Soluble CD23 Monomers Inhibit and Oligomers Stimulate IgE Synthesis in Human B Cells*

Received for publication, April 16, 2007, and in revised form, May 31, 2007. Published, JBC Papers in Press, June 18, 2007, DOI 10.1074/jbc.M703195200

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The low affinity IgE receptor, CD23, is implicated in IgE regulation and the pathogenesis of allergic disease. CD23 is a type II integral membrane protein, comprising a lectin “head,” N-terminal “stalk,” and C-terminal “tail” in the extracellular sequence. Endogenous proteases cleave CD23 in the stalk and the tail to release soluble fragments that either stimulate or inhibit IgE synthesis in human B cells. The molecular basis of these paradoxical activities is not understood. We have characterized three fragments of CD23, monomeric derCD23, monomeric exCD23, and oligomeric IzCD23. We show that the monomers inhibit and the oligomer stimulates IgE synthesis in human B cells after heavy chain switching to IgE. CD23 fragments could be targets for therapeutic intervention in allergic disease.

The low affinity IgE receptor, CD23, is implicated in the pathogenesis of autoimmunity and allergic disease. Levels of soluble CD23 are greatly elevated in rheumatoid arthritis (1, 2), systemic lupus erythematosus, and Sjögren’s syndrome (3), and the expression of CD23 is enhanced both as membrane and soluble forms in allergic patients (4, 5). Strikingly, levels of soluble CD23 are greatly elevated in rheumatoid arthritis (1, 2), systemic lupus erythematosus, and Sjögren’s syndrome (3), and the expression of CD23 is enhanced both as membrane and soluble forms in allergic patients (4, 5). Strikingly, levels of soluble CD23 are greatly elevated in rheumatoid arthritis (1, 2), systemic lupus erythematosus, and Sjögren’s syndrome (3), and the expression of CD23 is enhanced both as membrane and soluble forms in allergic patients (4, 5).

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‡1 Supported by Wellcome Trust Programme Grant 076343.

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The abbreviations used are: IL, interleukin; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

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soluble forms in allergic patients (4, 5). Strikingly, levels of soluble CD23 are greatly elevated in rheumatoid arthritis (1, 2), systemic lupus erythematosus, and Sjögren’s syndrome (3), and the expression of CD23 is enhanced both as membrane and soluble forms in allergic patients (4, 5). Strikingly, levels of soluble CD23 are greatly elevated in rheumatoid arthritis (1, 2), systemic lupus erythematosus, and Sjögren’s syndrome (3), and the expression of CD23 is enhanced both as membrane and soluble forms in allergic patients (4, 5).
EXPERIMENTAL PROCEDURES

Reagents

IgE Fc fragment Cε2-4 was produced and purified as described in Ref. 32. Humanized anti-CD23 antibody, lumiliximab, was kindly provided by M. Reff (Biogen-IDEC).

Production of derCD23, exCD23, and lzCD23

Recombinant derCD23 comprised amino acids Ser^{156–298} (Mr, 16,789), including the lectin domain and 16 amino acids (Asp^{283–298}) of the C-terminal tail of human CD23. exCD23 comprised amino acids Asp^{48–298} (Mr, 30,991), including the entire tail region. derCD23 and exCD23 were produced and purified as previously described (33). lzCD23 comprised amino acids Trp^{45–298} attached to the isoleucine zipper (Mr, 37,638). lzCD23 was produced and purified using the protocol described previously by (31) with the following alterations. lzCD23 was cloned into pet24a vector (Novagen), complete EDTA-free protease inhibitor mixture tablets (Roche Applied Science) were added to the refold buffer (1 m guanidine HCl, 2 mM EDTA, 100 mM (NH$_4$)$_2$SO$_4$, 0.4 m arginine, 1:2 mM reduced/oxidized glutathione, 50 mM CHES) to prevent degradation. A sample was analyzed by 12% SDS-PAGE, transferred to nitrocellulose (Schleicher & Schuell), probed with 10 µg/ml anti-CD23 (BU38 clone), and revealed with 1:2000 anti-mouse horseradish peroxidase (Dako) and ECL (Pierce) to check the purity and integrity of the protein. The oligomeric fragment was further purified by gel filtration using a Superdex-200 column (Amersham Bioscience), using PBS as the running buffer. Fractions containing the oligomer were pooled, concentrated to ~250 µg/ml, and stored at ~80 °C. All CD23 proteins contained <0.1 units/ml endotoxin measured by a commercial chromogenic limulus amebocyte lysate assay (Cambrex).

