

Inhibition of CD23 processing correlates with inhibition of IL-4-stimulated IgE production in human PBL and hu-PBL-reconstituted SCID mice

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Summary

Background CD23, the low affinity serum immunoglobulin E (IgE) receptor, is upregulated on B cells following interleukin (IL)-4 stimulation and is concomitantly cleaved to generate soluble CD23 (sCD23) fragments with cytokine-like activity.

Objective Compounds that selectively inhibit the proteolytic release of CD23 to generate sCD23 were assessed for their ability to inhibit IgE production in order to evaluate the contribution of sCD23 in the production of human IgE as well as the ability of such compounds to block IgE production.

Methods IgE production was measured in IL-4-stimulated human peripheral blood lymphocytes (PBL) and PBL-reconstituted SCID mice in the presence of a broad-spectrum matrix metalloprotease (MMP) inhibitor, a compound selective for inhibition of CD23 processing over MMPs and an anti-CD23 mAb, MHM6.

Results The two compounds were equipotent in inhibiting IgE production without inhibition of IgG production by IL-4/anti-CD40-stimulated PBL. Soluble CD23 release was also shown to precede IgE accumulation in the cell-free medium. Addition of compound at later times other than day 0 in the 14 day assay resulted in progressively less inhibition of both IgE and sCD23, and exactly paralleled the effect of an anti-CD23 mAb, MHM6 on IgE levels. Both compounds also inhibited the release of CD23 from human RPMI 8866 cells adoptively transferred i.p. to mice. Doses required for inhibition of CD23 correlated well with the doses required for inhibition of IgE production in IL-4-challenged hu-PBL-SCID mice. IgE was selectively inhibited over total IgG in the SCID mice as well.

Conclusions Inhibition of CD23 processing alone is sufficient to inhibit IL-4-stimulated IgE production both *in vitro* and *in vivo*.

Keywords: CD23, IgE, metalloprotease

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Introduction

Allergic or atopic disease is characterized in part by the excessive production of serum immunoglobulin E (IgE) in response to various allergens [1,2]. Results of recent clinical trials with anti-IgE monoclonal antibodies (mAbs) have

indicated a strong correlation between reduction in free serum IgE and improvement in allergic symptoms, providing the first experimental evidence that IgE modulation is clinically relevant [3,4]. These results suggest that therapeutic control of inappropriate IgE production may be more generally applicable to treatment of atopic disease.

In addition to the use of anti-IgE mAbs, interruption of IgE biosynthesis could also offer means of therapeutic control of IgE levels. Many steps in the detailed mechanism

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leading from antigen presentation to isotype switching and IgE production have been identified [5,6]. Isotype switching of a subset of mature B cells has been shown to require increased interleukin (IL)-4 secreted predominantly from Th2 cells as well as increased CD40L expression [7]. Additional costimulatory molecules in IgE production have also been identified, including CD23, the low affinity IgE receptor. Extensive *in vitro* studies with human CD23 as well as transgenic and knockout studies with murine CD23 confirm a role for this molecule in IgE production in response to IL-4 challenge [8–10]. In particular, it has been shown that human soluble CD23 (sCD23) stimulates B cell proliferation and germinal centre B cell survival by preventing apoptosis [11], and that certain anti-CD23 antibodies can inhibit IgE production *in vitro* [12]. The relative importance of membrane CD23 versus the cytokine-like activities of sCD23 is still under investigation.

Several recent reports have extended the laboratory relationship between CD23 and IL-4-stimulated IgE production to atopic disease, examining expression of CD23 both on the cell surface and in plasma in clinical settings. For example, expression of CD23 on lymphocytes from asthmatics was elevated 10-fold compared to normal patients, with virtually no overlap of the range of expression [13]. Furthermore, asthmatic patients receiving successful rush immunotherapy showed decreased CD23 expression over the course of their therapy [14]. These and similar studies strongly support a clinical role for CD23 in atopic disease, as predicted from the *in vitro* studies on human cells.

In studies of CD23 as a therapeutic target for modulation of atopic disease, CD23 has been shown to be processed from the cell surface by a metalloprotease to yield sCD23 soluble fragments [15]. Inhibitors of the metalloprotease prevent the release of sCD23 and result in accumulation of CD23 on the cell surface [16]. We describe here potent and selective inhibitors of CD23 processing and their characterization as inhibitors of IgE production from IL-4-stimulated human peripheral blood lymphocytes (PBL) and IL-4-stimulated SCID mice reconstituted with PBL from atopic donors. Importantly, we show that the inhibition by these compounds parallels closely the inhibition seen with an anti-CD23 Ab, providing strong evidence that inhibition of sCD23 production is sufficient for inhibition of IgE synthesis.

