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The low-affinity receptor for IgE (FcεRII or CD23) as a therapeutic target

D. H. Conrad, M. A. Kilmon, E. J. Studer and S.-w. Cho

Department of Microbiology and Immunology, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980678 MCV Station, Richmond, VA 23298, U.S.A.

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Introduction

One of the most straightforward methods of treating type I hypersensitivity is to block IgE synthesis; this is termed an 'isotype-specific' method of regulation. As initially suggested by Hirashima et al. [1], the 'switched' B-cell bearing surface IgE is a good target for isotype-specific regulation. One method of targeting this cell is to use an antibody (Ab) to IgE that is not anaphylactic; this Ab would target the sIgE⁺ B-cell and potentially induce its down-regulation or destruction. Evidence that such an Ab can be produced and does induce decreased IgE synthesis was discussed in this session and is also published elsewhere [2,3]. In addition, a second IgE-binding molecule, soluble FcεRIα chain, produced by molecular engineering [4,5], has been shown to block the passive cutaneous anaphylactic reaction and/or decrease IgE synthesis. Potential problems with this approach, using either the anti-IgE or the soluble FcεRIα chain, are the expense of the recombinant reagent and the half-life *in vivo*.

A third IgE-binding molecule that would be expected to be useful in this regard is FcεRII or CD23. Since its discovery [6], CD23 has been proposed to play a role in IgE regulation. When interleukin (IL)-4 was shown to up-regulate the expression level of both IgE [7] and CD23 [8], it led to the hypothesis that IL-4 induces both a positive effect, namely IgE production, and a negative regulator for IgE, namely CD23. However, evidence to date on this issue is limited. In one report, CD23 knockout mice were found to exhibit an enhanced antigen-specific IgE response [9]; however, this effect could not be repeated in other studies [10,11]. In contrast, mice transgenic for CD23 exhibited marked suppression of IgE production in response to both parasite and alum/antigen regimens ([12]; S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work).

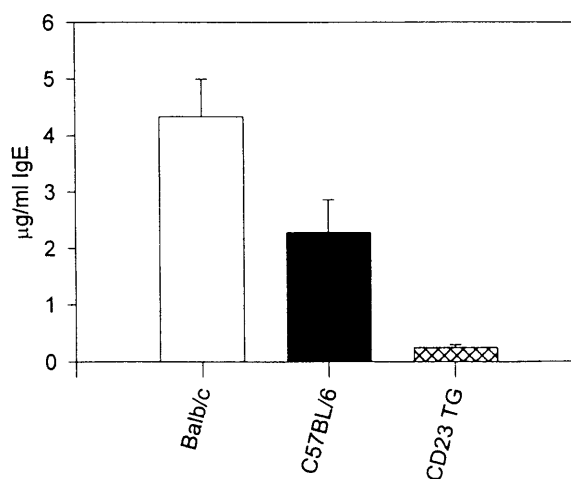
Results and discussion

Figure 1 shows analysis of serum IgE from mice injected with antigen dinitro-phenylated keyhole limpet haemocyanin (DNP-KLH). IgE responses were suppressed in CD23 transgenic mice. Only the 100 µg dose is shown, but suppression was seen at all levels of antigen used (S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work). In results not shown, antigen-specific IgE was also analysed and the same trend was seen. We next investigated whether the transgenic animals showed a decreased IL-4 or increased interferon γ (IFN-γ) response, since these cytokines would be expected to have an enhancing or suppressing effect on IgE production respectively. Lymph nodes were removed from immunized animals, and dispersed cells were cultured in the presence of increasing doses of antigen, similar to the procedure of McKnight et al. [15]. After 3 days of culture, supernatants were analysed for

Figure 1

IgE production is suppressed *in vivo* in CD23 transgenic mice

Balb/c, C57BL/6 and CD23 transgenic mice were given a primary immunization subcutaneously of 100 µg of DNP-KLH in an adjuvant consisting of 4 mg of alum and 2×10^8 heat-killed *Bordetella pertussis*. After 2 weeks the mice were given a secondary immunization of 100 µg of DNP-KLH, 2 mg of alum and 1×10^9 heat-killed *Bordetella pertussis* both in the feet and subcutaneously. On day 9 after the secondary immunization, the amount of total IgE present in the serum of these mice was determined by ELISA [14].



Abbreviations used: Ab, antibody; DNP-KLH, dinitro-phenylated keyhole limpet haemocyanin; IL, interleukin; INF-γ, interferon γ; ICAM, intracellular adhesion molecule; RT-PCR, reverse transcriptase PCR.

IL-4 by CT4S proliferation [16] and IFN- γ levels by ELISA. No significant differences in the production of either cytokine was evident between transgenic and control animals (results not shown; S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work).

