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THE FIRST TRANSMEMBRANE REGION OF THE BETA CHAIN STABILIZES THE TETRAMERIC FcεRI COMPLEX

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Abstract

The family of activating immune receptors stabilizes via the 3-helix assembly principle. A charged basic transmembrane residue interacts with two charged acidic transmembrane residues and forms a 3-helix interface to stabilize receptor complexes in the lipid bilayer. One family member, the high affinity receptor for IgE, FcεRI, is a key regulator of immediate allergic responses. Tetrameric FcεRI consists of the IgE-binding α-chain, the multimembrane spanning β-chain and a dimer of the γ-subunit (FcεRγ). Comparative analysis of these seven transmembrane regions indicates that FcεRI does not meet the charge requirements for the 3-helix assembly mechanism. We performed alanine mutagenesis to show that the only basic amino acid in the transmembrane regions, βK97, is not involved in FcεRI stabilization or surface up-regulation, a hallmark function of the β-chain. Even a βK97E mutant is functional despite four negatively charged acidic amino acids in the transmembrane regions. Using truncation mutants, we demonstrate that the first uncharged transmembrane domain of the β-chain contains the interface for receptor stabilization. *In vitro* translation experiments depict the first transmembrane region as the internal signal peptide of the β-chain. We also show that this β-chain domain can function as a cleavable signal peptide when used as a leader peptide for a Type I protein. Our results provide evidence that tetrameric FcεRI does not assemble according to the 3-helix assembly principle. We conclude that receptors formed with multispinning proteins use different mechanisms of shielding transmembrane charged amino acids.

Keywords

allergy; IgE; complex formation; multimeric immune recognition receptors; SNP

1. Introduction

FcεRI is an activating immune receptor of the immunoglobulin superfamily, like the T cell receptor, the B cell receptor and other Fc receptors, such as CD64 or CD89 (Call et al., 2002; Call and Wucherpfennig, 2004; Call and Wucherpfennig, 2007; Sigalov, 2005). Along with the IgE-binding α-subunit and a dimer of the signal-transducing γ-subunit (FcRγ), the β-chain

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forms the tetrameric isoform of the high affinity receptor for IgE, FcεRI. This tetrameric FcεRI (αβγ₂) complex is expressed by mast cells and basophils and is a key regulator of immediate allergic responses (Gould and Sutton, 2008; Kraft and Kinet, 2007). According to the literature, the β-chain provides two amplifier functions for the FcεRI receptor. The mere presence of the β-chain enhances surface expression of tetrameric FcεRI when compared to the trimeric receptor isoform (αγ₂) (Donnadieu et al., 2000b). In addition to increasing surface FcεRI expression, a C-terminal ITAM amplifies signals of the FcRγ dimer during receptor activation (Dombrowicz et al., 1998; Lin et al., 1996). The mechanism of increased surface display of αβγ₂ complexes remains poorly understood, but is important because allergic patients upregulate FcεRI in correlation with the severity of allergic symptoms (Kinet, 1999; Kraft and Kinet, 2007).

Among other structural features, activating immune receptors are thought to share a common basis for receptor assembly, referred to as the 3-helix assembly principle (Call and Wuchterpfennig, 2005; Call and Wuchterpfennig, 2007). This assembly mechanism proposes that one charged basic transmembrane residue in the ligand-binding subunit interacts with two charged acidic transmembrane residues in the signaling dimer, thereby forming a 3-helix interface, which allows for stable integration of the receptor complex into the lipid bilayer (Call et al., 2002; Call and Wuchterpfennig, 2004; Feng et al., 2005). However, analysis of the transmembrane regions of the α, β and γ chains shows that tetrameric FcεRI contains more acidic amino acids than the single basic amino acid K97 in the β-chain can shield. Thus, the unbalanced charges in the seven transmembrane regions of tetrameric FcεRI argue against the stabilization and assembly of this complex via the established 3-helix intramembrane assembly mechanism. This hypothesis is supported by studies that describe that the uncharged leucine in position 21 of the transmembrane region of the γ-chain is critical for stabilization of FcεRI (Hida et al., 2009; Wines et al., 2006).

