Binding of Anti-CD23 Monoclonal Antibody to the Leucine Zipper Motif of FcγRII/CD23 on B Cell Membrane Promotes Its Proteolytic Cleavage

EVIDENCE FOR AN EFFECT ON THE OLIGOMER/MONOMER EQUILIBRIUM*

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In the present study we have compared the binding of two monoclonal antibodies to CD23, EBVCS1 and mAb25, which recognize the stalk and the lectin domain, respectively, on the CD23 molecule. At 4 °C, EBVCS1 binds to about 10% of the receptors recognized by mAb25 on the B cell surface. At 37 °C, whereas mAb25 reaches its maximal binding within a few seconds, EBVCS1 requires 60 min to bind to the same extent. Stabilization of the oligomeric structure of CD23 with IgE strongly affects in a dose-dependent fashion the number of binding sites seen by EBVCS1 but not the t1/2 to reach them, suggesting that EBVCS1 binds to the coiled coil region through an allosteric mechanism. EBVCS1 rapidly down-modulates the membrane CD23 expression with a coincident increase of CD23-soluble fragments in the culture medium, an effect that is inhibited by IgE. In contrast, mAb25, as well as IgE, protects CD23 from proteolytic cleavage and stimulates its endocytosis. These results suggest that EBVCS1 unwarps the coiled coil structure of CD23, rendering it more susceptible to proteolytic attack. This supports the oligomeric model proposed previously (Gould, H., Sutton, B., Edmeades, R., and Beavil, A. (1991) Monogr. Allergy 29, 28–49). The biological significance of these observations is discussed.

The low-affinity receptor for IgE (FceRII), also known as CD23, is expressed on IgM+/IgD+ B cells, as well as on a variety of other cells, including monocytes, eosinophils, dendritic cells, platelets, and macrophages (1, 2). CD23 is a 45-kDa type II integral membrane glycoprotein that belongs to the C-type lectin family of adhesion molecules. The two isoforms of CD23, which vary by only a few amino acids in the N-terminal cytoplasmic tail (3) mediate different biological functions (4). A single membrane-spanning domain is followed by an extracellular domain that consists of three regions: the α-helical coiled coil stalk region, which mediates the formation of trimers (5–8), followed by the lectin head, which binds IgE (9), and, at the C terminus, a short tail containing an inverse RGD sequence. CD23 at the cell surface is turned over and yields a series of soluble fragments (sCD23)1 (10, 11) by a mechanism stimulated by triggering of CD20 (12, 13). In vitro experiments have shown that sCD23 has a variety of biological activities (2).

The modulatory effect of CD23 on IgE synthesis has been demonstrated in animals treated with CD23 antibody (14) and in those genetically deficient in CD23 (15–17).

Binding of IgE or IgE-immune complexes to the lectin region transduces a feedback-inhibitory signal that prevents further IgE synthesis (18, 19). In contrast, sCD23 up-regulates IgE production (20–22).

In addition to binding IgE, CD23 also interacts with CD21 (23). This interaction mediates most of the CD23 activities (24–26). CD23 has also been shown to regulate monocyte activation through an interaction with CD11b and CD11c (27), providing a new role of CD23 in inflammation (28).

The release of soluble fragments from membrane-bound FcεRII is inhibited by IgE (29), and IgE-immune complexes repress IgE synthesis (19). This is mimicked by most mAbs against the lectin domain (19, 20, 30, 31). In contrast, EBVCS1 CD23 mAb, which has been shown to bind to the membrane-to-lectin spacer segment (32), stimulates IgE synthesis induced by a combination of IL-4 and CD40 mAb or hydrocortisone (31). In addition, Gordon et al. (33) demonstrated that the ability of certain CD23 mAbs to deliver a growth-promoting signal to activated B cells is both epitope-restricted and independent of receptor cross-linking and proposed that an allosteric change in the protein might be responsible for signal transduction.

These observations prompted Gould and colleagues (6, 34) to suggest an allosteric mechanism by which binding of IgE or mAb to the lectin domain of the FcεRII would stabilize the coiled coil stalk region and would protect it against proteolysis, whereas other ligands would exert the opposite effect.

The present work shows that binding of EBVCS1 mAb to the stalk region of CD23 strongly stimulates the cleavage of FcεRII at the surface of B cells and provides evidence that this operates by unravelling the coiled coil structure.

