatially associated with HLA-DR antigens. *J. Exp. Med.* 176:57.
15. Yukawa, K., H. Kikutani, H. Howaki, K. Yamasaki, A. Yokota, H. Nakamura, E.L. Barsumian, R.R. Hardy, M. Suemura, and T. Kishimoto. 1987. A B-cell specific differentiation antigen, CD23, is a receptor for IgE (Fc epsilon R) on lymphocytes. *J. Immunol.* 38:2576.
16. Bonnefoy, J.-Y., J.P. Aubry, C. Peronne, J. Wijdenes, and J. Banchereau. 1987. Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD23 is a low affinity receptor for IgE. *J. Immunol.* 138:2970.
17. Bonnefoy, J.-Y., J. Shields, and J.J. Mermod. 1990. Inhibition of human interleukin-4-induced IgE synthesis by a subset of anti-CD23/FcE RII monoclonal antibodies. *Eur. J. Immunol.* 20:139.
18. Seed, B., and A. Aruffo. 1987. Molecular cloning of the CD2 antigen, the T-cell erythrocyte receptor, by a rapid immunoselection procedure. *Proc. Natl. Acad. Sci. USA.* 84:3365.
19. Liu, Y.J., D.E. Joshua, G.T. Williams, C.A. Smith, J. Gordon, and I.C.M. MacLennan. 1989. Mechanism of antigen-driven selection in germinal centres. *Nature (Lond.).* 342:929.
20. Graber, P., K. Jansen, S. Pochon, J. Shields, N. Aubonney, G. Turcatti, and J.Y. Bonnefoy. 1992. Purification and characterization of biologically active human recombinant 37 kDa soluble CD23/sFcERII expressed in insect cells. *J. Immunol. Methods.* 149:215.
21. Whelan, J., P. Ghersa, R. Hooft van Huijsduijnen, J. Gray, G. Chandra, F. Talabot, and J. DeLamarter. 1991. An NFκ B-like factor is essential but not sufficient for cytokine induction of endothelial leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Nucleic Acids Res.* 19:2645.
22. Goff, L.K., R.J. Armitage, and P.C.L. Beverley. 1988. Characterization of two CD23 monoclonal antibodies with reactivity distinct from other antibodies within this cluster of differentiation. *Immunology.* 65:213.
23. Kintner, C., and B. Sugden. 1981. Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus. *Nature (Lond.).* 294:458.
24. Bonnefoy, J.Y., J. Banchereau, J.P. Aubry, and J. Wijdenes. 1986. A flow cytometric micromethod for the detection of FcE receptors and IgE binding factors using fluorescent microspheres. *J. Immunol. Methods.* 88:25.
25. Bertho, J.M., C. Fourcade, A.H. Dalloul, P. Debré, and M.D.

Mossalayi. 1991. Synergistic effect of interleukin-1 and soluble CD23 on the growth of human $CD4^+$ bone marrow-derived T cells. *Eur. J. Immunol.* 21:1073.
26. Kikutani, H., S. Inui, R. Sato, E.L. Barsumian, H. Owaki, K. Yamasaki, T. Kaisho, N. Uchibayashi, R.R. Hardy, T. Hirano, S. Tsumasawa, F. Sakiyama, M. Suemura, and T. Kishimoto. 1986. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell.* 47:657.
27. Ludin, C., H. Hofstetter, M. Sarfati, C.A. Levy, U. Suter, D. Alaimo, E. Kilcherr, H. Frost, and G. Delespesse. 1987. Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:109.
28. Ikuta, K., M. Takami, L.W. Kim, T. Honjo, T. Miyoshi, Y. Tagaya, T. Kawabe, and J. Yodoi. 1987. Human lymphocyte Fc receptor for IgE: sequence homology of its cloned cDNA with animal lectins. *Proc. Natl. Acad. Sci. USA.* 84:819.
29. Johnston, G.I., R.G. Cook, and R.P. McEver. 1989. Cloning of GMP-140, a granule membrane protein of platelets and endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell.* 56:1033.
30. Lasky, L.A., M.S. Singer, T.A. Yednock, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel, and S.D. Rosen. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell.* 56:1055.
31. Bevilacqua, M.P., S. Stengelin, M.A. Gimbrone, and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160.
32. Van de Velde, H., I. von Hoegen, W. Luo, J.R. Parnes, and K. Thielemans. 1991. The B-cell surface protein CD72/Lyb-2 is the ligand for CD5. *Nature (Lond.).* 351:662.
33. Delespesse, G., M. Sarfati, C.Y. Wu, S. Fournier, and M. Letellier. 1992. The low-affinity receptor for IgE. *Immunol. Rev.* 125:77.
34. Akiyama, S.K., and K.M. Yamada. 1985. Synthetic peptides competitively inhibit both direct binding to fibroblast and functional biological assays for the purified cell binding domain of fibronectin. *J. Biol. Chem.* 260:10402.
35. Bettler, B., H. Hofstetter, M. Rao, W.M. Yokoyama, F. Kilcherr, and D.H. Conrad. 1989. Molecular structure and expression of the murine lymphocyte low affinity receptor for IgE (FcERII). *Proc. Natl. Acad. Sci. USA.* 86:7566.
36. Gollnick, S.O., M.L. Trounstine, L.C. Yamashita, M.R. Khery, and K.W. Moore. 1990. Isolation, characterization and expression of cDNA clones encoding the mouse Fc receptor for IgE (FcERII). *J. Immunol.* 144:1974.
37. Aubry, J.-P., S. Pochon, P. Graber, K.U. Jansen, and J.-Y. Bonnefoy. 1992. The CR2/EBV receptor, CD21, is a ligand for FcER2/CD23, and regulates IgE production. *Nature (Lond.).* In press.