Materials and Methods

Materials

Compounds A (*N*-[4-hydroxyamino-2-(*R*)-isobutyl-3-(*S*)-propargylthiomethylsuccinyl]-(*S*)-phenylalanine-*N'*-methylamide) and B (*N*-[3-(*S*)-hydroxy-4-hydroxyamino-2-(*R*)-(2-naphthylmethyl)succinyl]-(*S*)-tert-leucinamide) were synthesized at SmithKline Beecham Pharmaceuticals (King of

Prussia, PA, USA) as described (WO9743250, WO9743249). Recombinant sCD23 (residues 102–321 of the sequence in Genbank M14766) carrying an N-terminal peptide tag was expressed as a secreted molecule using Chinese Hamster Ovary (CHO) cells. A vector for expression of this molecule, with an enterokinase cleavage site between the peptide tag and sCD23, was constructed using vector pCTDND1D2 as described previously [17–18]. Medium from the CHO stable cell line was used for purification of peptide-tagged soluble CD23 by immunoaffinity and Ni-NTA chromatography. As previously reported [19], this sCD23 was unstable and yielded upon storage at 4 °C 25-kDa sCD23, >95% as determined by N-terminal sequencing. The 33-kDa CD23 with the correct N-terminus (residues 102–321) for use as an ELISA standard was expressed as a secreted molecule using a *Drosophila* cell culture system [20,21], purified from the extracellular medium as described above and the N-terminus was confirmed by sequencing as Leu-102.

Enzyme assays

Assays for inhibition of CD23 processing from RPMI 8866 cell membranes were performed as previously described [15]. In brief, RPMI 8866 cell membranes were incubated at 37 °C in the presence or absence of inhibitors (dissolved in dimethylsulphoxide, 2% final concentration) in a total volume of 50 µL PBS, containing 0.5–1.0 µg total protein. Reactions were quenched with 5 µM batimastat, membranes filtered on 0.2 micron filter plates and sCD23 in the filtrate determined by ELISA. Assays for MMP-1,2,3 were performed using fluorescent peptide substrates as previously described [22]. The substrate for MMP-1 and MMP-2 was SDP-3815 (Peptides International, Louisville, KY, USA) and that for MMP-3 was NFF-3 (Peptides International). All assays were performed in 50 µL total volume with buffers as described, and fluorescence intensity determined in the presence and absence of inhibitors either over time or after 30 min incubation at room temperature.

Peripheral blood leucocyte assays

Peripheral blood leucocytes (mononuclear cells) from consenting, atopic donors were purified from whole blood by Ficoll–Pacque gradient as described [16]. Cells were resuspended and cultures were maintained in HB101 medium (Irvine Scientific, Irvine, CA, USA) with 10% foetal bovine serum (FBS) at 37 °C in 5% CO₂ atmosphere. Cells were incubated in 24-well plates seeded at 1×10^6 cells/mL for up to 14 days in the presence of 50 ng/mL recombinant human IL-4 (rhIL-4, prepared at SmithKline Beecham), 50 ng/mL anti-CD40 Ab (Pharmingen), and compounds added to various concentrations from stock solutions of DMSO to a

final DMSO concentration of no more than 0.5%. At the end of the incubation period, cells were removed by centrifugation and IgE, IgG and sCD23 in the cell-free supernatant were determined by ELISA as previously described [15,16]. Alternatively, the amount of 33-kDa sCD23, which is a more direct measure of inhibition of CD23 processing by a metalloprotease, was determined also by ELISA, using identical conditions except substituting the 33-kDa CD23-specific antiserum previously described as the detection Ab [15] absolute levels in controls of this form of sCD23 in the PBL studies ranged from 3 to 5 ng/mL. All studies were performed a minimum of twice with two donors. Data are shown as mean $\pm$ SD ($n = 4$) for one representative donor.

IL-4-challenged hu-PBL-reconstituted SCID mice

C.B-17 SCID mice were bred and maintained under sterile conditions at SmithKline Beecham Pharmaceuticals. Mice were used prior to 12 weeks of age, and a small number of 'leaky' mice were excluded by determination of serum IgG before beginning experiments [23]. PBL from house dust mite sensitive, skin-test positive donors were purified as described above. Approximately 2×10^8 cells/donor were obtained and resuspended in serum-free RPMI 1640 medium. Mice were injected with 30×10^6 cells i.p. on day 0 and challenged with human rIL-4 (10 μ g/day) on days 1–5. Animals were orally-dosed daily by gavage with compounds between days 0 and 18 as indicated in the figure legends; controls received vehicle only consisting of 10% N,N-dimethylacetamide, 10% cremophore EL, 80% water. Orbital bleeds were performed weekly under general anaesthetic and serum human IgE, IgG and sCD23 determined as described previously [16].

Results

Enzyme inhibition

Two compounds of different selectivity with respect to matrix metalloproteinases (MMPs) were identified and used in these studies. Compound A and Compound B, as shown in Table 1, are, respectively, potent and weak inhibitors of MMP1–3, but are both potent inhibitors of CD23 processing. The profiles of these compounds with respect to enzyme inhibition compared with their *in vitro* and *in vivo* activities on IgE production, as detailed below, would indicate whether or not there is a significant contribution of MMPs to CD23 processing and subsequent effects on IgE production.