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1 The abbreviations used are: sCD23, soluble fragment of the CD23 molecule; EBV, Epstein-Barr virus; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody; RAM, polyclonal rabbit immunoglobulin anti-mouse immunoglobulins; IL, interleukin; PAGE, polyacrylamide gel electrophoresis.
medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 2 mM pyruvate, 5 × 10⁻⁵ M 2-mercaptoethanol in a humidified incubator under 6% CO₂ (Heraeus, Hanau, Germany).

**Antibodies, Drugs, and Chemicals—**CD23 mAb, mAb25 was obtained from Aster Biotecnologies (La Guade, France), polyclonal rabbit anti-mouse immunoglobulins (RAMs), and RAM-FITC (Dako, Glostrup, Denmark). Human polyclonal IgE and horseradish peroxidase-labeled BU38 mAb (CD23) were from The Binding Site (Grenoble, France). The EBVCS1 (35) murine hybridoma line producing antibody to human CD23 was a generous gift of Dr. B. Sugden (Mc Ardle Laboratory, Madison, WI); IgG was purified from ascites fluids and conjugated to FITC in our laboratory. Lactoperoxidase, elastase, and protease inhibitors (from Sigma, Na₂₃₅₁ and streptavidin-peroxidase were obtained from Amersham (Les Ulis, France). CNBr-activated Sepharose 4B and PD10 columns were from Pharmacia (St Quentin en Yvelines, France), and iodobeads were from Pierce. RPMI 1640 culture medium and fetal bovine serum were from Life Technologies, Inc.

**Immunofluorescence—**Cells (5 × 10⁵) were washed in cold phosphate-buffered saline + 0.1% bovine serum albumin, pH 7.3, and were incubated (45 min at 4°C) with an appropriate dilution of mAb25 or EBVCS1 in the same buffer. Cells were then washed, incubated (45 min at 4°C) with RAM-FITC, washed again, and fixed with 0.37% paraformaldehyde. The mean fluorescence intensity of 5000 cells was determined by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA).

**Soluble CD23 Determination—**CD23 present in the culture medium were determined by enzyme-linked immunosorbent assay essentially as described (36), with minor modifications. Briefly, 96 well enzyme-linked immunosorbent assay plates were coated with EBVCS1. After saturation, the plates were incubated overnight with serial dilution of samples. Horseradish peroxidase-labeled BU38 mAb was added, and incubation continued for 1 h. After addition of o-phenylenediamine (1 mg/ml), absorbance at 490 nm was measured.

**Surface Labeling and Immunoprecipitation—**Cells were ¹²⁵I-labeled on ice by the lactoperoxidase method as described previously (37). Surface-labeled cells were lysed in phosphate-buffered saline containing 0.5% Triton X-100 and a mixture of protease inhibitors as described (37). Immunoprecipitations were performed for 2 h at room temperature using anti-CD23 immunosorbent. After washing in lysis buffer, proteins were eluted by boiling immunoprecipitates in 3% SDS + 2-mercaptoethanol buffer, separated by SDS-PAGE according to Ref. 38, and revealed by autoradiography (BioMax or Tri Max films).

**Coupling of mAbs to CNBr-activated Sepharose 4B—**Coupling of mAb25 or EBVCS1 to CNBr-activated Sepharose 4B was performed at 5 mg/ml of gel as indicated by the supplier.

**Radiolabeling of mAbs—**¹²⁵I labeling of EBVCS1 and mAb25 was performed at room temperature using iodobeads as indicated by the supplier in the presence of ¹²⁵I. Then, iodinated antibody was separated from free ¹²⁵I by gel filtration through a PD10 column in phosphate-buffered saline + 0.1% bovine serum albumin. Binding of ¹²⁵I-labeled mAb25 or EBVCS1 to SKW6.4 cells was inhibited at 90% by prior incubation of cells with the corresponding nonradioactive monoclonal antibody.

**Internalization of CD23—**Endocytosis experiments were performed as described previously (37) with minor modifications. Briefly, ¹²⁵I-labeled cells were cultured at 37°C. At the indicated times, cells were washed and incubated with 50 μg/ml elastase for 15 min at 37°C. Proteolytic digestion was terminated by adding elastatin (final concentration, 200 μg/ml). Under these conditions, elastase removed CD23 with the maximal level increased as a function of the antibody concentration (data not shown), and the maximal level increased as a function of the antibody concentration (Table I). However, there was no significant variation in the time required to reach half-maximal binding (t₁/₂), indicating that the delay observed in the binding of EBVCS1 was independent of its affinity. This experiment was reproduced twice. This suggests that, in contrast to mAb25, which binds all of the CD23 molecules present at the cell surface, EBVCS1 binds CD23 molecules through an allosteric mechanism: at a steady state, only 10% of the CD23 molecules exhibit the EBVCS1 epitope, and binding of the mAb progressively drives the receptors to a new conformational state in which all these epitopes are exposed. This view is supported by evidence that EBVCS1 binds to the stalk region of the molecule (32) involved in the formation of oligomers (8).

