# Alternative splicing of myeloid IgA Fc receptor (FcαR, CD89) transcripts in inflammatory responses

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**Abstract More than 10 splice variants of the Fc receptor for IgA (FcαR, CD89) have been identified in human myeloid cells. In this study, we quantified FcαR splice transcripts ΔEC2 and Δ66EC2, which lack the entire and a part of the homologous immunoglobulin-like extracellular domain 2 (EC2), respectively. Tumor necrosis factor-α was found to specifically increase the ratio of ΔEC2 to the wild type CD89 in neutrophils and conversely decrease the ΔEC2 ratio in monocytes. We also observed a significant decrease in the neutrophil ΔEC2/CD89 ratio in pneumonia patients. These results suggest that ΔEC2 is differentially regulated and could be involved in immunoregulation of IgA-mediated host defense.**


*Key words:* IgA Fc receptor; CD89; Alternative splicing; Tumor necrosis factor-α; Myeloid cell; Pneumonia

## 1. Introduction

IgA is the predominant immunoglobulin (Ig) in external secretions of the mucosa. Human myeloid cells such as monocytes/macrophages and neutrophils express the Fc receptor for IgA (FcαR, CD89), and IgA-coated antigens trigger effector functions of these cells including phagocytosis, superoxide production, inflammatory cytokine release, and antibody-dependent cell-mediated cytotoxicity [1]. Although its role in mucosal immunoprophylaxis mechanisms is still poorly understood, recent studies with transgenic mice expressing human FcαR have provided direct evidence for FcαR function in host defense against bacterial infection [2,3].

The FcαR gene has been mapped to chromosome 19q13.4 [4], and consists of five exons spanning approximately 12 kb [5] (Fig. 1). Exons S1 and S2 encode the leader peptide, EC1 and EC2 each encode a homologous Ig-like extracellular domain, and TM/C encodes the transmembrane domain and cytoplasmic tail of the receptor. It has been reported so far that a number of diverse mRNA isoforms of FcαR are generated by deleting the entire or a part of each exon via alternative RNA splicing [6–11]. Among them, transcript Δ66EC2, which lacks 66 nucleotides in the portion encoding the EC2 domain, is expressed predominantly in alveolar macrophages and is the only variant that has been identified as a natural protein [7]. On the other hand, transcript ΔEC2 lacking the entire EC2 domain has been identified in granulocytes and monocytes [6–9], and Chinese hamster ovary cells transfected with this variant have been demonstrated to bind secretory IgA but not serum IgA, while transfectants expressing full-length CD89 bind both [8].

The expression and function of FcαR are modulated by lipopolysaccharide (LPS) [12] and several inflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor-α (TNFα), transforming growth factor-β (TGFβ), and IL-8 [12–15]. In addition, altered FcαR expression has been reported in allergic diseases [16], IgA nephropathy [17], HIV infection [18], bacterial infection [19], alcoholic liver cirrhosis [20], and ankylosing spondylitis [21]. While these reports suggest an important role of FcαR in mucosal inflammatory diseases, our knowledge of expression of FcαR splice isoforms in response to cytokines and in diseases has been poor. In this paper, we conducted quantitative analyses of the splice variants ΔEC2 and Δ66EC2 and demonstrated their differential expression in a cell type-specific manner and in response to TNFα. In addition, we observed an altered ΔEC2/CD89 ratio in neutrophils of patients with pneumonia.

