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Molecular analysis of expression and function of hFcγRIIbl and b2 isoforms in myeloid cells

Trupti Joshi [a], Latha P. Ganesan [c], Xianhua Cao [b], Susheela Tridandapani [a,b,c,*]

[a] *The Ohio State University Biochemistry Program, The Ohio State University, Columbus, OH 43210, USA*
[b] *Biophysics Program, The Ohio State University, Columbus, OH 43210, USA*
[c] *Department of Internal Medicine/Division of Pulmonary and Critical Care Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, USA*

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Abstract

The inhibitory receptor FcγRIIb becomes tyrosine phosphorylated and associates with the inositol phosphatase SHIP to downregulate phagocytosis. The two splice variants of FcγRIIb, b1 and b2, are differentially expressed in hematopoetic cells. Both isoforms of FcγRIIb are expressed in human myeloid cells although FcγRIIb2 predominates. In murine B cells FcγRIIb2 associates with clathrin-coated pits and undergoes endocytosis, whereas FcγRIIbl is excluded from the coated pits, indicating that the two isoforms serve partially differing functions. In humans, there are conflicting reports with regard to the ability of FcγRIIb2 to become tyrosine phosphorylated, and the functional capacities of the two isoforms are poorly understood. We, and others, have previously reported that the expression of FcγRIIb is upregulated in human monocytes by the anti-inflammatory cytokine IL-4. Here, we extend these findings to demonstrate that the IL-4-induced upregulation of FcγRIIb is synergistically enhanced by the addition of IL-10, both at the protein and the mRNA level. The upregulated receptors are functional as assessed by their ability to become tyrosine phosphorylated and to downregulate phagocytosis. Interestingly, both b1 and b2 isoforms are upregulated by anti-inflammatory cytokines. Transfection experiments expressing human FcγRIIbl or b2 in Raw 264.7 murine macrophage cells revealed that both isoforms are tyrosine phosphorylated and promote SHIP phosphorylation. Finally, both b1 and b2 isoforms of FcγRIIb downregulate phagocytosis to a similar extent. Thus we conclude that FcγRIIbl and b2 are both functional inhibitory receptors in the phagocytic process.

Keywords: Fcγ receptors; Phagocytosis; ITIM; Negative regulation; Signal transduction

1. Introduction

Fcγ receptor clustering on monocytes/macrophages initiates a series of signaling events that culminate in phagocytosis of IgG-coated particles, generation of inflammatory cytokines and the production of reactive oxygen and nitrogen radicals (reviewed in Aderem and Underhill (1999) and Sánchez-Mejorada and Rosales (1998)). Whereas FcγRI, FcγRIIa and FcγRIIIa are all activating receptors associated with immunoreceptor tyrosine-based activation motifs (ITAMs) and promote the phagocytic process, FcγRIIb is an inhibitory receptor that serves to downregulate phagocytosis (McKenzie and Schreiber, 1998).

The inhibitory potential of FcγRIIb is attributed to a tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail (Muta et al., 1994). Mutation of the tyrosine to phenylalanine or deletion of the ITIM abolishes the inhibitory potential of FcγRIIb. Upon co-ligation with an IT AM-containing receptor, FcγRIIb ITIM becomes tyrosine phosphorylated and recruits the inositol phosphatase SHIP via the SHIP SH2 domain (Liu et al., 1997; Ono et al., 1996; Tridandapani et

Abbreviations: FcγR, receptor for IgG; PBM, peripheral blood monocytes; BMM, bone marrow macrophages; PtdIns, phosphatidylinositol; ITAM, immnoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; SH, Src homology; IL, interleukin; IC, immune complex; IP, immunoprecipitation; IB, immunoblot

* Corresponding author. Tel.: +1 614 247 6768; fax: +1 614 247 8106.
E-mail address: tridandapani.2@osu.edu (S. Tridandapani).

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al., 1997; Chacko et al., 1996). Association of SHIP with the phosphorylated ITIM of FcγRIIb delivers SHIP to the membrane where it can access and hydrolyze its substrate PtdIns3,4,5P_3 to PTdIns3,4P_2, thereby downregulating the activation of downstream PtdIns3,4,5P_3-dependent enzymes. Deletion of SHIP abrogates the inhibitory effect of FcγRIIb indicating that SHIP is the effector molecule of FcγRIIb (Ono et al., 1996, 1997).

Both FcγRIIb and SHIP negatively regulate FcγR-mediated phagocytosis. Thus, FcγRH$^{-/-}$ macrophages and SHIP$^{-/-}$ macrophages display enhanced phagocytic efficiency in comparison to their wild-type counterparts (Clynes et al., 1999; Cox et al., 2001; Nakamura et al., 2002). In human monocytes FcγRIIb expression is upregulated by IL-4, and results in diminished phagocytic efficiency (Pricop et al., 2001; Tridandapani et al., 2002).