**EBVCS1 Enhances CD23 Cleavage—**We next tested whether the mAb could affect CD23 cleavage. For this purpose, SKW6.4 cells were ¹²⁵I-labeled and cultured with or without EBVCS1, and CD23 was immunoprecipitated from both culture medium and cell extract using immunosorbent mAb25 because the two antibodies do not cross-block (30). Results presented in the Fig.

**RESULTS**

mAb25 and EBVCS1 mAbs Do Not Recognize the Same Number of Binding Sites—Three B cell lines (SKW6.4, Alf, and RPMI 8866) were stained for CD23 by indirect immunofluorescence, using two CD23 mAbs, mAb25 and EBVCS1, that bind the lectin-like and leucine zipper domains, respectively. When the two antibodies were used at saturating concentrations, a 10-fold higher fluorescence was measured with mAb25 as compared with EBVCS1 (Fig. 1). This large difference was not due to the reactivity of RAM-FITC to the mAbs because (i) the two mAbs are IgG1, (ii) identical results were obtained when polyclonal goat immunoglobulin anti-mouse immunoglobulins-FITC was substituted for RAM-FITC, and (iii) Sepharose beads to which either mAb25 or EBVCS1 were chemically coupled were shown to bind the same amount of RAM-FITC (data not shown).

**Kinetics of EBVCS1 and mAb25 Binding—**We next analyzed the kinetics of binding of EBVCS1 and mAb25 to SKW6.4 cells. Cells were first incubated at 37°C with the mAb for various period of time. Bound mAbs were revealed with RAM-FITC at 4°C. Fig. 2 shows that binding of mAb25 was nearly maximal after a contact with the antibody as short as possible (a few seconds), whereas EBVCS1, at the same time, stained only 10% of the mAb25 binding sites, as already seen in the previous experiment, and reached the maximum after about 1 h. Thereafter, a decrease for both EBVCS1- and mAb25-associated fluorescence was observed (this point will be investigated in more detail below). Similar results were obtained with the two other EBV-transformed cell lines (data not shown).

We next performed kinetics of EBVCS1 binding at various antibody concentrations. Whatever the concentration, binding reached its maximum at about 60 min (data not shown), and the maximal level increased as a function of the antibody concentration (Table I). However, there was no significant variation in the time required to reach half-maximal binding (t₁/₂), indicating that the delay observed in the binding of EBVCS1 was independent of its affinity. This experiment was reproduced twice. This suggests that, in contrast to mAb25, which binds all of the CD23 molecules present at the cell surface, EBVCS1 binds CD23 molecules through an allosteric mechanism: at a steady state, only 10% of the CD23 molecules exhibit the EBVCS1 epitope, and binding of the mAb progressively drives the receptors to a new conformational state in which all these epitopes are exposed. This view is supported by evidence that EBVCS1 binds to the stalk region of the molecule (32) involved in the formation of oligomers (8).
show that under the control conditions, the receptor disappeared from the cell surface, and soluble forms of CD23 were detected in the culture medium, indicating that CD23 is spontaneously cleaved. EBVCS1 strongly affected the kinetics. The total radioactivity immunoprecipitated by mAb25 (mCD23 + sCD23) decreased with time, indicating that sCD23 is finally processed into fragments not recognized by mAb25. EBVCS1 also accelerated this processing. Interestingly, with EBVCS1, a major soluble fragment of 29 kDa was observed, whereas smaller species ranging from 25 to 12 kDa were not detected. This suggests that EBVCS1 affected the exposure and/or the sensitivity of the sites of proteolysis. In addition, EBVCS1 did not affect the amount of the 16-kDa fragment seen in the cell extracts after 16 h that results from intracellular degradation of internalized CD23 receptors (37). This suggests that EBVCS1 did not prevent immunoprecipitation of small CD23 fragments by mAb25.