## 2. Materials and methods

### 2.1. Subjects

Blood samples were collected from 18 healthy volunteers and four patients with pneumonia (all patients had Gram-negative bacteremia: *Klebsiella* sp., *Acinetobacter* sp., *Pseudomonas* sp., and Gram negative rod sp.). Septic shock and respiratory failure in patients with pneumonia occurred within 72 h after hospitalization, and were confirmed by microbiological and other laboratory tests. All the patients with pneumonia received antimicrobial chemotherapy; one patient was given ceftazidine 1.0 g twice daily for 3 days and the remaining patients were given imipenem/cilastatin sodium 0.5 g twice daily for 1 day. Each blood specimen was taken from the patients immediately after these treatments. None of the patients received corticosteroid and/or immunosuppressive drug treatment. In Fig. 5, sex and age were

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*Abbreviations:* FcαR, Fc receptor for IgA; HBBS, Hanks' balanced salt solution; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PMA, phorbol 12-myristate 13-acetate; TGFβ, transforming growth factor-β; TNFα, tumor necrosis factor-α

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matched between patients [three males and one female, ranging in age from 64 to 75 (mean age 68.5 ± 5.9 yr)] and healthy subjects [five males and two females, ranging in age from 61 to 79 (mean age 69.4 ± 8.0 yr)]. Healthy subjects in other figures were eight males and three females, ranging in age from 20 to 49 (mean age 30.2 ± 8.6 yr). Informed consent was obtained from all healthy subjects and patients.

### 2.2. Cell isolation

Neutrophils were isolated from heparinized peripheral blood by Polymorphprep (Nycomed, Oslo, Norway) gradient centrifugation, and the purity was greater than 95% as judged by Giemsa staining (Diff-Quik; Baxter, Dudingen, Switzerland); the remaining cells were mainly erythrocytes and rarely eosinophils. Human peripheral blood mononuclear cells (PBMC) were isolated by Lymphoprep (Nycomed) gradient centrifugation. Monocytes were purified from PBMC by negative indirect selection using the magnetic cell separation system MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) and the Monocyte isolation kit (Miltenyi Biotec) in accordance with the manufacturer's instructions, and the purity was 84–94% as judged by Diff-Quik; the remaining cells were mainly lymphocytes.

### 2.3. Cell culture

The human promonocytic cell line U937 was maintained in RPMI 1640 (Sigma, St. Louis, MO, USA) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For stimulation by TNFα, isolated monocytes were resuspended at $5\times10^5$ cells/ml in RPMI 1640 supplemented with 5 μg/ml polymyxin B (Sigma) to eliminate possible effects of LPS, and incubated with 20 ng/ml TNFα (R&D systems, Tokyo, Japan) for 24 h; isolated neutrophils were resuspended at $10^6$ cells/ml in $Ca^{2+}$- and $Mg^{2+}$-free Hanks' balanced salt solution (HBSS) supplemented with 0.1% gelatin, and stimulated with 20 ng/ml TNFα for 2 h.

### 2.4. RNA isolation and quantitative RT-PCR analysis

Total RNA was extracted from 4–15 × $10^6$ cells by the acid guanidine phenol chloroform method using Trizol (Gibco BRL, Gaithersburg, MD, USA). Specific oligonucleotide primers were used for amplification of full-length CD89 and each splice variant (forward primer (+), 5′-GTTCGTCATTGACCACATGG-3′; reverse primer (−), 5′-GAGGCTTCCTTGTTCAGTGC-3′ [9]). The isolated RNA (1 μg from monocytes and neutrophils, or 2 μg from U937 cells) was reverse transcribed using 10 pmol of reverse primer (−), 10 U of reverse transcriptase Superscript II (Gibco BRL), and 400 μM each dNTP in 50 μl of 2×reaction buffer (1×reaction buffer: 50 mM KCl, 10 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, and 0.01% gelatin) at 42°C for 30 min, followed by heating at 94°C for 5 min. After addition of 10 pmol of primer (+) and 1 U of Taq Gold polymerase (Perkin-Elmer, New Jersey), the sample was subjected to amplification (denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension

