The Importance of Lys352 of Human IgE in FcεRII/CD23 Recognition

Ian Sayers*, Jonathan E. M. Housden, Alan C. Spivey** and Birgit A. Helm

Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, S10 2TN.

*Division of Therapeutics & Molecular Medicine, Queens Medical Centre, Nottingham, NG7 2UH.

**Department of Chemistry, Imperial College, South Kensington Campus, London SW7 2AZ.

Note: The first two authors contributed equally to this study.

Correspondence to:
Dr. B. A. Helm
Krebs Institute
Department of Molecular Biology and Biotechnology
University of Sheffield
Sheffield, S10 2TN
Telephone: 44 114 2824375
FAX: 44 114 2795495
Email: B.Helm@sheffield.ac.uk
Summary

The interaction of immunoglobulin E (IgE) with its low affinity receptor (FcεRII/CD23) plays a central role in the initiation and regulation of type I hypersensitivity responses. We have previously identified the importance of amino acid residues in the A-B loop of the Cε3 domain of human IgE and implicated a region close to the glycosylation site at Asparagine 371 as contributing to IgE-CD23 interaction. These residues were now targeted by site directed mutagenesis. The IgE-CD23 interaction was assessed by semi-quantitative flow cytometry. Replacement of the entire Cε3 A-B loop (residues 341-356) with the homologous rat IgE sequence resulted in complete loss of human CD23 recognition, as did replacement of residues 346-353, indicating that class specific effector residue(s) are contained within these eight amino acids. Lysine 352 within the A-B loop was identified as contributing directly to human CD23 interaction, mutation to the rodent homologue Glycine, or Glutamate, resulted in a significant reduction in binding compared to native IgE, while conservative substitution with Arginine effected a small, but statistically significant, enhancement of CD23 binding. Mutation of the Cε3 glycosylation site at Asparagine 371 to Threonine or Glutamine did not significantly affect CD23 recognition. Our results yield new insights into the structural basis of the hIgE-CD23 interaction, and hold promise for the rational design of drugs that can manipulate IgE mediated regulation of the allergic response.
Introduction

Antibodies of the immunoglobulin E (IgE) isotype have a central role in the initiation and regulation of allergic disorders (1). IgE mediates these functions via the interaction of the Fc region of the molecule with its Fc receptors. The crystal structure of the high affinity receptor (FcεRI, $K_A \sim 10^{10} \text{M}^{-1}$) complexed to IgE-Fc has been determined, identifying critical residues involved in the interaction (2). FcεRII/CD23 has been identified as a low affinity receptor for human (h) IgE ($K_A \sim 10^7 \text{M}^{-1}$) (3). Structurally, CD23 is not a member of the Ig superfamily, but a type II transmembrane receptor expressed on a variety of cells of the immune system (4). In the absence of a high resolution structure, a composite molecular model has been proposed based on homology of the lectin head with the rat mannose binding protein (5,6). The model predicts that five heptadic repeats of hydrophobic Leu/Ileu residues form a trimeric α-helical stalk linking the N-terminal extracellular domain to the transmembrane and C-terminal cytoplasmic domain of the receptor (7).

Previous studies based on the interaction of hIgE-Fc with a soluble 16kDa form of the lectin homology domain expressed in NS-O cells identified this region as the containing the IgE binding site(s) (8). More recent studies indicate the necessity of the stalk region for IgE interaction (9). Using site-specific mutagenesis, the IgE binding region of CD23 has been mapped to two discontinuous segments between residues 165-190 and 224-256 (10). Although several other ligands for CD23 have been identified (11), the role of the receptor and receptor-derived fragments in the regulation of the IgE response has attracted particular interest (12,13). A greater understanding of the IgE-CD23 interaction, based on the identification of complementary binding regions on each molecule, could assist the development of strategies for the manipulation of the allergic response via this regulatory mechanism.

