A Pharmacokinetic Approach for Evaluating Cytokine Binding Macromolecules as Antagonists

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Purpose. Cytokine binding macromolecules such as antibodies and soluble receptors sometimes produce undesirable agonist-like activities instead of the expected antagonist-like effects when the cytokine binding macromolecule extends the half-life of a short-lived cytokine. The purpose of this paper is to identify the pharmacokinetic and physicochemical properties that can cause these agonist-like activities.

Methods. A simple pharmacokinetic model was used to determine whether a given cytokine binding macromolecule will function effectively as an antagonist in therapeutic situations in which cytokine is released chronically.

Results. The model proposed satisfactorily fits experimental data for soluble interleukin-4 receptor and for an anti-interleukin-4 monoclonal antibody under conditions in which agonist-like and antagonist activity are observed.

Conclusions. We show that the unexpected agonist-like activities result only when there is nonlinearity in the cytokine-cytokine receptor interaction and the cytokine binding macromolecule prolongs the half-life of the cytokine.

KEY WORDS: antibodies; soluble receptors; immunoadhesins, cytokines; pharmacokinetics.

INTRODUCTION

Cytokines play a central role in the pathogenesis of several autoimmune diseases and are attractive targets for the monoclonal antibodies, soluble decoy receptors, immunoadhesins and the host of immunoglobulin derivatives that are being tested as cytokine antagonists. Here, we refer to these cytokine binding proteins as cytokine binding macromolecules (CBM).

The application of CBM as cytokine antagonists has resulted in the realization that CBM administration may not be advantageous in certain circumstances (1). Several mechanisms cause these unexpected agonist-like results (2). For example, CBM binding can stabilize the tertiary structure of the cytokine and attenuate spontaneous activity decay (3) and they can act as carriers that prolong the half-life of short-lived cytokines (4-6). The pharmacokinetic determinants that cause these effects are the focus of this paper.

We use the Relative Exposure Index, a measure of cytokine receptor occupancy, to determine whether a CBM will act in an agonist-like manner *in vivo*. The equations are derived for situations in which the cytokine is chronically produced; e.g., in chronic viral infections.

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The agonist-like activity results when the relationship between the free cytokine levels and cytokine receptor occupancy is nonlinear and the CBM prolongs the half-life of the cytokine. The mathematical predictions of the approach are tested against *in vivo* data for two interleukin-4 CBM.

MATERIALS AND METHODS

The analysis assumes steady state conditions. This is a simplistic representation, but is reasonable for situations in which chronic cytokine release occurs.

Let [C], [CBM] and [CR] represent the effect compartment concentrations of free cytokine (C), free CBM and cytokine-occupied receptor (CR), respectively. If the CBM is present during cytokine release, the following reversible reaction that results in the formation of an inactive complex (C-CBM) occurs:

$$C + CBM \rightleftharpoons C\text{-}CBM$$

We assume that the system equilibriates rapidly, that cytokine binds its receptor reversibly with a dissociation constant of K, and that the dependence of receptor binding on cytokine concentration is described by a single site model.

To be useful as a cytokine antagonist, the presence of the CBM must reduce exposure of receptor to cytokine. The exposure is proportional to the probability that an released cytokine molecule will signal and for a one-compartment system at steady state, it is given by the product of the receptor occupancy relationship and the average time for which a cytokine molecule remains in the system.

In the absence of CBM, the average cytokine molecule circulates for one half life or τ_C seconds and the average steady state exposure is proportional to:

$$\frac{[CR]}{[CR]_{max}}\tau_C = \frac{[C]}{K + [C]}\tau_C$$

$[CR]_{max}$ is the concentration of cytokine-bound receptor at saturation. If the fraction of cytokine bound to CBM is f, and the CBM reduces the fraction of free cytokine available for receptor binding, then:

$$\left[\frac{[CR]}{[CR]_{max}}\right]_{\text{with CBM}} = \frac{[C](1 - f)}{K + [C](1 - f)}$$

The circulating time of the cytokine is increased or decreased by the CBM, depending on the relative clearances of free cytokine and CBM-bound cytokine. If the subscripts C and CB represent the free cytokine and the cytokine bound to CBM, respectively, the effective clearance CL_{mean} of cytokine in the presence of CBM is:

$$CL_{mean} = CL_C(1 - f) + CL_{CB}f$$

For a one compartment system, the mean time of exposure, τ_{mean}, in the presence of CBM is given in terms of the half-lives (τ_C and τ_{CB}) by:

