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Regulation of IgE Production Requires Oligomerization of CD23

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Here we describe the production of a rabbit polyclonal Ab (RAS1) raised against the stalk of murine CD23. RAS1 inhibits release of CD23 from the surface of both M12 and B cells resulting in an increase of CD23 on the cell surface. Despite this increase, these cells are unable to bind IgE as determined by FACS. CD23 has previously been shown to bind IgE with both a high (4–10 × 10^7 M^{-1}) and low (4–10 × 10^6 M^{-1}) affinity. Closer examination by direct binding of ^125I-IgE revealed that RAS1 blocks high affinity binding while having no effect on low affinity binding. These data support the model proposing that oligomers of CD23 mediate high affinity IgE binding. These experiments suggest that RAS1 binding to cell surface CD23 results in a shift from oligomers to monomers, which, according to the model, bind IgE with low affinity. These experiments also suggest that high affinity binding of IgE is required for IgE regulation by CD23 and is demonstrated by the fact that treatment of Ag/Alum-immunized mice treated with RAS1 results in a significant increase in IgE production similar to the levels seen in CD23-deficient mice. These mice also had significantly decreased levels of serum soluble CD23 and Ag-specific IgG1. RAS1 had no effect on IgE or Ag-specific IgG1 production in CD23-deficient mice. The Journal of Immunology, 2001, 167: 3139–3145.

The low affinity receptor for IgE, CD23, is a type II glycoprotein. The carboxyl terminus of CD23 contains a lectin domain, making it a member of the c-type lectin family (1). The lectin head is the site of interaction with the Ce3 domain of IgE (2, 3) and like other members of this family, this binding is calcium dependent (4, 5).

CD23 is found on a variety of cell types in humans including B cells, monocytes, eosinophils, and Langerhans cells (reviewed in Ref. 6). Two isoforms of CD23 exist in the human, CD23a and CD23b, which result from two different transcription initiation sites. The extensive distribution of CD23 on human cells is due to the use of the CD23b promoter. Only one isoform is found in the mouse that most closely resembles human CD23a (7). Therefore, expression of CD23 is relatively limited in the mouse and is found only on B cells and follicular dendritic cells (8).

CD23 is initially expressed as a membrane-bound protein, but it is cleaved by an unknown metalloprotease (9, 10) releasing the majority of the protein as a soluble protein (sCD23), which contains the stalk and lectin domain (11, 12). The initial fragment that is released has a molecular mass of 37 kDa in the human and 38 kDa in the mouse. Smaller fragments of sizes ranging from 33 to 12 kDa in the human and from 35 to 25 kDa in the mouse, all containing the lectin domain, are also seen.

The stalk region of CD23 is located between the lectin domain and the transmembrane region. The stalk of murine CD23 contains four 21-aa repeats (7), whereas the human has three (13). Beavil et al. (14) noted a periodic heptad repeat containing a hydrophobic amino acid (usually leucine) in the stalk, which is characteristic of a leucine zipper motif, and suggested that CD23 might form an α-helical coiled coil. Cross-linking of CD23 resulted in a molecule with a molecular mass consistent with trimer formation (15), and use of reversible cross-linkers showed that only the 49-kDa form of CD23 was present in the oligomers (16). Membrane CD23 has been shown to bind IgE with a dual affinity, from 4–10 × 10^6 M^{-1} to 4–10 × 10^7 M^{-1} (16). These observations led Gould et al. (1) to suggest a model where CD23 forms trimers and that oligomerization of CD23 mediates high affinity binding to IgE. A mutated form of murine CD23, containing only one of the 21-aa repeats, was capable of binding IgE with only a low affinity (16). These data indicate that in the oligomeric form, CD23 binds IgE with a high affinity (4–10 × 10^7 M^{-1}), and that the monomeric form of CD23 can only bind IgE with a low affinity (4–10 × 10^6 M^{-1}). More recently a chimeric protein was made which consisted of an isoluecine zipper (17) attached to the extracellular portion of CD23 (LZ-C1M-CD23), allowing the formation of a stable soluble trimer (18). This molecule bound IgE with a comparable affinity seen with membrane CD23, presumably because the leucine zipper enhances trimer formation. These data further suggest that CD23 must form a trimer to bind IgE with a high affinity.