The interaction of IgE with CD23 is species specific. Human IgE does not bind to the rodent receptor, and rat and mouse IgE will not engage the human homologue, although rat IgE recognises the mouse receptor (14). Previous studies have identified the hCε3 domain as the site of interaction with CD23 (15,16) although monoclonal antibodies that map to epitopes in the Cε2 and Cε4 domain have been described and are thought to inhibit receptor interaction by steric hindrance (15,17). Our own studies (18) and those by Nissim and co-workers (16) highlighted the importance of residues in the A-B loop region of hCε3 for binding to CD23.

The present investigation aimed to identify class specific effector residue(s) in the A-B loop influencing the hIgE-CD23 interaction. The A-B loop was targeted by site directed mutagenesis. Substitutions involved the
introduction of highly conservative changes, e.g. L to I or D to E in order to minimise potential structural perturbations caused by the mutagenesis. Residues targeted were also exchanged for rodent homologues in order to investigate the species specificity of the CD23 interaction (19).

The use of a eukaryotic expression system (20) facilitated an investigation of the role of hIgE glycosylation. IgE is heavily glycosylated (12-15%), however only N linked glycosylation has been detected to date. Glycosylation of IgE is not essential for CD23 recognition, in contrast with the interaction of this receptor with CD21, CD11b/c, which involves carbohydrate residues (21,22). The role of N-linked glycosylation in IgE is still unresolved, although it has been demonstrated that enzymatically deglycosylated IgE (PS) and hIgE-Fc fragments expressed in E.coli engage both Fc receptors, apparently enhancing the affinity of the CD23 interaction by ~10 fold (15). To clarify the role of glycosylation, the solvent exposed Cε3 glycosylation site at N371 was targeted and assessed for a role in the folding, processing and secretion of IgE variants in mammalian cells.

The functional consequences of site-specific mutagenesis were assessed by examining the binding of hIgE mutants to CD23 on the surface of the RPMI-8866 cell line employing a semi-quantitative flow cytometry assay. We show that substitution of Cε3 A-B loop residues in hIgE with rodent homologues is associated with loss of human CD23 recognition. Site directed mutagenesis of specific residues within the A-B loop, highlighted K352 as an effector determinant residue in human IgE-CD23 interaction.
Experimental Procedures

Gene Constructs and Site Directed Mutagenesis

Human epsilon (ε) gene variants were constructed by overlap extension mutagenesis PCR and sub-cloned into the mammalian expression vector pSV-VNP-He as described (20). The numbering scheme for the he-chain has been maintained as in previous publications (18). IgE variants were generated by transfection of expression vectors into the J558L cell line. Positive clones were selected and IgE was purified from cell culture supernatant by affinity purification on NIP-CAP Sepharose columns (19). All pSV-VNPHe variants were sequenced (Dyedodeoxy Sequencing, Applied Biosystems, Warrington, UK) to ensure the desired mutation had been engineered into the epsilon gene and spurious errors had not been introduced during the PCR reaction. The IgE variants were quantified using the BIAcore Biosensor (Pharmacia) as described (19).

Assessment of Cell Surface Receptor Interactions

Binding of IgE to cell surface receptors was assessed using flow cytometry. Three cell lines expressing different IgE receptors were used in this investigation; RPMI-8866 cells expressing human CD23 (23) Chinese hamster ovarian (CHO) cells transfected with mouse CD23 (Fc1.7 cells (24) - gift from D. Conrad) and Rat Basophilic Leukemia (RBL) cells transfected with human FcεRI alpha chain (25). Cells were maintained in culture as described previously (23-25). For RPMI-8866 cell analysis, cells were washed twice in wash buffer (Phosphate Buffered Saline (PBS), 1%FCS, 0.1% sodium azide) and re-suspended at 5x10⁶ cells/ml. 100 µl cell suspension was added to 20 µl recombinant IgE (0.125-5 µg). After incubation on ice for 30 minutes cells were washed twice with 1ml wash buffer then incubated with 100µl biotinylated anti IgE (Vector Laboratories, Ltd. Peterborough, UK) diluted 1/400 in wash buffer. Following a 30 minutes incubation on ice, cells were washed and incubated on ice with 100µl streptavidin R-phycoerythrin (PE) [1/20 dilution] (Sigma-Aldrich, Poole, UK) for 30 minutes. Before analysis by flow cytometry cells were washed twice and resuspended in 200µl wash buffer. To determine background levels of fluorescence PBS or control antibody (mouse IgE SPE7 (Sigma)) was used in place of the recombinant IgE. Fc1.7 cells or RBL cells transfected with human FcεRIα, cells were first detached from the culture dish using cell dissociation solution. Substitution of anti-IgE with biotinylated anti-λ antibody (Amersham Biosciences UK Limited, Buckinghamshire, UK) allowed direct comparison between mouse and recombinant human IgE binding.
using the Fc1.7 cells. Data were collected using a Coulter Etics Elite Flow Cytometer and analysed using Datamate (Dako, Ely, Cambridgeshire, UK).