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$$\tau_{mean} = \frac{\tau_C\tau_{CB}}{(1 - f)\tau_{CB} + f\tau_C}$$

The relative exposure index, R, is the ratio of the exposures:

$$R = \left(\frac{\tau_C\tau_{CB}}{(1 - f)\tau_{CB} + f\tau_C}\right)\left(\frac{[C](1 - f)}{K + [C](1 - f)}\right)\left(\frac{K + [C]}{[C]}\right)\left(\frac{1}{\tau_C}\right) \quad (1)$$

Alternatively:

$$R = \frac{1}{\left(1 - f + f\frac{\tau_C}{\tau_{CB}}\right)}\frac{(1 - f)}{\left(\frac{K}{[C]} + 1 - f\right)}\left(1 + \frac{K}{[C]}\right) \quad (2)$$

This definition of R is the starting point for the remaining analyses. If the CBM and the free cytokine interact with dissociation constant K_i, then:

$$K_i = \frac{[C][CBM]}{[C-CBM]} = \frac{(1 - f)[CBM]}{f} \quad (3)$$

Or:

$$f = \frac{1}{\frac{K_i}{[CBM]} + 1} \quad (4)$$

By substituting the Equation (4) in Equation (2), R is obtained in terms of two nondimensional concentrations ([C]/K and [CBM]/K_i) and the nondimensional half life (τ_C/τ_{CB}).

The nonlinear least squares fitting and graphing were done using Kaleidagraph™ on a Macintosh IIci computer.

RESULTS

The relative exposure index (R) value is a measure of the usefulness of a CBM as an antagonist because R values significantly greater than unity are likely to be associated with agonist-like activities and with side effects *in vivo*. A useful antagonist should therefore have R values of less than unity over a large range of f.

The R value is plotted as a function of fractional cytokine binding (f) using τ_C/τ_{CB} as a parameter for K/[C] values of 0.1, 1 and 10 in Figures 1A, B and C, respectively. As expected, all the curves have R values of unity, which corresponds to no activity, when no CBM is present (f = 0), and R values of zero, which corresponds to complete inhibition, when the cytokine is completely sequestered (f = 1).

Figure 2 shows the relationship between relative exposure index R, and the nondimensional CBM concentration, [CBM]/K_i. Overall, Figures 2A, B and C are very similar to Figures 1A, B and C because f is related to [CBM] via a monotonically increasing function. It is this representation that is used to test the model against experimental data.

We refer to a CBM as a "scavenger" if its τ_C/τ_{CB} is greater than unity and as a "carrier" if its τ_C/τ_{CB} is less than unity. As intuitively expected, the scavenger CBM act by facilitating cytokine clearance at low values of f and by the "sequestration" and scavenger mechanisms at high f values.

For carrier CBM, the R value is unfavorable unless a significant fraction of the cytokine is sequestered. The maximal values of R occur at intermediate values of f because