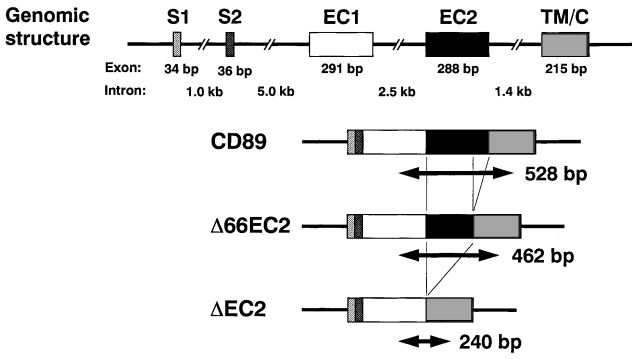


Fig. 1. Schematic representation of the organization of the FcαR gene and transcripts of the full-length form (CD89) and splice isoforms (Δ66EC2 and ΔEC2). DNA fragments of the indicated lengths are produced by RT-PCR using primers which correspond to the regions within the EC1 and TM/C domains.

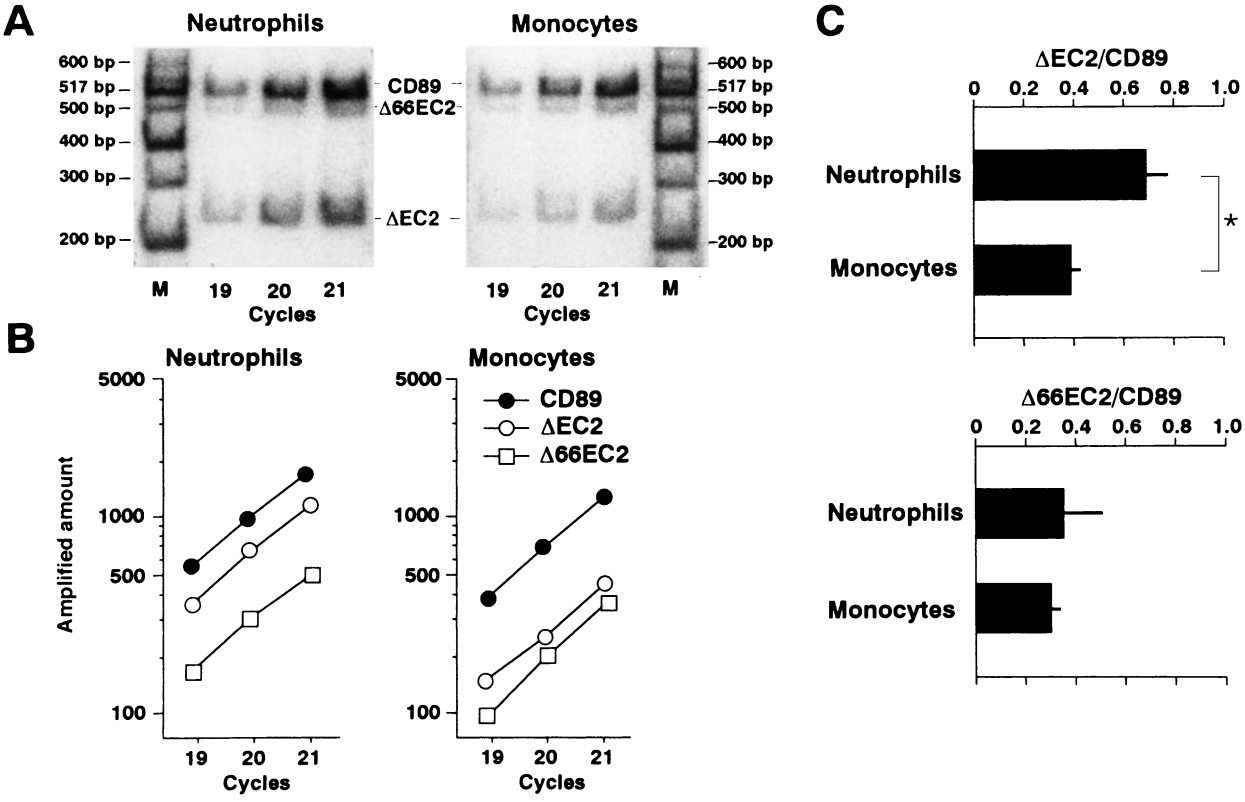


Fig. 2. PCR analysis of the FcαR transcripts in primary neutrophils and monocytes. A: Autoradiograms of the amplified products. M, a $^{32}P$-labeled 100 bp ladder (New England Biolabs) as molecular weight marker. B: Relative amounts of the amplified products. C: The ratios of each variant to CD89 at 20 cycles of amplification. Total RNA from neutrophils and monocytes of nine healthy individuals was analyzed by quantitative RT-PCR. Shown in the histograms are the mean ± S.E.M. of different donors. $^*P < 0.05$.