Data Analysis

Each assay was carried out using chimaeric IgE with wild type (WT) human Fcε sequence (Serotec, Kidlington, Oxford, UK) as a reference control. This commercial preparation of recombinant IgE was prepared using the pSV-Vγ8Hε expression plasmid and J558L host cell line, identical to that used in the current study. The median log channel number was used for the quantification of wild type (WT) and variant IgE receptor binding interactions. The mean values from at least three independent experiments were calculated and compared by ANOVA and 2 sample t-test. Statistical analysis was completed using Minitab (Minitab Inc, state College, PA), a p value < 0.05 was considered significant. To facilitate discussion, results were expressed as percentages of WT binding (100%).
Results

IgE variants generated, together with the rationale explaining the mutation, are shown (Table I). The positions of Cε3 A-B loop residues within the crystal structure of IgE-Fc´ are shown (Fig. 1) The interaction of IgE with cell surface high- and low-affinity Fc receptors was observed employing a semi-quantitative flow cytometry assay. Measurement of fluorescence over a range of IgE concentrations, allowed the construction of binding curves (Fig. 2 and 3) and comparison of IgE variants with wild type IgE (Table I). WT IgE binding to RPMI-8866 cells reached saturation at 2.5 µg/assay.

Insert Table I and Figure 1 here

Replacement of the entire Cε3 A-B loop (residues 341-356) in the hIgE Fc for the homologous rat IgE residues (variant R16) resulted in complete loss of hCD23 binding. Binding of the R16 variant was found to be not statistically different to the background level fluorescence (mean value 3% of wild type fluorescence at 2.5 µg IgE) and significantly reduced compared to wild type binding (p = 0.02, Table I). Throughout this study, FcεRI interactions were assessed in parallel as an indicator of potential structural perturbations, which might have occurred as a consequence of mutagenesis. Changes in FcεRI interaction observed for this variant (Fig. 2A) were not statistically different from the wild type molecule (88.7%, p=0.13, Table I).

The R16 variant was constructed prior to crystal structure determination of receptor bound IgE. When the structural co-ordinates for IgE/FcεRI became available (2) it could not be ruled out that mutation of residues within the β-strands of the immunoglobulin fold (S341, R342, T355 and I356) could induce significant structural perturbations affecting receptor recognition. To address this, a further IgE variant (R8) was generated with the more limited substitution of residues 346 to 353 of the Cε3 AB loop with the homologous rat residues. The binding characteristics of the R8 variant confirmed earlier results obtained with the R16 chimaera, the interaction with CD23 was completely inhibited (97% reduction (vs. WT p=0.02), identical to background, p=0.08). The FcεRI interaction (Fig. 2A) was modestly reduced with a marginal statistical significance for this variant (91%, p=0.03, Table I).

