Molecular Immunology 49 (2011) 329–337

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Differences in endocytosis mediated by FcγRIIA and FcγRIIB2

Christine Y. Zhang[a,b], James W. Booth[a,b,*]

[a] Department of Immunology, University of Toronto, Toronto, Ontario, Canada M5S 1A8
[b] Sunnybrook Research Institute, Toronto, Ontario, Canada M4N 3M5

ARTICLE INFO

Article history:
Received 5 January 2011
Received in revised form 15 August 2011
Accepted 5 September 2011
Available online 25 September 2011

Keywords:
Endocytosis
Protein trafficking
Fcγ receptors
Ubiquitylation

ABSTRACT

An important function of Fcγ receptors is the removal of IgG-containing immune complexes from the circulation. The activating receptor FcγRIIA and inhibitory receptor FcγRIIB2 are both expressed on human myeloid cells, and are both capable of mediating endocytosis of immune complexes. We studied endocytosis of these two receptors expressed by transfection in ts20 Chinese hamster fibroblasts. We find that while FcγRIIA-mediated endocytosis requires the participation of the ubiquitin-conjugating system, the endocytosis of FcγRIIB2 does not. Little if any ubiquitylation of FcγRIIB2 was observed in response to immune complex binding. FcγRIIB2 mediates internalization of immune complexes at a faster rate than FcγRIIA, and facilitates the endocytosis of FcγRIIA upon co-engagement of both receptors. This may represent a novel mechanism by which the inhibitory receptor can reduce signalling from the activating Fcγ receptor.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

In the immune system, the recognition of multivalent IgG-containing complexes is carried out by Fcγ receptors (FcγR) (Nimmerjahn and Ravetch, 2008). These receptors are essential for allowing a range of effector responses including antibody-dependent cellular cytotoxicity, phagocytosis and inflammatory mediator production. FcγRIIA and FcγRIIB are the activating and inhibitory members of the human FcγRII subfamily, respectively. Both receptors bind to the Fc portion of IgG with low affinity. The ratio of expression of FcγRIIA to FcγRIIB on different myeloid cells varies and can be regulated by cytokines (Boruchov et al., 2005; Liu et al., 2005; Pricop et al., 2001; Tridandapani et al., 2002). Present only in humans and other primates, FcγRIIA is widely expressed on leukocytes (Tan Sardjono et al., 2003). The intracellular domain of FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM). It is unique among Fc receptors in that the ITAM and the ligand-binding domain are contained in a single polypeptide. In addition, the ITAM is unusual, having 12 amino acid residues rather than the typical 7 separating the two YXXL sequences (Huang et al., 2004). These ITAM tyrosines are phosphorylated by Src family kinases upon receptor clustering to trigger downstream signalling cascades (Daeron, 1997). Whereas there is 92% sequence identity in the extracellular domains of FcγRIIA and FcγRIIB, the FcγRIIB intracellular domain diverges from that of FcγRIIA and contains an immunoreceptor tyrosine-based inhibitory motif (ITIM). When FcγRIIB is co-engaged with activating receptors, phosphatases are recruited to the ITIM and dampen ITAM-mediated cell activation (Gergely et al., 1999). The overall balance between positive and negative signals from activating and inhibitory FcγRs is important to maintain an appropriate level of effector responses to immune complexes. Imbalances in activating and inhibitory signalling causing exacerbation of FcγR-mediated effector responses have been associated with the manifestation of autoimmune disorders (Takai, 2005).

One important role FcγRs play during the course of immune responses is the removal of IgG-opsonized foreign material from the circulation. FcγRIIA is capable of mediating both phagocytosis of large antibody-coated particles and endocytosis of small soluble immune complexes (Indik et al., 1991; Booth et al., 2002). In contrast to phagocytosis, which requires ITAM-mediated receptor phosphorylation and actin rearrangement, FcγRIIA-mediated endocytosis of soluble immune complexes is phosphorylation-independent and proceeds via clathrin-coated pits. We have identified key residues within the atypical ITAM motif of FcγRIIA that are crucial for its endocytosis (Mero et al., 2006); these differ from the sequence requirements for internalization described for the canonical ITAM of the Fc receptor γ chain (Amigorena et al., 1992). The inhibitory FcγRIIB occurs as two isoforms generated by alternative splicing, with FcγRIIB1 present in B cells and FcγRIIB2 mainly expressed in myeloid cells. Both murine

Abbreviations: ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; aglgG, aggregated IgG.