Inhibition of IgE production from IL-4-stimulated PBL

The ability of compound A to inhibit IgE release into the

Table 1. Profile of selectivity of inhibition of sCD23 release from membranes and of representative MMPs

Compound	CD23 processing* (μ M)	MMP1† (μ M)	MMP2† (μ M)	MMP3† (μ M)
Compound A	0.05 (0.02)	<0.01	<0.01	<0.001
Compound B	0.02 (0.004)	> 10	> 1.0	> 10

*IC₅₀ determined as described in the Materials and Methods; †IC₅₀ determined using a peptide substrate as described in Materials and Methods.

medium from human PBL in the presence IL-4 was studied as a function of concentration of anti-CD40 antibody. The addition of anti-CD40 antibody as well as IL-4 provides a more consistent level of IgE production and more consistent EC₅₀ values for comparison of compounds. Figure 1(a) shows the IgE production at a constant level (50 ng/mL) of rhIL-4 with increasing amounts of anti-CD40 antibody, raising the IgE levels from 150 to 200 ng/mL with 10 ng/mL anti-CD40 to approximately 600 ng/mL at 200 ng/mL anti-CD40. Inhibition of IgE production by 1 μ M compound A was also determined across the range of anti-CD40 concentrations as shown in Fig. 1. There is a slight trend to higher inhibition at lower anti-CD40 concentrations, resulting in a twofold, but statistically not significant, shift of the ED₅₀. All subsequent studies were carried out with 50 ng/mL IL-4 and 50 ng/mL anti-CD40, to give consistent but less than maximal levels of IgE production. Under the conditions where anti-CD40 is present, as shown in Fig. 1(b), addition of exogenous sCD23 still augments the production of IgE as with IL-4 only stimulated IgE production showing that IgE production is still CD23 dependent.

The time course of IgE production relative to the time course of sCD23 release was also evaluated in this PBL assay system. As shown in Fig. 2, sCD23 is first measurable in the medium at day 3–4 following stimulation and the level continues to increase rapidly until day 7 followed by a slower increase out to day 14, whereas IgE is first measurable at day 5 and continues to increase until day 12–13. A single addition of 10 μ M compound A on day 0 inhibits release of both sCD23 and IgE out to day 14 at a consistent level of inhibition. Sufficient compound is still present in the cell-free supernatant until at least day 6 to inhibit release of sCD23 from RPMI 8866 membranes (data not shown).

A more detailed dose response of compound B inhibition of IgE and sCD23 only at day 14 was also determined (Fig. 3). Typical ED₅₀ values for inhibition by compounds A (not shown) and B were $0.22 \pm 0.02 \mu$ M and $0.33 \pm 0.09 \mu$ M, respectively. Figure 3(b) shows the comparison of inhibition by compound B of IgE and of 33-kDa CD23, the fragment

most completely characterized as a product of metalloprotease cleavage [15]. IgG release was not inhibited to a large extent in these experiments, even at 10-fold at the IC_{50} for IgE inhibition (for example, IL-4/anti-CD40, 849 ± 119 ng/mL; $10 \mu\text{M}$ compound B 712 ± 89 ng/mL, 16% inhibition).

