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# Expression of recombinant soluble FcεRI: function and tissue distribution studies

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## SUMMARY

Recombinant soluble IgE Fc receptors (rsFcεRI) are potent inhibitors of type I hypersensitivity reactions tested in a local inflammatory setting. However, the fate of these receptors *in vivo* is dependent on the cellular source of the rsFcεRI. We have produced these by transiently transfecting Cos-7 cells with a cDNA encoding the extracellular domains of human FcεRI α-chain. Following affinity purification, the rsFcεRI was characterized as 58 000 MW, which was reduced to 23 000 MW following endoglycosidase F treatment. The purified rsFcεRI could inhibit mouse IgE binding to FcεRI$^+$ transfected CHO-K1 cells *in vitro*, bind sIgE$^+$ B lymphoma cells *in vitro*, and inhibit the passive cutaneous anaphylaxis model *in vivo* in Sprague–Dawley rats. Pharmacokinetic studies *in vivo* involving intravenous injection of radiolabelled rsFcεRI in mice revealed the receptor to have a rapid initial blood clearance ($t_{1/2}$ early phase of 15 min) and to accumulate in the liver before being detected in urine. The localization of rsFcεRI in the liver could be blocked by administration of mannose glycosylated ovalbumin and mannan, demonstrating that liver uptake involved the mannose receptor that is expressed on liver sinusoid cells and Kupffer cells. The production of rsFcεRI using a stable expression system in CHO-K1 cells produced functional receptor of the same molecular weight as the Cos-7 system by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). However, biodistribution studies demonstrated differences; the CHO-K1 cell-produced material did not localize to the liver in comparison to the Cos-7-produced rsFcεRI.

## INTRODUCTION

Antibody-induced hypersensitivity reactions are responsible for extensive tissue damage and pathological processes in a variety of diseases. Some of the more common and serious consequences of immunoglobulin-mediated disease are allergy and asthmatic reactions mediated by IgE, IgA nephropathies, and IgG immune complex-induced glomerulonephritis or vasculitis in systemic lupus erythematosus (SLE) and other autoimmune diseases.[1–5]

As the activation of inflammation by antibody is dependent on the antibody's Fc portion and also on its interaction with specific, cell-surface Fc receptors (FcR), treatments that interfere with the Fc–FcR interaction would lead to inhibition of the inflammatory reaction.

One of the emerging concepts in the treatment of inflammatory disease is the use of recombinant soluble receptors to inhibit binding of ligand to its cellular receptor. Soluble CD4 has been used to regulate T-cell function and human immunodeficiency virus (HIV) infection, and soluble CRI has been used to modify inflammatory responses in animals and humans.[6–8]

As antibody-induced hypersensitivities involve Fc–FcR interactions, the use of recombinant soluble (rs)FcR to ameliorate inflammation is a potential approach to therapy. Our recent experiments have demonstrated that rsFcγRII is a potent inhibitor of the immune complex-induced Arthus reaction.[9]

IgE-mediated allergic reactions are also probable targets for receptor-based therapeutic strategies, and recent reports indicate that recombinant IgE receptors can modify IgE-induced type I hypersensitivity.[10,11] However, despite the potential use of recombinant receptors (especially FcεRI) for therapy of systemic disease, little is known of their fate *in vivo*. With increasing numbers and diversity of expression systems developing for the production of recombinant proteins, post-translational modifications may be important factors in determining how recombinant receptors behave *in vivo*. It is clear, for example, that glycosylation patterns differ between phyla and also species. Thus proteins produced in yeast, insect cells, rodent cells or primate cells may have distinct differences

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Abbreviations: rsFcεRI, recombinant soluble Fcε receptor I; TNBS, trinitrobenzene sulphonic acid; PMSF, phenylmethyl sulphonyl fluoride.

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in the addition of carbohydrates. This is certainly true for the distribution of α-galactosyl epitopes.[12]

In this study we examine the properties of human rsFcεRI produced by either transfected rodent or primate cells. We show that while the IgE-binding properties of the different sources of rsFcεRI are identical, their behaviour *in vivo* is quite distinct.