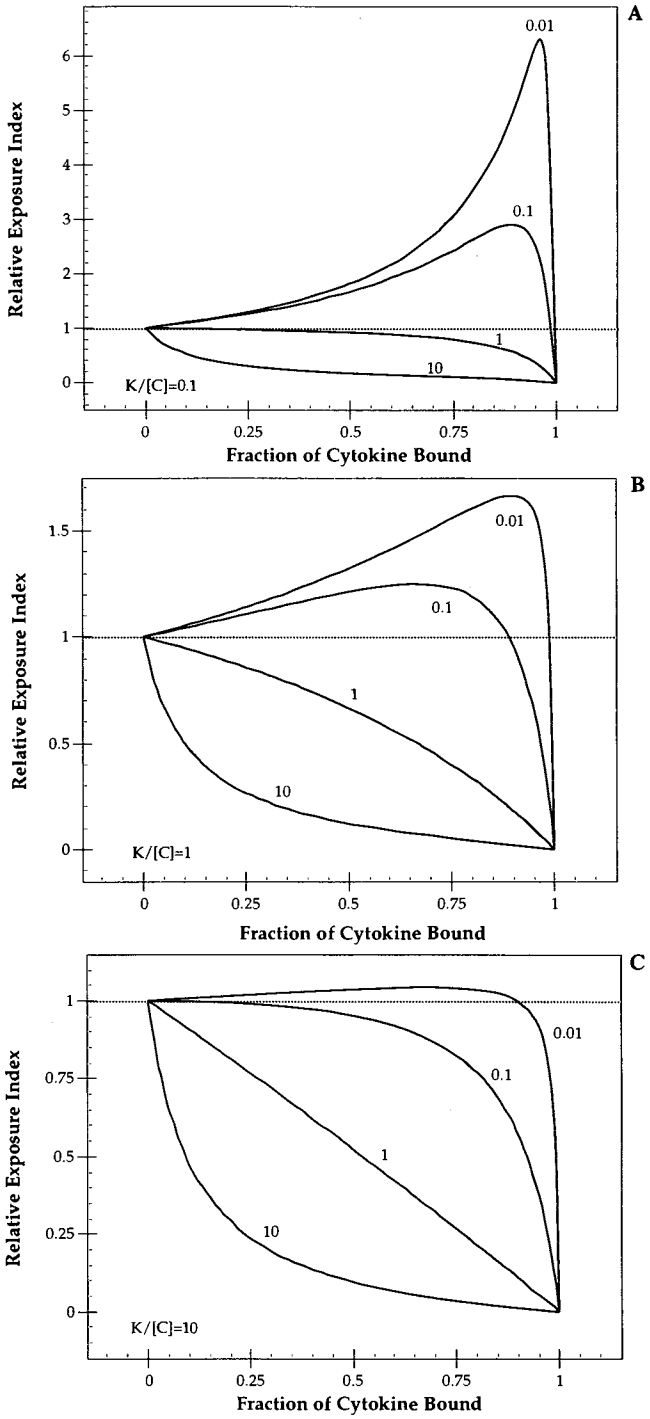

Fig. 1. Plots of R, the Relative Exposure Index vs. f, the fraction of cytokine bound by the CBM. The τ_C/τ_{CB} values are shown against the curves. Figure 1A, B and C are for K/[C] values of 0.1, 1 and 10, respectively.

the increases in mean cytokine half-life are not offset by sequestration and reduced receptor occupancy.

We analyzed the requirements for R to be less than unity for three limiting conditions to better delineate the underlying mechanisms of CBM activity.

Case I

Receptor Occupancy is in the Linear Part of the Binding Curve in the Presence and Absence of CBM, i.e., (K/[C]) >> 1 and Therefore (K/[C]) >> (1 − f). These constraints are reasonable when the cytokine is present at concentrations below the dissociation constant for receptor-cytokine binding. The value of of R is:

$$R = \frac{1}{\left(1 - f + f\frac{\tau_C}{\tau_{CB}}\right)} \frac{(1-f)}{\left(\frac{K}{[C]}\right)} \left(\frac{K}{[C]}\right)$$

$$= \frac{(1-f)}{\left(1 - f + f\frac{\tau_C}{\tau_{CB}}\right)} < 1 \text{ for activity} \qquad (5)$$

Because the denominator is greater than the numerator, R is always less than unity. Thus, in Case I, there are no constraints on the antagonist activity of CBM.

Case II

Receptor Occupancy is near Saturation in the Presence and Absence of CBM; i.e., (K/[C]) << (1 − f) and Therefore (K/[C]) << 1. This occurs when the cytokine is present at saturating concentrations and the CBM has a relatively low affinity for the cytokine. For this Case:

$$R = \frac{1}{\left(1 - f + f\frac{\tau_C}{\tau_{CB}}\right)} < 1 \textit{ for activity} \qquad (6)$$

The R value is less than unity only when the term, $[1 - f + f(\tau_C/\tau_{CB})]$, is greater than unity, or equivalently when, $\tau_C > \tau_{CB}$. Thus, only scavenger CBM can be antagonists under these conditions. It is reassuring that the model reflects this intuitive concept.