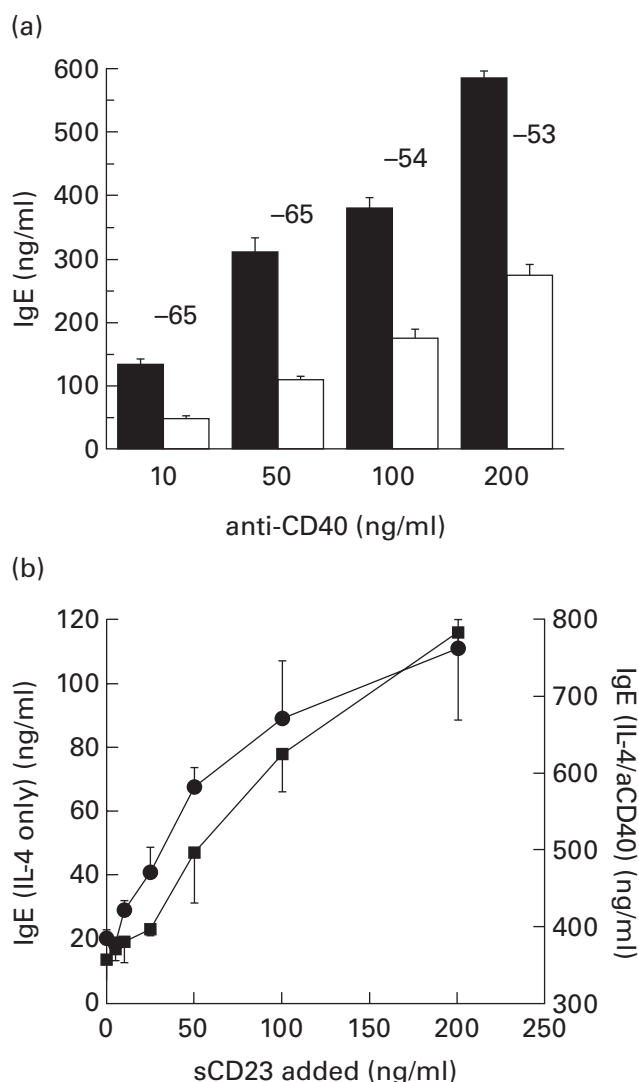


Fig. 1. (a) Dependence of IgE production from human PBL (solid bars) and inhibition of IgE production by compound A (open bars) in the presence of varying amounts of anti-CD40 antibody. Human PBL from atopic donors were cultured for 14 days with 50 ng/mL IL-4 and increasing amounts of anti-CD40 Ab with or without compound A and cell-free supernatants analysed for IgE per cent inhibition at $1 \mu\text{M}$ compound A is shown above each set of bars. Samples with compound A were compared with controls with no compound (Dunnett's post test, $P \leq 0.001$ for all conditions at $1 \mu\text{M}$ A). (b) Effect of addition of exogenous sCD23 on IL-4 (50 ng/mL, ■) or IL-4/anti-CD40 (50 ng/mL of each, ●) stimulated IgE production.

To investigate whether inhibition of IgE was consistent with the time course of sCD23 release observed in Fig. 2, compound B or anti-CD23 mAb, MHM6, was added at days from day 0 to day 12, rather than at day 0 as in the above experiments, and sCD23 and IgE levels in the culture medium were determined at day 14. As shown in Fig. 4(a), as the compound or mAb is added later in the incubation period, the percent inhibition of day 14 IgE decreases. Most compellingly, the steady decrease in inhibition by $3 \mu\text{g/mL}$ MHM6 and $1 \mu\text{M}$ compound B is virtually indistinguishable, supporting the hypothesis that the action of compound B is predominantly through inhibition of CD23 release. Figure 4(b) shows the comparison of inhibition of IgE and of 33-kDa CD23 with $1 \mu\text{M}$ compound B in the same study, demonstrating again a close correspondence of inhibition. Figure 4(c) compares the percentage inhibition of IgE by $1 \mu\text{M}$ compound B with the per cent of the total IgE measured at day 14 that is made after a given day of addition, which was calculated from the time course in Fig. 2. This comparison shows that essentially all of the IgE production that takes place after each day of addition is blocked by the addition of the compound on that day, up to the 70% maximum inhibition seen at this concentration of compound. That is, there is no time point at which there appears to be a committed pathway that can no longer be inhibited by addition of the compound or antibody.

In addition to *in vitro* studies, we investigated the inhibition of IgE production in IL-4-stimulated human PBL-reconstituted SCID mice. Suitable doses of compounds A and B were selected by evaluating the inhibition of CD23 release from RPMI 8866 cells injected i.p. in mice. Mice were predosed orally with varying concentrations of compound, then RPMI 8866-injected and allowed to release CD23 for 1 h. Lavage fluid was removed and sCD23 levels determined in order to evaluate effective doses for inhibition of CD23 release. Figure 5 shows the dose dependence of inhibition of CD23 release from RPMI 8866 cells for compound B, giving an ED_{50} of 5 mg/kg. A similar experiment with compound A gave an ED_{50} of 7 mg/kg. Duration of action was determined to be greater than 4 h for compound A and 8 h for compound B in similar studies extending the predosing time.

Based on this measure of inhibition of CD23 release *in vivo*, the ability of the compounds to inhibit IgE production in IL-4 stimulated hu-PBL-SCID mice was evaluated. A 15–18 day dosing period was chosen based on the time required to reach maximum levels of IgE in the *in vitro* assay. Figure 6(a–c) shows the inhibition of IgE over 6 weeks by MHM6, administered once on day 0, and compounds A and B, administered over the 15–18 day dosing period chosen based on the *in vitro* studies. Peak IgE was observed between weeks 3 and 4, with levels of IgE consistently as shown in the three independent experiments

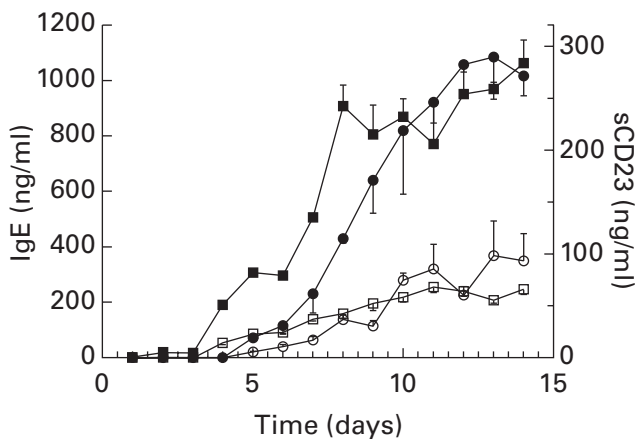


Fig. 2. Time course of sCD23 release and IgE production from human PBL. PBL were cultured with 50 ng/mL rhIL-4, 50 ng/mL anti-CD40 in the presence or absence of Compound A (10 μ M) for up to 14 days. Cell-free supernatants were collected on the day indicated and analysed by ELISA for sCD23 and IgE. IgE without (●) and with (○) compound A; sCD23 without (■) and with (□) compound A.

