

TRIPEPTIDE OF FC $\gamma$ RIIA

This application claims priority from Provisional Application No. 60/252,460, filed Nov. 22, 2000, the entire content of which is incorporated herein by reference.

## TECHNICAL FIELD

The present invention relates, in general, to phagocytosis and phagolysosomal fusion and, in particular, to a tripeptide of Fc $\gamma$ RIIA that mediates trafficking of targets phagocytosed via Fc $\gamma$ RIIA to the lysosomal compartment.

## BACKGROUND

Phagolysosome fusion is an important pathway in the degradation of internalized particles. Once a particle is internalized by phagocytosis it is directed toward the lysosomal compartment for degradation. Various studies have traced this sequence of events from binding and phagocytosis to eventual trafficking to lysosomes. In addition, the signaling machinery needed to perform many of these activities has been described. Recently, intracellular tyrosine-based activation motifs (ITAM) have taken center stage in the initiation and propagation of activation signals of phagocytic receptors.

ITAM motifs contribute to the ability of phagocytic receptors to efficiently internalize particles (Tuijnman et al, Blood 79:1651 (1992), Mitchell et al, Blood 84:1753 (1994)). ITAM motifs are composed of two YXXL motifs separated by a string of various amino acids. This motif forms a SH-2 binding domain for docking of signaling proteins such as Src and Syk, among others (Isakov Immunol. Res. 16:85 (1997), Isakov, J. Leuko. Biol. 61:6 (1997)). Specifically, upon ITAM phosphorylation, Fc $\gamma$ RIIA has been shown to signal through Syk (Indik, et al, Blood 86:4389 (1995), Matsuda et al, Mol. Bio. Cell 7:1095 (1996)). In addition, mutation of either of the ITAM tyrosines abolishes the phagocytic activity of Fc $\gamma$ RIIA (Mitchell et al, Blood 84:1753 (1994)). These YXXL sequences can also associate with adaptor proteins such as AP-1 and AP-2 in forming clathrin cups during phagocytosis.

Once a target is internalized, it can be sent to the lysosomal compartment for degradation. Di-leucine motifs in the cytoplasmic domain of various receptors are responsible for the trafficking of targets from phagosomes to lysosomes (Mayorga et al, J. Biol. Chem. 266:6511 (1991), Hunziker and Fumey, EMBO J. 13:2963 (1994), Letournier and Klausner, Cell 69:1143 (1992)). This motif is present in many receptors such as Fc $\gamma$ RIIB, the LDL receptor, and the mannose 6-phosphate receptor (Matter et al, J. Cell Biol. 126:991 (1994), Johnson et al, J. Biol. Chem. 267:17110 (1992)). Mutation of either or both of the leucine residues in these receptors significantly reduces or abolishes lysosomal delivery, respectively.

Fc $\gamma$ RIIA mediates phagocytosis through an ITAM motif and also mediates phagolysosomal fusion (Mitchell et al, Blood 84:1753 (1994)). However, there is no consensus di-leucine motif located in the cytoplasmic domain of Fc $\gamma$ RIIA. Therefore, another sequence in the cytoplasmic domain of Fc $\gamma$ RIIA must participate in lysosomal trafficking. The present invention relates to that sequence.

## SUMMARY OF THE INVENTION

The present invention relates to a tripeptide of Fc $\gamma$ RIIA that mediates trafficking of targets phagocytosed via Fc $\gamma$ RIIA to the lysosomal compartment.

Objects and advantages of the present invention will be clear from the description that follows.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. A distinct Fc $\gamma$ RIIA cytoplasmic domain sequence determines phagolysosomal fusion. CHO cells were transfected with WT Fc $\gamma$ RIIA (WT IIA, column 2) or with mutants of the Fc $\gamma$ RIIA cytoplasmic ITAM. Wt IIA contains the ITAM sequence Y2MTL-Y3LTL. The Fc $\gamma$ RIIA mutants contain the following ITAM sequences: Y2MTL-Y3ATL (designated Y3ATL, column 3), Y2MTL-Y3LTA (designated Y3LTA, column 4), Y2MTL-Y3ATA (designated Y3ATA, column 5) or F2MTL-F3LTL (designated Y2FY3F, column 6) (Y=tyrosine, M=methionine, T=threonine, L=leucine, A=alanine, F=phenylalanine). After 48 hrs, the transfected cells were loaded with rhodamine conjugated dextran and then incubated with IgG coated RBCs (EA). Following removal of externally bound EA, the phagocytic index (PI), the number of internalized EA/100 cells, was determined by bright field microscopy. Lysosomes labelled with rhodamine conjugated dextran were visualized by fluorescence microscopy. Phagolysosome fusion was analyzed by determining the co-localization of EA and rhodamine dextran and expressed as % co-localization. Column 1 represents sham transfected cells.

Mutation of either or both leucines in the Y3LTL sequence of the Fc $\gamma$ RIIA ITAM inhibits phagolysosomal fusion but does not inhibit phagocytosis of EA. It has been previously demonstrated that Fc $\gamma$ RIIA in the absence of ITAM tyrosines (Y2FY3F) does not mediate phagocytosis. However, phagocytosis of EA is partially restored for Y2FY3F by co-transfection with the complement receptor type 3 (CR3) (Worth et al, J. Immunol. 157:5660-5665 (1996)) as demonstrated in column 6. In co-transfected cells, Y2FY3F and CR3 interact and EA bound to Y2FY3F are phagocytosed through the cytoplasmic domain of CR3. 78% of the ingested EA mediated by CR3 and Y2FY3F co-localized with lysosomes (column 6), indicating that the ITAM tyrosines do not play a significant role in phagolysosomal fusion. Significant inhibition of phagolysosomal fusion (p<0.001) was observed for the mutants Y3ATL, Y3LTA and Y3ATA, while the ingestion of EA (phagocytosis) was unaltered (columns 3-5). Thus the LTL sequence of the Fc $\gamma$ RIIA cytoplasmic domain targets the phagosome for fusion with lysosomes whereas the tyrosines of the ITAM sequence are essential for the initial stage of phagocytosis.

FIG. 2. Mutation of the novel L-T-L motif in the cytoplasmic domain of Fc $\gamma$ RIIA inhibits phagolysosome fusion.

FIG. 3. L-T-L motif mediates specific targeting of internalized targets to fuse with lysosomes.

FIG. 4. L-T-L motif inhibits fusion events leading to phagolysosome formation but not protein colocalization.

FIG. 5. Inserting the L-T-L motif into a receptor that normally does not mediate efficient phagolysosome formation increases the ability to form phagolysosomes.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the realization that the cytoplasmic domain of Fc $\gamma$ RIIA mediates lysosome fusion subsequent to phagocytosis. This L-T-L motif is found at the C-terminal of the ITAM motif of Fc $\gamma$ RIIA.

Chinese hamster ovary (CHO) cells provide a good model system for studying phagocytosis and intracellular traffick-