Case III

Receptor Occupancy is near Saturation in the Absence of CBM, i.e., (K/[C]) << 1. However, in the Presence of CBM, Receptor Occupancy is in the Linear Part of the Binding Curve and, (K/[C]) >> (1 − f). Using these constraints in Equation (2), we obtain:

$$R = \frac{1}{\left(1 - f + f\frac{\tau_C}{\tau_{CB}}\right)} \frac{(1-f)}{\left(\frac{K}{[C]}\right)} < 1 \text{ for activity} \qquad (7)$$

Under these conditions R is less than unity only if:

$$f > \frac{\left(\frac{[C]}{K} - 1\right)}{\left(\frac{[C]}{K} + \frac{\tau_C}{\tau_{CB}} - 1\right)} \qquad (8)$$

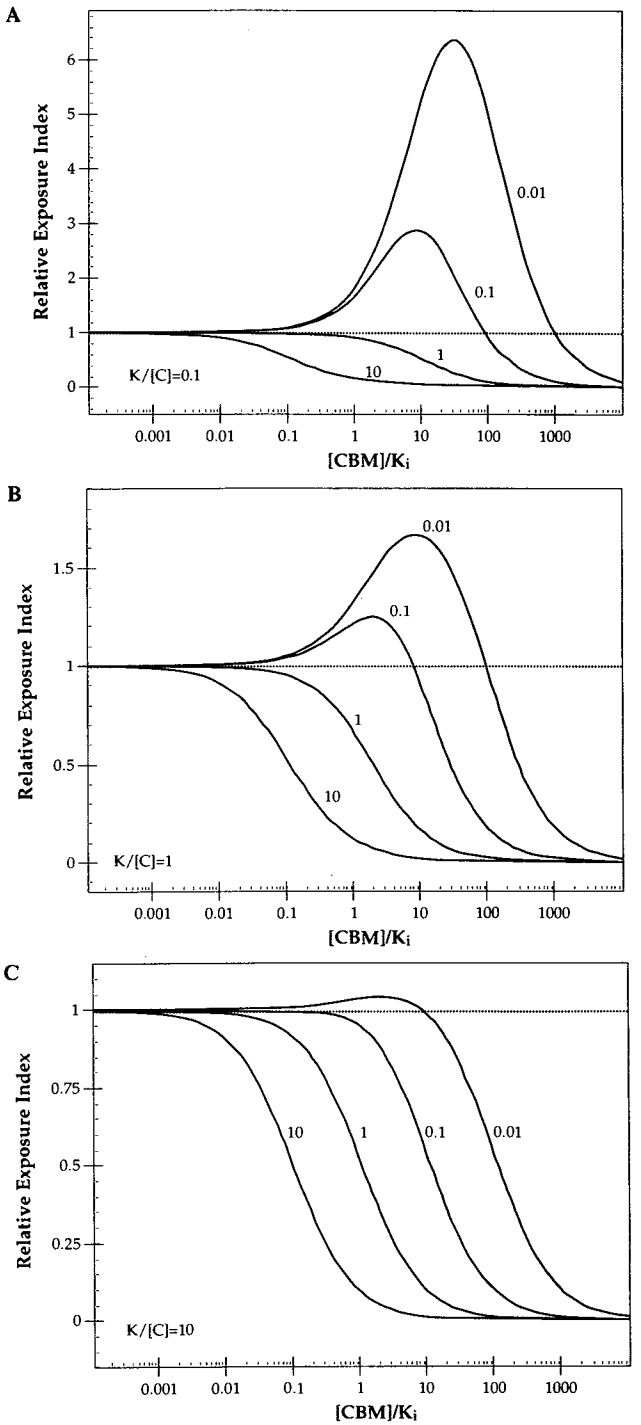

Fig. 2. Plots of the Relative Exposure Index vs. nondimensional CBM concentration, $[CBM]/K_i$. The τ_C/τ_{CB} values are shown against the curves, and Figures 2A, B and C were obtained for K/[C] values of 0.1, 1 and 10, respectively.