with different donors in Fig. 6(a–c). At the peak time of IgE production, inhibition by MHM6 or either compound at the higher concentrations was greater than 90% using four separate donors. In contrast, no inhibition of IgG production was observed at the peak time of IgE production, as indicated in the figure legend. It is also of interest that compounds are dosed out to 18 days, and the inhibition of IgE is seen out to week 4. In one experiment with compound A (not shown), IgE levels recovered at weeks 4 and 5, following the end of the dosing period. Soluble CD23 levels were also measured in separate control experiments (IL-4 alone) at early times (day 11, 0.3 ng/mL; day 15, 5.8 ng/mL) but could not be consistently measured in samples from animals where the compound had been administered.

Discussion

The steps involved in IgE production in a Th2 dependent antigen-response include both the production of soluble mediators such as IL-4 and/or IL-13 and the cognate interaction between molecules on the surface of T and B cells (e.g. of CD40/CD40L). These interactions led to induction of isotype switching with upregulation of the germline ϵ message. The steps downstream, leading to release of IgE from plasma cells are less clearly understood. One downstream event is upregulation of CD23 message expression, which is directly upregulated through STAT6 activation upon IL-4 receptor engagement [24]. The correspondence of inhibition of IgE and of sCD23 release by compounds A and B shows that not only is sCD23 able to

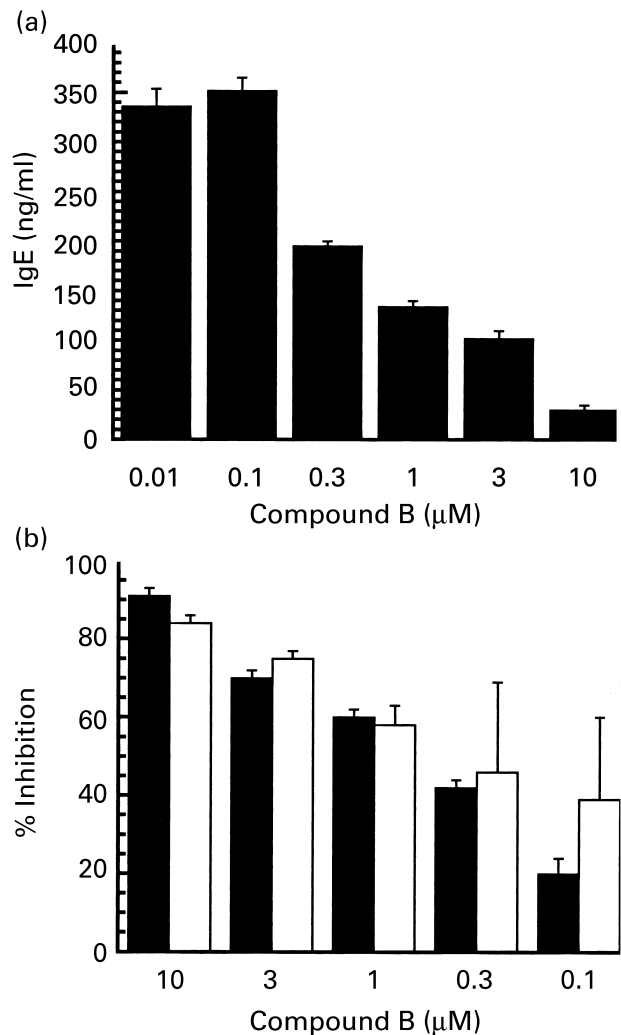


Fig. 3. Dose response of inhibition of IgE production and sCD23 release by compound B. (a) PBL were cultured for 14 days with 50 ng/mL IL-4, 50 ng/mL anti-CD40 in the presence of varying concentrations of compound B. IgE levels in the cell-free supernatant were determined at day 14. (b) Under the same conditions using PBL from a second donor, the per cent inhibition of IgE (solid bars) and 33-kDa CD23 (open bars) at day 14 were compared. Absolute levels of IgE and 33-kDa CD23 were consistent with those given in Materials and Methods.

enhance IgE production, but at least under the conditions of IL-4 stimulation, is an obligatory co-factor for IgE production.

