

Short communication

An enzyme-linked immunosorbent assay specific for transient (29–37-kDa) fragments of soluble CD23/IgE-binding factors

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The low-affinity IgE receptor (Fc ϵ RII) of B cells and monocytes – also known as CD23 – is released from the cell surface by proteolytic cleavage to yield a series of soluble fragments which can accumulate in cell culture supernatants and body fluids. Of these, the most stable is a 25-kDa molecule which is generated from transient intermediates ranging in size from 29 to 37 kDa. It has been claimed that these latter species act as IgE-promoting factors while the 25-kDa molecule is endowed with various cytokine-like activities which are independent of IgE binding. We describe here a novel enzyme-linked immunosorbent assay (ELISA) which allows for the distinction between these two classes of soluble CD23. It is based on the observation that the CD23 antibody EBVCS1 can capture recombinant 29-kDa and 37-kDa fragments of CD23 but does not bind to the 25-kDa species: when EBVCS5 is used as the capture antibody, all three fragments are bound. The availability of these differential ELISA should facilitate investigations on the biological properties of CD23 fragments in health and disease.

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CD23 is the low-affinity receptor for IgE (Fc ϵ RII) on B cells and monocytes: it is also expressed by follicular dendritic cells, Langerhans' cells, some T cells, and natural killer (NK) cells (9, 10). Expression by eosinophils and platelets has also been described (1, 4, 5). At least on activated B cells, the 45-kDa type II membrane-bound glycoprotein is known to be cleaved by active proteolysis to yield first a 37-kDa fragment, followed by a 33-, a 29-, and finally a stable 25-kDa species (14). Under conditions of cell stress (for example, in serum-free medium) a 14–17-kDa component can also be generated (6). Soluble CD23 has been detected not only in cell-culture supernatants but also in normal serum and urine, and it is present at elevated levels in patients with B-chronic lymphocytic leukaemia, rheumatoid arthritis, systemic lupus erythematosus, certain allergic disorders, and in patients receiving autologous bone-marrow transplants (reviewed by Gordon (8)).

The transient fragments of CD23 have been re-

ported to act as IgE-potentiating factors, a property apparently lost in the stable 25-kDa molecule (7). Although 25-kDa CD23 has been described as an IgE-binding factor, its capacity for binding IgE is very low, and a role for this fragment in the modulation of IgE synthesis has been questioned (11). On the other hand, recombinant and natural 25-kDa CD23 is clearly endowed with cytokine-like properties which are quite independent of IgE binding. Thus, particularly in synergy with interleukin (IL)-1, recombinant 25-kDa CD23 can serve as a thymocyte-differentiation factor, a growth and differentiation factor for early myeloid cells, and a survival factor for centrocytes participating in germinal centre responses. Natural 25-kDa CD23 has been reported to act as a migration inhibition factor for U937 monocytic cells and a histamine-releasing factor on mast cells, and it may also possess pyrogenic activity (reviewed by Gordon (8)). The wide-ranging action of soluble CD23 (sCD23) may relate to its being a

member of the C-type lectin family, a fact which could potentially allow for a large number of ligands (9).

Given the apparently dichotomous behaviour of transient and stable fragments of sCD23 and the controversy that surrounds their function, the availability of a simple assay for discriminating between the two is desirable. Such an assay is now described.

Material and methods

Reagents

The EBVCS1, EBVCS4, and EBVCS5 murine hybridoma lines producing antibodies to human CD23 were the generous gift of Dr B. Sugden (McArdle Laboratory, Madison, WI, USA). Monoclonal IgG antibody was purified by DEAE-cellulose ion-exchange chromatography from ascites fluid generated with the EBVCS1 and EBVCS5 clones. Murine EBVCS4 – an IgM antibody – was used as diluted ascites fluid: we had noted previously that this antibody becomes unstable on purification. Horseradish peroxidase (HRP)-labelled sheep antimouse IgM (μ -chain specific) was purchased from Sigma (Poole, Dorset, UK).