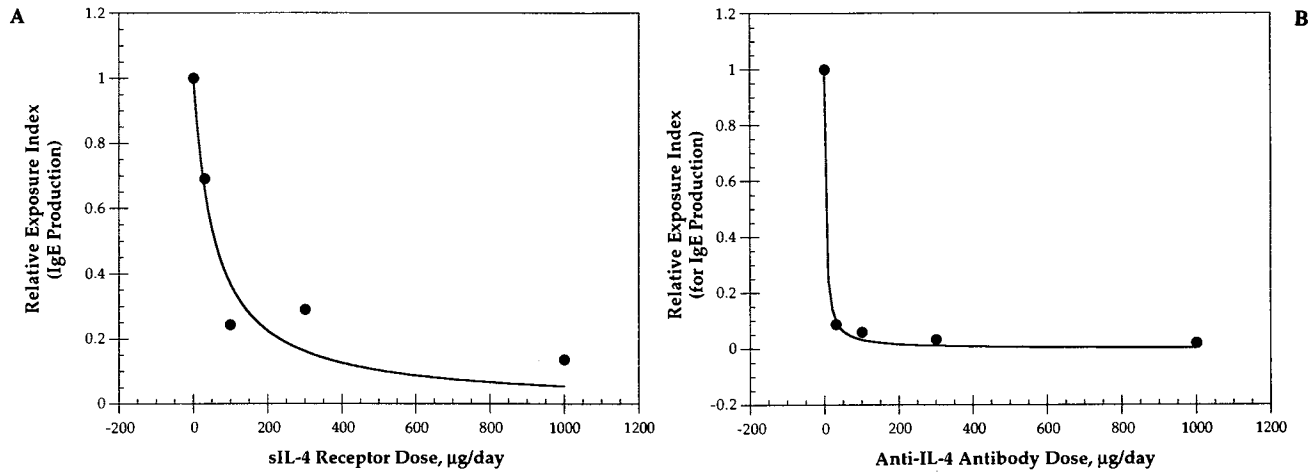

Fig. 3. Plots of Relative Exposure Index for IgE levels in serum on Day 9 as a function of dose for the soluble IL-4 receptor (Figure 3A) and the anti-IL-4 monoclonal antibody (Figure 3B). The IgE induction was elicited (7) in BALB/c mice using anti-IgD antibodies treated with 100 µg of anti-IgD antibodies on Day 1, and with two i.p. injections of the indicated doses of CBM on days 3, 4 and 5. The R value was calculated using R = IgE(Actual) – IgE(None,No anti – IgD)/IgE(None,Anti – IgD) – IgE(None,No anti – IgD). The curve represents the fit obtained using the model. For the soluble receptor, a τ_C/τ_{CB} value of 0.0833 was used and a value of 0.0319 was used for anti-IL-4 antibody.

In this Case, both scavenger and carrier CBM can be effective antagonists. However, the dosing criterion in Equation (8) must be exceeded. The f values required for antagonist activity increase at lower τ_C/τ_{CB} values and at higher dimensionless cytokine concentrations, [C]/K.

Upon comparing Case I to Cases II and III, we see that the nonlinear dependence of the cytokine-receptor interaction on cytokine concentration contributes to unexpected increases in relative exposure index.

The General Case

To determine the conditions under which R is greater than unity, we used Equation (2) and expanded the inequality.

$$R = \frac{1}{\left[(1-f) + f\frac{\tau_C}{\tau_{CB}}\right]}\frac{(1-f)}{\left[\frac{K}{[C]} + (1-f)\right]}\left[1 + \frac{K}{[C]}\right] > 1$$

Upon expansion, this yields:

$$f\left[\left(1 + \frac{K}{[C]}\right)\frac{\tau_C}{\tau_{CB}} - 1 + f\left(1 - \frac{\tau_C}{\tau_{CB}}\right)\right] < 0$$

For $\tau_C < \tau_{CB}$, the following condition follows:

$$f < 1 - \frac{K}{[C]}\left(\frac{\tau_C}{\tau_{CB} - \tau_C}\right) \quad (9)$$

If

$$\frac{K}{[C]}\left(\frac{\tau_C}{\tau_{CB} - \tau_C}\right) > 1,$$

then R is less than unity for $0 < f < 1$. If not, the "critical" CBM concentration that must be exceeded for R values less than unity is obtained using Equation (3) as:

$$[CBM] = K_i\left[\frac{[C]}{K}\left(\frac{\tau_{CB}}{\tau_C} - 1\right) - 1\right] \quad (10)$$

By partial differentiation of R with respect to f and by evaluating the second derivative, we determined that the maximum value of R occurs at a f value given by:

$$1 - f = \left[\frac{K}{[C]}\left(\frac{\tau_C}{\tau_{CB} - \tau_C}\right)\right]^{1/2} \quad (11)$$

Thus, a maximum occurs between $0 < f < 1$ if:

$$0 < \frac{K}{[C]}\left(\frac{\tau_C}{\tau_{CB} - \tau_C}\right) < 1 \quad (12)$$

Testing the Model

To test the model, we identified a report (7) in which agonist-like and antagonist activities were found with the use of CBM directed against interleukin-4 (IL-4). IL-4 is an important T cell and mast cell derived mediator of B cell function that is required for the stimulation IgE production by anti-IgD antibodies.