The role of CD23 in IL-4-stimulated IgE production both *in vitro* using human PBL and *in vivo* in hu-PBL-SCID mice has been evaluated in detail. *In vitro* IgE production was studied in the presence of anti-CD40 mAb in order to provide a more reproducible IgE response, as previous results had indicated that although IL-4 alone was capable of stimulating IgE synthesis by PBL, this was inconsistent

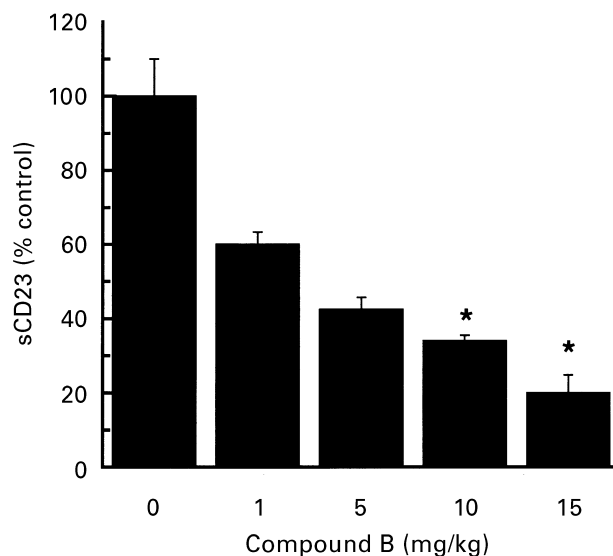
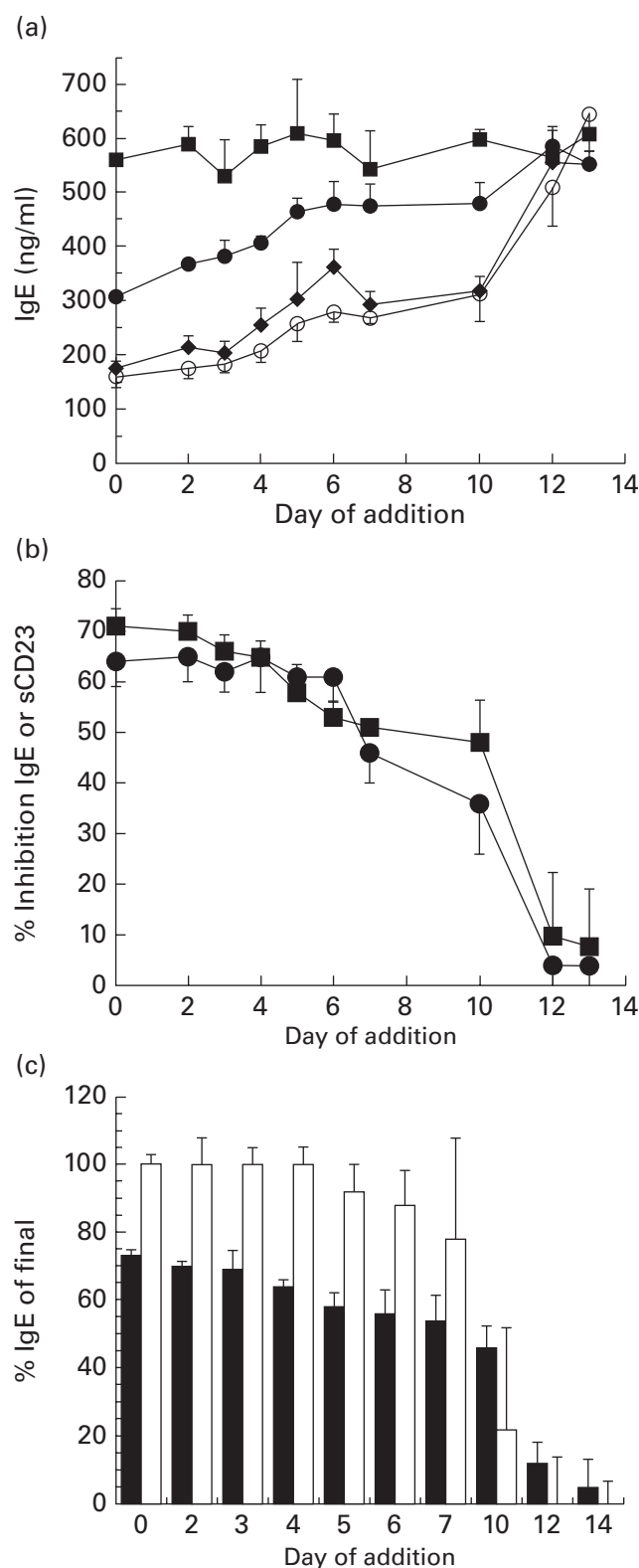


Fig. 5. Inhibition of sCD23 release from RPMI 8866 cells injected i.p. by orally-dosed compound B. Compound was administered 30 min prior to injection of 2×10^6 cells i.p.; after 1 h, peritoneal lavage was performed with 1 mL PBS and the samples analysed for sCD23 by ELISA; absolute levels of sCD23 with no compound added were 7.4 ± 1.4 ng/mL. Samples at each dose were compared directly to control samples with no compound (Dunnett's post test, $P \leq 0.05$).

and varied within the same donor on different occasions. Inhibition of IgE synthesis by compound A was shown to be minimally dependent on the amount of anti-CD40 used strongly suggesting that the compound was working by a mechanism independent of the signalling of CD40. *In vivo* IgE production from engrafted hu-PBL in C.B-17 SCID mice was studied using only IL-4 stimulation. As has been previously shown [23], there is a robust IgE response in addition to production of total IgG to very high levels, as would be expected from an authentic antigen-driven Th2 response.

