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Short communication

Molecular characterization of the dimer formation of Fcα/μ receptor (CD351)

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ABSTRACT

Fcα/μR (CD351) is an Fc receptor for both IgA and IgM and forms an atypical dimer that is resistant to reduction by 2-mercaptoethanol or boiling. We previously demonstrated that the cytoplasmic portion of Fcα/μR is required for dimer formation and for its efficient cell-surface expression. However, the biochemical nature of these phenomena has not been determined. By using a BW5147 mouse cell line expressing deletion mutants of the cytoplasmic region of Fcα/μR, we found that the region spanning amino acids 504–523 was required for efficient cell-surface expression, whereas the region spanning amino acids 481–490 was required for dimmer formation. Immunoblotting analyses of transfectants simultaneously expressing Flag-tagged Fcα/μR and hemagglutinin-tagged Fcα/μR suggested that Fcα/μR does not form homodimers. Instead, our data suggest that Fcα/μR forms heterodimers with an as-yet-unknown molecule with a molecular weight of 60–70 kDa.

1. Introduction

Fc receptors recognize the Fc portion of immunoglobulins and mediate a variety of immune responses upon binding to immune complexes of immunoglobulins and antigens (Daeron, 1997). Several Fc receptors for IgG and IgE have been shown to play pivotal roles in IgG- or IgE-mediated immune responses, such as antibody-dependent cellular cytotoxicity, mast cell degranulation, phagocytosis, cell proliferation, antibody secretion, and antigen presentation (Daeron, 1997; Lin et al., 1994; Ravetch, 1997). In contrast, the functional characteristics of the Fc receptors for IgM are not as well understood. We previously identified Fcα/μR (CD351), an Fc receptor for IgA and IgM (Sakamoto et al., 2001; Shibuya et al., 2000). Because the Fcα/μR gene is located near the polymeric IgR on chromosome 1 (1F in mice and 1q32.3 in humans), these receptors seem to be closely related (Kaetzel, 2005; Shibuya et al., 2000; Shimizu et al., 2001). Fcα/μR is expressed not only on hematopoietic cells such as B cells and macrophages but also on follicular dendritic cells (FDCs) within follicles of lymphoid organs (Honda et al., 2009). Fcα/μR mediates endocytosis of the ligands IgA and IgM, for which the cytoplasmic portion of Fcα/μR is responsible (Shibuya et al., 2000; Yang et al., 2009). By using *Fcα/μR*-deficient mice, we reported that Fcα/μR negatively regulates T-independent antigens retention by FDCs, leading to suppression of humoral immune responses against T-independent antigens (Honda et al., 2009).

We have also reported that the cytoplasmic portion of Fcα/μR is important for the formation of dimers with a molecular weight of ~130 kDa. These dimers are resistant to boiling in SDS and to reduction by 2-mercaptoethanol (2-ME). The cytoplasmic portion is also important for the efficient cell-surface expression of Fcα/μR (Cho et al., 2010). In this study, we further examined this cytoplasmic region by using BW5147 transfectants expressing deletion mutants of the cytoplasmic portion of Fcα/μR. We also examined the formation of Fcα/μR dimers and show that Fcα/μR does not form homodimers, as we expected, but instead forms heterodimers with a molecule that has a molecular weight of 60–70 kDa.

2. Materials and methods

2.1. Cells and transfectants

BW5147 transfectants stably expressing wild-type Fcα/μR or mutant Fcα/μR lacking the cytoplasmic portion from amino acids 481–535 (ΔCyt.Fcα/μR), 491–535 (Δ490.Fcα/μR), 504–535 (Δ503.Fcα/μR) or 524–535 (Δ523.Fcα/μR) and tagged with Flag or hemagglutinin (HA) at the N-terminus were established as described previously (Cho et al., 2010; Shibuya et al., 2000). To generate site-directed mutations of the Fcα/μR di-Leucine motif at residues Leu519 and Leu520 and of the Gln482 and Gln486 residues within the cytoplasmic region spanning amino acids 481–490, PCR primers were designed that contained a codon for Ala instead of these residues. The PCR products were subcloned into a pMX

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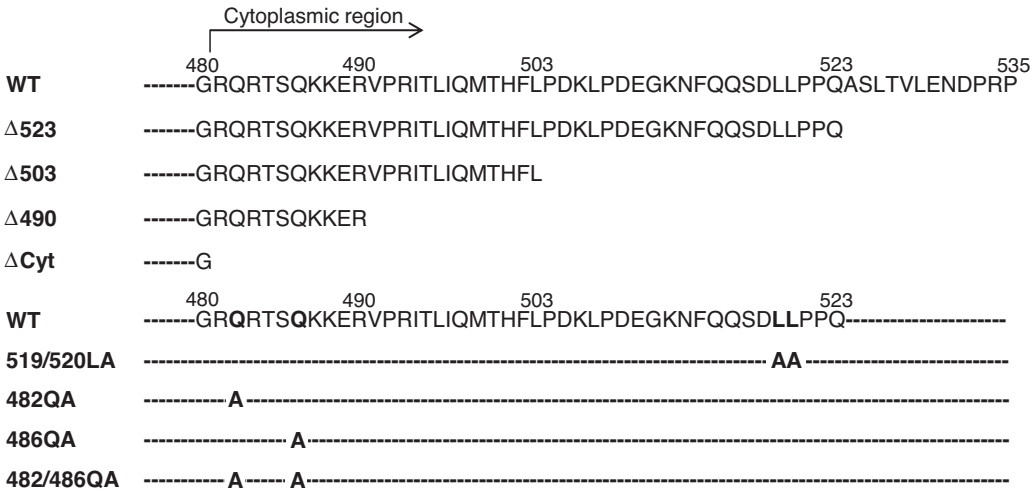

Fig. 1. Schematic presentation of Fcα/μR mutations in the cytoplasmic region. Amino acid sequences in the cytoplasmic region of wild-type (WT) Fcα/μR, and various deletion (Δ) and site-directed (substitution of Ala for Leu [LA] or Gln [QA]) mutants of Fcα/μR.

retrovirus vector with IRES-GFP or GFP sequences as described previously (Cho et al., 2010; Shibuya et al., 2000).