## MATERIALS AND METHODS

*cDNA constructs*

*Soluble FcεRI.* A termination codon was inserted into the human α FcεRI cDNA 5′ of the sequence encoding the transmembrane region by replacing the codon encoding proline 173 with TGA, using splice overlap extension polymerase chain reaction (PCR).[13] The rsFcεRI PCR product was subcloned into the transient expression vector pKC3,[14] which contains a simian virus 40 (SV40) early promoter. RsFcεRI was transfected transiently into Cos-7 cells using diethylaminoethyl (DEAE) dextran, as described elsewhere.[13] The rsFcεRI cDNA was also subcloned into pEE6/HCMV/GS (Celltech Ltd, Slough, UK). Recombinant soluble FcεRI was stably transfected into CHO-K1 cells using $CaPO_4$ as described elsewhere,[16] and a stable CHO-K1 cell line (SE-7) was established that secreted rsFcεRI at high levels.

*Cell-surface FcεRI construct for generation of FcεRI$^+$ cell line.* FcεRI α-chain (IgE-binding chain) cannot be expressed on the cell surface without FcεRI γ-chain co-expression.[15] To circumvent this problem, a chimeric receptor was generated that consisted of the extracellular domain of FcεRI and the transmembrane and cytoplasmic tail of FcγRII.[13] This construct was subcloned into the pEE6/HCMV/GS expression vector and transfected into CHO-K1 cells using the $CaPO_4$ method.[16] Methionine sulphoximine-resistant colonies were screened for IgE binding and a positive cell line was established (EEG–CHO).

*Purification of rsFcεRI from tissue culture supernatant*

The supernatant from rsFcεRI-transfected cells was harvested, centrifuged at 2000 *g* to pellet cellular debris, and stored at −20°. The rsFcεRI produced by Cos-7 cells (rsFcεRI–Cos) and rsFcεRI produced by CHO-K1 cells (rsFcεRI–CHO) were recovered from culture supernatant by affinity chromatography. Supernatant was loaded onto a human IgE–Sepharose affinity column and was recirculated over the column for 48 hr at 4°. After washing the 2-ml column with 1 l of phosphate-buffered saline (PBS), bound material was eluted with 0·1 M glycine, pH 2·7. The elutant was neutralized with 3 M Tris–HCl, pH 8·0, and dialysed against PBS.

*Detection of rsFcεRI*

Mouse IgE (αTNBS–ATCC TIB142; 9 μg; Rockville, MD) was radiolabelled with $^{125}$I (Amersham Int., Amersham, UK) using chloramine T (1 mg/ml) in PBS. Twofold dilutions of supernatant (either rsFcεRI–Cos, mock or rsFcεRI–CHO), or purified fractions of rsFcεRI, were incubated overnight at 4° with an equal volume (25 μl) of membrane (m)IgE at 480 ng/ml. EEG–CHO cells ($10^5$) were incubated with the supernatant/IgE mix for 2 hr at 25°, washed repeatedly with DME medium and the cell pellets counted in an LKB 1260 multi gamma-counter (LKB Pharmacia, Uppsala, Sweden). As one rsFcεRI molecule binds one IgE molecule,[10] the amount of rsFcεRI in the supernatants was calculated by inhibition titres on known amounts of radioiodinated IgE. Recombinant soluble FcεRI–Cos culture supernatant contained approximately 150 ng/ml rsFcεRI, while rsFcεRI–CHO culture supernatant contained approximately 18·75 μg/ml rsFcεRI (125-fold more).

*Protein biochemistry and N-linked glycosylation analysis*

Purified rsFcεRI–Cos (230 ng) was radiolabelled with $^{125}$I using chloramine T. Unincorporated iodine was removed using a PD-10 column (Pharmacia). The radiolabelled rsFcεRI–Cos was incubated with human IgE or human IgG-conjugated Sepharose 4B beads at 4° for 1 hr. The beads were washed five times with PBS and three times with endoglycosidase F (Endo F) buffer (0·1 M $Na_2HPO_4$, 50 mM EDTA, 1% Tween 20, 1 mM PMSF, pH 6·1). All samples were resuspended in 20 μl of Endo F buffer, and to one IgE sample 2 U Endo F (Boehringer–Mannheim, Mannheim, Germany) was added and beads were incubated at 37° for 16 hr prior to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions.

*Recombinant soluble FcεRI binding to IgE$^+$ lymphoma*

Radiolabelled rsFcεRI–Cos was incubated with $1 \times 10^5$ I29E$^+$ cells (membrane IgE$^+$ B lymphoma)[17] or A3 cells (membrane IgG$^+$ hybridoma[18]) at 4° for 2 hr, and then washed thrice with DME medium. Cell-bound counts were measured using a gamma-counter as above.