The β-chain is a multipass transmembrane protein with four predicted membrane-spanning domains and both N- and C-termini facing the cytosol (Kinet et al., 1988). The presence of a C-terminal immunoreceptor tyrosine-based activation motif (ITAM) excludes this protein from the tetraspanin family (Hemler, 2005; Levy and Shoham, 2005a). Several single nucleotide polymorphisms (SNPs) in the β-chain have been linked to allergy (Hill and Cookson, 1996; Kraft et al., 2004; Shirakawa et al., 1994). So far studies on the consequences of these C-terminal SNPs on FcεRI function and regulation revealed no effects on the two amplifier functions of the β-chain (Donnadieu et al., 2000a). Recently, other transcriptional control mechanisms were proposed in the context of these SNPs (Nishiyama et al., 2004). Our understanding of how β-chain polymorphisms regulate the function of tetrameric FcεRI *in vivo* is however very limited and awaits further analysis.

A unique feature of FcεRI is that this receptor complex requires cotranslational assembly of its subunits in the ER, while other receptors like the T cell receptor can assemble in several consecutive steps (Call et al., 2002). The formation of stable IgE-binding FcεRI tetramers is a crucial quality control step in the pathway leading to cell surface expression of FcεRI (Fiebiger et al., 2005). Proper ER assembly is required to overcome the multicomponent intracellular retention signal in the α-chain of FcεRI (Cauvi et al., 2006). It has been proposed that the β-chain controls receptor assembly, while IgE regulates receptor degradation via trapping properly formed complexes at the cell surface (Bruhns et al., 2005). Accumulating evidence suggests that, in general, transmembrane domains are sufficient to mediate assembly and that ionizable transmembrane residues are critical interaction sites (Call and Wuchterpfennig, 2005; Call and Wuchterpfennig, 2007; Gosse et al., 2005), although some evidence also shows the importance of membrane proximal domains (Xu et al., 2006; Xu et al., 2008). Along the same line, one can argue that the charged amino acids that form the interaction sites in the transmembrane regions in FcεRI ultimately control receptor assembly and expression.

Given the importance of the β -chain in amplifying receptor surface expression as well as receptor signaling, an understanding of these transmembrane interaction sites could provide a new target for the design of interference strategies to modulate Fc ϵ RI expression and function in allergic patients. Therefore we studied the transmembrane assembly requirements of the β -chain with its other partners, $\alpha\gamma_2$, to form tetrameric Fc ϵ RI. We generated several mutant β -chains and analyzed their ability to form tetrameric complexes with the Fc ϵ RI α -chain and common γ -chain dimer. Here we show that the basic charged residue, lysine 97, in the second transmembrane region of the β -chain is not involved in stabilization of tetrameric Fc ϵ RI. In fact, the first transmembrane region of the β -chain contains the interface for stabilization of the tetrameric complex despite the absence of charged residues. The first transmembrane region also acts as the intrinsic signal peptide for this multispinning membrane protein.

2. Materials and methods

2.1. Antibodies

Anti-Fc ϵ RI α mAb 19-1 was kindly provided by Dr. J.-P. Kinet (Laboratory of Allergy and Immunology, Beth Israel Deaconess Medical Center, Boston, MA) and used as published (Maurer et al., 1994). PE-conjugated anti-Fc ϵ RI α mAb CRA1 and mIgG2b isotype control mAb were purchased from eBioscience. Anti-Fc ϵ RI γ polyclonal serum was purchased from Millipore and peroxidase-conjugated anti-HA (3F10) was from Roche. Anti-actin mAb AC-15 and peroxidase-conjugated goat-anti-mouse IgG and goat-anti-rabbit IgG were from Sigma.

2.2. Plasmids and cDNA modifications

Plasmids—cDNAs encoding the Fc ϵ RI α -, β -, and γ -chains have been previously published (Fiebiger et al., 2005). All of the following β -chain constructs contain an N-terminal HA tag and were subcloned into pcDNA3.1 and pIRES2-EGFP expression vectors and sequence verified (Sequegen).

Site-directed mutagenesis—Fc ϵ RI β -chain point mutants, β K97A and β K97E, were generated using the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's guidelines.