at 72°C for 1 min) in 100 μl of 1×reaction buffer. PCR was performed in linear conditions as shown in Fig. 2B, and unless otherwise indicated, results at 20 cycles of amplification are shown in the figures. After adding 0.5 pmol of 5′-$^{32}$P-labeled forward primer (+), the sample was further amplified once by extension at 72°C for 5 min following denaturation and annealing, and electrophoresed on 5% native polyacrylamide gels. After electrophoresis, the gel was dried, and radioactivities of amplified bands were evaluated using the Image Analyzer Fujix BAS 2500A (Fuji Film, Tokyo, Japan). Statistical significance of the results was determined using the Student's *t*-test. Differences were considered significant at $P < 0.05$.

## 3. Results and discussion

We measured the relative amount of three FcαR transcripts using a pair of oligonucleotide primers complementary to exons encoding the EC1 and TM/C domains, as described previously [9] (Fig. 1). The full-length form CD89 and splice isoforms Δ66EC2 and ΔEC2 can be detected as fragments of 528, 462 and 240 bp, respectively. We first examined cell type specificity for expression of each FcαR splice variant. Total RNA was prepared from freshly isolated blood neutrophils and monocytes, and subjected to the quantitative RT-PCR analysis as described in Section 2.4. As displayed in Fig. 2A,B, the amount of DNA increased proportionally at least up to 21 cycles when 1 μg of RNA from neutrophils and monocytes was used. When compared under these conditions, the ratio of ΔEC2 to CD89 in neutrophils is about two-fold higher than that in monocytes, while no significant differences in the Δ66EC2/CD89 ratio were observed between these cell types (Fig. 2C).

Because the results shown in Fig. 2 suggested that expression of FcαR splice isoforms is differentially regulated in neutrophils and monocytes, we next examined expression of these variants during myeloid differentiation. Immature myeloid cell line U937 develops into monocyte/macrophage-like phenotype upon induction of differentiation with phorbol 12-myristate 13-acetate (PMA). As shown in Fig. 3A, when U937 cells were cultured with PMA, steady-state levels of the CD89 transcript increased as described previously [22]. At that time, the extent of increase in the ΔEC2 transcript was smaller than that in CD89 (Fig. 3A), which resulted in a decrease in the ratio of ΔEC2 to CD89 (Fig. 3B). This is consistent with the observation of lower ΔEC2 ratio in mature monocytes compared with neutrophils, and suggests the involvement of ΔEC2 in certain cell type-specific functions of FcαR. On the other hand, we could detect no Δ66EC2 mRNA in this cell line.

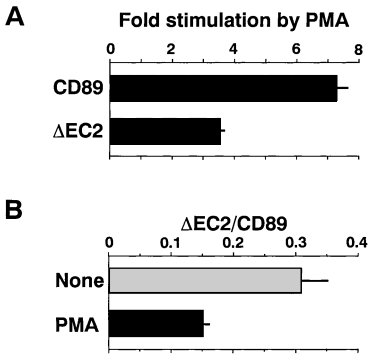


Fig. 3. Effect of PMA on expression of the FcαR splice variants in immature myeloid cell line U937. Total RNA from U937 cells differentiated with 100 nM PMA or vehicle (ethanol) for 72 h was analyzed by quantitative RT-PCR. Data represent the mean ± range of values of two independent experiments. A: Induction of the FcαR splice variants in response to PMA. B: Effect of PMA on the ratio of ΔEC2 to CD89.