Since the levels of the two cytokines most heavily implicated in IgE regulation were not significantly affected, we next considered whether the B-cells from the transgenic animals produced less IgE under optimal stimulating conditions. As reported elsewhere [17], IgE produced in culture *in vitro* is highly dependent on B-cell level. Once a critical threshold number of B-cells is present, IgE production is inversely proportional to B-cell number. Thus different numbers of B-cells were stimulated with optimal levels of CD40L-trimer (a gift from W. Fanslow [18]) plus IL-4 and IL-5, and IgE production was determined by ELISA. Six experiments of this type were performed in which IgE production in B-cells from Balb/c or C57BL/6 mice were compared with the levels produced by transgenic B-cells. IgE production by the transgenic B-cells was generally lower than by the B-cells from Balb/c or C57BL/6 mice (results not shown; S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work). The trend was seen at all input levels of B-cells, arguing against the effect being due to a shift in the B-cell dose-response curve. However, in some of the experiments, the effect was of marginal significance. A possible explanation for this variation is the observation by Texido et al. [12] that transgene expression in B-cells is relatively minimal because of the choice of Thyl promoter for the CD23 transgene.

In order to confirm this suppression, we investigated the effect of culturing B-cells from Balb/c or C57BL/6 mice with CD23-transfected fibroblasts (CHO cells). To set up an *in vitro* model for this suppression, CHO transfectants with CD23⁺, CD23⁺/intracellular adhesion molecule (ICAM-1)⁺ or ICAM-1⁺ were prepared. These cells are termed 'support cells'. ICAM-1 was chosen because of the strong B-cell adhesion capacity shown for this system [19] and was expected to further enhance any CD23 effects.

Modulation of CD40-dependent B-cell activation by CD23 was investigated by culturing B-cells with either CHO or CHO transfected with CD23, ICAM-1 or both ICAM-1 and CD23 in the presence of CD40L⁺-CHO cells plus excess IL-4 and IL-5. Support cells and CD40L⁺

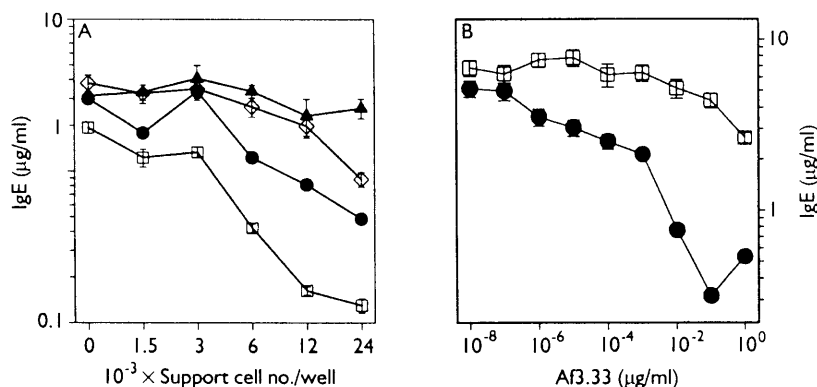
cells were treated with mitomycin-C to suppress their proliferation. Figure 2(A) shows IgE production after 8 days of culture from a typical experiment of this design. Note that there was a dose-dependent decrease in IgE production, based on support cell concentration, especially when double-positive (ICAM-1⁺/CD23⁺) cells were used, and a smaller trend with CD23⁺ CHO. At high support cell concentration (12000 and 24000 respectively), some decreased production of IgG1 and IgM was also seen (S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work) (results not shown). A dose-dependent decrease in IgE production was evident, approaching zero at the higher doses of double-positive (ICAM-1⁺/CD23⁺) cells. The generalized effect of high-dose CD23⁺ cells on immunoglobulin production suggested that B-cell proliferation may be influenced. This was not evident in experiments that used a relatively low input of B-cells as in Figure 2; however, when the B-cell input was increased to 50000 cells/well, a dose-dependent decrease in B-cell proliferation was observed (S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work) (results not shown). Note that both double-positive (ICAM-1⁺/CD23⁺) and single-positive (CD23⁺) cells caused a dose-dependent decrease in B-cell proliferation. In this experiment neither CHO cells nor ICAM-1⁺ single-positive cells had any effect on B-cell proliferation (results not shown).