Fc ϵ RI β -chain truncations—A series of β -chain truncations containing transmembrane (TM) domains 1, 1-2, or 1-3 were generated by introduction of a stop mutation following each TM region using PCR cloning. Truncations contained the first 89, 126 or 165 amino acids and were designated β 1TM, β 2TM, and β 3TM, respectively. An additional β -chain mutant lacking the first TM domain but containing TM domains 2-4, denoted β 2-4TM, was generated by PCR cloning. β 2-4TM begins at residue 86.

Fc ϵ RI β 1TM- α -chain fusion protein—The signal peptide of the Fc ϵ RI α -chain was replaced with the first TM domain of the β -chain resulting in a fusion protein of amino acids β 1-89 and α 25-257. This protein was generated with a 2-step PCR cloning strategy according to the literature (Horton et al., 1993) and denoted β 1TM- α .

2.3. Generation of stable cell lines and transient transfections

293 and HeLa cells were maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 2 mM L-glutamine. Stable expression of human Fc ϵ RI α - and γ -chains was achieved with the Retro-X Q retroviral vector transduction system (Clontech). DNA encoding human Fc ϵ RI α - and γ -chains was subcloned into the pQCXIH and pQCXIP retroviral vectors, respectively. Following the manufacturer's protocol, FuGENE 6 (Roche) was used to transiently transfect α -chain DNA into the Phoenix Ampho 293T-derived packaging cell line (provided by the Nolan Laboratory, Stanford University School of

Medicine, Stanford, CA). Cell-free viral supernatant was harvested at 48 h post transfection and used to infect parental 293 and HeLa cells in the presence of 5 µg/ml polybrene (VWR) to enhance infection efficiency. Empty control vector transfections (ø293 and øHeLa cells) were carried out in parallel throughout all retroviral transduction protocols. Selection resistant (hygromycin 1.0 mg/ml) clones were screened by flow cytometry for intracellular α -chain expression using mAb CRA1. The retroviral transduction protocol was repeated with stable α 293 and α HeLa cells using γ -chain DNA under expression of retroviral vector pQCXIP. Stable γ -chain lines were selected on the basis of puromycin resistance (0.5 µg/ml). Stable double transfectants ($\alpha\gamma$ 293 and $\alpha\gamma$ HeLa cells) were confirmed by analysis of cell surface expression of Fc ϵ RI by flow cytometry.

Transient transfections—Lipofectamine 2000 (Invitrogen) was used to transiently transfect wild type and mutant β -chains into $\alpha\gamma$ HeLa, øHeLa, γ 293 or ø293 cells following the manufacturer's protocol. Cells were harvested by trypsinization at 24 or 48 h post transfection. Where indicated, the proteasome inhibitors lactacystin (10 µM, Sigma) or ZL₃VS (20 µM, generously provided by Dr. H.L. Ploegh) were added to the culture medium for 2-4 h (Fiebiger et al., 2002). DMSO was used as solvent control.

2.4. Immunoprecipitation and immunoblotting

Cells were solubilized in lysis buffer (0.5% Brij 96, 20 mM Tris, pH 8.2, 20 mM NaCl, 2 mM EDTA, 0.1% NaN₃) containing protease inhibitors (Complete, Roche) for 30 min on ice. Immunoprecipitation was performed with 3 µg of anti-Fc ϵ RI α mAb 19-1 as previously described (Fiebiger et al., 2005). Beads were eluted in non-reducing Laemmli sample buffer and samples were run on 12% non-reducing SDS-PAGE gels, transferred to PVDF membrane (Pierce) and probed with anti-Fc ϵ RI α (CRA1) followed by peroxidase-conjugated goat-anti-mouse IgG for detection of precipitated α -chain. Reducing gels were run for detection of co-precipitated β - and γ -chains with either peroxidase-conjugated anti-HA (3F10, Roche) or anti-Fc ϵ RI γ followed by peroxidase-conjugated goat-anti-rabbit IgG. Peroxidase was detected using SuperSignal chemiluminescent substrate reagents (Pierce).