* Corresponding author at: Department of Immunology, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8. Tel.: +1 416 763 6716; fax: +1 416 978 1938.
E-mail addresses: James.Booth@utoronto.ca, jameswdbooth@gmail.com (J.W. Booth).

0161-5890/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2011.09.003

FcγRII isoforms have been reported to support phagocytosis of large particles (Joiner et al., 1990); the ability to mediate phagocytosis depends on the presence of a tyrosine residue C-terminal to the ITIM motif (Daeron et al., 1993). Human FcγRIIB lacks this extra tyrosine and does not support phagocytosis (Hunter et al., 1998; Van den Herik-Oudijk et al., 1994). Both mouse (Miettinen et al., 1992) and human FcγRIIB2 (Engelhardt et al., 1990; Van den Herik-Oudijk et al., 1994) can mediate endocytosis of soluble immune complexes, which depends on the presence of a dileucine motif that lies within the ITIM (Hunziker and Fumey, 1994; Matter et al., 1994). FcγRIIB1 does not mediate endocytosis; it is excluded from clathrin-coated pits due to the presence of an amino acid insertion in its cytoplasmic domain (Miettinen et al., 1992).

Ubiquitylation has been shown to be an important factor regulating the trafficking of many membrane proteins (Staub and Rotin, 2006). It has been implicated in the endocytosis of several receptors, including those for epidermal growth factor and growth hormone (Stang et al., 2004; Strous et al., 1996). We found that ubiquitylation is also important for endocytosis of soluble immune complexes mediated by FcγRIIA (Booth et al., 2002).

While FcγRIIA and FcγRIIB2 are both competent for endocytosis, the extent to which endocytosis via these two receptors proceeds by similar or distinct mechanisms is unclear. In this work, we compare the requirements for ubiquitylation and the internalization kinetics of these two endocytic pathways. We also investigate how receptor endocytosis is affected when FcγRIIA and FcγRIIB2 are co-engaged. We find that FcγRIIB2, in contrast to FcγRIIA, can internalize immune complexes in a ubiquitylation-independent manner. This endocytosis is more rapid than that mediated by FcγRIIA, and coengagement of FcγRIIA with FcγRIIB2 allows for the enhancement of FcγRIIA internalization and downregulation.

2. Materials and methods

2.1. Reagents and antibodies

Fetal bovine serum, α-minimal essential medium, and G418 were from Wisent (St. Bruno, Quebec, Canada). Mouse anti-ubiquitin antibody P4G7 and mouse anti-Myc antibody 9E10 were from Covance (Berkeley, CA). Rabbit anti-myc antibody A-14 was from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-FcγRIIA antibody IV.3 was purified from hybridoma supernatants. Mouse anti-FcγRIIB antibody 4F5 (Su et al., 2007) was a kind gift from Dr. Robert Kimberly (University of Alabama at Birmingham). Anti-FcγRII antibody AT10 was from Abcam. Rabbit polyclonal antibody specific to the intracellular domain of FcγRIIB2 was generated as described (Zhang and Booth, 2010). Cy3-, Cy5-, and horseradish peroxidase-conjugated secondary antibodies were from Jackson Immunoresearch Laboratories. Alexa488-conjugated secondary antibodies were from Invitrogen. Paraformaldehyde was from Canemco (16%, EM grade). MG132 was from Calbiochem. IL-4 and IL-10 were from BioSource International (Camarillo, CA). Supersignal West Pico chemiluminescent substrate, Restore Western Blot Stripping Buffer, and Ultralink protein G beads were from Thermo Scientific. Human IgG, protease inhibitors and other chemicals were from Sigma–Aldrich.

