

Anti-CD23

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Abstract

CD23, the low-affinity immunoglobulin (Ig)E receptor (FcεRII), is widely distributed on the surface of various human cells. CD23 mediates numerous IgE-related immune responses (including allergen focusing) by enhancing IgE antigen complex presentation, regulating IgE synthesis, influencing cell differentiation and growth of both B- and T-cells, and stimulating production of pro-inflammatory mediators from monocytes/macrophages, eosinophils, and even airway smooth muscle cells. Both membrane and soluble CD23 play an important role in allergic reactions. Cellular contacts and cytokines modulate its expression in a concerted manner as needed for allergic reactions. Expression of CD23 and soluble CD23 has been associated with allergic diseases. Targeting CD23 with monoclonal antibody (MAb) is a promising candidate therapy in allergic diseases. A newly developed agent known as Lumiliximab, which is an anti-CD23 MAb (Lumiliximab), was demonstrated to be a well-tolerated agent in a phase I clinical trial (a placebo-controlled study with allergic asthma). Adverse events were mild, and no relationship was apparent between the dose of Lumilixilab and the frequency, severity, or type of event. Sustained and dose-dependent decreases in mean serum total IgE concentrations were noted. The serum half-life of Lumilixilab increased from 2 to 10 d with increasing doses. Blocking antigen presentation, preventing costimulation signals, and reducing production of pro-inflammatory mediators are some of the potential mechanisms involved for anti-CD23 activity. Although the safety and clinical efficacy of Lumilixilab in allergic asthma and rhinitis require confirmation, the observed data imply that anti-CD23 is a promising candidate therapy option for future treatment of allergic diseases.

Index Entries

IgE; IgE low affinity receptor (CD23); anti-CD23; allergic diseases.

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Introduction

Immunoglobulin (Ig)E has long been considered to be a key molecule in allergic diseases. Binding of IgE antigen complex to its two receptors, high-affinity IgE receptor (FcεRI) and low-affinity IgE receptor (FcεRII/CD23), which are located on various cell types, initiates immune and inflammatory reactions in allergy. Activation of FcεRI is involved in acute allergic responses to allergens mainly through mast cells and basophils. CD23 appears to mediate various forms of IgE-mediated immunity. Expression of CD23 on blood lymphocytes, alveolar macrophages, airway smooth muscle cells, and even serum soluble CD23 have been linked to allergic diseases. Immunotherapy, which has been suggested as an efficient therapy option for allergic diseases, and other systemic treatments significantly reduced their expression (1–6). In CD23-deficient transgenic mice, allergic airway hyperresponsiveness was significantly reduced compared with wild-type mice. Anti-CD23 reduced allergic airway inflammation, IgE levels, and eosinophilia and normalized allergen-stimulated airway responsiveness in wild-type mice (7,8). Recent studies have also indicated that genetic polymorphisms of CD23, as well as their expression levels, could be another important contributor among candidate gene variations to influence the occurrence, severity, and treatment of atopic diseases (9). Targeting CD23 is expected to affect multiple aspects of allergic disorders. CD23 antagonists, such as anti-CD23 antibodies, could be useful in the treatment of allergic diseases, including allergic rhinitis, allergic asthma, and so forth. Therefore, a primatized, IgG1, anti-CD23 monoclonal antibody (MAb) known as Lumiliximab was constructed, consisting of primate (cynomolgus macaque) variable regions and human constant regions (IDEC Pharmaceuticals, San Diego, CA). The in vitro and clinical studies have demonstrated its safety profile and downregulation of serum IgE level. This article

provides a brief summary about CD23 and its role in allergic diseases, the preliminary results of anti-CD23 concerning its safety, future potential clinical usage, and the rationale behind its potential mechanisms.