*Biosynthetic labelling of rsFcεRI–CHO*

CHO-K1 cells transfected with rsFcεRI cDNA (SE-7) and CHO-K1 cells transfected with the expression vector alone (V15) were incubated in cysteine/methionine-free DME for 1 hr at 37° before 0·5 mCi of $^{35}$S-labelled cysteine/methionine (ICN, Sydney, Australia) was added, and incubated for 4 hr. The supernatant was harvested and centrifuged at 13 000 *g* to remove cellular debris, prior to incubation with either human IgE- or IgG-coated Sepharose beads.

*Inhibition of passive cutaneous anaphylaxis*

Sprague–Dawley rats were shaved and sensitized by intradermal injections of 5 ng of mouse IgE anti-TNBS per site, with or without rsFcεRI–Cos at various concentrations beginning at 1/10 (460 ng/ml). RsFcεRI–Cos was incubated with IgE (anti-TNBS) at 4° overnight prior to sensitization. The antigen (TNBS–BSA (bovine serum albumin); 28:1) was administered intravenously 16 hr later in the presence of Evans' blue dye. Type I hypersensitivity reaction was assessed by the extravasation of the dye at the injection site.

*Blood clearance and biodistribution studies in mice*

(CBA × BALB/c) $F_1$ mice were used to estimate the *in vivo* half-life and tissue distribution of rsFcεRI delivered by an intravenous route. Recombinant soluble FcεRI (Cos or CHO; 500 ng) was radiolabelled with $^{125}$I using chloramine T, and free $^{125}$I was removed using a PD-10 Sephadex column (Pharmacia). Groups of three mice were injected intravenously with radiolabelled rsFcεRI and were killed at various time-points. Blood, urine and tissues were weighed and measured for radioactivity. The blood volume was calculated as 7% of the body weight and the half-life of rsFcεRI obtained from a

 

logarithmic plot of percentage injected dose versus time, where $t_{1/2}$ = ln2/gradient.[9] Radioactivity in the tissues was expressed as percentage injected dose per gram of tissue.

*Inhibition of liver localization by ovalbumin and mannan*

Inhibition of liver uptake of the rsFcεRI was performed as described previously.[19] Briefly, (CBA × BALB/c) $F_1$ mice were injected intravenously with 10 mg of ovalbumin (mannose terminated) and 5 mg of mannan two min prior to intravenous administration of iodinated rsFcεRI. Mice were killed after 15 min and liver samples were weighed and radioactivity measured. Data are presented as percentage injected dose per gram of liver.

## RESULTS

Two sources of rsFcεRI were produced using two established expression systems. First, primate fibroblasts (Cos-7) were used where rsFcεRI was transiently produced (rsFcεRI–Cos); second, rodent fibroblasts (chinese hamster ovary cells; CHO-K1) were used where rsFcεRI was stably produced (rsFcεRI–CHO). In both cases identical cDNA encoding rsFcεRI was used for both, but in different expression vectors.

### Identification of functional rsFcεRI

The presence of functional rsFcεRI in the supernatants of transiently transfected Cos-7 cells was determined by assessing the extent of inhibition of IgE binding to membrane FcεRI expressed on the cell line EEG–CHO (Fig. 1a). Supernatant at low dilution completely inhibited IgE binding to cells. This inhibition was specific as 'mock'-transfected supernatant was unable to inhibit IgE binding. Moreover, less than 1% of counts bound non-specifically, as determined by binding to the FcεRI cell line V15. Following affinity chromatography of supernatant, the rsFcεRI–Cos was enriched 125-fold (based on the inhibition titre).

Similarly, functional rsFcεRI–CHO was present in the culture supernatant of stably transfected CHO cells (Fig. 1b). This supernatant contained approximately 125 times more