These data strongly suggest that the conformation of CD23 induced by EBVCS1 is a better substrate for the extracellular proteolytic enzymes.

**Antagonistic Effects of IgE**—IgE has been reported to stabilize the oligomeric structure of CD23 (7, 39) and to protect both human (40) and murine (29) receptors from the proteolytic cleavage. In addition, EBVCS1 does not affect IgE binding to CD23 (30). Therefore, we have tested the effects of IgE on the kinetic of EBVCS1 binding and the formation of sCD23 induced by this mAb.

Preincubation of cells with IgE did not significantly affect mAb25 binding (not shown) but strongly decreased the binding of EBVCS1 (Fig. 4). Furthermore, the effect of IgE was dose-dependent, as shown in the inset of Fig. 4, with an estimated IC_{50} of 100 nM, which is close to the dissociation constant reported for the binding of IgE to CD23 (7, 41). The fact that IgE did not affect mAb25 binding although mAb25 blocks IgE binding (30) might be explained by an higher affinity of mAb25, which could dissociate IgE from its binding site. This is also supported by the observation that IgE does not prevent immunoprecipitation of mCD23 by immunosorbenent mAb25.

We next tested the effects of IgE on the cleavage of CD23 induced by EBVCS1. 125I-Labeled SKW6.4 cells were cultured with or without EBVCS1. At the indicated times, cells were separated from culture medium by centrifugation. Cell extracts and medium were incubated with immunosorbed CD23 (mAb25). Immunoprecipitated proteins were eluted and separated by SDS-PAGE under reducing conditions. Upper panel, autoradiogram of immunoadsorbed proteins from cell extracts and culture medium. Lower panel, radioactivity associated with immunoadsorbed proteins from cell lysates (triangles) or culture media (squares) from control (closed symbols) or EBVCS1-treated (open symbols) cells was determined and plotted as a function of time.
presence of submaximal concentrations of EBVCS1 (8 μg/ml) and high concentrations of human IgE (100 μg/ml). Control experiments were performed with each effector alone. Membrane-associated and soluble forms of CD23 were immunoprecipitated and separated by SDS-PAGE. As expected, a down-regulation of mCD23 in the presence of EBVCS1 and an enhanced production of soluble fragments were observed (Fig. 5). IgE inhibited the spontaneous cleavage. In 11 independent determinations, the inhibition ranged from 43 to 17%, with an average of 26%. Clearly, IgE also decreased EBVCS1-induced degradation of the mCD23 and the amount of sCD23 detected in the medium. This was not due to a competition of IgE with mAb25 in the immunoprecipitation step because addition of IgE in the medium from control cells prior to immunosorbent mAb25 did not affect the recovery of sCD23 (Fig. 5, Inset). The experiment was performed as in Fig. 2 in the absence or presence of IgE. Inset, dose-response curve of IgE on EBVCS1 binding.

In a confirmatory experiment, we found that IgE blocked the EBVCS1-induced down-regulation of cell surface CD23, as assessed by staining mCD23 with mAb25-FITC (data not shown).

**mAb25 and EBVCS1: Two Pathways for CD23 Regulation**

The binding curves for both EBVCS1 and mAb25 exhibit a dose-response curve (Inset, Fig. 2). We have previously shown that mAb25 protects CD23 from extracellular cleavage but stimulated the endocytosis of the receptor and its intracellular degradation leading to a quite stable 16-kDa fragment (37). The data presented above showed, in contrast, that EBVCS1 stimulated the cleavage at the cell surface, and this accounts for the decay observed with the EBVCS1. Moreover, it did not affect the intracellular formation of the 16-kDa fragment (Fig. 3). To further analyze the effects of EBVCS1 and mAb25 on mCD23 endocytosis, 125I-labeled cells were incubated at 37 °C for various periods of time with or without the mAb. Extracellular CD23 was removed by treatment with elastase, and the presence of CD23 or its fragments protected from proteolytic digestion (i.e., endocytosed receptors) was assessed by immunoprecipitating Triton extracts with mAb25. Fig. 6 shows that elastase, when added immediately after labeling, effectively removed 90% of mCD23. In control cells, the fraction of receptors protected from elastase digestion as well as the amount of 16-kDa fragments increased with time, demonstrating the internalization of mCD23. In the presence of EBVCS1, endocytosis was less pronounced due to the competition between internalization and extracellular cleavage. In the presence of mAb25, the fraction of endocytosed receptor increased drastically, as we had already observed. It is interesting to note that intracellular degradation in SKW6.4 cells proceeded more rapidly than in RPMI 8866 cells, in such a way that unless the receptor is massively internalized (with mAb25), intracellular receptors are rapidly converted into its 16-kDa fragment. This could be due to differences in the lysosomal enzymatic equipment. The effects of EBVCS1 and mAb25 on CD23 endocytosis have been confirmed by two other techniques. Cells were incubated with either 125I-mAb25 or 125I-EBVCS1 at 37 °C. Cell-associated and internalized antibodies were determined after washing the cells in either normal or acidic medium. Internalized acid resistant fraction increased with time in the presence of mAb25, whereas it remained nearly undetectable with EBVCS1 (not shown). In addition, fibroblasts transfected with CD23a have been incubated with either EBVCS1 or mAb25 and then with RAM-FITC, cultured for various periods of time, and observed with a confocal microscope. The experience clearly showed bright and dull intracellular staining with mAb25 and EBVCS1, respectively (not shown). Altogether, it is clear that mAb25 stimulates CD23 endocytosis, whereas EBVCS1 does not. In contrast, EBVCS1 but not mAb25, stimulates extracellular cleavage. Thus, two completely different mechanisms account for the decay observed for the binding curves of both EBVCS1 and mAb25, depicted in Fig. 2.