CD23 as a Potential Target for Therapy

Structure and Distribution

CD23 is a type II integral membrane single-chain glycoprotein with an apparent molecular weight of 45 kDa, composed of 321 amino acids (10–12). It is widely distributed in various human cells, including B-cells (10), monocytes and macrophages (13–15), eosinophils (16–18), platelets (16,19), epidermal Langerhans' cells (20–22), follicular dendritic cells (23,24), keratocytes (25), and even airway smooth muscle cells (6). Two subtypes, FcεRIIa/CD23a and FcεRIIb/CD23b, have been identified. They differ in tissue-specific gene expression and regulation. FcεRIIa is believed to be constitutively expressed only in normal B-cells, B-cell lines (perhaps follicular dendritic cells), and eosinophils, whereas FcεRII b is induced in various cell types (13,17,26,27). The human *FcεRII/CD23* gene has been mapped to chromosome 19p13.3 (28,29). It spans 13 kb and consists of 11 exons. FcεRII appears to be generated using different transcription initiation sites and alternative RNA splicing. CD23a transcripts contain exons I, II, and III–XI, whereas CD23b transcripts comprise exons b and III–XI (26). The differences in transcripts result in six to seven different amino acids in the N-terminal cytoplasmic side between CD23a and CD23b but shared common C-terminal extracellular regions (26). CD23 may exist on cell membranes as a dimer or trimer (30–32). Six structural features for the human CD23 trimer were discussed by Kijimoto-Ochiai (33):

1. C-type lectin domain for IgE binding.
2. Cytoplasmic domain differing between CD23a and CD23b.

3. Leucine zipper structure.
4. The protease cleavage sites.
5. The reverse Arg-Gly-As (RGD) sequence near the C-terminus.
6. The "RGD binding inhibition peptide" at the root of the *N*-linked carbohydrate chain.

Immune Modulation and Pro-Inflammatory Functions

It is well-known that antigen presentation and pro-inflammatory responses driven by allergen exposure play a crucial role in immune responses and inflammatory reactions in allergic diseases. CD23 may function in these diversified immunological activities by enhancing antigen presentation, activating antigen-presenting cells (APCs), improving cell differentiation and growth, and regulating IgE synthesis (34–41).

Antigen Presentation

CD23 mediates IgE-specific antigen presentation, as evidenced by selectively blocking CD23 with an anti-CD23 MAb (34). Other studies reported that anti-CD23 antibody induces B- and T-cell tolerance signals *in vivo* and that genetically engineered CD23-deficient mice do not display IgE-dependent augmentation of an immune response (42,43). CD23 enhanced IgE antigen complex presentation 100-fold more than either unfocused or IgG-bound antigen in animal studies (44–46). The proposed role for CD23 in antigen presentation might include the binding of antigen–IgE antibody complexes, internalization of the complexes, transport to compartments of the endosomal network containing proteolytic enzymes, interaction with major histocompatibility complex (MHC) class II antigens, and interaction with CD21 at points of contact on the B- and T-cell surface (refs. 47 and 48; see Fig. 1).

Binding of CD23 to IgE is believed to occur between the CH3 domain of IgE and within the lectin homology region of CD23. Binding of IgE to CD23 via protein or protein–carbohydrate interaction leads to antigen uptake through

both CD23a and CD23b (27,35). CD23a in B-cells appears to mediate endocytosis, whereas CD23b mediates phagocytosis because of their difference at the N-terminus with six or seven amino acids (47,48). CD23 is associated with MHC class II molecules on the cell surface of APCs. CD23 was observed in cytoplasmic organelles that resemble "the compartments for peptide loading" or "class II vesicles." These are considered components of the class II transport network, with characteristics of endosomal compartments and the lysosomal network (47). This might be expected to facilitate peptide loading of human leukocyte antigen-DR (HLA-DR) and, therefore, the process of antigen presentation. The return of CD23 with HLA-DR to the cell surface may help to stabilize interactions between B- and T-cells, contributing to T-cell activation via contact with CD21 (49,50).

T-Cell Activation and Differentiation

Uptake of allergen by APCs (B-cells, monocytes/macrophages, dendritic cells, etc.) modulates immune and inflammatory reactions in several aspects. After interaction with MHC class II, antigen is presented to naïve T-cells by interacting with the T-cell receptor (TCR) and initiates T-cell activation and differentiation. Meanwhile, interactions of cellular molecules between APCs and T-cells (e.g., CD40 on APCs with CD154 on T-cells and CD86 or CD80 on APCs with CD28 on T-cells) contributes to this process by sending accessory signals and enhancing development of APCs. Other signals may come from cytokines and other pro-inflammatory mediators produced from activated APCs. Complicated stimulations promote naïve T-cells to differentiate into more specific T-cells (T-helper [Th]2 and Th1; see Fig. 1). Differentiated T-cells produce different subsets of cytokines in driving cellular and humoral immunity. Activated naïve Th0 cells primarily produce interleukin (IL)-2, which stimulates T-lymphocyte growth in an autocrine fashion. Th1 cells produce interferon (IFN)- γ and tumor necrosis factor (TNF)- β ,