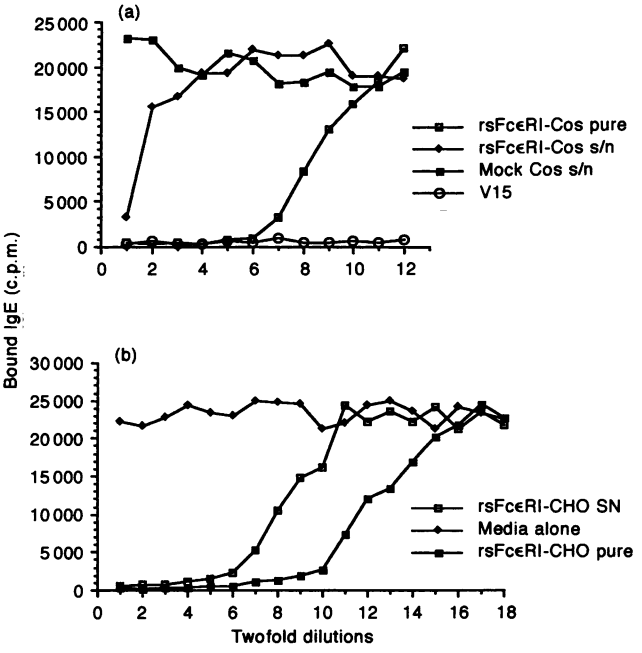


**Figure 1.** Inhibition of radiolabelled mIgE binding to EEG–CHO cells by rsFcεRI–Cos (a) and rsFcεRI–CHO (b). Radioiodinated IgE was incubated with serial dilutions of rsFcεRI$^+$ culture supernatant (SN), affinity-purified rsFcεRI (pure), mock culture supernatant or media alone, before further incubation with membrane FcεRI$^+$ EEG–CHO cells or FcεRI$^-$ V15 cells (to measure background IgE binding; a). After washing, the levels of cell-bound radioiodinated IgE was assessed.

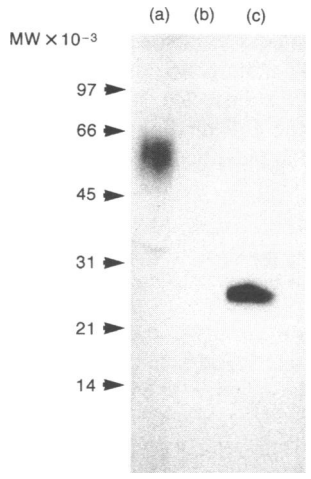


**Figure 2.** SDS–PAGE analysis of rsFcεRI–Cos after affinity purification. Radioiodinated rsFcεRI–Cos was incubated with human IgE-coated Sepharose (a and c) and human IgG Sepharose (b). The Sepharose samples were washed with Endo F buffer and incubated without (a and b) or with (c) Endo F for 16 hr at 4° prior to 13% SDS–PAGE (reducing conditions) and autoradiography.

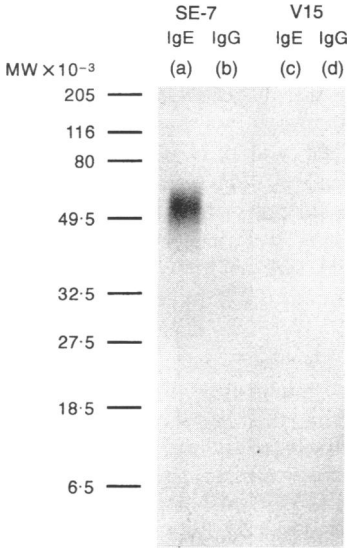


**Figure 3.** SDS–PAGE analysis of rsFcεRI–CHO from biosynthetically labelled culture supernatant. SE-7 cells (secreting rsFcεRI–CHO; a and b) or V15 cells (expression vector alone; c and d) were incubated with [$^{35}$S]methionine and cysteine for 4 hr, before the culture supernatant was incubated with either human IgE Sepharose (a and c) or human IgG Sepharose (b and d). The samples were then analysed by 13% SDS–PAGE under reducing conditions prior to autoradiography.

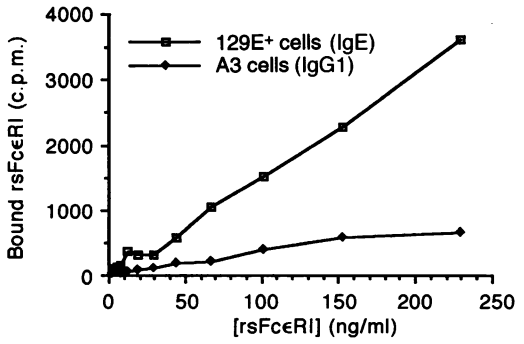


**Figure 4.** rsFcεRI–Cos binds to a IgE$^+$ B-cell line. Radioiodinated rsFcεRI–Cos was incubated with either a membrane IgE$^+$ B lymphoma (I29E+) or a membrane IgG$^+$ hybridoma (A3) before the cells were washed and the amount of bound [$^{125}$I]rsFcεRI–Cos determined.

rsFcεRI than the Cos-7 cell supernatants (measured by IgE inhibition titre, data not shown). The rsFcεRI was subsequently affinity purified on a human IgE column.