Kinetics of Interaction with IgE-Fc Cε2-4

Surface plasmon resonance was used to measure the kinetics of interaction of der-, ex-, and lzCD23 with IgE Fc (Cε2-4). All experiments were performed at 25 °C on an automated instrument (Biacore 3000; GE Healthcare). The methods for derCD23 and exCD23 were outlined previously (33). A similar protocol was used for determining the kinetics of interaction of lzCD23 with Cε2-4. 500–700 RU of biotinylated Cε2-4 was immobilized to a streptavidin-coated chip, derCD23 or lzCD23 was injected over the chip in duplicate at a variety of concentrations (1000, 500, 250, and 125 nM) at a flow rate of 20 µl/min in HBS/2 mM CaCl$_2$ with an association phase of 6 min and dissociation phase of 8 min. Regeneration of the chip was performed using 30-s pulses of 10 mM EDTA in HBS and 0.2 M glycine, pH 2.2, followed by a 120-min recovery.

Sedimentation Equilibrium Studies

Analytical ultracentrifugation studies were performed using a Beckman XL-A analytical ultracentrifuge as described previously (34). exCD23 and derCD23 were dialyzed into Tris-buffered saline plus 2 mM CaCl$_2$ and spun at 4 °C and 17,500, 20,000,
and 24,000 rpm (derCD23) or 10,000, 14,000, and 17,000 rpm (exCD23). Data were analyzed using the Beckman analysis software running under Microcal Origin version 3.78 in terms of a single ideal solute to obtain the buoyant molecular mass, $M(1 - i\rho)$. Residuals were calculated by subtracting the best fit of the model from the experimental data. In all cases, a random distribution of the residuals around zero was noted as a function of the radius. To determine the molecular mass of the CD23 fragments, values for partial specific volume were calculated from the sequence, again using SEDNTERP. These included self-association up to hexamers and also mixtures of noninteracting species.

Attempts were made to fit the exCD23Ce2-4 data with this and a number of other models (e.g. considering exCD23 as a monomer, dimer, or trimer), but none was found that adequately describes the data obtained.

**Isolation and Culture of Human B Cells**

Human B cells were isolated from tonsillar tissue from donors undergoing routine tonsillectomies (ethical approval from Guy’s, King’s and St. Thomas Hospital Trust). Tonsillar mononuclear cells were separated by density on a Ficoll gradient. B cells were then isolated using AET-treated sheep red blood cells. B cells were routinely >98% CD20-positive and ≤2% CD3-positive as determined by flow cytometry. B cells were cultured in 24-well plates (Nunc) at 0.5×10^6 cells/ml in RPMI with penicillin (100 IU/ml), streptomycin (100 μg/ml), 2 mM glutamine (Invitrogen), 10% fetal bovine serum (Hyclone, ECACC, UK) was used to construct a standard curve. IgE binding was detected by mouse anti-human IgE conjugated to horseradish peroxidase (DakoCytomation) diluted 1:1000 in 1% Marvel PBS-T for 4 h at room temperature. The color reaction was developed with OPD (Sigma). Sensitivity was 10 ng/ml. It was possible that recombinant CD23 interferes with the detection of IgE by ELISA. We investigated this by incubating CD23 fragments with NIP-IgE at 37 °C, employing the same culture
IgE—Maxisorp plates were coated with polyclonal goat anti-human IgG (Oxford Biotechnology Ltd.) diluted 1:1000 in carbonate buffer, pH 9.8, for 16 h at 4 °C. Unbound sites were then blocked with 2% Marvel in PBS-T for 30 min at room temperature. Samples were added at appropriate dilutions as described previously, and the plates were incubated for 16 h at 4 °C; total IgG (Sigma) was used to construct standard curves. IgG binding was detected by goat anti-human IgG horseradish peroxidase (Sigma) diluted 1:1000 in 1% Marvel PBS-T for 4 h at room temperature. The color reaction was developed with OPD (Sigma). Sensitivity was 4 ng/ml.