Previous observations suggested K352 within the A-B loop may be a key effector residue, with mutation to G, the homologous residue in rodent IgE, resulting in a considerable reduction in receptor interaction (50% decrease (vs. WT p=0.0007), Table I) (11). We interpret the effect of this substitution as identifying K352 as a class specific
effector residue contributing directly to the binding of hIgE to CD23, possibly facilitating docking via an electrostatic interaction. The elimination of this interaction appeared to account, at least in part, for the observed loss of CD23 binding associated with the R16 and R8 variants, which contained this substitution. Of all the A-B loop variants generated within this study the most prominent change in the IgE-CD23 interaction was observed for the K352G substitution. Point mutations involving D347N, D347E, L348I or R351K had no significant effect on the IgE-CD23 interaction (Table I).

Mutation of P345 to A resulted in an apparent increase in CD23 binding compared to the wild type molecule, although statistical analysis shows this is of marginal significance (12.5% increase, p = 0.09, Table I). The molecular basis of this observation is currently uncertain, but could be due to a potential role of P345 in the restriction of structure and maintenance of the rigid conformation of the A-B loop. An increase in loop flexibility may account for the apparent enhancement of the docking process of hIgE and CD23, while at the same time suggesting that P345 residue is not critical for the overall maintenance of the A-B loop CD23 binding conformation.

To substantiate the identification of K352 within the AB loop as a key effector residue involved in the IgE/CD23 interaction, further point mutations were generated (Fig. 2B). Conservative substitution of K352 with R resulted in a small, but statistically significant, increase in the CD23 receptor interaction (14% increase, p = 0.0006). Substitution with the negatively charged E residue effected the complete loss of CD23 receptor interaction (98% decrease (vs. WT p=0.0019), not significantly different to background, p=0.61, Table I).

These results substantiate a role for residue K352 in the interaction of hIgE with CD23 and suggest the possibility of an electrostatic interaction between K352 of IgE and the receptor. Mutation of K352 to R or E maintained the FcεRII interaction, and a binding level similar to the wild type molecule was observed (Table I).

Variant R16/K352 (K352 of hIgE restored within the R16 variant) was generated to evaluate the contribution of residue 352 to the loss of interaction seen with replacement of the entire AB loop. This variant showed a limited, but significant degree of restoration of CD23 recognition (binding level 39% (vs. WT p=0.043), significantly different to background p=0.025, Table I). The specificity of the interaction was confirmed by the finding that this interaction could be inhibited using anti CD23 mAb (MHM6) (data not shown) which is known to
inhibit hIgE/CD23 binding. As observed for the R16 variant, the FcεRI binding levels for the R16/K352 were similar to WT binding, supporting a localized effect of mutagenesis (Table I, Fig. 2).

To evaluate whether substitution with homologous rat A-B residues conferred the ability to interact with the rodent receptor, IgE variants were screened by flow cytometry analysis using CHO cells transfected with mouse CD23 (Fc1.7 cells). Neither the R16 chimaera or hIgE-G352 variants conferred binding to rodent CD23, however the R8 variant was found to have an increased interaction with Fc1.7 cells (20% compared to WT mIgE, (Fig. 3)). While these data indicated that the R8 variant was able to interact with rodent CD23, further analysis showed that this interaction was observed with parental CHO cells (Fig. 3) indicating that this interaction was not specific to CD23.

Insert Figure 3 here

Two additional variants were generated to assess the effect of N371 linked glycosylation on the IgE-CD23 interaction (Fig 2C). T371 and Q371 aglycosylated variants both demonstrated a modest decrease in CD23 binding (84%, p=0.024, and 88%, p=0.15 respectively, Table I) indicating that enhanced binding reported for deglycosylated IgE cannot be attributed to a lack of glycosylation at this residue.
Discussion

In the present study, we confirm the role of the Cε3 A-B loop of human IgE in the IgE-CD23 interaction and identify K352 as an important determinant in CD23 receptor recognition, although the scope of flow cytometry is limited due to the fact that it does not yield kinetic data for the interaction studied. These data are in agreement with our previous study (18) and the study of Nissim and co-workers, who replaced residues within the N-terminal of the hIgE Cε3 domain with the homologous mouse residues (16). Replacement of residues 330-346 (variant C3BX) maintained the interaction, however replacement of residues 330-356 (variant C3HD) dramatically reduced activity suggesting a role for this region in the CD23 interaction (16). It is unlikely that the substitutions generated within the R16 variant have affected the overall Cε3 domain conformation with concomitant loss of CD23 recognition, since FcεRI interaction, which is dependent on residues in hCε3, was fully maintained in this study (Table I, Fig. 2) and in a previous study which used surface plasmon resonance to assess the interaction of IgE variants with soluble truncated FcεRIα (19).