This study used a polyclonal Ab made against the stalk region of CD23 to examine the role oligomerization of CD23 plays in IgE

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5. Abbreviations used in this paper: sCD23, soluble CD23; RAS1, polyclonal rabbit anti-CD23 stalk Ab; nRfG, normal rabbit IgG; muCD23(70–175), murine CD23(70–175)–KLH, keyhole limpet hemocyanin; CHO, Chinese hamster ovary.
The HT was calibrated with the same buffer. The column was rinsed with 250 ml of the sodium phosphate (pH 7.4) followed by 3.5 ml of a 10 mg/ml bovine thymonin stock solution. Cleavage was complete and quenched after 22 min with 6.0 ml of a 0.5 M benzamidine hydrochloride solution. The HT–muCD23 50-175 was concentrated to a final volume of 25 ml on an Amicon (Beverly, MA) concentrator using a YM1 membrane (Amicon) and purified in two batches by gel filtration (Sephacryl S-200 HR, XK26 100-cm column; Pharmacia) with 5 M guanidine hydrochloride and 50 mM sodium phosphate (final pH = 6.0). The fractions containing the HT–muCD23 50-175 were identified by SDS-PAGE, pooled, and dia
dyed exhaustively (SpectraPor 7, molecular weight cutoff 2000 membranes) against 2 liters of water at 4°C. The peptide was then lyophilized and stored as a white powder at −20°C. Analysis by 15% SDS-PAGE showed that the HT–muCD23 50-175, comprising muCD23 50-175 and an N-terminal GSH, was >99% pure. A 4-liter fermentation yielded ~75 mg of the peptide.

Preparation of anti-stalk rabbit polyclonal Ab

The immunogen (mouse stalk) was emulsified in CFA and injected into a New Zealand White rabbit. Subsequent boosts were given in IFA. The antistalk antiserum was purified by precipitation with 40% ammonium sulfate and ion exchange chromatography on DE-52 cellulose performed with 0.175 M phosphate, pH 6.3. The polyclonal anti-stalk Ab will be further referred to as RAS1 (rabbit anti-stalk 1).

Cell culture and B cell preparation

CD23-expressing Chinese hamster ovary (CHO) K1 cells, Fc1.7, were made as previously described (16) and grown in DMEM-glutamine supplemented (25), was purchased from American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 medium with 10% FBS, 100 U/ml penicillin in 200 ml of 300 mM NaCl and 50 mM sodium phosphate (pH 8.0). The solution was clarified by centrifugation, and the pellet was extracted a second time to yield 200 ml of a solution containing HT–muCD23 50-175, namely, the muCD23 50–175 peptide containing an N-terminal histidine tag.

Recombinant HT–muCD23 50-175 was expressed in BL21(DE3) Esch
cerichia coli. Transformants were grown overnight at 37°C in Luria-Bertani medium containing 100 μg/ml of ampicillin and diluted 40-fold into 4 liters of Ecpn1 medium containing 100 μg/ml ampicillin. The E. coli were grown with aeration at a pH of 7.0 and 37°C for 5 h in a 4-liter fermentor (Maestra; SL Biolafitte, Saint Germain en Laye, France) as described (23). Protein expression was induced with 1.0 mM isopropyl β-thiogala
taric acid, and 0.1 mg/ml M15 (anti-CD40 ligand trimer mAb) (19). To determine the effect of RAS1 on surface CD23 and CD23 release, 1 × 10 7 B2 cells were stimulated as indicated above in 24-well plates (Corning Costar, Cambridge, MA) in a final volume of 1 ml. On day 2, cells were washed and resuspended in medium with stimulators, and indicated concentrations of RAS1 or normal rabbit IgG (nRIgG; Sigma) as an isotype control. The cells were also cultured with or without IgE (100 μg/ml). Eighteen hours later, cells and supernatants were harvested. CD23 surface levels were determined by FACS analysis, and CD23 release was measured by ELISA. IgE binding capability was determined by incubating cells with mouse IgE (10 μg/ml) for 30 min and then detecting bound IgE with FITC-rat anti-mouse IgE (BD PharMingen).

Scatchard analysis

The affinity for CD23 with or without the presence of RAS1 was deter
dined as previously described (16). Briefly, 5 × 10 7 Flc1.7 cells were added to tubes containing increasing amounts of cold IgE and RAS1 or nRIgG for concentration titrations. After a 30-min incubation, 125 I-labeled IgE (0.5 or 5.0 μg) was added, resulting in a final concentration range of 1.0–400 μg/ml. These tubes were incubated for 60 min on ice, and the free and cell-bound 125 I-IgE was separated on a phalate oil mix
ture (29). Nonspecific binding was determined by adding 100-fold excess cold IgE, and the value was subtracted to obtain specific binding. Addition of nRIgG did not inhibit binding of IgE. Binding affinities were determined by linear regression analysis.