Sato et al. (7) reported the dose-response curves for soluble IL-4 receptor and anti-IL-4 monoclonal antibody on IgE production elicited with either anti-IgD antibodies or with anti-IgD antibodies plus exogenous IL-4 administration in mice. Both soluble receptor (Figure 3A) and anti-IL-4 antibody (Figure 3B) inhibited anti-IgD induced IgE production. However, when anti-IgD antibodies plus exogenous IL-4 was used to elicit IgE production, agonist-like effects were observed (Figures 4A-B and Figures 5A-B). We will show that the predictions of the model are consistent with the data of (7).

Because a multiple dosing regimen was used, we assume that steady state conditions are approximated. The half-lives of free IL-4, soluble receptor bound IL-4 and anti-IL-4 antibody bound IL-4 were reported to be 1.5, 18, and 47 minutes, respectively (7). These yield τ_C/τ_{CB} values of 0.0833 and 0.0319 (for soluble IL-4 receptor and anti-IL-4 antibody, respectively) that were used in our fitting procedure as non-adjustable parameters.

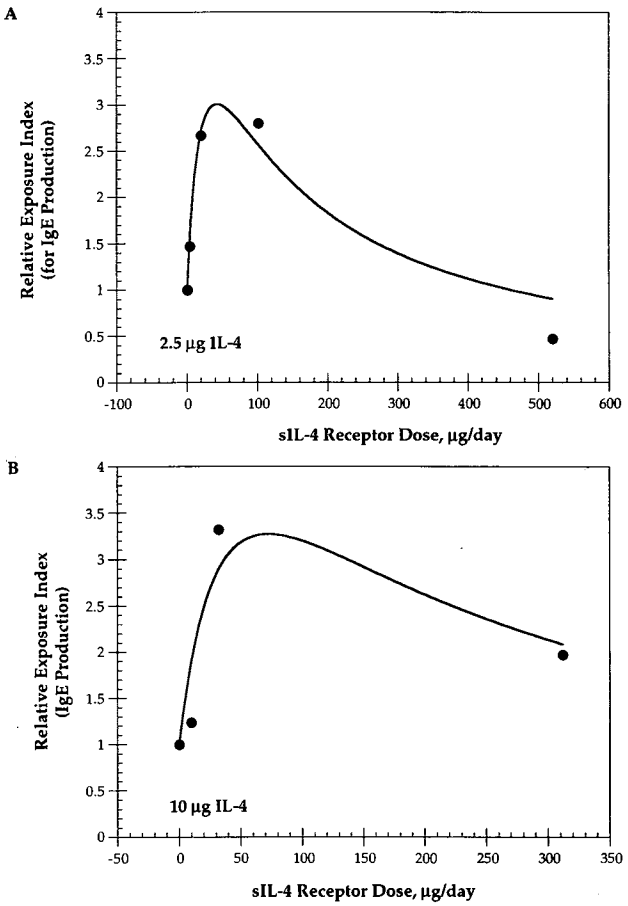

Fig. 4. Plots of Relative Exposure Index for IgE levels in serum on Day 9 as a function of dose for the soluble IL-4 receptor. The R values were calculated from Figure 4A of (7) using R = IgE(Actual)/IgE(No Inhibitor). The IgE induction was elicited using either 2.5 μg/day (Figure 4A) or 10 μg/day (Figure 4B) exogenous IL-4. BALB/c mice were treated with 100 μg of anti-IgD antibodies on Day 1, and with two i.p. injections of the indicated doses of premixed IL-4 and CBM on days 3, 4 and 5. The curve represents the fit obtained with the model using a τ_C/τ_{CB} value of 0.0833.

Figures 3A and 3B contain data from Table I of (7) fitted parsimoniously to the equations for Case I. The R value is:

$$R = \frac{(1 - f)}{(1 - f) + f\left(\frac{\tau_C}{\tau_{CB}}\right)} = \frac{K_i}{K_i + [CBM]\left(\frac{\tau_C}{\tau_{CB}}\right)} \quad (13)$$

Here, K_i is the only parameter determined by fitting and the fitted curves, K_i values and correlation coefficients are shown in Figures 3A and 3B. The units of K_i are the same as the units used for CBM dose. The fit is fairly satisfactory in both Figure 3A and Figure 3B.