Fig. 4. Dependence of inhibition of IgE or sCD23 production on day of addition of inhibitor. (a) PBL were cultured with 50 ng/mL rhIL-4, 50 ng/mL anti-CD40 alone (■) or in the presence of compound B (1 μM, ○, 0.3 μM, ●) or MHM6 (3 μg/mL, ◆) added on the day indicated following the start of incubation (day 0). IgE levels from separate samples were generated for each day of addition. Cell-free supernatants were all collected on day 14 and analysed by ELISA for sCD23 and IgE. (b) Comparison of inhibition of IgE and sCD23 release at each day of addition at 1 μM compound B (■, IgE; ●, sCD23). (c) Comparison of inhibition of day 14 IgE from A with time course of production in Fig. 2. Open bars show per cent of day 14 IgE that is made after each day, calculated from Fig. 2; solid bars show percent inhibition of day 14 IgE using 1 μM compound B, from A.

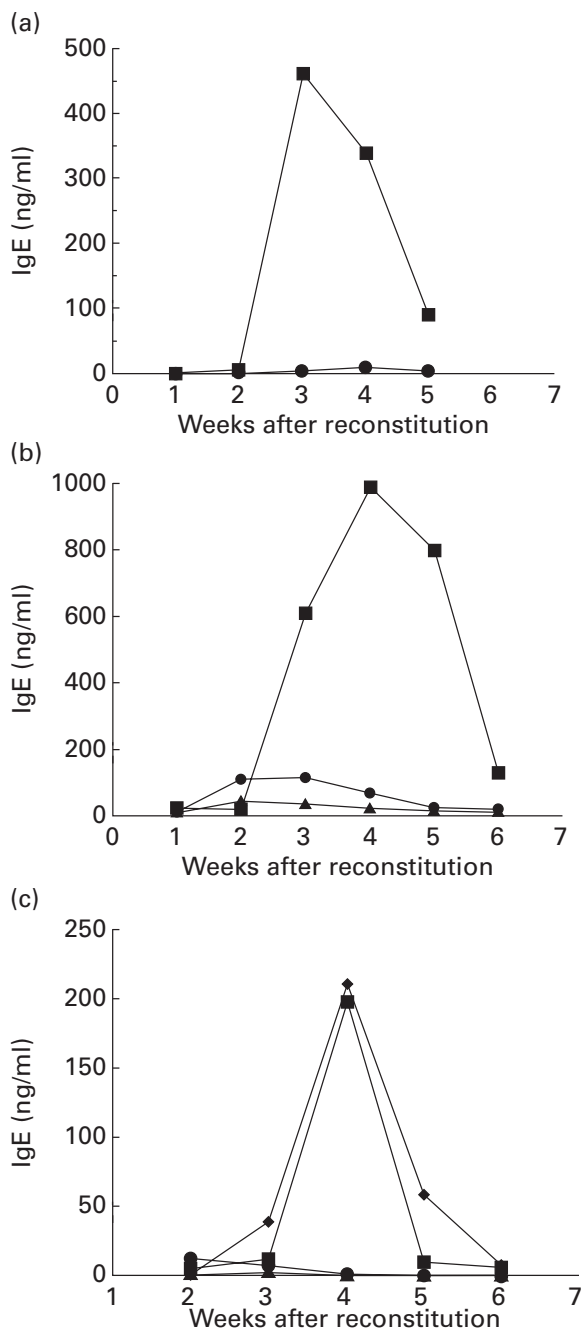


Fig. 6. Inhibition of IgE production in IL-4 challenged SCID mice reconstituted with hu-PBL from atopic donors. Data are representative from a set of mice reconstituted from a single donor. IL-4 (■) 10 μ g/mouse/day, days 1-4 (a) MHM6, 0.5 mg/mouse, once on day 0 (●); (b) Compound A, 10 (▲) or 20 (●) mg/kg, p.o., b.i.d., days 0-18 (c) Compound B, 1 (◆), 5 (◇), 12 mg/kg (●), p.o., once/day, days 0-15. IgG values at peak IgE production: IL-4, week 3, 1026 μ g/mL, week 4, 2862 μ g/mL; Compound A, 10 mg/kg, week 3, 1862, 2780 μ g/mL, week 4, 3467, 2461 μ g/mL; 20 mg/kg, week 4, 2434, 1734 μ g/mL; IL-4, week 3, 360 μ g/mL, week 4, 748 μ g/mL; compound B, 12 mg/kg: week 3, 532, week 4, 1008 μ g/mL.