### Biochemical characterization of rsFcεRI

Affinity-purified rsFcεRI–Cos was radioiodinated and immunoprecipitated with human IgE (Fig. 2a, c). A 48 000–58 000 MW moiety was precipitated with human IgE (Fig. 2a). The rsFcεRI–Cos retained its specificity as it bound IgE but not IgG (Fig. 2b). Following digestion with Endo F, the rsFcεRI–Cos had a molecular weight of 23 000 MW (Fig. 2c), which is consistent with the predicted molecular size of the peptide core of the extracellular domains which have seven potential N-linked glycosylation sites.

The rsFcεRI–CHO was also approximately 48 000–58 000 MW and specifically bound to IgE, and not IgG as expected (Fig. 3a, b, respectively). Furthermore, no IgE-binding material was precipitated from the control supernatant (V15 cells; Fig. 3c, d).

### Recombinant soluble FcεRI-Cos binds to IgE$^+$ B cells

The observations that the rsFcεRI–Cos could bind IgE in solution raised the question about the potential interaction with naturally expressed membrane IgE of B cells. Radioiodinated rsFcεRI–Cos was incubated with the mouse IgE$^+$ I29E$^+$ B lymphoma or mouse IgG1$^+$ A3 hybridoma. The rsFcεRI–Cos bound to the membrane IgE of the I29E$^+$ B lymphoma but not to the hybridoma expressing membrane IgG1 (Fig. 4). This binding was dose dependent.

### The rsFcεRI inhibits type I hypersensitivity reactions

Type I hypersensitivity reactions are dependent on antigen crosslinking of cell-surface FcεRI bound IgE, which leads to mast cell degranulation, release of a variety of mediators and induction of inflammation. The rsFcεRI has obvious potential as an inhibitor of this reaction *in vivo*. The passive cutaneous anaphylaxis model (PCA) was used to evaluate the *in vivo* efficacy of rsFcεRI. In this model, IgE-dependent type I reactions were identified by the extravasation of Evans' blue dye at the site of IgE/antigen challenge (Fig. 5).

The rsFcεRI–Cos completely inhibited the PCA reaction and was dose dependent; 460 ng/ml and 92 ng/ml completely inhibited the PCA. This was specific as the soluble IgG receptor rsFcγRII did not inhibit the PCA. Quadruplicate controls of IgE and buffer indicated that the PCA reactions were reproducible and detectable. Moreover, human IgE (which cannot bind to rat FcεRI and therefore cannot induce degranulation) did not induce a detectable lesion as expected (data not shown). This clearly establishes the inhibitory activity of the rsFcεRI in a local inflammatory setting.

### Origin of rsFcεRI influences pharmacokinetics and biodistribution

The studies of the fate of the rsFcεRI *in vivo* yielded surprising results (Fig. 6a, b). Blood clearance of radioiodinated rsFcεRI–Cos showed a rapid early phase, with a $t_{1/2}$ of