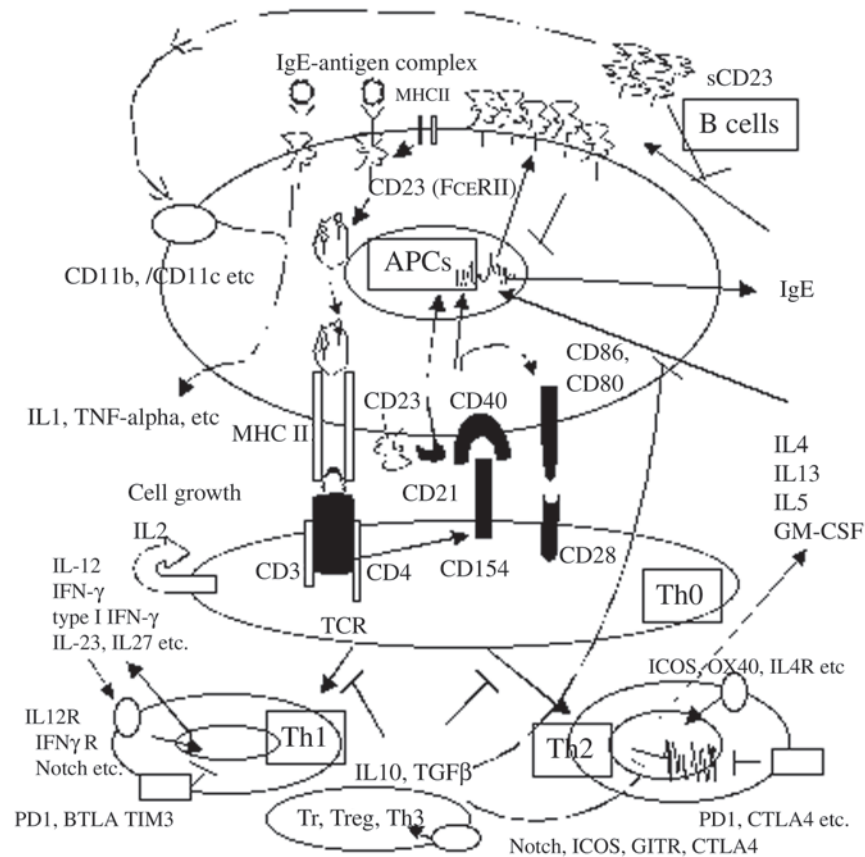


Fig. 1. Hypothetic scheme for the role of CD23 in allergic reaction.

rather than IL-4 and IL-5, and promote cellular immunity, which is critical to control of intracellular pathogens and phagocytosis by macrophages. Th2 cells predominantly produce IL-4, IL-5, IL-13, and IL-9, which induce allergic immune responses, including IgE production and eosinophil activation. A third class of Th lymphocytes (known as Th3 or T-regulatory [Tr]1) has also been identified and produces the immunosuppressive cytokines transforming growth factor (TGF)- β and IL-10. These lymphocytes are important in actively suppressing or terminating immune response and then inducing allergen tolerance. CD25⁺ is found on another subset of CD4⁺ Tr cells. These cells are characteristically anergic to stimulation by TCR in the absence of exogenous IL-2 and in their ability to suppress the activation of other T-cells in a cell contact-dependent manner, rather than via IL-10 or TGF- β .

Reduction of IL-4-producing cells by anti-CD23 was reported in an *in vivo* animal study (47). Production of IL-4 and granulocyte macrophage-colony stimulating factor (GM-CSF) may occur following CD23-mediated IgE antigen presentation (34,42,51). We believe that blocking of CD23 effectively influences T-cell activation and Th2-cell differentiation, accounting for its downregulation of IgE. The role of CD23 in this process at the level of Th1, Th3 (or Tr1), or Tr cells are not yet known and require future studies.