2.5. FACS analysis

Cells were stained with either PE-conjugated anti-Fc ϵ RI α (CRA1) or isotype control mAb (1.0 µg/10⁶ cells) and analyzed on a FACScan flow cytometer (Becton Dickinson) using CellQuest software for acquisition. For staining of intracellular Fc ϵ RI α , cells were fixed and permeabilized using Caltag Fix & Perm reagents (CALTAG Laboratories) prior to staining. Analysis of CRA1 surface expression on EGFP-gated cells was carried out with either WinMDI 2.9 (Scripps Research Institute) or CellQuest Pro 5.2.1 (Becton Dickinson) software.

2.6. In vitro transcription and translation

Both methods were performed as previously described (Fiebiger et al., 2005). In brief, β -chain cDNA was linearized with the indicated restriction enzymes and *in vitro* transcriptions were performed using T7 polymerase (Promega). RNA was capped as previously described (Bijlmakers et al., 1994; Huppa and Ploegh, 1997) and stored as alcohol precipitations at -80° C. RNA was decapped prior to translation. Optimal RNA amounts were determined empirically for each construct and each stock of RNA. Optimal *in vitro* translation reaction time was determined empirically as 1 h. Reticulocyte lysate was purchased from Promega. Microsomes were prepared from astrocytoma cells and pelleted after *in vitro* translations for further analysis as previously described (Fiebiger et al., 2005; Furman et al., 2002).

2.7. Data analysis

FcεRIα surface expression was represented as the mean fluorescence intensity (MFI) ± the standard deviation of triplicate values. Empty vector control transfectants were compared to either wild type or mutant β-chain transfectants using the Student's *t* test. Statistical significance was defined at $p < 0.05$.

3. Results

3.1. The lysine residue 97 in the second transmembrane region of the β-chain is not required for formation of FcεRI tetramers

It has previously been shown that basic charged residues shield acidic amino acids in transmembrane regions of activating immune receptors and allow proper stabilization of receptor complexes. Comparison of the seven transmembrane domains of FcεRI αβγ₂ (Fig. 1) shows that this receptor does not contain balanced charges that would allow for complex stabilization according to the 3-helix assembly principle (Call and Wucherpfennig, 2004; Call and Wucherpfennig, 2005; Call and Wucherpfennig, 2007). We defined the lysine at position 97 in the second transmembrane domain of the β-chain (βK97) as the only candidate for shielding the negatively charged aspartic residues in the α and γ-chains. βK97 was modified by site-directed mutagenesis to examine whether mutated β-chains can still form tetrameric complexes. Lysine 97 was replaced with an alanine to change charge and polarity (βK97A). In addition, we replaced lysine 97 with glutamic acid to reverse charge while maintaining polarity (βK97E). Transient expression of βK97A and βK97E in αγHeLa cells was comparable to that of wild type β-chain (Fig. 2A). Immunoprecipitation of FcεRI complexes was carried out using mAb 19-1 which recognizes the properly folded mature form of FcεRIα. Immunoprecipitation was performed following transient expression of wild type β, βK97A or βK97E in αγHeLa cells (Fig. 2B). Trimeric FcεRI was precipitated from αγHeLa cells transfected with empty vector as a control. Like wild type β, both βK97 mutants were able to form stable FcεRI tetramers, as demonstrated by co-precipitation of the mutant β-chains with the FcεRI α- and γ-chains (Fig. 2B). These results demonstrate the stability of FcεRI tetrameric complexes in the absence of βK97.

3.2. Upregulation of FcεRI surface expression does not depend on βK97

The next question we wanted to address was whether the βK97 mutants would be able to function like the wild type protein. One of the amplification functions of the β-chain is its ability to enhance FcεRI surface expression (Donnadieu et al., 2000b). We examined the ability of βK97A and βK97E to enhance cell surface expression of FcεRI. αγHeLa cells were transiently transfected with wild type β, both βK97 mutants and empty control vector. Use of pIRES2-EGFP expression vectors allowed analysis of transfected cells by the selection of only those cells co-expressing EGFP. Compared to control vector transfectants, which express trimeric FcεRI at the cell surface (MFI 65.23 ± 6.14), transfection with wild type β significantly increased expression of FcεRI (MFI 97.43 ± 9.47 ; $p = 0.008$, $n = 3$; Fig. 2C). This increase is in agreement with published findings using a similar transient transfection system (Donnadieu et al., 2000b). We next compared the increase in FcεRI surface expression induced by wild type β with that of βK97A and βK97E. No statistically significant differences in FcεRI surface expression levels were detected between these three β constructs (β MFI 97.43 ± 9.47 ; βK97A MFI 101.2 ± 7.34 ; βK97E MFI 90.17 ± 9.03 ; $n = 3$, Fig. 2C). These results show that the ability of the β-chain to upregulate FcεRI surface expression is independent of βK97. This set of data is in line with our results that βK97 is not involved in the stabilization of the tetrameric complex as shown by co-immunoprecipitation