Polyacrylamide Gel Electrophoresis and Western Blotting
Purity and apparent molecular weight of CD23 fragments were determined by 12% SDS-PAGE under reducing conditions. Protein was visualized by a Coomassie Blue stain. Western blotting was used to confirm CD23 identity, using anti-CD23 (BU38 clone) and anti-mouse IgG horseradish peroxidase. Bound antibodies were visualized by enhanced chemiluminescence (Pierce).

Statistics
One-tailed, paired Student’s t tests were used to calculate p values, significance < 0.05.

RESULTS
Self-association of derCD23, exCD23, and lzCD23—The CD23 fragments were prepared as described under “Experimental Procedures.” Each of the three fragments exhibited a principal species of the expected size on SDS gel electrophoresis (Fig. 1a) and was confirmed to be CD23 by Western blotting (data not shown). The CD23 fragments were studied by sedimentation equilibrium in the analytical ultracentrifuge. Fig. 1, c and d, shows the data obtained for derCD23 and exCD23. The data for both fragments could be fitted using a single ideal species model, giving residuals randomly distributed around zero, revealing the fragments to be monomeric. The molecular masses calculated were 18,085 ± 350 Da for the derCD23 and 29,990 ± 906 Da for the exCD23. The structure of derCD23 has been determined by NMR spectroscopy (33) and found to be monomeric at 1.7 mg/ml (100 μM), although a small proportion of oligomer could be detected at extremely high concentration, 20 mg/ml (1.2 mM). exCD23 exhibited the propensity to form dimers and trimers at 1 mg/ml (32 μM) in sucrose gradient sedimentation velocity experiments (36). Taken together, these results suggest that the lectin head alone (derCD23) and the head plus the stalk (exCD23) both have the potential for self-association at sufficiently high concentrations.

To compare the activity of exCD23 as a monomer and an oligomer in our assays, we expressed exCD23 with an isoleucine zipper attached to the N terminus to generate lzCD23 (31) (calculated molecular mass for a monomer, 37,638 Da). The isoleucine zipper was previously characterized as a trimeric -helical coiled-coil by x-ray crystallography (30). As shown in Fig. 1b, lzCD23 eluted from a size exclusion column as a monodisperse protein with an apparent molecular mass of 20 kDa. However, the presence of a 15-nm -helical coiled-coil stalk, extended to 20 nm by the addition of the zipper (4.8 nm), would lead to a highly asymmetric structure and possibly an overestimation of its size by gel filtration. To obtain an unambiguous determination of the size of lzCD23, we used sedimentation equilibrium in the analytical ultracentrifuge (Fig. 2). The data could not be fitted to a species of a single size (data not shown);
therefore, data from three concentrations were fitted simulta-
neously to a wide range of self-association and mixture models. The residuals for the best fit are shown (Fig. 2b); this is for a monomer-trimer-hexamer equilibrium and yields residuals randomly distributed about zero. In addition, fits for monomer-
trimer equilibrium (Fig. 2c) and a noninteracting mix of mono-
mers, trimers, and hexamers (Fig. 2d) are shown for compari-
son, and these are clearly poorer fits to the data, with systematic residuals in both cases. The same result was obtained using data collected at two other speeds and at a lower range of concen-
trations (data not shown). The effective $K_d$ values for the mon-
omer to trimer and monomer to hexamer equilibria are 3.5 and 14.4 $\mu$M, respectively, which, for a total concentration of 12.5 $\mu$M lzCD23, the highest concentration examined, leads to cal-
culated values of 3.4 $\mu$M for monomeric, 3.0 $\mu$M for trimeric,
and 2.3 nM for hexameric lzCD23. The proportion of lzCD23
molecules as hexamer is thus extremely low, but their contri-
bution is essential to achieve a good fit to the data. The fraction
of lzCD23 molecules in the trimeric form is clearly substantial
at all concentrations tested: 73% at 12.5 $\mu$M, 68% at 9.3 $\mu$M, and
60% at 6.4 $\mu$M total concentration.