2.2. Antibodies

Anti-Fcα/μR antibodies (TX7 and TX61) were generated in our laboratory, as described previously (Cho et al., 2006). Anti-Flag and anti-HA antibodies were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Roche (Indianapolis, IN, USA), respectively.

2.3. Biochemistry

Cells were lysed in 1% NP-40 lysis buffer containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 10 U/mL aprotinin [Sigma–Aldrich]). Immunoprecipitation was performed by using M280 beads (Sigma–Aldrich) coupled with anti-Flag, anti-HA, or TX61 antibodies. Immunoblotting experiments were performed with anti-Flag, anti-HA, or TX7 antibodies as described previously (Cho et al., 2010).

2.4. Flow cytometry analysis

Cells were stained with a biotinylated anti-Fcα/μR antibody (TX61), followed by allophycocyanin (APC)-conjugated streptavidin. The expression of Fcα/μR on the cell surface relative to the total amount of Fcα/μR, as determined by GFP expression, was calculated as described previously (Cho et al., 2010).

3. Results and discussion

3.1. The region spanning amino acids 504–523 of the cytoplasmic portion is necessary for the efficient cell-surface expression of Fcα/μR

In previous reports, we showed that the cytoplasmic portion of Fcα/μR is required for both dimer formation and efficient cell-surface expression (Cho et al., 2010). To determine the region responsible for these features, we established a BW5147 transfectant expressing mutated Fcα/μR, in which the cytoplasmic portion of Fcα/μR was deleted after the 503rd amino acid (Δ503.Fcα/μR) or the 523rd amino acid (Δ523.Fcα/μR) (Fig. 1). Consistent with our previous report (Cho et al., 2010), flow cytometry analyses showed that much less ΔCyt.Fcα/μR than WT.Fcα/μR was expressed on the cell surface (Fig. 2A). Although the cell-surface expression of Δ503.Fcα/μR was even lower than that of ΔCyt.Fcα/μR, Δ523.Fcα/μR was expressed on the cell surface at a level comparable to that of WT.Fcα/μR (Fig. 2A). Thus, the region spanning amino acids 504–523 of the cytoplasmic portion is necessary for the efficient cell-surface expression of Fcα/μR. The cytoplasmic region spanning amino acids 504–523 comprises at position 519 and 520 a di-Leucine motif that might be internalization of Fcα/μR (Shibuya et al., 2000). Similar motifs are known to be implicated in endosome and lysosome targeting of diverse proteins (Gabilondo et al., 1997). To examine whether the di-Leucine motif is involved in the efficient cell-surface expression of Fcα/μR, we established a BM5147 transfectant stably expressing mutated Fcα/μR in which the di-Leucine residues were replaced with Ala residues (Fig. 1). This mutant was expressed on the cell surface at a level comparable to that of WT.Fcα/μR (Fig. 2B). Thus, the di-Leucine motif is not responsible for the efficient cell-surface expression of Fcα/μR.

3.2. The region spanning amino acids 481–490 of the cytoplasmic portion is necessary for the formation of Fcα/μR dimers

To determine the region responsible for the formation of Fcα/μR dimers, we carried out an immunoblotting analysis of transfectants stably expressing WT or mutated Fcα/μR. Fcα/μR lacking the cytoplasmic portion (ΔCyt.Fcα/μR) existed as a monomer with a molecular weight of ~65 kDa in BW5147 cells (Fig. 3A), consistent with our previous report (Cho et al., 2010). However, Δ490.Fcα/μR, Δ503.Fcα/μR, and Δ523.Fcα/μR, as well as WT.Fcα/μR, formed dimers (Fig. 3A), suggesting that the region spanning amino acids 481–490 in the cytoplasmic portion is required for dimer formation. Because the Fcα/μR dimers are remarkably stable and resistant to reduction by 2-ME (Cho et al., 2010), we hypothesized that a posttranslational modification might be involved, such as tissue transglutaminase-mediated cross-linkage between a Gln and a Lys residue to form an ε-(γ-glutamyl) lysine isopeptide bond between the cytoplasmic portions (AbdAlla et al., 2004; Griffin et al., 2002). Indeed, there are two Gln residues, Gln482 and Gln486, within the region spanning amino acids 481–490. To examine the possible involvement of transglutaminase-mediated cross-linkage in the formation of Fcα/μR dimers, we established BW5147 transfectants stably expressing Fcα/μR with substitutions of Ala for Gln482 and Gln486. An immunoblotting analysis showed that Fcα/μR mutants with substitutions of each Gln residue still formed dimers (Fig. 3B), indicating that transglutaminase might not be involved in the formation of Fcα/μR dimers. Collectively, these results indicate that