FcγRIIb is expressed as two common isoforms, b1 and b2. The two differ in their cytoplasmic tails where b1, in humans, contains a 19 amino acid insert, encoded by exon C1, spliced out of the b2 isoform (Brooks et al., 1989). ITIM sequences, downstream, are retained unmodified in both. In mouse, the insert is longer but the molecules are otherwise analogous (Ravetch et al., 1986; Hibbs et al., 1986; Lewis et al., 1986). Deglycosylated forms of the two proteins can be readily distinguished by size on SDS-PAGE (Weinrich et al., 1996; Tridandapani et al., 2002; Lyden et al., 2001). Both isoforms are expressed in all cells expressing FcγRIIb although the b1 form predominates in B cells and mast cells whereas the b2 form predominates in cells of mononuclear phagocyte lineage (Cassel et al., 1993; Ravetch and Kinet, 1991). Previous studies have examined the functional differences between the two isoforms in B cells. Thus, it has been reported by several groups that both human FcγRIIb2 and murine FcγRIIb2 are capable of endocytosis (Miettinen et al., 1989, 1992; Van Den Herik-Oudijk et al., 1994), whereas FcγRIIbl is not. In other studies, Budde et al. observed that in B cells human FcγRIIb2 ITIM tyrosine is not phosphorylated although its ability to downregulate antigen receptor-induced calcium mobilization was comparable to that of FcγRIIbl, which is tyrosine phosphorylated (Budde et al., 1994b). Although the molecular details of this paradoxical observation are not fully understood, it is clear that FcγRIIbl and b2 likely serve some non-overlapping functions in B cells. Of note, there are no studies to date comparing the functional capacity of hFcγRIIbl and b2 in macrophages. Hunter et al. used a COS-1 fibroblast model to study the inhibitory potential of hFcγRIIb. In this study, it was clearly demonstrated that hFcγRIIbl can downregulate FcγR-mediated phagocytosis. However, it is not clear whether hFcγRIIb2 can function in a comparable manner (Hunter et al., 1998). Likewise, it is not known if both FcγRIIbl and b2 are tyrosine phosphorylated in macrophages and function as inhibitory receptors during phagocytosis.

Here, we have examined the regulation of expression and the function of hFcγRIIbl and b2 in myeloid cells. Our data demonstrate that the previously reported IL-4-induced upregulation of total FcγRIIb expression is synergistically enhanced in the presence of IL-10. This upregulation occurs at the mRNA level. We further demonstrate that both isoforms of FcγRIIb, b1 and b2, are upregulated under these conditions. In order to test the functional characteristics of the two isoforms, we have expressed hFcγRIIbl and b2 in Raw 264.7 murine macrophage cells. We report that both isoforms of hFcγRIIb are equivalently surface expressed, suggesting that the presence or absence of the 19 amino acid insert has no influence on surface expression of these receptors. Interestingly, unlike in B cells, both isoforms are tyrosine phosphorylated upon FcγR clustering. We extend these findings to show that both isoforms are equally capable of inducing SHIP activation and downregulating FcγR-mediated phagocytosis.

2. Materials and methods

2.1. Cells, antibodies and reagents

U937 human monocytic cells and Raw 264.7 murine macrophage cells were obtained from ATCC and maintained in RPMI with 10% fetal bovine serum. Phospho-FcγRIIb and pSHIP antibodies were purchased from Cell Signaling Technology (Beverly, MA). Actin antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Absolutely RNA RT-PCR Miniprep Kit for total RNA purification was purchased from Stratagene (La Jolla, CA). SuperScript First-Strand Synthesis System for RT-PCR Kit for cDNA synthesis was purchased from Life Technologies (Carlsbad, CA). TaqManUniversal PCR Master Mix was obtained from PE Applied Biosystems (Branchburg, NJ). Human FcγRIIb probe (5′-6FAM-CTCATGCACCCGGATGCTCTGGA-MGBNFQ-3′), hFcγRIrb forward primer (5′-GCTGAGA-ACACAATCACCTATTCACT-3′), and hFcγRIIb reverse primer (5′-CGGTTCTGGTCAGGCTC-3′) were purchased from PE Applied Biosystems (Foster City, CA). Anti-FcγRII mAb KB61 was obtained from Dr. D. Mason, Oxford, UK. Anti-FcγRIIb rabbit polyclonal antibody, Ab163, was from Dr. Jean-Luc Teillaud, and was raised against a GST fusion protein of the cytoplasmic tail of FcγRIIbl. mAb IV.3, and 22.2 were obtained from Medarex (Annandale, NJ). mAb FLI8.26 was purchased from BD Pharmingen (San Diego, CA). All FcyRII antibodies used have been previously described (Tridandapani et al., 2002).

2.2. Isolation of peripheral blood monocytes (PBM)

CD14-positive PBM were isolated as previously described (Tridandapani et al., 2003). Briefly, peripheral blood mononuclear cells (PBMCs) were first isolated by density gradient centrifugation over Histopaque (Sigma). Monocytes were then purified from the PBMCs by negative selection using the MACs Monocyte Isolation Kit (Miltenyi Biotech). For this, PBMCs were first treated with FcR blocking Reagent