Stoichiometry of the Interaction of CD23 Fragments with C2-4—To investigate the interaction of derCD23 and
exCD23 with IgE-Fc (C2-4), we used sedimentation equilibrium as pre-
viously described for a similar monomeric CD23 fragment
binding to C2-4 (35). Fig. 3a shows the data for the derCD23/C2-4
interaction fitted to the same 1:1 and 2:1 interaction model used in Shi et al. (35), and it is clear that this is a good fit
to the data. Using a larger fragment of IgE-Fc thus has no effect
on the stoichiometry of the interaction.

The interaction between exCD23 and IgE-Fc is more com-
plex, although the exCD23 alone is also monomeric. Fig. 3b
shows the same 1:1 and 2:1 model fitted to the data, and it is
clearly a poor fit. Despite trying an extensive range of other
models, we were unable to find one that fits these data. It is
clear, however, that the complex (or mixture of complexes)
formed is larger than would be expected for the 1:1 and 2:1
interaction, implying that exCD23 must be oligomerizing upon
binding to the IgE-Fc.

Kinetics of Interaction of CD23 Fragments with C2-4—To investigate the influence of oligomer formation on the kinet-
ics of interaction with IgE, we immobilized the IgE-Fc
(C2-4) on a Biacore sensor chip and exposed this surface to
derCD23 or lzCD23 (Fig. 4). Surface plasmon resonance was
measured as a function of time to measure the rate of asso-
ciation with C2-4 and, after stopping the flow of CD23, to
measure the rate of dissociation of the complex. As shown in
Fig. 4a, the interaction of derCD23 with C2-4 was
monophasic and exhibited relatively fast on and fast off rates
and micromolar ("low") affinity ($K_D = 1000$ nM). In contrast,
lzCD23 exhibited biphasic kinetics ($K_{D1} \sim 2000$ and 100 nM)
(Fig. 4b). Previous analysis of exCD23 yielded binding curves
and association constants similar to those of lzCD23, ($K_D$
values $\sim 1000$ and 40 nM) (33).

Inhibition of IgE Synthesis by Anti-CD23—Having character-
ized all three CD23 fragments using biophysical techniques, we
next investigated their biological activity with respect to IgE
synthesis. First, as a control, we examined the antibody, lumil-
iximab, an anti-CD23 antibody produced as a potential therapy
for allergic asthma. Results from in vitro studies clearly showed
Soluble CD23 and IgE Regulation

**TABLE 1**

Lumiliximab (anti-CD23) at 1 μg/ml inhibits IgE production from tonsillar B cells stimulated with anti-CD40 (1 μg/ml) and IL-4 (200 IU/ml) for 10 days

| Expt. | IgE ± S.D. (n ≥ 3) | Anti-CD23 |
|-------|-------------------|------------|
|       | N o addition | 17.9 ± 1.3 | 14.1 ± 0.6 |
| Expt. 1 | 37.3 ± 4.5 | 17.9 ± 1.3 | 14.1 ± 0.6 |
| Expt. 2 | 420.8 ± 88.6 | 287 ± 73.9 | 14.1 ± 0.6 |
| Expt. 3 | 1487.3 ± 528.2 | 642.9 ± 14.1 | 14.1 ± 0.6 |
| Expt. 4 | 351.9 ± 64.1 | 177.5 ± 18.9 | 14.1 ± 0.6 |

*Expt., experiment.

**FIGURE 5.** Soluble CD23 fragments influence IgE production. Human tonsillar B cells were cultured for 10 days with 1 μg/ml of anti-CD20 and 200 IU/ml IL-4, with or without CD23 fragments, derCD23 (circles), exCD23 (triangles), and lzCD23 (squares) for 10 days. IgE was measured in cell supernatants by specific ELISA.