In vivo studies

BALB/c mice were treated with 2 mg RAS1 in PBS i.p. on days −2, −1, 0, and 7. Another group of mice received nRIgG (Sigma) as a control. On day 0, mice were immunized s.c. with 100 μg keyhole limpet hemocyanin

Materials and Methods

Reagents and animals

Balb/cusloucators containing recombinant murine IL-4 was a gen-
erous gift from Dr. W. Paul (National Institutes of Health, Bethesda, MD); recombinant murine IL-5 was purchased from R&D Systems (Minneapolis, MN). CD40 ligand trimer and M15 (mouse IgG1 anti-CD40 ligand trimer) (19) were obtained from Immunix (Seattle, WA). FITC-anti-CD23 (B3B4) was obtained from BD PharMingen (San Diego, CA) and FITC-goat anti-rabbit IgG was purchased from Cappel (Duham, NC). Monoclonal mouse IgE anti-DNP (20) was purified from ascites as described (21). BALB/c mice were purchased from the National Cancer Institute (Frederick, MD). CD23+/− mice (22) were a gift from Dr. H. Van der Putten (Novartis Pharma, Basel, Switzerland). All animals used in experiments were between 6 and 10 wk of age and were kept in an accredited animal facility.

Cloning, expression, and purification of muCD23 50–175

DNA for the expression of muCD23 50–175 was amplified by PCR from a
muCD23 cDNA containing plasmid (sfsCD23 in pCDNA-1 amp; Ref. 7) using the following primers: CCGCTTCCATATGGAAACGAGA GAACTCAA and CGGATCTTATATCCGCAAGCGTTTGC

This yields the plasmid pRG15b:muCD23:50–175:1, which codes for HT–muCD23 50–175, namely, the muCD23 50–175 peptide containing an N-terminal histidine tag.

The recombinant protein was purified by nickel affinity chromatography. The HT–muCD23 50–175 was expressed in BL21(DE3) Esch
cerichia coli. Transformants were grown overnight at 37°C in Luria-Bertani medium containing 100 μg/ml of ampicillin and diluted 40-fold into 4 liters of Ecpn1 medium containing 100 μg/ml ampicillin. The E. coli were grown with aeration at a pH of 7.0 and 37°C for 5 h in a 4-liter fermentor (Maestra; SL Biolafitte, Saint Germain en Laye, France) as described (23). Protein expression was induced with 1.0 mM isopropyl β-thioga
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In vivo studies

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production. The results support the hypothesis that only oligomers of CD23 can bind IgE with high affinity. Surprisingly, these studies also indicate that only membrane CD23 in the form of oligomers and not monomers can regulate IgE production. These data also suggest that it is the trimeric form of CD23 that plays a role in enhancing Ag processing and presentation of IgE/Ag complexes.
(KLH)-DNP in an adjuvant consisting of 4 mg Alum and 110 ng pertussis toxin (Sigma). Mice were bled on days 14 and 20. Levels of total IgE as well as KLH-DNP-specific IgG1 in the serum were determined by ELISA. sCD23 levels in the serum were also determined by ELISA. Serum IgE, KLH-DNP-specific IgG1, and sCD23 levels of experimental mice were normalized to the levels in control (BALB/c) mice. Statistical differences were determined by Student’s t test.

ELISAs
sCD23 was determined as previously described (18). Immulon ELISA plates (Dynex Technologies, Chantilly, VA) were coated with 2G8 (anti-CD23). EC-CD23 was used as the standard (18). EC-CD23 consists of the extracellular portion of CD23 and was made in E. coli as a denatured protein. EC-CD23 was renatured as described (18), and the concentration was determined by spectrophotometry. Samples and standards were detected using a rabbit polyclonal anti-mouse CD23 followed by goat anti-rabbit IgG-HRP (Southern Biotechnology Associates, Birmingham, AL).