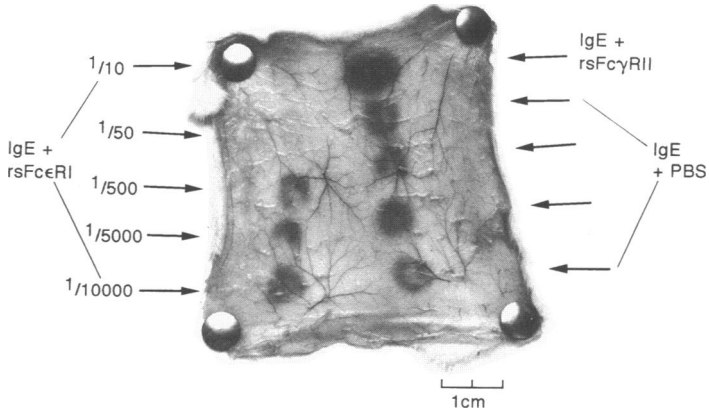


**Figure 5.** rsFcεRI–Cos inhibits the passive cutaneous anaphylaxis model. Sprague–Dawley rats were shaved and anaesthetized prior to intradermal sensitizing injections of mouse IgE (anti-TNBS) in the presence (a) or absence (b) of rsFcεRI–Cos. The dilution of rsFcεRI–Cos administered is indicated; 1/10 (460 ng/ml),1/50 (92 ng/ml),1/500 (9·2 ng/ml), 1/5000 (0·9 ng/ml), 1/10000 (0·4 ng/ml). A negative control recombinant protein (rsFcγRII) was administered at 1 mg/ml and showed no inhibition. PBS was administered with IgE repeatedly to demonstrate reproducibility of the lesion.

 

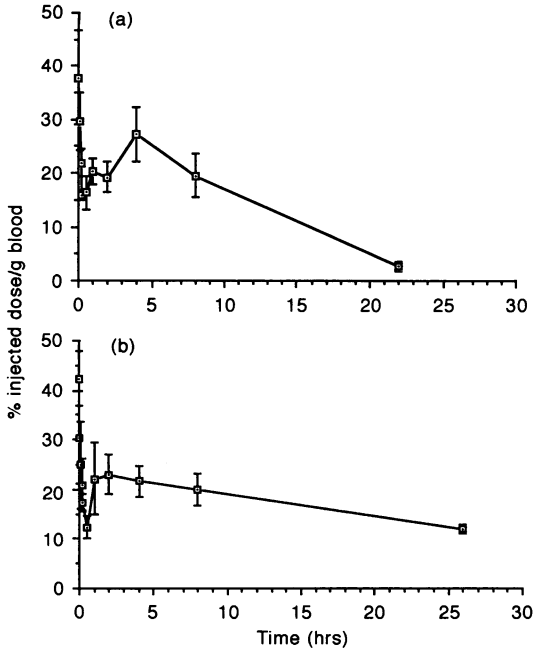


**Figure 6.** *In vivo* blood clearance of rsFcεRI with time. Groups of three (CBA × BALB/c)$F_1$ mice were injected intravenously with radioiodinated rsFcεRI–Cos (a) or rsFcεRI–CHO (b) and blood samples were taken at various time-points, weighed and counted.

15 min (Fig. 6a). Similarly, the early phase $t_{1/2}$ of rsFcεRI–CHO was also approximately 15 min. However, the later phase of clearance from blood of both sources of receptor showed distinct differences. Following the rapid early phase there was a rise in the levels of rsFcεRI–Cos which reproducibly peaked at 4 hr post-injection. This was followed by a second later phase of blood clearance with a $t_{1/2}$ of 11 hr. The rsFcεRI–CHO also showed a rise in blood levels after the early phase but surprisingly this peaked much sooner (1–2 hr) than the rsFcεRI–Cos (4 hr). Furthermore, the $t_{1/2}$ of the second later phase of clearance was substantially extended, to approximately 26 hr for rsFcεRI–CHO versus 11 hr for rsFcεRI–Cos.

The tissue localization of the two receptor preparations also showed distinct differences. The rsFcεRI–Cos predominantly localized to the liver (up to 20% injected dose/g tissue) and was present in smaller quantities in lung, spleen, kidney and heart (5–8% injected dose/g tissue) (Fig. 7a). The localization of rsFcεRI–CHO was strikingly different, where only 4–6% of the injected dose was found in these tissues, including the liver (Fig. 7b).

As the rsFcεRI protein in both the rsFcεRI–Cos and rsFcεRI–CHO was identical, i.e. derived from the same cDNA, the difference in localization may relate to differences in post-translational modification. Certainly the human FcεRI contains seven potential sites for N-glycosylation, which may result in different patterns of carbohydrate addition, depending on cell type and/or species in which the receptors are produced.

The uptake of natural or recombinant glycoproteins by hepatic mannose receptors has been documented widely.[19–21] To establish if liver uptake of rsFcεRI–Cos might be mediated by mannose receptors, *in vivo* blocking studies were performed (Fig. 8). Mice were treated with a mixture of glycoprotein containing terminal mannose (ovalbumin 10 mg) mixed with 5 mg of the yeast polymannose oligosaccharide, mannan. This treatment resulted in a 50% reduction in uptake of the rsFcεRI–Cos by liver after 15 min (Fig. 8) and a 30% reduction after 60 min (data not shown).