As a first approach to address the involvement of FcαR splice variants in myeloid functions in inflammatory process, we examined their expression in the presence of various inflammatory cytokines, which have previously been reported to modulate FcαR expression and functions [12–15,23]. In our survey using U937 cells, induction of ΔEC2 by TNFα was found to be significantly lower than that of CD89, while no significant changes in the induction or downregulation levels were observed between ΔEC2 and CD89 upon treatment with IL-1β, 1-α,25-dihydroxyvitamin $D_3$ and TGFβ (Fig. 4A). Therefore, we next examined the effect of TNFα on the ratios of splice variants to CD89 in primary neutrophils and monocytes. As shown in Fig. 4B, when monocytes were incubated with TNFα for 24 h, a significant decrease in the ratio of ΔEC2 to CD89 was observed similar to that seen in U937

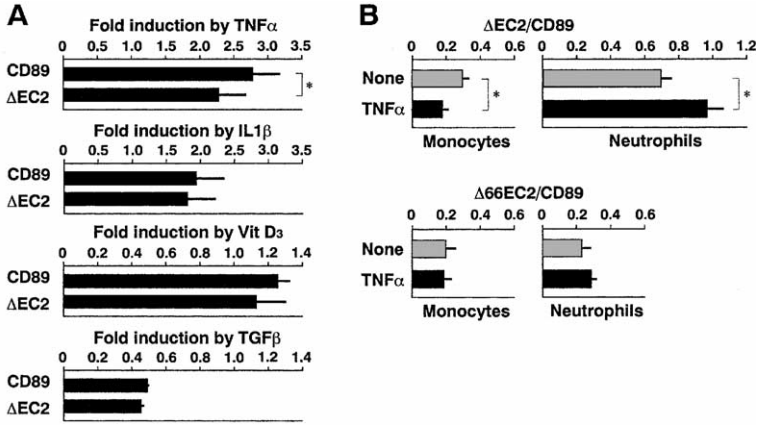


Fig. 4. Effect of inflammatory cytokines on FcαR alternative splicing. A: Induction of ΔEC2 and CD89 by cytokines in U937 cells. Total RNA from U937 cells treated with 20 ng/ml TNFα, 10 ng/ml IL-1β, 100 nM 1-α,25-dihydroxyvitamin $D_3$ (Vit $D_3$) and 20 ng/ml TGFβ for 24 h was analyzed by quantitative RT-PCR. Data represent the mean ± S.E.M. of two to seven independent experiments. *$P < 0.01$. B: Effect of TNFα on the ratios of splice variants to CD89 in primary neutrophils and monocytes. Neutrophils and monocytes from five and four healthy individuals, respectively, were treated with or without TNFα, and total RNA was analyzed by quantitative RT-PCR. Data represent the mean ± S.E.M. of different donors. *$P < 0.05$.

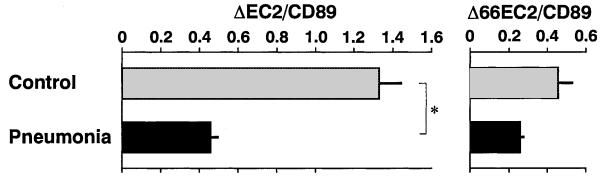


Fig. 5. PCR analysis of the FcαR transcripts from neutrophils of patients with pneumonia. Total RNA from neutrophils of seven healthy individuals as controls or four patients with pneumonia was analyzed by quantitative RT-PCR. Data represent the mean ± S.E.M. of different donors. $*P < 0.05$.

cells. Conversely, 2-h treatment with TNFα increased the neutrophil ΔEC2 ratio, although such short-term treatment did not affect the monocyte ΔEC2 ratio (data not shown). In addition, the opposite effect of TNFα on the ΔEC2 ratio in neutrophils and monocytes is unlikely to be due to differences in culture conditions, because an increased ratio in neutrophils in response to TNFα was also observed when RPMI 1640 medium and HBSS containing $Ca^{2+}$ were used (data not shown). In contrast, neither neutrophils nor monocytes showed significant changes in the ratio of Δ66EC2 to CD89 (Fig. 4B).