B-Cell Activation, Differentiation, and IgE Synthesis

B-lymphocytes function in antigen presentation and humoral immunity. The direct influence of CD23 on B-cell activation and differentiation may occur through multiple mechanisms, including ligation of membrane CD23,

soluble CD23 (sCD23), and crosslinking of CD23. Membrane CD23 has been demonstrated to be cleaved through various endogenous and exogenous enzymes, producing sCD23 molecules (52–54). Different sizes of fragments of 17, 25, 27 to 29, 33, and 37 kDa can be observed, corresponding to cleavage sites around Ser 299, Ser 156, Arg 149, Lys 102, and Glu 81, respectively (53,55). The sCD23 may act as an autocrine growth factor for B-cells (39,56). As a differentiation factor, sCD23 (in synergy with IL-1) not only encourages the survival of germinal center B-cells but also promotes their differentiation toward a plasmacytoid morphology through prevention of apoptosis (57–59). Binding of sCD23 to various known surface molecules, such as CD21, CD11b/CD18, CD11c/CD18, or other as yet unknown receptors, may be involved in this process (59). However, co-crosslinking CD23 with surface IgD was demonstrated to inhibit mouse B-cell proliferation. This may explain a negative feedback loop for IgE regulation, in which higher levels of antigen-specific IgE may downregulate B-cell activation and IgE synthesis (60).

Activating CD23 on B-cells with a MAb can either stimulate or inhibit cell growth, depending on the epitope involved (38,61,62). It has been suggested that a pertussis toxin-insensitive G protein couples membrane CD23 to phospholipase C in human B-cells following CD23 activation. Consequently, the generation of inositol (1,4,5)-triphosphate (IP₃) results in calcium release from intracytoplasmic stores following phospholipase C-dependent hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP₂) (63). Subsequent events might stimulate activation of genes encoding various cytokines and inducible nitric oxide synthase via nuclear factor- κ B, leading to cell activation, differentiation, and cell death.

The regulation of IgE synthesis by CD23 has been well-documented, although other antigen-specific antibodies may also be modulated by CD23 (44,45,47). Multiple mechanisms may be involved. First, increased cytokine pro-

duction, such as IL-4, and augmented accessory signals after antigen presentation may contribute to its enhancing effect on IgE production. Second, sCD23 molecules produced from cleavage of membrane CD23 were observed to be key molecules for potentiating spontaneous synthesis of IgE (64–66). They are believed to be composed of bigger molecules of 37, 33, and 25 to 27 kDa. The interaction of sCD23 and CD21 may provide accessory signals to IL-4 and CD40-dependent IgE synthesis machinery or stabilize interactions between T- and B-cells (67–69). The smaller 16-kDa molecule appears to downregulate the ongoing and IL-4-stimulated synthesis of IgE (70). Alternatively, a promoting effect of activating CD23 on B-cell growth and differentiation may be another mechanism that enhances IgE synthesis.

Pro-Inflammatory Effects

In addition to its regulatory effects on innate and adaptive immunity, CD23 also plays an important role in inflammation through promotion of the release of pro-inflammatory mediators and cell adhesion activity. CD23 has been reported to stimulate production of pro-inflammatory mediators, including cytokines (TNF- α , IL-1, IL-16, IFN- γ), oxidant burst, lipid mediators (such as thromboxane B₂), and chemokines (such as IL-8, macrophage inflammatory protein-1 α , and macrophage inflammatory protein-1 β) from monocytes and macrophages (40,71–74). Ligation of membrane CD23 and interaction of sCD23 with cell surface molecules, such as CD11b or CD11c and vitronectin receptor and CD47, are involved in this process. Multiple signaling pathways, including p38 mitogen activated protein kinase (MAPK), JNK, and Erk pathways may be involved in the regulation of pro-inflammatory mediators (40,72,75–78).

Eosinophils are believed to play a major role in allergic asthma (79). Expression of both types of CD23 has been detected in human eosinophils—mainly in the hypodense subpopulation (17). Once activated through CD23,

eosinophils release various products that amplify the inflammatory reaction via production of toxic oxygen species and TNF- α (80–82), as well as the increased cell surface receptor and adhesion molecule expression to mediate migration of eosinophils (18,83,84).