2.2. Cell culture

Chinese hamster ts20 cells were grown at 34 °C in 5% CO_2 in α-minimal essential medium +10% fetal bovine serum. To abolish ubiquitylation, the E1 ubiquitin activating enzyme was inactivated by pre-incubation of the cells at a non-permissive temperature (42.5 °C) for 2 h. Alternatively, cells were pre-treated for 2 h with MG132 by addition of the inhibitor directly to the culture medium at a concentration of 20 μM. Human monocytes were isolated from peripheral blood of healthy donors and enriched by negative selection using RosetteSep Monocyte Enrichment Cocktail (StemCell Technologies, Vancouver, British Columbia, Canada) according to the manufacturer's instructions. Monocytes were cultured overnight at 37 °C at 0.5×10^6 cells/ml in RPMI 1640 with 30 ng/ml of both IL-4 and IL-10 for upregulation of expression of FcγRIIB (Wijngaarden et al., 2004).

2.3. DNA constructs and transfection

Plasmids containing cDNAs for FcγRIIA (His131 variant) and FcγRIIB2 in pcDNA3.1/Myc-His for expression with C-terminal Myc-His tags were previously described (Mero et al., 2006; Zhang and Booth, 2010); the plasmid encoding His-tagged lysine-mutated FcγRIIA was a kind gift of Dr. Alan Schreiber (University of Pennsylvania). Transfections were performed with FuGENE 6 (Roche Applied Science) or Lipofectamine 2000 (Invitrogen) according to the manufacturers' instructions. Stable cell lines expressing FcγRIIA, FcγRIIB2 and mutant receptors were selected with G418 (0.5 mg/ml).

2.4. Endocytosis assays

Human IgG (10 mg/ml) was aggregated at 62 °C for 20 min in phosphate-buffered saline (PBS), followed by centrifugation at 16,000 × *g* for 10 min to precipitate insoluble IgG aggregates; supernatants containing soluble aggregates were used at 1:100 dilution to induce endocytosis. Internalization of aggregated IgG was assessed by microscopy. For quantitation of internalization of FITC conjugated-heat aggregated IgG (FITC-agIgG) by flow cytometry, FITC was conjugated to IgG (~4 FITC per IgG), then this FITC-IgG was mixed with unlabeled IgG at a ratio of 1:4 before heat aggregation. Cells were incubated with FITC-agIgG for 20 min on ice before transfer to 34 °C medium to initiate endocytosis. Surface FITC-agIgG was quenched with 0.1% trypan blue solution for 10 min at room temperature before fixation of the cells and analysis.

Alternatively, endocytosis was assayed by engaging receptors with anti-FcγR antibodies, followed by measuring disappearance of antibodies from the cell surface by flow cytometry, as previously described (Booth et al., 2002). Anti- FcγR antibodies (IV.3 or AT10) were bound to cells for 20 min at 4 °C, then cells were washed. Cells were then incubated with goat anti-mouse secondary antibodies for 20 min at 4 °C to cluster receptors, then washed and warmed to 34 °C to allow endocytosis. Receptor-expressing ts20 cells were detached from culture dishes, dispersed in PBS, washed, fixed with 2% paraformaldehyde and stained with Cy5 donkey anti-goat antibodies to follow the disappearance of antibodies from the cell surface. In some experiments no clustering antibody was added, in which case disappearance of AT10 from the cell surface was measured with Cy5-anti-mouse antibodies. For analysis of mean fluorescence intensities, background subtraction of fluorescence of untransfected cells was performed. For experiments with monocytes, endocytosis was performed at 37 °C and cells were stained with Cy5 anti-goat antibodies before fixation. Flow cytometry was performed using a FACSCalibur (Becton Dickinson).

2.5. Immunofluorescence and microscopy

Cells were washed, fixed with 4% paraformaldehyde for 30 min and then permeabilized with 0.1% Triton X-100 at room temperature for 20 min. In some experiments, heat aggregated IgG (agIgG) remaining on the cell surface was detected by incubating for 10 min on ice with fluorophore-labeled anti-human secondary antibodies before fixation. To detect FcγRs, cells were blocked with 5% BSA

Download English Version:

https://daneshyari.com/en/article/5917503

Download Persian Version:

https://daneshyari.com/article/5917503

Daneshyari.com