Our findings indicate that induction of ΔEC2 by TNFα is differentially regulated in a cell type-specific manner. Especially the observation of the increased ΔEC2 ratio in neutrophils could reflect some physiological roles of this variant in FcαR functions in this cell lineage. A recent study using polysaccharide-specific IgA has suggested that FcαR represents an important leukocyte receptor on neutrophils to exhibit antipneumococcal cellular effector function against *Streptococcus pneumoniae* [24]. Moreover, enhanced expression and function of neutrophil FcαR have recently been reported in bacteremia including pneumonia [19]. Therefore, we isolated neutrophils from patients with pneumonia and examined the expression of FcαR splice transcripts. Similar to the previous report [19], increased neutrophil FcαR expression was observed in pneumonia (data not shown). In addition, we found that the ratio of ΔEC2 to CD89 in neutrophils of the patients was significantly lower compared with healthy controls, whereas no significant differences in the Δ66EC2/CD89 ratio were observed (Fig. 5). The higher ΔEC2 ratio observed in neutrophils from healthy controls compared with the results in Fig. 2C is likely due to aging, because we collected blood samples from older volunteers to be matched with the patients by age (see Section 2.1). The ΔEC2 expression pattern in pneumonia is contrary to the TNFα response of neutrophils in which the ΔEC2 ratio increased (Fig. 4A). Chiamolera et al. [19] have reported that although serum TNFα levels increase in patients with bacteremia, this does not significantly correlate with increased FcαR levels observed in these patients. Therefore, the mechanism underlying a decrease of the ΔEC2/CD89 ratio in pneumonia might involve a factor(s) in addition to or other than TNFα.

At present, the function of ΔEC2 is unknown. We and others have previously demonstrated the physical association of FcαR with the FcRγ subunit [25–28], which contains a common ITAM (immunoreceptor tyrosine-based activation motif) in its cytoplasmic tail and is essential for signaling through FcαR [25,27]. Based on the molecular structure, the ΔEC2 protein, which lacks a part of the extracellular portion, is thought to be capable of associating with FcRγ, because FcRγ interacts with FcαR through the FcαR transmembrane/cytoplasmic domains [27]. Therefore, one can speculate that ΔEC2 competes with the full-length CD89 for FcRγ. In this context, Launay et al. [29] have recently demonstrated that FcαR is expressed with or without FcRγ on monocytes and neutrophils, and FcRγ-associated and FcRγ-less FcαRs have different functions. Moreover, Chiamolera et al. [19] have shown that the FcαR molecule whose expression increases in bacteremia is the FcRγ-associated form. Therefore, the decreased ΔEC2 ratio in pneumonia observed in Fig. 5 may lead to an increase in FcRγ-associated FcαR through relieving competition for FcRγ. In addition, although the EC1 domain rather than the EC2 domain has been identified as the IgA-binding domain of FcαR [30,31], Pleass et al. [8] have shown that the ΔEC2 protein cannot bind serum IgA. In this context, the ΔEC2 variant might act as a dominant negative form of FcαR and selectively affect serum IgA-triggered FcαR functions. The results presented in Fig. 4 showing opposite effects of TNFα on the monocyte and neutrophil ΔEC2/CD89 ratios may reflect the switching of serum IgA-responsive cells from neutrophils to monocytes during the inflammatory process.

In conclusion, we suggested in this study that expression of the FcαR splice isoform ΔEC2 but not Δ66EC2 is differentially regulated in a cell type-specific manner and in response to inflammatory and/or infectious processes. FcαR splice isoforms with deletion at the EC2 domain could represent a physiological mechanism to generate its functional diversity in different cell types, which has a critical role in IgA-mediated host defense.

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