The liver uptake of the rsFcεRI–CHO was clearly lower than that of the rsFcεRI–Cos as seen in Fig. 7. However, the treatment of mice with the ovalbumin/mannan mixture could also reduce this already low level of uptake. Presumably a smaller proportion of the rsFcεRI–CHO was cleared by a 'mannose' dependent route. It is also clear that the ovalbumin/mannan mixture did not completely inhibit rsFcεRI–Cos uptake, implying the existence of other removal mechanisms. Alternatively, the incomplete inhibition of uptake may have been due to only partial blockade of available hepatic mannose receptors.

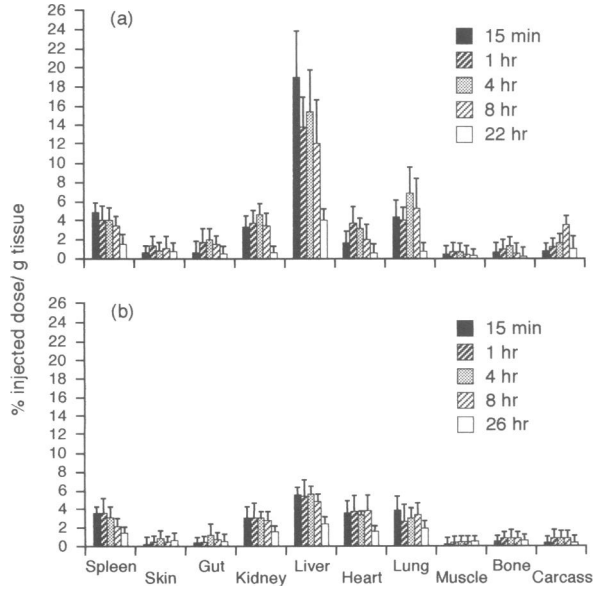


**Figure 7.** *In vivo* tissue biodistribution of rsFcεRI. Groups of three (CBA × BALB/c)$F_1$ mice were intravenously injected with radioiodinated rsFcεRI–Cos (a) or rsFcεRI–CHO (b) before tissue samples were taken at the five time-points listed. The tissue samples were bathed in saline to remove excess blood before being weighed and counted.

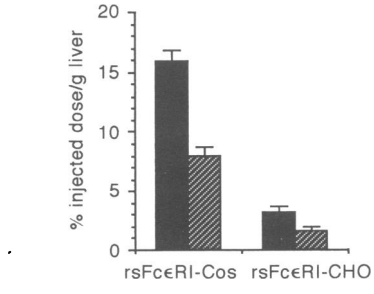


**Figure 8.** Blockade of liver uptake of rsFcεRI. Groups of three (CBA × BALB/c)$F_1$ mice were injected intravenously with 10 mg of ovalbumin (containing terminal mannose) and 5 mg of mannan 2 min prior to intravenous administration of either rsFcεRI–Cos or rsFcεRI–CHO (hatched bars). Livers were removed 15 min later, weighed and counted. The localization of rsFcεRI–Cos and rsFcεRI–CHO to the liver without injection of excess mannose is seen in the closed bars.

## DISCUSSION

Recombinant soluble forms of human FcεRI are clearly potent inhibitors of IgE binding and mast cell activation *in vitro* and *in vivo*, as described here. The ability of rsFcεRI to bind to $IgE^+$ B cells also means the potential is there for modulating *de novo* IgE synthesis at the B-cell level. However, we have not investigated this directly.

It is clear, with the development of molecular genetics and protein expression systems, that the potential for the use of recombinant receptors as therapeutic modalities in the prevention of ligand-induced activation of cells is becoming a reality. Indeed, not only have we shown here that the IgE receptor can inhibit IgE-induced inflammation, as have others,[10,11] but we have also demonstrated the principle of the application of soluble FcR to the inhibition of autoimmune inflammation by using soluble recombinant FcγRII.[9]

The recombinant soluble FcεRI produced either in Cos cells or CHO cells has similar IgE-binding activity based on the inhibition of IgE binding to cells. It was clear that the production systems yielded very different quantities of the material, with stably transfected CHO cells producing significantly more material (125-fold) in the supernatants of cell cultures. The molecular sizes of the secreted receptors, however, were similar in both cases, as assessed by SDS–PAGE.