Figure 4 (adapted from Figure 4A of (7)) shows the effect of treatment with soluble IL-4 receptor on IgE production induced by either 2.5 μg (Figure 4A) or 10 μg (Figure 4B) of exogenously added IL-4. Figures 5A and 5B (adapted from Figure 4B of (7)) are the corresponding dose response curves for anti-IL-4 antibody. The definition for R can be combined with Equation 4 to yield:

$$R = \frac{K_i\left(1 + \frac{K}{[C]}\right)(K_i + [CBM])}{\left(K_i + [CBM]\left(\frac{\tau_C}{\tau_{CB}}\right)\right)\left((K_i + [CBM])\left(\frac{K}{[C]}\right) + K_i\right)} \quad (14)$$

This equation contains only three parameters besides the dependent variable (R) and the independent variable ([CBM]). The τ_C/τ_{CB} values were kept at 0.0833 (for Figures 4A and B) and 0.0319 (for Figures 5A and 5B) and the values of K_i and K/[C] were determined by fitting. The results are summarized in Table I. The fitted curves were all quite satisfactory except in Figure 5A. The poor fit in Figure 5A can be attributed to the fact that the R value decreases with dose before increasing at the highest doses. Equation (5) does not represent such features well and only 3 out of the 6 data points are "usable" for fitting. However, this may not be a problem if the value of IgE level at a zero CBM dose is kept as an adjustable parameter in the fitting.

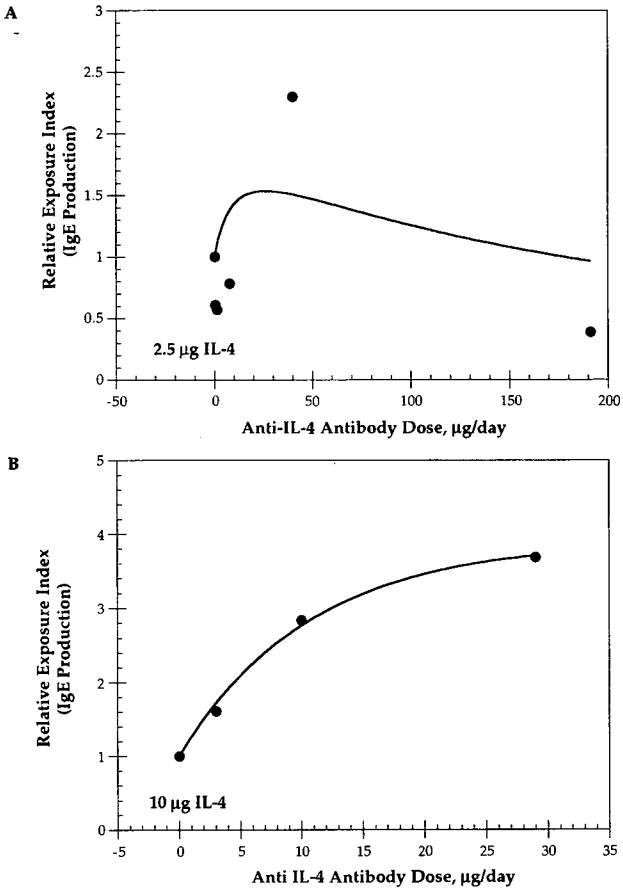

Fig. 5. Plots of Relative Exposure Index for IgE levels in serum on Day 9 as a function of anti-IL-4 monoclonal antibody dose. The R values were calculated from Figure 4B of (7) using R = IgE(Actual)/IgE(No Inhibitor). The IgE induction was elicited using either 2.5 μg per day (Figure 5A) or 10 μg per day (Figure 5B) exogenous IL-4. BALB/c mice were treated with 100 μg of anti-IgD antibodies on Day 1, and with two i.p. injections of the indicated doses of CBM on days 3, 4 and 5. The curve represents the fit obtained with the model using a τ_C/τ_{CB} value of 0.0319.