**DISCUSSION**

Gould and co-workers (5) have proposed a structural model in which the α-helical coiled coil spacer region of the CD23 molecule is responsible for the formation of dimers or trimers. In this model, the protective effect of IgE on the initial cleavage of CD23 (29, 40) results from a stabilization of the integrity of
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the coiled coil structure, which contains the sites of cleavage, through shifting the equilibrium toward oligomerized CD23. The model predicts, in contrast, that unraveling of the superhelical structure would render it more susceptible to proteolytic attack.

The present work supports this model. Indeed, we show that binding of EBVCS1, a mAb that has been shown to recognize the leucine zipper domain of CD23 (32),\(^3\) induces the conformational change of the receptor and promotes its proteolytic cleavage. The model is the following: under normal conditions at steady state, CD23 molecules at the cell surface predominantly form oligomers and only a few monomers. Both oligomers and monomers are equally recognized by mAb25, but they can be distinguished by their ability to bind EBVCS1. About 10% of the CD23 molecules recognized by mAb25 exhibit the epitope for EBVCS1. Upon binding, EBVCS1 progressively drives the population of molecules to a new steady state in which all of the EBVCS1 epitope is exposed. Several lines of evidence support this model. EBVCS1 recognized 10-fold less membrane CD23 than mAb25 at 4 °C. When binding was performed at 37 °C, in contrast to mAb25, which reached maximal binding within a few seconds, EBVCS1 required about 1 h to have access to the same number of sites. This is not related to the affinity of the mAbs because variation in EBVCS1 concentration affected the number of binding sites but not the half-time required to reach them. In addition, IgE, by stabilizing the oligomeric form of CD23, strongly affects the binding of EBVCS1, which is reminiscent of the effects of inhibitors of allos- teric enzymes.

The present report also shows that EBVCS1 drastically stimulated the cleavage of membrane CD23, and this also was inhibited by IgE. Taken together, these data strongly suggest that the conformational change induced by binding of EBVCS1 to the leucine zipper region exposes the cleavage sites. In addition, unravelling oligomers also renders the lectin domain more susceptible to proteolytic attack leading to fragments unrecognized by mAb25.

Another interesting point is the effect of EBVCS1 on the pattern of soluble fragments released in the culture medium. In the presence of the mAb, a major form of 29 kDa was observed, whereas the species of lower molecular mass were not detected. This is indicative that the proteolytic site located at the boundary of the stalk and the lectin domains is hidden by EBVCS1. The presence of large amount of 29-kDa fragment very early, together with the 37-kDa component, suggests that binding of EBVCS1 both uncovers the cleavage site generating the 29-kDa component and inhibits its processing into the 25-kDa species. This is supported by the demonstration that this mAb recognizes 29-kDa and higher molecular mass CD23 fragments but not the 25-kDa sCD23 (32, 37).