A significant role for the A-B loop in IgE function has previously been proposed by Wurzburg et al (26). Based on crystal structures of IgE Fc fragments, Wurzburg and colleagues describe how interactions of A-B loop residues facilitate transition between an open conformation when bound to FcεRI, and a closed conformation when uncomplexed. Wurzburg proposes that this conformational flexibility may allow IgE to form optimal interactions with both FcεRI and CD23.

It could be argued that substitution with homologous rat residues in the R16 and R8 constructs has effectively constricted domain movement and locked the molecule in a non binding conformation with respect to CD23. However maintenance of normal FcεRI interactions would argue in favour of a functionally active molecule. In addition, the absence of noteworthy contacts made by K352 within the available IgE structures, and the considerable loss of receptor interaction observed with the G352 variant point to direct involvement in the interaction with CD23. We propose therefore that residues within CD23 bind directly to K352 in hIgE, probably involving electrostatic interaction, and concomitant conformational changes to provide optimal docking.

The IgE-CD23 interaction has been proposed to have a 2:1 binding stoichiometry, with two lectin heads of the trimeric receptor interacting with the two ε chains of an individual IgE molecule (27), and dimerisation of the hIgE molecule is required (15). Dual kinetics have been reported for IgE binding to CD23 (24). The most straightforward explanation for this phenomenon in the context of this model is that one lectin head would initially
dock with low affinity, providing the basis for a second interaction of higher affinity. This could involve two sets of distinct interactions, possibly involving different points of contact in the IgE molecule and/or within the receptor. The IgE binding site on CD23 has been mapped to two discontinuous segments between residues 165-190 and 224-256 (10). Our observation demonstrating that mutation of a single residue has a profound effect on receptor recognition suggests that K352 makes a definitive contribution, either by establishing an initial point in the docking with CD23 and/or by contributing to the stability of the interaction. While these data strongly suggest a dominant role for K352 (e.g. mutation to E resulted in an equivalent loss of CD23 binding as observed for the replacement of the entire AB loop in the R16 and R8 variants), we cannot exclude the contribution of other residues in the AB loop including residues at position 346, 349 and 353, which were not evaluated directly in the current study.

Abrogation of the higher affinity interaction using an anti-CD23 stalk region antibody has been shown to result in cells that appear negative for IgE binding as determined by flow cytometry, while low affinity binding is still detectable using 125I-labelled IgE (28). The use of flow cytometry to assess IgE-CD23 interaction does not allow us to conclude whether loss of binding observed with mutants in the present study results from abrogation of one or both of these interactions.

The localisation of N371 on the same face as K352 and our preliminary data regarding interaction of mutants inhibiting glycosylation, led us to propose a role of glycosylation at N371 in the inhibition of hIgE-CD23 interaction. Our present study shows however that N371 glycosylation is not involved in this process. Others have suggested that glycosylation of the Cε2 domain accounts for this effect. Deglycosylation of IgE has been shown to expose epitopes on the Cε2 domain (29) and a role for N265 has been postulated (30) to which our results would lend some tentative support.