Co-activation of B-cells with CD40L and multivalent anti-IgD was also shown to suppress IgE production [17]. The model used was designed to be analogous with secondary germinal centres where IgG-antigen complexes would interact with Fc γ receptors and give a multivalent signal to B-cells through the sIg receptors. The model system used Fc γ RI-transfected CHO cells and anti-IgD (IgG2a) monoclonal antibodies. This activation system required nanogram quantities of anti-IgD for efficient B-cell activation [17] and the dose-response to anti-IgD was similar to anti-IgD-dextran, used by others [17,22]. When the capacity of B-cells to produce immunoglobulin was analysed, significant levels of IgM and IgG1-production were seen; this required both IL-4 and IL-5 [17]. IgE production, however, was never seen with this signalling procedure. In addition, Figure 2(B) shows the results of an experiment where B-cells were co-activated with both CD40L and multi-

Figure 2

IgE production is suppressed by (A) activation of B-cells in the presence of CD23⁺ cells and (B) co-activation of B-cells with CD40L and anti-immunoglobulin

In (A) B-cells (Balb/c mice) were purified [20] and cultured in the presence of the indicated number of 'support' cells. Untransfected CHO (▲), ICAM-1⁺-CHO (◇), CD23⁺-CHO (●) or CD23⁺, ICAM-1⁺-CHO (□) cells were treated with mitomycin c [17] and added to 96-well culture plates containing CD40L⁺-CHO and B-cells (6000 and 10000/well respectively), 10000 units/ml IL-4 and 5 µg/ml IL-5. After 8 days of culture, IgE levels were determined by ELISA [14]. In (B), B-cells from C57BL/6 mice cultured at 10⁵/well were co-activated by incubation with the indicated levels of anti-IgD (AF.3.33 [21]) bound to FcγRI⁺-CHO (●) and CHO control (□). Both transfected CHO cell lines and CHO controls were treated with mitomycin c before use and were added at 6000/well [17]. All wells contained IL-4/5 as in (A). After 8 days of culture, IgE levels were determined by ELISA [14].



valent anti-IgD and, as seen, co-activation resulted in strong inhibition of IgE₁ production. Co-activation had no effect on B-cell proliferation or IgG1 or IgM production (results not shown) [17].

In order to investigate the mechanism for this suppression, we analysed B-cells activated in the presence of high CD23-expressing cells and those co-activated with CD40L and multivalent anti-IgD for germline ϵ -transcripts. Inhibition of ϵ -transcript production would indicate that isotype switching is inhibited, whereas no inhibition of the ϵ -transcripts, combined with inhibition of IgE synthesis, would imply that differentiation of the switched B-cell is inhibited.

The protocol used was based on the procedure of Warren and Berton [23]. Primers for the ϵ -germline transcript were as published [23]. Initial attempts to use either hypoxanthine-guanine phosphoribosyltransferase as used by Warren and Berton [23] or actin-specific primers as the housekeeping control proved unsuccessful because of cross-reaction of the primer with the hamster counterpart from the support cells. For this reason, primers for CD22, a B-cell-specific protein that maintains a relatively constant expression level through different stages of

B-cell activation [24], were chosen. Primers for CD22 were upstream, 5'-AACTCCACTACC-CAAGG-3', and downstream, 5'-AGGTGATCTTAGAATCTTCC-3', and encode a 419 bp PCR product from 650 to 1069 in murine CD22 cDNA (Genebank). B-cells were activated in 96-well tissue culture plates in the presence of either FcγRI⁺-CHO and anti-IgD [17] or CD23⁺ support cells. After 3 days of culture, RNA was isolated and reverse transcriptase (RT)-PCR was performed. For the PCR step, 15 cycles were chosen in order to be on the linear portion of the PCR expansion curve [23]. Figure 3 shows the result of this RT-PCR experiment. A clear suppression of IgE transcript level is apparent after co-activation, but this level remains relatively constant in the presence of excess CD23. The bottom part of Figure 3 shows a bar graph analysis of the data, which were normalized for the levels of CD22 present. Again the data lead to the conclusion that co-activation results in an inhibition of isotype switching, and high CD23 levels inhibit the differentiation of switched B-cells.

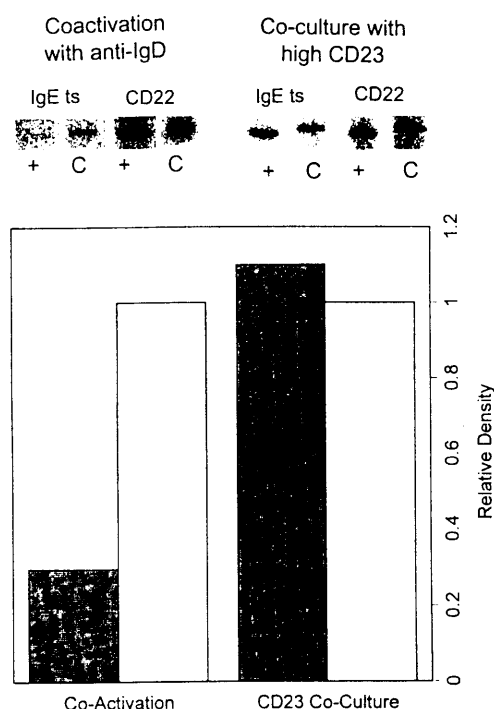
The general observation from these studies was that when membrane CD23 was present, either on mitomycin-C-treated CD23⁺ or