We reported previously that ligation of mAb25 to the lectin domain stimulates the endocytosis and inhibits the extracellular cleavage of CD23 (37). In addition, it has been shown that type a of CD23 mediates the endocytosis of IgE-coated particles, and type b mediates the phagocytosis of soluble IgE complexes (4). This provides a molecular basis for the role of CD23 in IgE- or CD23 mAb-dependent antigen focusing (42–44). The present report confirms that binding of mAb25 to the lectin domain stimulates CD23 endocytosis. In contrast, triggering of the stalk region with EBVCS1 has an opposite consequence: no effect on endocytosis and stimulation of the extracellular cleavage. Hence, the two mAbs provoke a decay of mCD23 (Fig. 2) but through completely different mechanisms. Like EBVCS1, a putative natural ligand for the stalk region would regulate the functions of CD23 toward the cytokine side and reduce the properties associated to the membrane receptor.

As mentioned above, a large body of evidence indicates that CD23 plays a central role in the regulation of IgE synthesis. Both the membrane-bound FcεRII and its soluble fragments are involved. On one hand, cross-linking FcεRII at the cell surface by IgE delivers a negative feedback for IgE production and inhibits the release of sCD23. On the other hand, sCD23 fragments larger than 25 kDa that retain a part of the stalk region promote continuing IgE production by at least two mechanisms: (i) sCD23 directly stimulates IgE production by triggering of CD21 (24, 25), and (ii) sCD23, by its ability to trap IgE in the medium, prevents negative feedback through membrane-bound FcεRII. Our finding that EBVCS1 decreases the expression of the FcεRII at the surface of B cells and induces the release of fragments, together with the observation that it enhances IgE synthesis by human B cells stimulated in vitro by a combination of IL-4 and CD40 mAb or hydrocortisone (31), further supports this model. It is interesting to point out that among the sCD23 fragments, the 29-kDa species, which is one of the major components produced by EBVCS1-stimulated cells, has a strong stimulatory activity on IgE production (2, 23).

This effect of EBVCS1 on CD23 is not limited to B cells. In U937 cells in which CD23 expression was induced by IL-4 and IFN-γ for 48 h, EBVCS1 strongly stimulated the shedding of CD23 (data not shown).

Interestingly, sCD23 activates monocytes to produce proinflammatory mediators, including IL-1β, IL-6, and tumor necrosis factor-α but not IL-10 (27, 45), whereas IL-10 is produced by triggering of membrane-bound CD23b (46). Strikingly, both IL-6 (47) and tumor necrosis factor-α (48) enhance IL-4-induced IgE synthesis, whereas IL-10 suppresses it (49, 50). Altogether, these observations support the view that the stimulatory effect of sCD23 and the inhibitory role of membrane CD23 on IL-4-induced IgE synthesis could be mediated to some extent by monocytes.

Increasing evidence indicate that the release of sCD23 is associated with physiopathological situations. Leishmaniasis is known to be associated with a high IgE level and increased

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\(^3\) O. Munoz, C. Brignone, and J.-L. Cousin, unpublished data.
FceRII expression. Interestingly, *Leishmania chagasi* has been shown to selectively down-modulate CD23 expression on B cells and monocytes with a coincident increase of sCD23 cleavage (51). A parasite-derived protease does not seem to be responsible for the shedding, but a direct contact between promastigote and B cell was required. An effect of the protozoan on the three-dimensional structure of CD23 is a candidate mechanism. The fact that different strains of *Leishmania* are able to down-modulate both human and mouse FceRII (51) in which proteolytic sites are not conserved supports this view.

Recently, it has been shown that Der p I, the major house dust mite allergen, disrupts the IgE network by selectively splitting CD23 and CD25 (for review, see Ref. 52). Upon cleavage, the lectin domain of CD23 was removed, whereas the stalk region remained associated with the cell membrane and therefore only become accessible upon dissociation of the oligomer. The mechanism that we propose for FceRII-induced CD23 cleavage completely fits in this model.

Recently, we found that triggering of CD20 with mAbs decreases the expression of CD23 by stimulating the cleavage of extracellular CD23 on the surface of EBV-transformed B cells and IL-4-stimulated B cells. Whether this occurs through a conformational change in the CD23 molecule is currently being investigated.

In conclusion, we provide evidence that binding of EBVCS1 to the stalk region of the FceRII results in a destabilization of the oligomeric structure of the receptor that renders it more susceptible to a proteolytic attack.

We speculate that any agent, of autologous or exogenous origin, that is able to interact with the leucine zipper domain of CD23 would disrupt the IgE regulatory mechanism, thereby increasing IgE synthesis. Additionally, the sCD23 produced would provoke the release of proinflammatory mediators.

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