At present, limited structural information is available concerning the interaction of hIgE and CD23. In the absence of high-resolution structural data, we identify key regions of the hIgE molecule involved in CD23 recognition, which provide new insights into the structural nature of this interaction. The A-B loop in Cε3 was identified as a critical region for the interaction, with K352 as a class specific residue. Acquiring information concerning the structure of complementary binding sites on IgE and its receptors holds potential for the rational design of drugs, which could modulate the allergic response.
References


Footnotes

This work was supported by grants from the BBSRC, Medical Research Council and The Wellcome Trust.
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Figure Legends

Figure 1. The Position of Cε3 A-B loop residues within IgE-Fc. Upper panel - Solid 3D rendering of IgE Cε3 and 4 in open conformation (2). Cε4 domains are shown a shade darker than Cε3 domains. A-B loop residues within Cε3 are highlighted close to the Cε4. Lower panel - Arrangement of A-B loop residues within the closed conformation crystal structure (26). Other residues of the Cε3 and 4 domains are in solid rendering with Cε4 in darker grey. Residues 341 – 356 were targeted by mutagenesis. K352, an exposed residue, was identified as a key effector residue of the human IgE-CD23 interaction.

Figure 2. Interactions of Human IgE Variants with Human CD23 and FcεRI. Cells bearing CD23 (RPMI-8866 cells) and cells bearing FcεRIα (transfected RBLs) were harvested and washed three times in cold wash buffer (PBS with 0.1% NaN3, 1% FCS), then resuspended in the same at 5x10^6/ml. 100μl cell suspension was added to 20μl IgE (0.125 – 5μg) incubated on ice for 30mins, then washed (2 x 1ml wash buffer). Cells were incubated with 100μl biotinylated anti-IgE antibody (1/400) for a further 30 mins, washed as before, then finally incubated for 30 mins with 100μl streptavidin R-phycoerythrin (1/25 dilution) and washed again. Cells were resuspended in 200μl wash buffer and analysed by flow cytometry. For a positive control, chimaeric human IgE (Serotec) was used and the background fluorescence determined in the absence of IgE and using mouse IgE (SPE7) on RPMI-8866 cells or parental RBLs. Median channel numbers were standardised relative to the WT IgE control (100%) and show the mean of three separate determinations (+/- SEM).

Figure 3. Interactions of Human IgE variants With Rodent CD23. CHO cells bearing mouse CD23 (Fc1.7 cells) were harvested and washed three times in cold wash buffer (PBS with 0.1% NaN3, 1% FCS), then resuspended in the same at 5x10^6/ml. 100μl cell suspension was added to 20μl IgE (0.125 – 5 μg) incubated on ice for 30 mins then washed (2 x 1ml wash buffer). Cells were incubated with 100μl biotinylated anti-lambda antibody (1/400) for a further 30 mins, washed as before, then finally incubated for 30 mins with 100μl streptavidin R-phycoerythrin (1/25 dilution) and washed again. Cells were resuspended in 200μl wash buffer and analysed by flow cytometry. For a positive control, mouse IgE (SPE7) was used and the background fluorescence determined in the absence of IgE. Control experiments using parental CHO cells are also shown. Median channel numbers were standardised relative to the positive control (100%) and show the mean of three separate determinations (+/-SEM).
Tables

Table I. Summary of IgE-CD23 interaction data.
IgE variants generated along with the rationale for construction are listed together with an evaluation of effect of receptor interaction. IgE binding was compared to WT using median channel numbers at 2.5 µg/assay and expressed as a percentage of the WT binding (designated 100%). The median channel numbers from three independent experiments were compared by t-test. P values < 0.05 were considered significant *. FcεRI interactions for some variants were not determined by flow cytometry (marked n.d.) but have previously been shown to be as wild type (19).
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Table I