Airway smooth muscle (ASM) is another important target in allergic asthma. CD23 is expressed in human ASM tissues. Increased expression occurs in inherently asthmatic ASM tissue. Activation of CD23 through IgE complex stimulated asthmatic ASM tissues to produce pro-inflammatory cytokine IL-1 (6). More studies are expected to further delineate its role in the asthmatic process.

The wide distribution of CD23 implies multiple roles in allergic reactions. Anti-CD23 is expected to beneficially impact multiple allergic inflammatory processes and to facilitate immune modulation.

Regulation of CD23

CD23 expression is strictly regulated for its multiple functions by various stimuli. CD23 expression has been reported as absent or at very low levels in T-lymphocytes, monocytes, macrophages, eosinophils, and ASM cells from nonatopic subjects compared with atopic subjects or compared with other diseases. In human B-cells, low-level expression of CD23 is a constitutive feature of IgM⁺ IgD⁺ B-cells in the periphery, whereas immature μ^+ and Δ^+ B-cells in bone marrow do not express CD23. B-cells lose CD23 expression after undergoing isotype switch to γ -, α -, or ϵ -bearing cells. In tonsils, B-cells in the follicular mantle are CD23⁺, and those in the germinal center are CD23⁻ (15,38). Both extracellular ligands and humoral stimulation deliver necessary signals to effectively modulate CD23 expression.

Cellular contacts have been demonstrated to play an important role in potentiating or suppressing CD23 expression. CD40 ligands on T-cells bind CD40 and activate resting B-cells to elevate expression of CD23 together with MHC class II (85). JAK–signal transducers and

activators of transcription (STAT) and p38 MAPK are believed to be the pathways involved (86). Cytokines produced from Th cells include a large repertoire of signals capable of regulating CD23—especially in allergic immune responses. IL-4, IL-5, IL-13, and IL-9 are prototypic Th2 cytokines that induce allergic immune responses (87). Elevation of CD23 expression is one of the IL-4- and IL-13-induced allergy-related biological activities. CD23b is considered to be at least the main form stimulated by IL-4 and IL-13 (13,88). The STAT6 pathway, cyclic adenosine monophosphate, and transient inositol lipid hydrolysis—but not protein kinase C and Ca⁺⁺ pathways—are believed to mediate CD23 regulation (89,90). IL-5 and GM-CSF with shared γ_c receptor have been reported to enhance CD23 expression alone or in synergy with IL-4 (13,85,91,92). Upregulated CD23 by GM-CSF mediates IgE-induced macrophage activation, making cells susceptible to IgE-dependent activation (13,92). IFN- γ showed conflicting effects, with inhibition of expression on B-cells (15,93,94), whereas it induced CD23 expression on monocytes and human epidermal Langerhans' cells (13,92,95). IFN- γ was also shown to increase the release of sCD23 from the surface of monocytes and epidermal Langerhans' cells (94–97). The magnitude of the enhancing effect of IFN- γ on the secretion of sCD23 appears to be more than that expected from its capacity to upregulate CD23 expression. Therefore, IFN- γ might accelerate the cleavage of surface CD23 or increase the intracellular breakdown of this molecule into its secreted form.

Although corticosteroids have been widely used as potent anti-allergic and anti-inflammatory drugs, their activities in allergic diseases remain controversial (98,99). However, steroids have been consistently demonstrated to downregulate expression of CD23 (98,100,101). They decrease human CD23 expression synergistically with IFN- γ in mononuclear cells and B-cells via downregulation of IL-4-induced receptor expression and STAT6 activation. The

inhibition of tyrosine phosphorylation of JAK1, IL-4 receptor, and STAT6, as well as subsequent nuclear translocation and DNA binding might be the inhibition mechanism (101).

Similarly to corticosteroids, TGF- β was identified to suppress CD23 surface expression on the human eosinophilic cell line EoL3 and human monocytic cell line U937 at baseline and after stimulation by IL-4, IFN- γ , and platelet-activating factor (102,103). Almost 90% of enhanced surface CD23 was inhibited in the presence of TGF- β . The inhibitory effects also occurred in isolated tonsil human B-lymphocytes (104). Additionally, we confirmed the suppressive effect of TGF- β on CD23 expression in human peripheral blood mononuclear cells and lung macrophages (unpublished data). The mechanisms involved in this process require further exploration.