Table I. Summary of fitting results

Figure/CBM	Equation Used	$\frac{\tau_C}{\tau_{CB}}$	Parameter[a] ± Error	f[b]	χ^2	r[c]
Figure 3A, Soluble Receptor	Eqn. (11)	0.0833	K_i = 4.8 ± 1.4	0 – 0.99	0.040	0.96
Figure 3B, Antibody	Eqn. (11)	0.0319	K_i = 0.11 ± 0.023	0 – 0.99	0.0017	0.99
Figure 4A, Soluble Receptor + 2.5 μg IL-4	Eqn. (12)	0.0833	K_i = 4.7 ± 1.0 K/C = 0.11 ± 0.019	0 – 0.99	0.28	0.97
Figure 4B, Soluble Receptor + 10 μg IL-4	Eqn. (12)	0.0833	K_i = 7.2 ± 2.8 K/C = 0.091 ± 0.034	0 – 0.98	0.65	0.89
Figure 5A, Antibody + 2.5 μg IL-4	Eqn. (12)	0.0319	K_i = 5.4 ± 7.9 K/C = 0.90 ± 0.86	0 – 0.97	1.8	0.52
Figure 5B, Antibody + 10 μg IL-4	Eqn. (12)	0.0319	K_i = 3.0 ± 0.30 K/C = 0.14 ± 0.0095	0 – 0.91	0.018	0.99

[a] Units of K_i are μg/day; K/C is nondimensional.
[b] f is the fraction of cytokine bound. Calculated from Equation (4).
[c] r is the correlation coefficient.

DISCUSSION

In this paper, we use the relative exposure index, R, for determining the potential of antibodies and other macromolecules as antagonists for cytokines. The nonlinearity of the interaction between the cytokine and its receptor can cause undesirable increases in relative cytokine exposure index with otherwise potent cytokine binding macromolecules. The model fits experimental data obtained using IL-4 inhibitors and as predicted, maximal agonist-like activity occurs at intermediate concentrations of CBM.

The simplicity of this model represents both its strengths and its weaknesses. The usefulness of the model may be limited by the variability in *in vivo* data and by the simplifying assumptions such as steady state, equilibrium and single site binding that were made in the mathematical development. Also, feedback regulation of cytokine release and receptor expression should be negligible. If the cytokine exerts biological effects after internalization, the τ_C measurement methodology used should account for both intracellular and extracellular cytokine. Because Equation (4) implicitly assumes that receptor binding does not influence f, these results establish a lower limit for R. In practice, the receptor will compete with CBM, cause a reduction of f, and result in increased R for the three Cases. However, for Case II, when $\tau_C < \tau_{CB}$, R decreases with a reduction of f.

Many cytokines, e.g., IL-1β and IL-6, bind α_2 macroglobulin by reversible or irreversible mechanisms. For such systems, α_2 macroglobulin should be treated as an "indigenous CBM" that can potentially alter the half-life of the cytokine or compete for receptor occupancy. Qualitative insights into the effects of CBM can be obtained from the model provided the binding isotherms and the K, K_i, and τ_C values used reflect a milieu containing α_2 macroglobulin. However, a more comprehensive model may be warranted if the cytokine modulates expression of α_2 macroglobulin.

The K_i values for the antibody and soluble receptor are similar (Range: 3.02 – 7.22 μg; see Table I), and this is consistent with the *in vitro* properties of these CBM (7). However, the K_i value from Figure 3B deviates from this pattern. This deviation may be the result of the limited data availability—almost no data are available over the dose range in which substantive changes in R occur. We estimate that K_i values obtained by fitting are about 1.7 times higher than the IC_{50} values in (7) and about 60 times higher than the dissociation constant reported for the soluble receptor (8). The discrepancies may be due to the simplifying assumptions in the model and the use of CBM dose instead of the CBM free concentration for fitting.

For evaluating cytokine antagonists, Klein and Brailly (2) propose the use of the parameter $T_{1/2}$(RTf/RTc), where $T_{1/2}$ is the dissociation half life of the cytokine-CBM complex and (RTf/RTc) is ratio of the *in vivo* half lives of the free CBM to cytokine-CBM complex. The parameter estimates potency and may be useful for determining dosage. The R value approach does not directly address dosing requirements because the clearance of the CBM does not enter the analysis. Instead, the method focuses on the extent of the antagonistic activity that can be expected.

We believe that the model will be useful for interpreting experimental data and as an intuitive aid in decision making during drug design and development.

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