**TABLE 2**

IgE production from tonsillar B cells stimulated with IL-4 (200 IU/ml) and anti-CD40 (1 μg/ml) in the presence or absence of soluble CD23 for 10 days

| Expt. | IgE ± S.D. |
|-------|------------|
|       | No addition | derCD23 | exCD23 | lzCD23 |
| Expt. 1 | 1527 ± 420 | 622 ± 94 | 1654 ± 731 | 2016 ± 578 |
| Expt. 2 | 48 ± 7 | 35 ± 1 | 48 ± 2 | 262 ± 22 |
| Expt. 3 | 114 ± 10 | 61 ± 4 | ND* | 174 ± 9 |
| Expt. 4 | 405 ± 46 | 452 ± 18 | 573 ± 47 | 652 ± 145 |
| Expt. 5 | 444 ± 23 | 268 ± 4 | 276 ± 18 | 262 ± 8 |
| Expt. 6 | 85 ± 4 | 63 ± 1 | 55 ± 3 | 272 ± 26 |
| Expt. 7 | 86 ± 7 | 75 ± 5 | 76 ± 3 | 79 ± 7 |
| Expt. 8 | 243 ± 34 | 259 ± 41 | 267 ± 42 | 271 ± 40 |

*Expt., experiment.

**Effects of derCD23, exCD23, and lzCD23 on IgE Synthesis—**Preliminary experiments were carried out to determine the concentrations of CD23 fragments required for their maximum positive or negative effects on IgE synthesis in our system. In all cases, maximum activity was observed at concentrations equal to or greater than 100 ng/ml (data not shown). We therefore used 100 ng/ml in subsequent experiments. It is notable that 100 ng/ml (6 nm for derCD23, 3 nm for exCD23, and 0.8 nm for lzCD23), is 2–3 orders of magnitude below the observed Kd values for the interaction of these fragments with Ce2-4. B cells stimulated with anti-CD40 and IL-4 for 10 days produced variable amounts of IgE, depending on the donor (Fig. 5 and Table 2). The addition of derCD23 at 100 ng/ml significantly inhibited IgE synthesis. The percentage inhibition varied between donors but was unrelated to the level of IgE synthesis. Of eight donors tested, six demonstrated inhibition of IgE by derCD23 (mean = −24%; range −59 to +10%). The addition of lzCD23 at 100 ng/ml to the tonsil B cells, stimulated with anti-CD40 and IL-4, significantly up-regulated IgE in six of eight donors (mean +99%, range −41 to +446%) (Fig. 5 and Table 2). The level of stimulation, like that of inhibition by derCD23, varied between donors but was not related to that of IgE production. The addition of exCD23 at 100 ng/ml had no significant effect on anti-CD40- and IL-4-stimulated IgE synthesis (mean −3%, range −38 to +41%) (Fig. 5 and Table 2). Since CD23 binds to both secreted IgE and membrane IgE, it was possible that recombinant CD23 interferes with the detection of IgE by ELISA. This was tested by competitive ELISA and found not to be the case (data not shown). It was also possible that CD23 fragments might be degraded during the lengthy incubations with B cells. Western blot analysis of CD23 in parallel cultures revealed that no degradation occurred (data not shown). A further consideration is that incubation of the B cells with IL-4 up-regulates the expression of CD23 and the release of endogenous soluble fragments. In fact, we observed variable concentrations (30–50 ng/ml) of soluble CD23 in the culture medium after incubation of B cells with IL-4 and anti-CD40 in the absence of added CD23 fragments (results not shown), but this is at least 50% less than the concentrations of the recombinant CD23 fragments added to the cultures.

**Effects of derCD23 and lzCD23 on IgG Production—**To investigate whether the effect of soluble CD23 on IgE production was
isotype-specific or a general effect on immunoglobulin levels, we analyzed IgG production in the presence of derCD23 and IZF23. Neither fragment, added to human B cell cultures with

![Image](https://example.com/image.png)

**FIGURE 6. Proposed mechanisms of IgE regulation by soluble CD23.** a. model of trimeric soluble CD23 cross-linking IgE and CD21 on the B cell surface, leading to the formation of large networks, as shown in d, and the up-regulation of IgE synthesis. b. derCD23 competition with trimeric soluble CD23 for binding to IgE and CD21, preventing the formation of a network, and thus the up-regulation of IgE synthesis. c. IgE (Ce3 dimer shown in black) binding to two derCD23 heads (hatched), which can also bind to CD21 (domains D1D2 shown in white), in a defined complex, that cannot form larger aggregates. d. trimeric soluble CD23 molecules (shaded) binding up to three IgE molecules (one or two only are shown) and CD21 (D1D2), leading to the formation of large networks of CD23-IgE-CD21. It is this event that we propose leads to the up-regulation of IgE synthesis by trimeric soluble CD23 observed in this study. This model satisfies our present knowledge of the stoichiometry of the complexes that can be formed between these molecules.