3.3. The first transmembrane region of the β -chain is sufficient for the stabilization of Fc ϵ RI tetramers

We next wanted to define the transmembrane domain(s) involved in the formation of tetrameric Fc ϵ RI. A series of C-terminal β -chain truncations were generated. These mutants contain transmembrane regions 1 to 3, 1 to 2, or the first transmembrane region only. We refer to these constructs as β 3TM, β 2TM, and β 1TM, respectively (scheme in Fig. 3A). Expression of the β -chain truncations was detectable in cell lysates following 24 h transient transfection and 2 or 4 h treatment with the proteasome inhibitor ZL₃VS (Fig. 3B). Moderate to low levels of truncated β proteins were detectable in the absence of proteasome inhibition. Inhibition of proteasomal degradation showed a substantial increase in the amount of detectable protein (Fig. 3B). This shows that these modified proteins are significantly less stable than the wild type protein, a feature fairly common for truncation mutants (Brodsky, 2007; Todd et al., 2008; Vembar and Brodsky, 2008). We next examined the ability of the β -chain truncations to form stable Fc ϵ RI tetramers. Immunoprecipitation was carried out following transient transfection with wild type or truncated β -chains after treatment of the culture with ZL₃VS for 2 h. mAb 19-1 was used to pull down only properly folded complexes. Trimeric Fc ϵ RI complexes were precipitated from α γ HeLa cells transfected with control vector (Fig 3C, lanes 3, 6 and 9). Like the wild type β -chain (Fig 3C, lanes 2, 5 and 8), β 3TM, β 2TM, and β 1TM (Fig 3C, lanes 1, 4 and 7, respectively) were able to form stable tetrameric complexes, as demonstrated by the presence of co-precipitated β - and γ -chains. Thus a β -chain construct that contains only the first of the four TM regions is sufficient for the formation of stable Fc ϵ RI complexes. This finding suggests that the first transmembrane region of the β -chain is the main interface for stabilization of the tetrameric receptor.

3.4. The first transmembrane region of the β -chain is required for Fc ϵ RI stabilization

Our co-immunoprecipitation experiments show that β 1TM associates with the α - and γ -chains and forms stable Fc ϵ RI complexes. We next asked whether the presence of the first TM region of the β -chain was a requirement for complex formation. An N-terminally truncated β -chain that lacks the first transmembrane domain, but contains transmembrane regions 2-4 was generated and expressed in α γ HeLa cells (β 2-4TM, Fig. 4A and B). Using the same immunoprecipitation strategy, we analyzed whether β 2-4TM could form a stable complex with α γ 2. As opposed to wild type β , β 2-4TM did not co-precipitate with the Fc ϵ RI complex (Fig. 4C). We can conclude that the first transmembrane region of the β -chain is not only sufficient, but also required for the formation of Fc ϵ RI tetramers.