CD23 preparations

Recombinant 37-, 29-, and 25-kDa CD23 proteins were the generous gift of Dr J.-Y. Bonnefoy (Glaxo, Geneva, Switzerland). The 37- and 25-kDa fragments were derived from supernatants of insect cells transformed with the appropriate vector by the baculovirus expression system, as previously described (13). The 29-kDa species was produced in COS cells. "Neat" preparations in this study contained approximately 100 ng/ml CD23 protein. A CD23 standard derived from bulk culture supernatants of RPMI 8866 lymphoblastoid cells which is used in a commercial ELISA kit (The Binding Site, Birmingham, UK) was provided by Dr C. Sykes (IDRL, Birmingham, UK). Lysate CD23 was generated from RPMI 8866 cells at 10^6 /ml in the presence of protease inhibitors as previously, described (4).

Description of the ELISA assays

Purified capture antibodies to CD23, EBVCS1, or EBVC5 were coated onto 96-well ELISA plates (GIBCO BRL, Paisley, UK) at 20 μ g/ml in 100 μ l of phosphate-buffered saline (PBS) pH 7.2. After overnight incubation at 4°C, the wells were washed three times in PBS containing 0.05% Tween 20 (washing buffer) and flicked dry. Samples were applied at 100 μ l per well in duplicate and incubated for 2 h at 37°C. Plates were washed three times in

washing buffer and flicked dry, and EBVCS4 ascites fluid, diluted 1:100 in washing buffer, was added at 100 μ l per well. We had previously found that the use of EBVCS4-containing ascites fluid generated no higher a background than that seen with purified antibody in such assays (3); moreover, when isolated free of other proteins contained in the ascites fluid, the IgM antibody tended to be labile. After 2 h at 37°C, plates were washed three times and 100 μ l HRP-conjugated sheep antimouse IgM diluted 1:5000 was added to each well for a further 2 h at 37°C. The plates were then washed as before. Substrate buffer comprising 13 ml 0.2 M Na_2HPO_4 , 12 ml 0.1 M citric acid, and 0.012% H_2O_2 plus 10 mg *o*-phenylenediamine was added at 100 μ l per well. The plates were left at room temperature to develop the reaction, which was stopped by the addition of 25 μ l per well of 20% H_2SO_4 . The optical density (OD) was read on an ELISA plate reader (Anthos Labtec 2001) at 492 nM. Results were expressed as means of duplicate readings which were always within 5% of each other.

Results

The EBVCS1 antibody is known to react outside the IgE-binding domain which is contained within the 25-kDa fragment of the CD23 molecule (2). In initial studies, we found that while EBVCS1 was efficient at binding CD23 isolated from RPMI 8866 cells lysed in the presence of protease inhibitors, it reacted poorly as a capture antibody for sCD23 from culture supernatants when compared either with EBVCS5 from our previously described "in-house" CD23 ELISA (3) or with the BU38 monoclonal antibody (mAb) which is used in a commercially available kit (The Binding Site, Birmingham, UK). From other studies, we know that the lysate-derived CD23 will be primarily in the intact 45-kDa form, whereas the bulk of soluble material will exist as the 25-kDa species (4).

Recombinant fragments of CD23 which had been engineered so as to correspond to the naturally generated 25-, 29-, and 37-kDa species were introduced into ELISA established by using either EBVCS1 or EBVCS5 as the capture antibody: EBVCS4 was used as the common detection mAb in the two systems. It is clear from Fig. 1a that when EBVCS1 is coating the plates, 25-kDa CD23 remains undetected while a strong reaction is obtained in the EBVCS5-based assay. In marked contrast, both the EBVCS1 and EBVC5 mAb provide an efficient capture base for the 29- and 37-kDa recombinant fragments (Fig. 1b and c). This pattern of detection was observed over numerous experiments with different preparations of recombinant proteins. As mentioned above, intact 45-kDa CD23 contained within RPMI