In addition to regulation of cellular expression, regulation of proteolysis is specific for CD23 regarding its functional activities in immunology compared to other Ig receptors, because sCD23 has cytokine-like properties. The culture supernatant from CD23⁺ B-cells has been long reported to be capable of potentiating the spontaneous synthesis of IgE, but not IgG or IgM, by B-cells from allergic donors (66). Subsequent studies identified the fact that sCD23 molecules in the culture supernatant (shed from the cell surface) account for this activity (105–107). The cleavage of CD23 occurs on the cell surface rather than through an intracellular posttranslational process. The 37- and 33-kDa fragments are short-lived intermediates that are cleaved into more stable 25- to 27-kDa molecules (53). Numerous endogenous and exogenous enzymes have been reported to catalyze the cleavage processes. Chymotrypsin and elastase are among those that cleave CD23 at Glu 81-Ala 80, releasing a 37-kDa fragment (108,109). It was recently demonstrated that purified human leukocyte elastase and cathepsin G (a chymotrypsin-like proteinase in human neutrophils) efficiently cleaved membrane CD23 on B-cells, accounting for neutrophil-

derived production of sCD23 in inflammatory reactions. The proteolytic fragments released, 37 or 25 kDa, may be responsible for the activation of monocytes, enhancing oxidative stress and secretion of TNF- α (110). The release of the 33-kDa sCD23 fragments from cell membranes has been reported to be mediated by one or several membrane-bound matrix metalloproteases, because metalloprotease inhibitors 1,10-phenanthroline and imidazole and the matrix metalloprotease broad spectrum inhibitor batimastat antagonize this process (111). A recent report identified ADAM8, ADAM15, and, especially, ADAM28 (MDC-L), but not ADAM17, as members of the ADAM family that play an important role in “ectodomain shedding” of membrane CD23 (54). ADAM33 may be another member of ADAM family capable of catalyzing CD23 cleavage (unpublished observations). Regarding the wide distribution of ADAM proteases in various cell types, the release of CD23 might be related to allergic reactions in different tissue types (111). *Der p 1*, a cysteine protease representing a major allergen of the house dust mite *Dermatophagoides pteronyssinus*, has been shown to cleave CD23 from the surface of cultured human B-cells (52,112,113). The cleavage sites are at Ser-Ser (155–156) and Glu-Ser (298–299). A 17-kDa fragment containing the lectin domain and part of the C-terminus tail is released. The question regarding whether the hypersensitivity in patients with allergic to *Der p 1* results from its proteolytic activity remains unanswered.

Studies regarding the mechanisms involved in the cleavage of CD23 are important for seeking appropriate agents to target its proteolysis in allergic disease treatment. CD20 is a B-cell-specific surface molecule that may be involved in the regulation of activation, proliferation, and differentiation (114). One MAbs to CD20 (B1) has been found to decrease the expression of CD23 by stimulating the cleavage of extracellular CD23 from the surface of Epstein-Barr virus-transformed B-cells and IL-4-stimulated normal B-cells rather than at the messenger

RNA level (115). The exact mechanism between CD20 engagement and CD23 shedding is not clear. Extracellular adenosine triphosphate (ATP) was recently shown to mediate shedding of L-selectin and CD23 from the surface of both normal and leukemic lymphocytes via activation of the P2Z/P2X7 purinoceptor of these cells (24,116). The P2Z/P2X7 receptor, but not P2X, is expressed on the surface of human dendritic cells, T- and B-lymphocytes, natural-killer cells, monocytes, and macrophages (24,117,118). Activated P2X7 may stimulate the activation of metalloproteases to mediate the shedding of L-selectin and CD23. Different membrane metalloproteases appear to be involved in the shedding of L-selectin and CD23 because ATP and BzATP induced shedding of CD23 in B-cells at smaller rates and the inhibition kinetics showed differences between the two molecules (24,119). Based on this information, researchers should look for appropriate matrix metalloprotease inhibitors, as well as anti-CD23 antibodies that diminish proteolytic processes of CD23. Such efforts have resulted in reduced IgE production in human tonsillar B-cells stimulated with IL-4 plus anti-CD40, human peripheral blood cells, and even with in vivo mice experiments (120–123).