The time course of CD23 production and IgE production *in vitro* also supports the hypothesis that CD23 is a significant cofactor for IgE production. CD23 can be measured in the cell-free supernatant at times before IgE can be detected and peak sCD23 precedes peak IgE production. Inhibition of IgE and sCD23 were compared in several assays, using inhibition of 33-kDa CD23, the form of CD23 best characterized as a product of metalloprotease cleavage, as the measure of inhibition of CD23 cleavage by compounds A and B. In a study of the time course where compound B was added at progressively later times in the assay, the correspondence of IgE and sCD23 inhibition over the time of addition, as well as the agreement of IgE inhibition by compound B and the anti-CD23 mAb, MHM6, strongly supports the hypothesis that compound B works through inhibition of CD23 release. Analysis of the IgE produced over time in these experiments (Fig. 4c) shows that subsequent IgE synthesis is halted following each day of addition of inhibitor or antibody, suggesting that continual stimulation by CD23 is required to maintain the plasma cells in an IgE-producing state, rather than for an early, irreversible selection of such a state.

The similar potency of IgE inhibition obtained with compounds A and B is evidence for the lack of direct involvement of MMPs, as compound B is not a broad-spectrum MMP inhibitor in contrast to compound A. A number of other cell surface molecules have been shown to be shed by a metalloprotease(s) with properties similar to that of CD23 processing. Of these, TNF release and L-selectin release have been shown to be inhibited by compounds similar to A [25-27]. L-selectin release is not thought to be relevant in the PBL-IgE assay as it is primarily expressed on neutrophils, whereas TNF levels were measured across the 14-day time course of IL-4/anti-CD40-stimulated PBL and found to be low and constant throughout (<50 pg/mL), making TNF unlikely to be an essential contributor to IgE production in this assay.

Inhibition of *in vivo* production of human IgE was studied in SCID mice reconstituted with human immune cells [23]. As it is impractical to obtain large numbers of reconstituted mice for extensive dose response studies, doses and dose frequency were chosen based on *in vivo* inhibition of i.p. injected human RPMI 8866 cells. Once again, CD23 processing inhibition correlated with *in vivo* inhibition of IgE production. For example, the ED₅₀ for compound B for CD23 release was 5 mg/kg and a 5-mg/kg dose in the hu-PBL-SCID model was effective in completely inhibiting IgE whereas a 1-mg/kg dose was not effective. Statistical analysis of data from the hu-PBL-SCID model is also hampered by the small numbers of mice that can be reconstituted with cells from one donor, but repeat studies show a consistent correlation between the CD23 processing inhibition results (adoptively transferred RPMI 8866 cells)

and the SCID IgE results, as well as a good comparison with the complete inhibition of IgE by MHM6. Finally, both *in vitro* and *in vivo*, there is specific inhibition of IgE production relative to total IgG production by both compound B and MHM6. The specificity of IgE inhibition again points to a requirement for CD23 in order to maintain IgE production.

The precise role of CD23 in the regulation of IgE production has been studied extensively, but remains uncertain. We have previously shown that inhibition of CD23 processing results in the accumulation of surface CD23 as well as the loss of sCD23. Studies in murine CD23 deficient and transgenic mice suggest that overexpression of full-length CD23 does impair IgE production in response to repeat antigen challenge [28–30]. In contrast, there is evidence that CD23 knockout mice develop higher levels of IgE under similar conditions and also demonstrate a higher rate of development of bronchial hyperreactivity [31]. There was no effect on IgE levels of overexpression of a truncated, soluble form of murine CD23, but experiments directly comparable to those using the knockout have not been reported. Therefore, the evidence from the murine system suggests that higher levels of membrane CD23 negatively regulate IgE production.

Human CD23 appears to function by a somewhat different mechanism where sCD23 interaction with receptor(s) is required. The anti-CD23 antibody, MHM6, appears to function identically to a CD23 processing inhibitor in these studies. We have not been able to demonstrate that this antibody can directly inhibit release of CD23 from RPMI 8866 membranes (data not shown), although it is possible that an antibody could modify the rate of release in a more physiological setting, as has been suggested for cross-linked IgE in both human and murine cell studies [32–33]. It is entirely possible that MHM6 functions simply by preventing the binding of sCD23 to its receptor(s). There is significant evidence for a receptor(s) for sCD23, such as CD11b/c on monocytes [34], and possibly CD21 on B cells [35]. The CD23/CD21 interaction has been implicated in response of B cells to antigen [36] as well as in the IgE response to IL-4 stimulation [37]. A recent report describes the ability of sCD23 to protect against low-density-induced apoptosis in a B cell line lacking any of the known receptors, including CD21, suggesting that there is an unidentified receptor that is important in maintaining B cell functions [38]. CD21 has been shown to be a receptor for human CD23, and not for murine CD23 [36], consistent with the lack of function of murine sCD23, but it is not been clearly demonstrated that sCD23 binds to human CD21 with sufficiently high affinity to signal through the CD19/CD21/BCR complex. Thus it remains possible that there is an additional receptor. We would propose that any sCD23 receptor is involved in signalling that maintains B plasmacytes in an

IgE-producing state, likely by modification of a default path to apoptosis or by enhancement of the steps in production of mature IgE following message induction during isotype switching. We anticipate that further studies will elucidate these pathways. In conclusion, modulation of CD23 release from membranes appears to be sufficient to inhibit IL-4-induced IgE production both *in vitro* and *in vivo*.

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