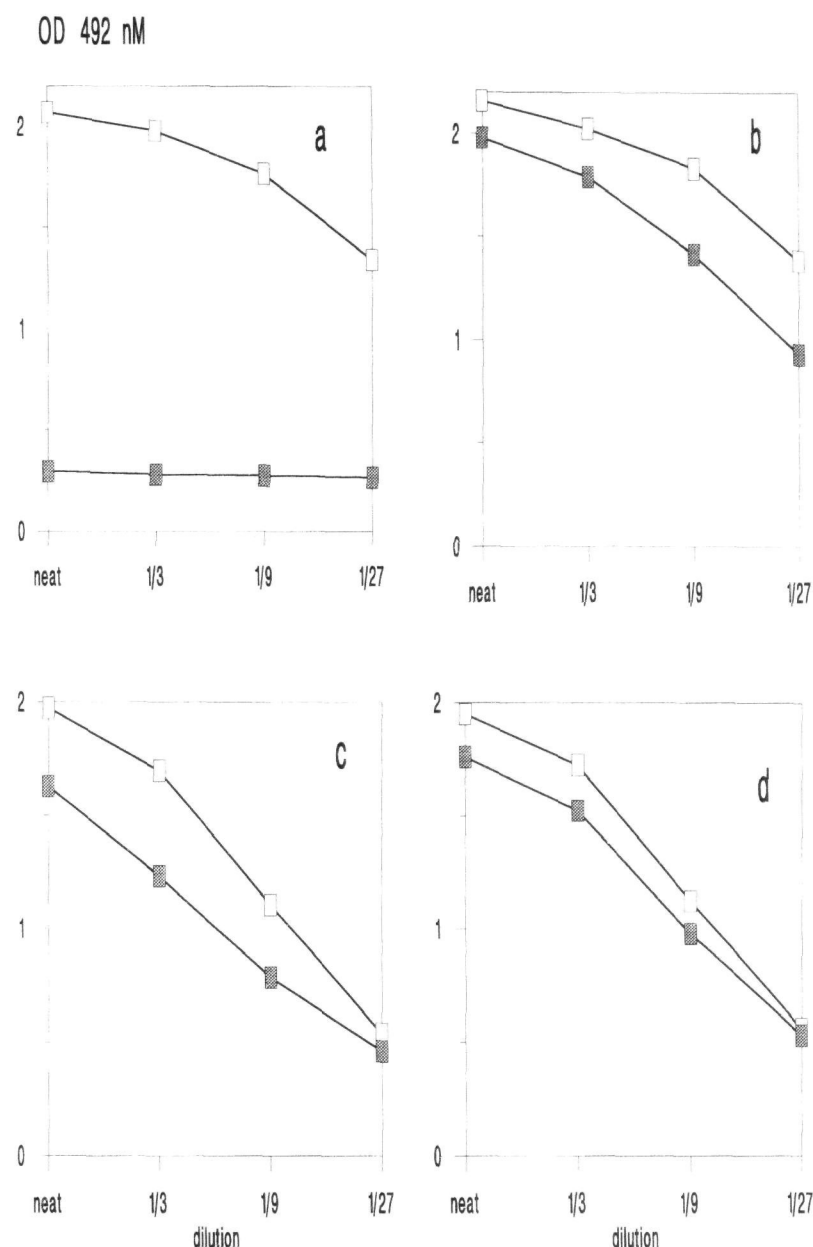


Fig. 1. Capacity of different CD23 species to be recognized in ELISA. ELISA were established with either EBVCS1 (■) or EBVCS5 (□) as capture antibody, as described in Material and methods. Samples were added and amount of EBVCS4 subsequently binding was determined as described, with OD of resulting reaction read at 492 nM. OD readings in blanks (controls) were 0.244 for the EBVCS1-based and 0.180 for the EBVCS5-based assay, respectively. Results are given as means of duplicate determinations and are representative of several similar experiments: a) 25-kDa CD23; b) 29-kDa CD23; c) 37-kDa CD23; d) lysate (45-kDa) CD23.

8866 cell lysates was readily detected in both of the assay systems (Fig. 1d).