Therapeutic Experiences With Anti-CD23 (Lumiliximab)

In the single-dose, dose-escalating, placebo-controlled study with 24 patients with mild-to-moderate persistent allergic asthma, 3 to 6 patients received single intravenous infusions of either placebo or lumiliximab; 0.05, 0.25, 1, 4, 10, or 15 mg/kg on day 1. During the 12 wk following the treatment, the data showed that lumiliximab was well tolerated. The serum half-life of lumiliximab increased from 2 to 10 d with increasing doses. Peak concentration in serum reached 658 mg/mL (124). These results suggest that a weekly or monthly dosing schedule will be feasible for multiple-dose studies. A phase II, randomized, single-

blind, placebo-controlled, multiple-dose study in 131 patients with allergic rhinitis was also performed. Patients received four monthly intravenous infusions of 0.25, 1, 2, or 4 mg/kg of lumiliximab. Further analysis is underway.

Clinical Activities

Sustained and dose-dependent decreases in mean serum total IgE concentrations were demonstrated after a single dose of intravenous infusion ranging from 0.05 to 15 mg/kg (see Fig. 2). IgE appears to decrease as early as day 2, and for doses of 1 mg/kg or greater, IgE drops about 30% from baseline by 6 wk and remains about 25% less than baseline by 12 wk. For the highest dose (15 mg/kg), mean IgE concentration remains at about 40% less than baseline at 12 wk. Long-time reduction of IgE concentrations through the use of an anti-CD23 MAb (perhaps with multiple doses) is likely to be beneficial in allergic asthma and other allergic diseases.

Patients with allergic asthma were the subjects in the phase I, single-dose study. Clinical symptoms and pulmonary functions related to anti-CD23 treatment were examined. No apparent dose-dependent change in forced expiratory volume after 1 s or morning peak expiratory flow rate was observed. Other indicators, such as mean number of asthma or rhinitis symptom-free days per week, number of nighttime awakenings caused by asthma, or the use of on-demand (rescue) β -agonist or asthma medication, were also not affected. Because of several limitations in the phase I study (such as small sample size, single-dose treatment, and lack of allergen challenge), more studies are exploring clinical improvement. The favorable reduction in serum IgE concentration indicates that targeting of CD23 by MAbs might provide a positive clinical effect in patients with allergic asthma.

Safety of Lumiliximab

Based on human clinical studies in allergic subjects performed up to date, lumiliximab was

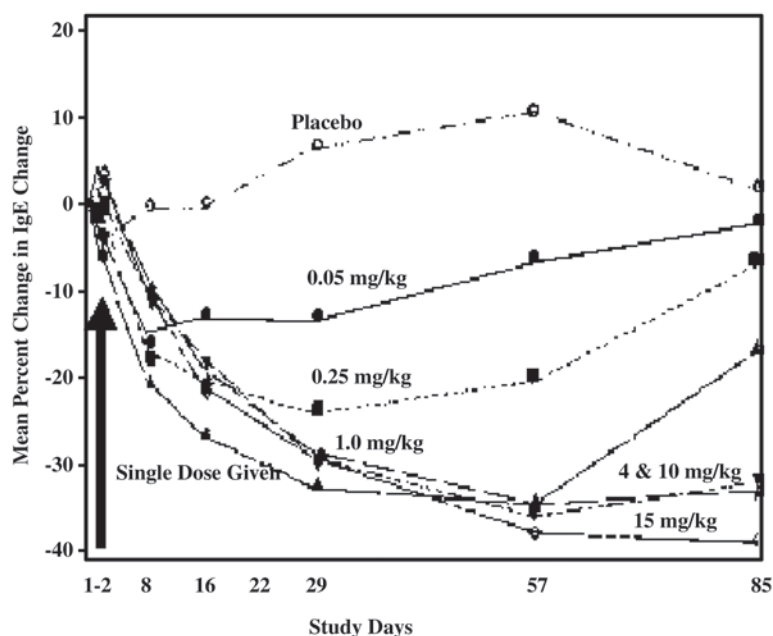


Fig. 2. Dose-dependent decrease in total immunoglobulin-E with the anti-CD23 monoclonal antibody (IDEC-152). (From ref. 124.)