double-positive (CD23⁺/ICAM-1⁺) CHO cells, a dose-dependent down-regulation of IgE production occurred. Untransfected CHO cells or ICAM-1 single transfected cells had minimal effect on both immunoglobulin production and proliferation. Indeed, at high CD23⁺ cell levels, a general down-regulation of immunoglobulin production ensued and, if the number of B-cells was increased so as to more accurately determine B-cell proliferation, this proliferation was decreased also in concordance with CD23⁺ cell number.

Figure 3

IgE germline transcription is inhibited by co-activation with anti-IgD and CD40L but not by CD23⁺ co-culture

The relative levels of κ -germline transcript (IgE ts) were determined by RT-PCR as described ([23]; S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work). For co-activation, B-cells were activated with CD40L alone (C) or with anti-IgD and CD40L (+) as described in Figure 2. For co-culture, B-cells (Balb/c) were activated with CD40L⁺-CHO as described in Figure 2 in the presence of either CHO cells (C) or double-positive ICAM-1⁺, CD23⁺-CHO cells (+). Total RNA was isolated after 3 days by the Trizol procedure as recommended by the manufacturer (Gibco), and RT-PCR was performed using the GeneAmp RNA PCR kit (Perkin-Elmer) as described [23], using 15-cycle PCR. Note that the PCR product is directly ³²P-labelled by inclusion of [³²P]dATP in the protocol and the product is run on a 6% polyacrylamide gel and analysed with a PhosphorImager (Molecular Dynamics). To control for RNA yield, RT-PCR for CD22 was performed and the IgE ts and CD22 bands are labelled. The bar graph represents the relative level of IgE ts after normalization to the level of CD22 transcript recovered in both control and experimental conditions.



The latter decrease suggests the intriguing possibility that high levels of CD23 may induce B-cell apoptosis – clearly a subject for further study. The ligand or counter receptor that interacts with CD23 is not clear from these studies. One attractive hypothesis is that the high-level CD23 interacts with sIgE on stimulated B-cells and increases this apoptosis. In this sense, CD23 would be acting much like the models proposed for the anti-IgE antibody that blocks IgE synthesis [3]. The second current candidate for a 'CD23 ligand' is CD21, shown by the Bonnefoy's group to interact with CD23 [25]. They also reported evidence that this interaction (in the human system) actively enhances IgE synthesis [25]. Clearly, the interaction of CD21 with TAPA-1 and CD19 in the B-cell receptor (see [26,27] for review) suggests that an agent that interacts with CD21 simultaneously with anti-Ig (antigen-sIg) would enhance this stimulation. However, the interaction reported here is via CD40L, not anti-sIg B-cell stimulation, and the effects of co-cross-linking of CD21 and CD40 are not known at present. So, although no positive results for mouse CD23-CD21 interaction have been reported, this remains a possibility, as does another as yet undiscovered ligand. In this regard, our laboratory is attempting to prepare a soluble CD23 that exerts a similar biological effect to that of the membrane form. Such a molecule would greatly enhance our capability for intensifying alternative ligands.

These studies suggest a rationale for why IgE is produced in relatively low amounts. Stimulation of B-cells with an activating agent, e.g. CD40L, in conjunction with IL-4 induces efficient isotype switching to IgE, but also induces high levels of CD23, which in both transgenic animals ([12]; S.-w. Cho, M. A. Kilmon, E. J. Studer and D. H. Conrad, unpublished work) and in *in vitro* models ([17]; this study) results in lower IgE production. This finding returns us to the hypothesis that IL-4 induces both positive and negative regulatory elements for IgE. These data suggest that an attractive allergy treatment would be to inhibit the protease that cleaves membrane CD23 into soluble CD23; this would be expected to increase expression of membrane CD23 and inhibit differentiation of IgE-switched cells into IgE-producing plasma cells. In addition, in the secondary response, germinal centres would contain antigen-antibody (IgG) complexes. The B-cell down-regulatory action of FcγRII is now a clas-

sical model [28] but the co-activation studies [17] further indicate that co-activation with anti-Ig and CD40L is inhibitory for the isotype switch to IgE. Thus this provides additional mechanisms for the control of IgE levels.

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