A standard used in a commercially available kit for CD23 was assessed for its reactivity in the two assays. As can be seen from Table 1, the standard preparation was detected in both of the assays: by reference to the readings obtained using the recombinant 37-kDa CD23, it seems that a minor but appreciable fraction of the standard was represented by transient species of soluble CD23. The ability to detect relatively high levels of otherwise unstable CD23 fragments is probably due to the prior addition of protease inhibitors to the standard preparation (Dr C. Sykes, IDRL, Birmingham, UK). It can be noted that addition to the standard of soluble

Table 1. Detection of commercial CD23 standard and recombinant proteins in different ELISA

Dilution	OD at 492 nM ^a			
	Standard ^b	Standard plus EBVCS1 ^c	r25-kDa ^d	r37-kDa
EBVCS1-capture assay				
Neat	0.924	0.589	0.386	2.212
1/3	0.731	0.477	0.372	1.708
1/9	0.534	0.372	0.374	1.135
1/27	0.442	0.374	0.369	0.708
Blank	0.385			
EBVCS5-capture assay				
Neat	2.349	2.839	2.523	2.593
1/3	1.703	2.736	2.357	2.066
1/9	0.815	1.983	2.051	1.313
1/27	0.485	0.751	1.302	0.728
Blank	0.442			

^a ELISA were established with either EBVCS1 or EBVCS5 as capture antibody, as described in Material and methods.

^b "Standard" as supplied in CD23 ELISA assay, Binding Site, Birmingham, UK.

^c EBVCS1 was added at 25 µg/ml.

^d Recombinant CD23 proteins of size indicated were generated as described in Material and methods.

EBVCS1 mAb at the concentration shown reduced its detectability in the EBVCS1-based ELISA by around 85% (Table 1), while increasing the amount of antibody resulted in greater blocking (not detailed): surprisingly, the presence of soluble EBVCS1 enhanced the capacity of the EBVCS5-based ELISA to detect CD23 in the commercial standard. We have since shown that the addition of EBVCS1 increases the ability of EBVCS5 to bind transient forms of sCD23 while, as would be predicted, leaving binding to 25-kDa CD23 unchanged (unpublished observations). Again, 25-kDa recombinant CD23 was undetectable in the EBVCS1-based ELISA (Table 1). It should be noted that the commercial ELISA kit for CD23 detected all of the natural and recombinant forms of the molecule used in this study (data not detailed).

Discussion

EBVCS1 was shown previously to be distinct from other CD23 antibodies inasmuch as it failed both to inhibit IgE binding to CD23 and to block IL-4-dependent IgE synthesis in cultures of peripheral blood mononuclear cells (2). IgE appears to bind within the lectin-homology domain of CD23, as do those antibodies which influence CD23 function (9). In epitope-mapping studies, EBVCS1 failed to interfere with the binding to CD23 of other mAb including EBVCS4 and EBVCS5 (11). Our finding in this study that EBVCS1 can bind 29- and 37-kDa CD23, but not 25-kDa CD23, is consistent with the earlier observations and makes this antibody unique

in binding outside the lectin-homology domain which is contained entirely within the stable 25-kDa fragment.

The sequence spanning the lectin head to the cell surface in membrane-bound CD23 is composed of an extended leucine-zipper motif encoded by a series of amplified exons. All of this structure is present in the initial 37-kDa cleavage product and some will be retained in the 29-kDa fragment. Modelling studies predict that this stalk region should encourage trimer formation both in membrane CD23 and in the intermediate soluble products (12). The lack of this potential in 25-kDa CD23 may account for the biological differences reported for transient and stable forms of sCD23. It was of interest that incubation of transient fragments of sCD23 with EBVCS1 led to a higher level of detection in the EBVCS5-based ELISA. This suggests the possibility of an allosteric mechanism whereby epitopes within the lectin domain which may be hidden when the molecule is oligomerized are made more accessible on binding of a ligand within the leucine-zipper region, perhaps through an unravelling of the trimeric complex.

The availability of differential ELISA capable of discriminating between two categories of sCD23 species should prove useful in further elucidating the biological role of this complex molecule. Undoubtedly, the sensitivity of these ELISA could be improved upon, and it will then be of interest to re-analyse body fluids for transient and stable species in those disease states in which overall elevations in sCD23 have been described. Finally, these results should emphasize that caution should be adopted when interpreting results from assays of soluble CD23 based upon commercially available kits where EBVCS1 comprises the capture antibody (for example, see Wilhelm et al. (15)).

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