3.5. The first TM region of the β -chain contains an ER insertion signal

Tetrameric Fc ϵ RI forms cotranslationally (Fiebiger et al., 2005). Thus, we hypothesized that the interface for receptor stabilization might concur with the sequence of the β -chain that regulates ER insertion. We used *in vitro* translation as a method to address whether the first transmembrane region of the β -chain contains a signal sequence for ER insertion. Linearization of β -chain cDNA was performed with *Rsa*I to generate a truncated cDNA at position 245 for transcription and consecutive translation. This truncated protein contains the first transmembrane region of the β -chain. Microsomal fractions from astrocytoma cells were used as membrane-supplements to mimic the ER environment for translation supported by rabbit reticulocyte lysates. With this cDNA, a 9 kD β -chain truncation after the serine residue in position 81 was translated in the presence (+) or absence (−) of microsomes (β 81; data not shown and Fig. 5A). For comparison, the wild type version of the β -chain was generated by *in vitro* transcription/translation after linearization of the cDNA with *Eco*RI (Fig. 5A, lane 3). When analyzing the microsomal fraction of the β 81 *in vitro* translation, we found that the β 81 fragment inserted into the microsomal transmembrane equivalent like the 30kD wild type β -chain, (Fig. 5A, lanes 1 and 3). A small amount of wild type β -chain was found in β 81

translations, likely due to incomplete linearization of the cDNA by *RsaI*. This experiment shows that the first transmembrane region of the β -chain contains the ER insertion signal for this multispanning transmembrane protein.

If the first transmembrane region of the β -chain is an actual signal peptide, this protein stretch should serve as a leader peptide for ER insertion of a type I membrane protein (Higy et al., 2005; Higy et al., 2004; Wickner and Lodish, 1985). To test this idea, we exchanged the endogenous signal peptide of the Fc ϵ RI α -chain with the first 89 amino acids of the Fc ϵ RI β -chain, which contain the N-terminal cytosolic tail and first transmembrane domain. This fusion protein of residues 1-89 of the β -chain and residues 25-257 of α was referred to as β 1TM- α . Transient expression of β 1TM- α in γ 293 cells resulted in cell surface expression of Fc ϵ RI, as demonstrated by surface staining for Fc ϵ RI α (Fig. 5B, upper left panel). In the absence of the γ -chain (ϕ 293), the α -chain did not reach the cell surface (Fig. 5B, upper right panel). Intracellular staining for Fc ϵ RI α confirmed robust expression of the α -chain in both γ 293 cells and ϕ 293 following transient expression of β 1TM- α (Fig. 5B, lower panel). Only α -chain that properly inserted into the ER can form a complex with the γ -chain, which is in turn required for surface expression of the receptor. Therefore, our results demonstrate that the first transmembrane domain of the Fc ϵ RI β -chain is a bona fide signal peptide.

4. Discussion

Our study shows that proper stabilization and surface transport of tetrameric Fc ϵ RI is not dependent on the balancing of charged transmembrane amino acids according to the 3-helix assembly principle. We demonstrate that the first transmembrane domain of the β -chain, which does not contain any charged amino acids, is sufficient and necessary for formation of the tetrameric complex. As this isoform of Fc ϵ RI can assemble properly despite three unbalanced negatively charged aspartic acids in its seven transmembrane regions, it is fair to conclude that the 3-helix assembly principle is not applicable for this activating immune receptor. This conclusion is further supported by earlier studies that show that the uncharged leucine 21 in the FcR common γ -chain is critical for Fc ϵ RI stabilization. One explanation for our findings might derive from the fact that the 3-helix assembly principle was established for receptors that are formed with single membrane spanning proteins of the Type I or Type II family (Call et al., 2002; Call and Wucherpfennig, 2004; Call and Wucherpfennig, 2005; Call and Wucherpfennig, 2007). In contrast, four of the seven transmembrane regions of Fc ϵ RI come from a multipass transmembrane protein (Higy et al., 2004; Wickner and Lodish, 1985), the β -chain. Depending on the topology of the proteins that form the receptor complex, other mechanisms to balance charged amino acids in transmembrane regions might apply.