IgE was determined as previously described (30) using a pair of rat anti-mouse mAbs, B1E3 and R1E4 (both purified from ascites), as the capture and biotinylated secondary Ab, respectively. IgG1 (30) was determined using an unlabeled primary goat anti-mouse IgG1 Ab and detected using a goat anti-mouse IgG1 coupled to alkaline phosphatase (both obtained from Southern Biotechnology Associates). All ELISAs were performed in Immulon ELISA plates (Dynex Technologies).

Results
Analysis of a polyclonal anti-mouse CD23 stalk Ab
A portion of the stalk of CD23 corresponding to amino acids 50–175 and produced in E. coli was used as the immunogen to produce a polyclonal antiserum. The purified IgG fraction from the rabbit antiserum (RAS1) was tested for CD23 binding to Fc1.7 cells, CD23-overexpressing CHO cells. As shown in Fig. 1, RAS1 did bind to the Fc1.7 cells but not to control CHO cells. To further confirm specificity of RAS1 for CD23, cell lysates from 125I-labeled Fc1.7 and CHO cells were immunoprecipitated with RAS1. RAS1 specifically bound CD23 in the Fc1.7 cells but not proteins of any other size and did not immunoprecipitate any proteins from the control CHOK1 cells (Fig. 1, inset).

Effect of anti-stalk Ab on sCD23 release
To determine whether RAS1 binding to CD23 had any effect on CD23 cleavage, M12 cells, a B cell lymphoma, were stimulated with IL-4 and LPS to induce CD23 expression. Activated cells were then cultured in the presence of RAS1 overnight. Fig. 2A shows that M12 cells cultured alone or with nRIgG (control) both were then cultured in the presence of RAS1 overnight. Fig. 2B shows that M12 cells cultured alone or with RAS1 exhibited increased CD23 cleavage. M12 cells cultured with either 200 or 400 μg/ml RAS1 demonstrated no IgE binding. However, when RAS1 was added at 100, 200, or 400 μg/ml, CD23 expression was increased on the surface of these cells dose dependently.

Increased cell surface CD23 levels do not correspond to increased IgE binding
Because addition of RAS1 decreased the amount of sCD23 released by CD23-expressing cells, the amount of CD23 on the surface of these cells was examined. As shown in Fig. 3A, nRIgG had no effect on the amount of CD23 on the surface of B cells, nor did the addition of 10 μg/ml RAS1 to the cells (data not shown). However, when RAS1 was added at 100, 200, or 400 μg/ml, CD23 expression was increased on the surface of these cells dose dependently.

Effects of RAS1 on IgE production in vivo
To further examine the effect RAS1 had on IgE binding to CD23, saturation analysis was performed. Fig. 4 shows that Fc1.7 cells incubated with a large range of IgE concentrations exhibited the same dual-affinity IgE binding (Kd = 9.9 × 10^7 M^-1 and 1.4 × 10^6 M^-1) previously seen with cells expressing CD23 (16). However, addition of RAS1 to these same cells resulted in a single low affinity binding (Kd = 2.1 × 10^6 M^-1). Inhibition of high affinity IgE binding to CD23 by RAS1 was also temperature sensitive. At 37°C, 10-fold less RAS1 was needed to give 100% inhibition of IgE binding as compared with the amount needed to inhibit IgE binding at 4°C (data not shown). These data suggest that RAS1 binds to the stalk of CD23 and causes dissociation of CD23 trimers allowing only low affinity binding of IgE to monomers of CD23. The presence of nRIgG did not affect IgE binding.

Effect of RAS1 on IgE production in vivo
To determine whether RAS1 would have an effect on IgE production in vivo, mice were immunized with KLH-DNP in alum with pertussis toxin. This treatment stimulates a strong IgE response. Mice were also treated with either nRIgG or RAS1 on days −2, 0, and 7, and serum was collected on days 14 and 20. BALB/c mice
treated with 2 mg RAS1 during the IgE induction phase produced significantly higher amounts of IgE than mice that received nRIgG (Fig. 5). Lower doses were tested for their ability to influence IgE production, and RAS1 was found to be effective at increasing IgE levels at a dose as low as 0.5 mg/mouse; however, lower doses (0.01 and 0.1 mg/mouse) did not change IgE levels as compared with the control group (data not shown). There was no difference in the amount of IgE produced by CD23−/− mice whether or not they received RAS1, proving that the increase in IgE production seen in BALB/c mice was mediated by CD23. Also of importance is that the CD23−/− mice produced higher amounts of IgE than the BALB/c mice, 4313.1 ± 1478.1 and 1887.1 ± 518.1 ng/ml (day 14), respectively, which is consistent with a previous report (32) where the lack of CD23 results in higher IgE synthesis. KLH-DNP-14), respectively, which is consistent with a previous report (32)