Although the functional and biochemical studies indicated that the rsFcεRI derived from the primate (Cos) or rodent (CHO) cells were very similar, additional *in vivo* experiments indicated that the fate of the receptors was very different depending on which cell type was used to produce the recombinant soluble receptor. It was clear from the biodistribution experiments that the half-life of the receptor produced from Cos cells was short and was rapidly cleared from the blood, but slowly re-emerged to then be rapidly cleared from the blood a second time. By comparison, the material produced from CHO cells had the same initial rapid clearance from the blood but very rapidly reappeared in the circulation and slowly disappeared with a greatly increased half-life. An analysis of the tissue distribution showed that the Cos cell-produced material predominantly accumulated in the liver, whereas the CHO cell material showed little and equal accumulation in the kidney, liver, heart and lung. The accumulation of the Cos cell-derived soluble FcεRI in the liver was significantly reduced by a pretreatment of the mice with a large excess of ovalbumin and the polymannosaccharide, mannan, implying that the principal mode of liver uptake was via mannose receptors. Indeed, the rapid clearance from the circulation of glycoproteins via mannose receptors (for glycoproteins terminating in mannose of N-acetyl glucosamine) on hepatic sinusoidal cells is well documented.[19–21]

The implications of these findings on the use of recombinant proteins *in vivo* for therapy are interesting. In the studies described here, the two rsFcεRI preparations had the same protein core but markedly different fates *in vivo*. Presumably, since the receptors have the same protein core, the *in vivo* differences relate to the post-translational modification of the peptide core. As a combination of mannan and the glycoprotein ovalbumin inhibited liver uptake of the receptor, it seems likely that in fact the differences *in vivo* relate at least in part to differences in the N-linked carbohydrates that are added by the different cells used to produce the rsFcεRI.

It is interesting to note, therefore, that the material produced in the rodent cells had a much longer half-life in mice than the material produced in the heterologous cells, i.e. the primate Cos cells, and it is interesting to speculate that recombinant receptors used *in vivo* in a particular species will persist in the circulation longer if they have been made in cells related to that species, a function that is determined by the nature of the carbohydrate on the recombinant protein. The precedent for this may exist in humans, where naturally occurring antibodies in the circulation of humans and greater apes are responsible for the rapid recognition and destruction of organ xenografts. These antibodies detect carbohydrate antigens, specifically a Galα(1,3)Gal linkage which is present on the vast majority of mammalian cells but absent from humans and greater apes. Clearly, glycoproteins produced in cells of the non-human species would probably acquire the Galα(1,3)Gal epitope, which in turn would render them susceptible to binding of the natural antibodies in humans. Indeed, the rsFcγRII that was successfully used in rats and produced in rodent cells had the Galα(1,3)Gal epitope (M. Powell, personal communication).[9] These different glycosylation patterns may cause rapid clearance of recombinant receptors *in vivo* by natural antibodies binding to such epitopes, or by specific clearance through glycosyl receptors such as the mannose receptor. The importance of heterologous glycosylation in therapeutic agent efficacy is yet to be determined and will become clear as more recombinant proteins are tested in humans.

Another factor to consider is that different glycosylation patterns may produce antibodies reactive with the recombinant receptor, and it is unknown whether these antibodies would cross-react with the native glycosylated FcεRI on the cell surface. The production of antibodies to allotypic determinants may also arise as a potential problem; however, there are no known polymorphisms of the human FcεRI α-chain to date.

Other factors will also influence the *in vivo* efficacy of rsFcεRI. The PCA model revealed that rsFcεRI can inhibit IgE-mediated PCA reactions when present in equimolar amounts. Extrapolation of this model to systemic anaphylaxis means that if rsFcεRI was present in equimolar or greater concentrations to that of circulating and already bound IgE it may inhibit the triggering of anaphylaxis. There are, however, three factors that need to be taken into account. (1) As the $t_{1/2}$ of the rsFcεRI (Cos and CHO) in the circulation is short, then administration of substantial quantities would be required to maintain levels of rsFcεRI that are sufficient to influence IgE-mediated reactions. (2) The levels required are in turn influenced by the rate of *de novo* synthesis of IgE. (3) The 'appropriate' levels would have to be maintained for a sufficient length of time, as the dissociation rate of IgE from the cell-surface FcεRI is extremely slow.

It is clear from this work that recombinant human IgE receptors are potent inhibitors of IgE-mediated inflammation but, like all recombinant proteins, their efficacy *in vivo* remains to be fully determined by further investigation.

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