Our hypothesis that additional mechanisms for the stabilization of receptor complexes must exist is supported by the fact that Fc ϵ RI can also exist as a trimeric isoform in absence of the β -chain, despite the three charged aspartic acids in its three transmembrane regions (Fig. 1). *In vitro* transcription-translation experiments show that the trimeric receptor stabilizes co-translationally when only these two receptor subunits are translated (Fiebiger et al., 2005). Detergent solubilization studies also argue for the existence of a trimeric isoform of human Fc ϵ RI (Kinet, 1999; Ravetch, 1997; Ravetch and Kinet, 1991). The fact that both of the proteins that form the trimeric Fc ϵ RI are Type I membrane proteins argues against the hypothesis that multispanning proteins allow for receptor stabilization by shielding transmembrane charged amino acids. Alternatively, one might reason that a trimeric Fc ϵ RI does not exist *in vivo*. Several reports have shown that members of the tetraspanin family associate with Fc ϵ RI and other Fc receptors and modulate their function (Fleming et al., 1997; Kraft et al., 2005; Moseley, 2005). CD63 and CD9 are tetraspanins commonly expressed by human dendritic cells (Mantegazza et al., 2004), a cell type which also constitutively expresses trimeric Fc ϵ RI (Maurer et al., 1996). It is conceivable that Fc ϵ RI *in vivo* exists as a tetra or multimer with a cell type specific

tetraspanin substituting for the basophil and mast cell-specific β -chain. Alternatively, a truly trimeric Fc ϵ RI could reside in tetraspanin-enriched microdomains (Hemler, 2003; Hemler, 2005; Levy and Shoham, 2005b). These microdomains of the tetraspanin web might have different ways to shield transmembrane charges than lipid rafts or the cholesterol-rich microdomains, in which the TCR-CD3 complex is commonly found (Lillemeier et al., 2006; Schamel et al., 2005).

We show here that the first transmembrane region of the β -chain contains not only the interface for receptor stabilization but also the signal peptide sequence for proper insertion of β into the ER. The same domain of the protein is thus responsible for proper ER orientation as well as complex stabilization. This set of data supports our earlier observation that formation of Fc ϵ RI proceeds via a co-translational assembly event (Fiebiger et al., 2005). This feature differentiates Fc ϵ RI from other activating immune receptors, which stabilize in a series of sequential post-translational assembly events. For the TCR/CD3 complex, four preformed dimers, the ligand-binding TCR $\alpha\beta$ heterodimer along with three signaling dimers, assemble in three consecutive steps (Call et al., 2002). This difference in assembly requirements adds to the evidence that Fc ϵ RI and TCR/CD3 complexes, despite similar modular structures, vary substantially with regards to mechanisms of complex assembly and stabilization.

Several polymorphisms linked to allergy have been identified in the Fc ϵ RI β -chain. Among them, an I181L/V183L double mutation in the fourth TM domain and an E237G mutation in the C-terminal cytosolic tail have been described (Hill and Cookson, 1996). Disappointingly, neither mutation interfered with the β -chain's ability to positively regulate Fc ϵ RI surface expression or modulate γ -chain signalling (Donnadieu et al., 2000a; Kraft et al., 2004). The latter finding can be explained by the fact that none of the point mutations modified the ITAM of the β -chain. We show that neither the fourth transmembrane region nor the C-terminal tail of the β -chain is involved in receptor stabilization or surface transport of Fc ϵ RI. Our results therefore explain why Donnadieu et al. did not observe any alterations in the amplifier functions of the β -chain. Hence, the physiological role for the C-terminal polymorphisms remains to be elucidated.

Much effort in the field has been focused on defining unifying principles of assembly and stabilization of immune activation receptors. Here we show that Fc ϵ RI, a receptor of this family and a key regulator of allergic responses, substantially differs in its assembly requirements from the described 3-helix assembly principle. This new understanding of the transmembrane signaling events that regulate Fc ϵ RI formation might lead to new intervention strategies to destabilize complex stability, which could ultimately prove to be a method for modulating allergic responses.

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Fig. 1.

Topology model of tetrameric FcεRI. FcεRIβ is a multispanning protein that contains a basic lysine residue in its second transmembrane region. The IgE-binding alpha chain, FcεRIα, is a Type I membrane protein that forms the core of the tetrameric complex. FcεRIα belongs to the immunoglobulin family and contains an acidic aspartic acid in the transmembrane region. The FcεRγ signaling dimer is formed by two Fc receptor common γ-chains, which are also Type I membrane proteins, and contains a pair of acidic aspartic acids in the N-terminal segment of the transmembrane domain.

Fig. 2.