well tolerated. In the phase I study (124), adverse events were mild, and no grade 3 or 4 reactions (as defined by National Cancer Institute common toxicity criteria) were observed with lumiliximab. No relationship was apparent between the dose of lumiliximab and the frequency, severity, or type of event. The most common adverse events possibly related to lumiliximab include ecchymosis at the infusion site, peripheral edema, and nausea. Patients did not have abnormal platelet counts or bleeding times. Although infections, such as sinusitis, colds, bronchitis, pneumonia, otitis media, upper respiratory infection, and viral infection, were observed in almost 50% of patients, they were also common in placebo groups at similar rates and, therefore, might not be associated with lumiliximab treatment. B-cell counts remained in normal ranges with low doses of lumiliximab, whereas doses, such as 4, 10, and 15 mg/kg, decreased B-cell counts. A maximum of 55% decrease from baseline was observed in subjects receiving a dose of 10 mg/kg. However, a clear dose response was not observed. There is a trend for slow return back

to the normal range over time with high-dose groups. CD23 is expressed on a fraction of normal B-cells. B-cells are probably one of the main targets for anti-CD23 treatment. The clinical significance of this B-cell transient reduction requires further clinical studies. Mean CD4⁺ T-cell counts did not show obvious changes during the treatment. No subjects developed detectable levels of anti-lumiliximab antibodies. Preliminary safety information has been favorable in patients treated with anti-CD23 antibody for ragweed-induced seasonal allergic rhinitis. Further analysis is underway (125). To date, evidence supports the safe use of anti-CD23, but additional studies both with more subjects and multiple dosing are needed to verify its safety.

Potential Mechanisms Involved

Anti-CD23 Induces T-Cell Tolerance in Ex Vivo Experiments

To further understand the mechanisms involved, allergen-induced cell proliferation was determined in freshly isolated blood

mononuclear cells from allergic patients by incubating with anti-CD23 (unpublished data). After 6 d of cell culture, proliferation reflected from the stimulation index showed that addition of anti-CD23 at both 10 and 100 $\mu\text{g}/\text{mL}$ significantly reduced the allergen-induced cell proliferation. This result suggested that lumiliximab induced allergen tolerance, possibly by affecting membrane CD23 or sCD23.

Anti-CD23 Inhibits Co-Accessory Signals

Co-stimulation signals from APCs to T-cells are important in allergen-induced cell proliferation and differentiation. CD86 and CD80 are examples of co-stimulatory surface molecules. Blocking CD86 or CD80 and MHC class II with MAb significantly suppresses the allergen-induced cell proliferation. It appears that CD86 is more potent than CD80 in altering the allergen-mediated reaction. Anti-CD23 at 100 $\mu\text{g}/\text{mL}$ has been demonstrated to significantly reduce CD86 surface expression stimulated by IL-4 plus GM-CSF in the U937 cell line (unpublished data). This indicates that blocking CD23 by lumiliximab can inhibit co-accessory signals. Meanwhile, we have examined apoptosis using Annexin V. Anti-CD23 did not induce significant U937 cell apoptosis after 6 h of incubation, but understanding its role in apoptosis requires further study.

Anti-CD23 Inhibits Pro-Inflammatory Mediator Production

Pro-inflammatory mediators are important in regulating various cellular functions. To determine the effect of anti-CD23 on pro-inflammatory mediator production, anti-CD23 was incubated with blood mononuclear cells in the presence of allergen for 48 h. IL-1 β was measured in supernatants using a specific enzyme-linked immunosorbent assay. At 100 $\mu\text{g}/\text{mL}$, anti-CD23 significantly reduced the production of IL-1 β , suggesting that a suppressive effect of anti-CD23 on pro-inflammatory mediator production could be another mechanism involved in its effect.

Conclusion

With respect to the complicated role of CD23 in immune modulation and pro-inflammatory functions, anti-CD23 might be expected to induce tolerance, block antigen presentation, suppress T-cell differentiation, and diminish activation of APCs in allergic reactions. More studies are underway to clarify the role of CD23 in allergic reactions in ex vivo experiments and to explore the mechanisms involved by applying advances in immunological sciences. We also believe these studies will provide more information about the process of allergic reactions and will help us understand the mechanisms involved. Anti-CD23 may be a promising therapeutic option for allergic diseases in the future.

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