Discussion

This study examines the effect of using a polyclonal Ab directed against the stalk region of mouse CD23 on IgE binding to CD23 and IgE regulation by CD23. RAS1 specifically binds an epitope on CD23-transfected cells, but not on control CHOK1 cells. When added to cultures of activated M12 or B cells, RAS1 inhibits sCD23 release by both types of cells. This suggests that the cleavage site is protected by RAS1. The protection from cleavage that RAS1 provides corresponds to an increase of CD23 on the cell surface. However, the increase in surface CD23 does not lead to an increase in IgE binding. In fact, the cells treated with the highest doses of RAS1 exhibit negligible IgE binding when measured by FACs analysis.

CD23 binds IgE with both a high and low affinity. This dual affinity was explained by Gould et al. (1) to be related to the oligomerization of CD23. According to this model, low affinity binding occurs when one molecule of IgE is bound to one CD23 molecule, and high affinity binding of IgE to CD23 occurs when a molecule of IgE is bound by multiple CD23 receptors at the same time. The higher affinity binding seen in this case is really a measure of higher avidity because multiple receptors are involved in binding to one molecule of IgE. We further examined the lack of IgE binding when RAS1 is bound to CD23 by Scatchard analysis. Fc1.7 cells, expressing a native form of CD23, exhibited the expected dual affinity binding to IgE (Kd = 9.9 × 10⁻⁷ M⁻¹ and 1.4 × 10⁻⁶ M⁻¹) previously seen. The addition of RAS1 to these cells resulted in only low affinity binding. Because RAS1 recognizes the stalk of CD23, which mediates oligomerization of CD23, it is most

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** RAS1 dose dependently increases CD23 surface levels while decreasing IgE binding ability. Resting B cells were stimulated as in Materials and Methods and then cultured in the presence of the indicated concentrations of RAS1 or nRIgG for 18 h. The cells were then harvested and stained for CD23 expression (A), or IgE (10 µg/ml) was added to determine the amount of IgE that was able to bind the cells and was detected using FITC-rat anti-mouse IgE (B).
likely inhibiting high affinity binding by destabilizing CD23 trimers. Inhibition of IgE binding to CD23 by RAS1 was shown to be temperature dependent in that 10-fold less RAS1 was required to completely inhibit high affinity binding at 37°C as compared with 4°C. This suggests that the stalk must uncoil for some of the epitopes to become available for RAS1 to bind and inhibit IgE binding.

CD23 has been proposed to have several functions. Among these include IgE regulation and enhancement of processing and presentation. The first suggestion that CD23 might be a regulator of IgE came from the observation that IL-4 stimulates both B cell switching to IgE and production of CD23 (reviewed in Ref. 33). However, only recently has more substantial data in support of this concept been reported. Culture of B cells with CHO cells expressing high levels of membrane CD23 resulted in a decrease in IgE production by those B cells (34). The availability of CD23 transgenic and knockout mice further supports this idea of CD23 as a negative regulator. CD23-deficient mice produced more Ag-specific IgE in response to Ag/alum treatment (32). The decrease in Ig production was limited to the IgE isotype. One line of CD23 transgenic mice with CD23 expression under the control of the Thy1.1 promoter showed a decrease of IgE production when challenged with Ag/alum (35). A second line of transgenics, with CD23 expression under the control of the MHC class I promoter with expression limited to lymphocytes, produced barely detectable levels of IgE in response to Ag and alum and infection with Nippostrongylus brasiliensis (36). Adoptive transfer studies using these mice suggest that it is the CD23 on nonlymphoid cells, possibly on follicular dendritic cells, that is important in regulating IgE (37). These data show that membrane CD23 functions as a negative regulator for IgE production.