Lysine 97 in the second transmembrane domain of the β -chain is not required for the formation of Fc ϵ RI tetramers or cell surface upregulation of the tetrameric complex. **A.** Western blot analysis of whole cell lysates from $\alpha\gamma$ HeLa cells transiently transfected with empty control vector (CTRL) or cDNA encoding β , β K97A, or β K97E. HA-tagged β -chains were detected with an anti-HA mAb 3F-10. Lower blot shows an actin loading control. **B.** Co-Immunoprecipitation of Fc ϵ RI tetrameric complexes using the anti-Fc ϵ RI α mAb 19-1 from lysates of $\alpha\gamma$ HeLa cells transfected with control vector or the various β -chain constructs. Top blot shows precipitated α -chain (mAb CRA1), middle blot shows the co-precipitated β -chains (anti-HA mAb 3F-10), the lower blot shows coprecipitated Fc ϵ R γ (anti- γ polyclonal serum). **C.** Surface expression of Fc ϵ RI α is upregulated by both β K97 mutants. Anti-Fc ϵ RI α staining with PE-conjugated mAb CRA1 after transfection of $\alpha\gamma$ HeLa cells with empty control vector or the indicated β -chains. Data are represented as mean fluorescence intensity (MFI) $\pm$ SD, n=3. Statistical significance (*p<0.05) was determined by Student's *t* test. ns, not significant.

Fig. 3.

The first transmembrane region of the β -chain is sufficient for the stabilization of Fc ϵ RI tetramers. **A.** Schematic of wild type β and C-terminal β truncations, which contain the transmembrane domains 1 to 3, 1 to 2, or the first transmembrane domain only. Constructs are labeled as β 3TM, β 2TM and β 1TM respectively. Terminal amino acid positions and estimate molecular weights (kD) are given for all constructs on the right. **B.** β truncations are rapidly degraded by the proteasome. α HeLa cells were transiently transfected and expression levels of β , β 3TM, β 2TM and β 1TM were analyzed in the absence or presence of the proteasome inhibitor ZL₃VS. **C.** C-terminal truncations of the β -chain do not exclude the mutant proteins from tetrameric Fc ϵ RI. α HeLa cells were transfected with the truncation mutants and co-immunoprecipitation of Fc ϵ RI tetramers was performed with anti-Fc ϵ RI α mAb 19-1. Co-precipitation of mutants β 3TM, β 2TM, and β 1TM is shown in lanes 1, 4 and 7, respectively. Transfection of wild type β was used as a positive control in lanes 2, 5, and 8. Empty control vector transfections (CTRL) are shown in lanes 3, 6, and 9. Western blots are shown for co-precipitated β -truncations (upper blots) and Fc ϵ R γ (lower blots).

Fig. 4.

The first transmembrane region of the β -chain is required for assembly of stable Fc ϵ RI tetrameric complexes. **A.** Comparative schematic of wild type β and a N-terminal β -truncations, which lacks the first transmembrane region. The protein is referred to as β 2-4TM. Amino acids and estimate molecular weights (kD) are shown. $\alpha\gamma$ HeLa cells were transfected with β 2-4TM or β . **B.** Detection of β 2-4TM in whole cell lysates. **C.** β 2-4TM does not co-precipitate with Fc ϵ RI $\alpha\gamma$. Immunoprecipitation was performed with anti-Fc ϵ RI α mAb 19-1. Control co-immunoprecipitations with β and empty vector are shown.

Fig. 5.

The first transmembrane region of the β -chain contains an ER insertion signal. **A.** *In vitro* translations and ER insertion experiment. Linearization of β -chain cDNA was performed with *RsaI* to generate a β 81 truncation that contains the first transmembrane region (lanes 1 and 2). For the translation of wild type β , *EcoRI* was used for linearization. Microsomal fractions from astrocytoma cells were used as ER equivalents (+, lanes 1 and 3). The β 81 protein is found in the microsomal fraction like the wild type β -chain (compare lanes 1 and 3). **B.** The first transmembrane region of the β -chain can replace the endogenous signal peptide of the FcεRIα-chain. Surface and intracellular staining for FcεRIα γ 293 cells and ø293 transfected with β 1TM- α . γ 293 cells express FcεRIα (upper left panel). In ø293, FcεRIα does not reach the cell surface (upper right panel). Both cell lines show intracellular expression of FcεRIα (lower panel).