**TABLE 3**

| IgG production from tonsillar B cells stimulated with IL-4 (200 IU/ml) and anti-CD40 (1 μg/ml) in the presence or absence of soluble CD23 for 10 days | ng/ml |
|---|---|---|
| Expt." 1 | No addition | derCD23 | IZF23 |
| | 220 ± 53 | 271 ± 35 | 246 ± 52 |
| | p = 0.2 | p = 0.3 | 0% |
| | +23% | +12% | 0% |
| Expt. 2 | 37 ± 6 | 39 ± 3 | 37 ± 2 |
| | p = 0.3 | p = 0.5 | 0% |
| | +4% | 0% | 0% |
| Expt. 3 | 84 ± 8 | 68 ± 2 | 89 ± 2 |
| | p = 0.0004 | p = 0.1 | 0% |
| | −19% | +6% | 0% |
| Expt. 4 | 48 ± 6 | 48 ± 6 | 43 ± 2 |
| | p = 0.5 | p = 0.1 | 0% |
| | 0% | +2% | 0% |

*Expt., experiment.

**DISCUSSION**

This is the first study to compare the effects of structurally well characterized monomeric and trimeric human CD23 fragments upon IgE synthesis in human B cells. We have demonstrated that derCD23, containing the head domain of CD23, is a monomer, and exCD23, with the head and stalk, is also a monomer, whereas IZF23, which differs from exCD23 only by the attachment of an isoleucine zipper at the N terminus, forms an equilibrium mixture of monomers and trimers. The use of sedimentation equilibrium in the analytical centrifuge determines the self-association state of the three CD23 fragments unambiguously.

We have studied the kinetics of binding of these fragments to IgE-Fc. The interaction of derCD23 is monophasic with “low” affinity (KD ~ 1000 nM), whereas the interactions of both exCD23 and IZF23 are biphasic with “low” and “high” affinity components (KD values of ~1000 and 100 nM). This biphasic kinetic behavior is similar to that observed for IgE binding to membrane-bound murine CD23, which also exhibits “low” and “high” equilibrium binding affinities with similar KD values (13). Protein-protein cross-linking revealed that membrane-bound CD23 can form trimers (13, 39), and thus the interpretation of the biphasic kinetics is that the interaction with a single CD23 head domain accounts for the “low” affinity component, whereas interaction with the trimer (and engagement of two head domains) accounts for the “high” affinity component of binding to IgE. Since we have shown that exCD23 is monomeric yet displays biphasic kinetics, we propose that oligomerization of exCD23 occurs upon binding to IgE-Fc. The analytical ultracentrifugation data for exCD23:Fce2-4 complexes reported here support this proposition.

We have previously shown by protein-protein cross-linking studies of a fragment similar to exCD23 in solution that it has the capacity to form trimers (39). Furthermore, we have shown here that the Ce2-4 fragment of IgE-Fc binds two molecules of derCD23. This provides a molecular mechanism to explain the ligand-induced oligomerization. When Ce2-4 was added to exCD23 in the analytical ultracentrifuge, we observed the formation of high
molecular weight complexes, consistent with oligomerization of the exCD23 and formation of extended aggregates with the divalent IgE-Fc. It was not possible to define the size distribution of these extended complexes, but the analytical ultracentrifugation data show clearly that although exCD23 and derCD23 are both monomeric when unliganded, they behave very differently in the presence of IgE-Fc. exCD23 forming large aggregates, whereas derCD23 forms only 1:1 and 2:1 complexes (CD23-IgE-Fc).

Most importantly, we have correlated the structure and IgE interaction kinetics of the CD23 fragments with their effects on IgE synthesis. The results clearly show a correlation between the capacity of fragments to form trimers and the stimulation of IgE synthesis in this system. derCD23 inhibits, exCD23 has no apparent effect upon, and lzCD23 stimulates IgE synthesis. The apparent lack of activity of exCD23 is probably due to competition between stimulatory trimeric and inhibitory monomeric fragments binding to IgE on the B cells. Furthermore, the observed effect was specific to IgE, since no change in IgG synthesis was seen.