Several studies have examined the effect of anti-CD23 Abs on IgE binding to CD23 and their influence on the regulation of IgE. In the human, Abs that inhibit IgE binding and, therefore, most likely bind to the lectin domain have been shown to inhibit IgE production by PBMC stimulated with IL-4 alone or in combination with anti-CD40 mAb or hydrocortisone (38). An in vivo study by Flores-Romo et al. (39) examined the effect of a polyclonal Ab to the lectin domain of CD23 on IgE production. Rats immunized with OVA and injected with the anti-lectin domain Ab produced 90% less total IgE as well as less Ag-specific IgE. The inhibition observed was also isotype specific in that only IgE was affected. Both intact and Fab anti-lectin were capable of inhibiting IgE production. However, in another study, only the intact anti-CD23 mAb was capable of inhibiting IgE production and not the F(ab’)_2, suggesting that the Fc region of the anti-CD23 mAb may be important (40). In contrast to the results obtained with the Abs directed against the lectin domain, an Ab directed against the stalk

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**FIGURE 4.** Scatchard analysis for IgE binding to Fc1.7 in the presence of RAS1. Fc1.7 cells were incubated with increasing concentrations of cold IgE with or without RAS1 for 30 min, followed by 125I-labeled IgE. After 1 h, cell-bound cpm was determined on duplicate aliquots of cells. ●, IgE binding to Fc1.7; △, binding to Fc1.7 in the presence of RAS1. The lines represent regression analysis.

**FIGURE 5.** In vivo treatment of mice with RAS1 results in augmented IgE production and is mediated by CD23. BALB/c or CD23-deficient mice were injected with 2 mg RAS1 or nRIgG on days −2, 0, and 7 and immunized with KLH-DNP in alum on day 0. Mice were bled on day 14 (●) and day 20 (■). Total IgE, KLH-DNP-specific IgG1, and sCD23 levels were determined by ELISA. Total amounts of IgE, KLH-DNP-specific IgG1, and sCD23 varied between experiments: IgE, 1.04–8.1 µg/ml; IgG1, 73–876 µg/ml; sCD23, 2.61–190 ng/ml. Statistical analysis was performed using Student’s t test and represents significant differences between the nRIgG-treated BALB/c mice and the other groups. Values of p were ≤0.2 when CD23−/− mice (plus or minus RAS1 treatment) were compared with BALB/c mice that received RAS1 (∗, p ≤ 0.05; ∗∗, p ≤ 0.01).
portion of human CD23 (EBVCS-1) enhanced IgE production by PBMC stimulated with a combination of either IL-4 plus anti-CD40 mAb or hydrocortisone (38). This Ab did not inhibit IgE binding to CD23 (38), but IgE did inhibit binding of EBVCS-1 to CD23. This same Ab was later shown to enhance cleavage of CD23. The enhancement of proteolysis by EBVCS-1 could be decreased by the addition of IgE (41).

The effects of RAS1 on IgE production were studied using an in vivo model. Treatment of mice with RAS1 results in an increased IgE production in response to Ag/alum treatment. RAS1 also greatly reduces sCD23 found in the serum of these same mice suggesting that it is again stabilizing CD23 on the cell surface greatly reduces sCD23 found in the serum of these same mice.

Interestingly, mice treated with RAS1 produce less Ag-specific IgG1 than control animals. The levels of KLH-DNP-specific IgG1 are similar to those produced by CD23-deficient mice. Therefore, it seems likely that high affinity binding to IgE is needed not only to regulate IgE production but also for CD23 to play a role in enhancement of Ag presentation of IgE/Ag complexes.

These data support the work presented by Cousin et al. (41) who concluded that the mAb EBVCS1 enhanced cleavage of CD23 by interfering in oligomerization. This manuscript also supports the hypothesis of Cousin et al. that any molecule that could destabilize CD23 trimers would cause an increase in IgE production.

CD23 is being considered as a potential target for treatment of allergic diseases (9). The principle behind the idea is that by increasing CD23 expression on the cell surface, IgE production would be decreased. This work suggests that not only is it important for there to be high levels of surface CD23, but the oligomeric conformation of CD23 must be present to allow for high affinity IgE binding for IgE production to be regulated.

Acknowledgments
We thank Anne E. Shelburne for excellent technical assistance.

References
11. Heyman et al. (46) published that it is the CD23 on B cells that is responsible for the enhancement of IgE-mediated Ag presentation. Interestingly, mice treated with RAS1 produce less Ag-specific IgG1 than control animals. The levels of KLH-DNP-specific IgG1 are similar to those produced by CD23-deficient mice. Therefore, it seems likely that high affinity binding to IgE is needed not only to regulate IgE production but also for CD23 to play a role in enhancement of Ag presentation of IgE/Ag complexes.

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