<table>
<thead>
<tr>
<th>hIgE variant</th>
<th>Rationale for mutation</th>
<th>Receptor Interaction Compared to WT (%) ± SEM</th>
<th>Significance (t-test, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce3 AB Loop</td>
<td></td>
<td>FcεRI CD23 FcεRI CD23</td>
<td></td>
</tr>
<tr>
<td>R16</td>
<td>Homologous rat (aa341-356) grafted into the human Fc region to replace loop AB. Is loop AB a critical determinant for the CD23 interaction?</td>
<td>88.7 ± 4.6 3.0 ± 1.8 0.13 0.02*</td>
<td></td>
</tr>
<tr>
<td>R8</td>
<td>Homologous rat (aa346-353) grafted into the human Fc region to replace loop AB. Limited substitutions based upon crystal structure.</td>
<td>91.2 ± 1.6 3.2 ± 1.2 0.03* 0.02*</td>
<td></td>
</tr>
<tr>
<td>P-A345</td>
<td>P345 is conserved in human, mouse and rat IgE. Mutation to A is computed to remove fixed bend while maintaining the hydrophobicity and size of the residue.</td>
<td>n.d. 118 ± 2.4 - 0.09</td>
<td></td>
</tr>
<tr>
<td>D-N347</td>
<td>D347 is conserved in human, mouse and rat IgE. Mutation to N maintains the size of the amino acid side chain but alters the charge.</td>
<td>n.d. 101.3 ± 2.9 - 0.79</td>
<td></td>
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<tr>
<td>D-E347</td>
<td>Mutation to E maintains the charge of the residue but increases the length of the side chain.</td>
<td>n.d. 111.5 ± 2.9 - 0.14</td>
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<tr>
<td>L-I348</td>
<td>L348 is conserved in human, mouse and rat IgE. Mutation to I is highly conservative, changing only the position of a methyl group on the side chain.</td>
<td>n.d. 92.0 ± 4.6 - 0.27</td>
<td></td>
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<tr>
<td>R-K351</td>
<td>R351 is not conserved in mouse and rat IgE. The rodent homologue is N. Mutation to K maintains the positive charge of the residue while decreasing the length of the side chain.</td>
<td>n.d. 105.7 ± 2.4 - 0.28</td>
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<tr>
<td>K-G352</td>
<td>K352 is not conserved, the rodent homologue is Gly.</td>
<td>96.5 ± 2.7 50.0 ± 4.53 0.32 0.0007*</td>
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<tr>
<td>K-R352</td>
<td>Conservative substitution to maintain charge.</td>
<td>95.1 ± 1.2 114.6 ± 0.5 0.06 0.0006*</td>
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<tr>
<td>K-E352</td>
<td>Non-conservative substitution alters charge and size of residue.</td>
<td>94.7 ± 5.9 2.4 ± 6.0 0.55 0.0019*</td>
<td></td>
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<tr>
<td>R16/K352</td>
<td>Human residue restored within the R16 variant.</td>
<td>105 ± 1.6 38.6 ± 8.1 0.09 0.043*</td>
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<td>N371 Glycosylation Site</td>
<td></td>
<td>FcεRI CD23 FcεRI CD23</td>
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<tr>
<td>N-T371</td>
<td>N371 is conserved in human and mouse IgE, the rat homologue is T. Mutation to T may change the glycosylation from type N to O or inhibit glycosylation</td>
<td>98.7 ± 1.5 84.2 ± 2.7 0.48 0.024*</td>
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<td>N-Q371</td>
<td>Mutation to Q will inhibit glycosylation.</td>
<td>96.1 ± 3.8 87.7 ± 5.3 0.37 0.15</td>
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Figure 1
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Figure 2

A

CD23 Interaction

FcεRI Interaction

Fluorescence (arbitrary units)

0 2 4 6

IgE (µg/assay)

WT □ G352 ▲ R16 ■ R8 ● background

B

CD23 Interaction

FcεRI Interaction

Fluorescence (arbitrary units)

0 2 4 6

IgE (µg/assay)

WT □ E352 ▲ R16/K352 ■ R352 ● background

C

CD23 Interaction

FcεRI Interaction

Fluorescence (arbitrary units)

0 2 4 6

IgE (µg/assay)

WT □ T371 ▲ Q371 ● background
Figure 3

Rodent CD23 Interaction

Parental CHO Controls

Fluorescence (arbitrary units)

IgE (µg/assay)

mIgE hIgE-WT R16 R8 G352 background