CD23 has two ligands expressed on IgE class-switched B cells, IgE (40) and CD21 (41). Although IgE binds to a site in the lectin head, CD21 binds to the C-terminal tail, and NMR studies revealed that monomeric derCD23 is able to bind IgE and CD21 simultaneously (33). Murine CD21 has been shown to mediate the signals of oligomeric C3dg and related fragments of the C3 component of complement in stimulation of the immune response (42). Pierce (43) further demonstrated that this is accompanied by the formation of signaling platforms in the B cell membrane. Signaling through IgM and the CD19-CD21 complex stimulates the synthesis of Bcl-xL and Bcl-2, respectively, and these two signals act synergistically to promote B cell survival and the production of antigen-specific antibodies in the immune response (44). Co-ligation of IgM and CD21 on human B cells leads to the rescue from Fas-induced apoptosis through the induction of Bcl-2 (45). CD23 binding to CD21 and IgE in IgE-switched B cells might be analogous to antigen-C3dg complexes binding to IgM and CD21 in unswitched naive B cells, thus promoting IgE synthesis in the allergic response (Fig. 6a).

In this present study, we have demonstrated that lzCD23 up-regulates IgE synthesis from human B cells, and we propose that lzCD23 may act by co-ligating IgE and CD21 on IgE-switched B cells (Fig. 6a). In fact, trimeric CD23 could form a large array of such complexes (shown schematically in Fig. 6a), which could act as a signaling platform, similar to that observed for IgM and CD21 (43). The extended complexes formed between dimeric IgE-Fc and trimeric CD23 (Fig. 6d) are consistent with the observation of large complexes formed between Fce2-4 and exCD23 in the analytical ultracentrifuge. In contrast, derCD23 was found to inhibit IgE synthesis, and the mechanisms responsible may be understood by taking into account the presence of endogenous CD23. Clearly, endogenous CD23 is involved in IgE synthesis induced by CD40 ligation and IL-4, since anti-CD23 (lumiliximab) blocks IgE synthesis. derCD23 may therefore act by blocking the binding of endogenous trimeric soluble CD23 to CD21 and IgE (Fig. 6b), limiting the size of the signaling platform induced by the trimeric soluble CD23, since derCD23 only forms 2:1 complexes with IgE-Fc (Fig. 6c), and thereby preventing up-regulation of IgE synthesis.

Further support for this model comes from earlier work, employing less fully characterized CD23 fragments, which showed that soluble fragments of CD23 target ongoing IgE synthesis from committed B cells (28, 46–48). These observations are consistent with a role for membrane IgE in the regulation of IgE synthesis. Membrane IgE is associated with the same αβ signaling subunits as IgM (49), and cytoplasmic sequence of the ε-chain is required for the survival of IgE-expressing B cells (50). A role for CD21 has also been established. Antibodies that bind to the same region of CD21 as CD23 stimulate IgE synthesis, whereas antibodies that bind to other regions do not (15, 51). Experiments are under way to discover how the interaction of CD23 with IgE and CD21 up-regulates IgE synthesis.

In conclusion, our biophysical studies provide insight into the biological effects of different fragments of human CD23 that have puzzled earlier workers. We have for the first time demonstrated the crucial importance of the oligomeric nature of human CD23 fragments in the regulation of IgE synthesis in isolated B cells. These results have implications for the design of agents to prevent the release of soluble CD23 from cells or to modulate the oligomeric state and thus the activity of soluble CD23 fragments, in the treatment of allergic disease. As exemplified by lumiliximab, the inhibition of soluble CD23 fragments binding to membrane IgE and CD21 on B cells is a promising strategy for dampening the allergic response.

Acknowledgments—We thank Dr. Andrew Beavil for help in optimizing refolding conditions and guidance in purification of recombinant CD23 proteins. We thank Rodolfo Ghirlanda for valuable discussions relating to the analysis of the analytical ultracentrifugation data and Mitchell Reff for providing lumiliximab.

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