Identification of TOSO/FAIM3 as an Fc receptor for IgM

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Transmitting Editor: T. Kurosaki
Received 22 October 2009, accepted 18 November 2009

Abstract

Fc receptors specifically bind to the Fc region of Igs to mediate the unique functions to each class of Igs. To identify a novel Fc receptor for IgM, we searched expressed sequence tag database for molecules containing Ig domains with homology to those of known Fc receptors for IgM, Fc\textsubscript{a}/\mu R and polymeric Ig receptor. As a result, we identified TOSO/Fas apoptotic inhibitory molecule 3 (FAIM3) as a possible Fc receptor for IgM. HeLa cells transfected with a TOSO/FAIM3-expression vector bound to IgM but not IgG and were able to internalize IgM-conjugated beads but not IgG-conjugated beads, suggesting that TOSO/FAIM3 is indeed a receptor for IgM (Fc\textsubscript{M}R). Fc\textsubscript{M}R protein was expressed predominantly on B-lineage cells; expression of the Fcmr transcripts was observed from the pre-B-cell stage and maintained thereafter during B-cell development. These results identify TOSO/FAIM3 as a receptor for IgM and suggest that Fc\textsubscript{M}R may serve as an uptake receptor for IgM-opsonized antigens by B cells.

Keywords: B cell, Fc receptor, IgM, TOSO/FAIM3

Introduction

Antibodies define humoral immunity and translate specific antigen recognition to diverse effector functions in the immune system. There are two major functional domains in Igs that were originally defined by proteolytic digestion studies, the Fab (fragment antigen binding) and the Fc (fragment crystallizable). The Fab fragment determines the antigen specificity for a given Ig, whereas the Fc fragment confers antibody effector functions, e.g. by binding to cellular Fc receptors. Igs are divided into five main classes in mammals based on the differences in amino acid sequence of their Fc fragments, namely IgM, IgG, IgA, IgE and IgD. Fc receptors have been identified that specifically recognize each class of Ig, except for IgD. Each Fc receptor is distinctly distributed mainly among hematopoietic cells and has been shown to possess unique immunological functions (1–3).

Among them, two receptors have been shown to bind to IgM, namely Fc\textsubscript{a}/\mu R (4) and the polymeric Ig receptor (plgR) (5, 6). Both Fc\textsubscript{a}/\mu R and plgR can bind to IgA and IgM with intermediate affinity, and the genes encoding these receptors are present within the same chromosomal region on chromosome 1 both in humans and mice. Fc\textsubscript{a}/\mu R is expressed by mature B cells, macrophages and follicular dendritic cells in the secondary lymphoid organs such as lymph nodes and Peyer’s patches in the intestine and is suggested to possess a range of immunoregulatory function (4, 7, 8). On the contrary, plgR is predominantly expressed on the basolateral plasma membrane of mucosal epithelial cells and plays a role in the delivery of secretory IgA and IgM across the epithelial layer to the mucosal lumen (6, 9, 10). Although the existence of an Fc receptor uniquely specific for IgM has long been suggested (1,11–22), its genetic identity has not been elucidated.

In search of as yet unidentified Fc receptors for IgM, we took advantage of large databases of expressed sequence tags (ESTs) to identify genes encoding Ig domains with homology to those of plgR and Fc\textsubscript{a}/\mu R. In this paper, we report the identification and molecular characterization of TOSO/Fas apoptotic inhibitory molecule 3 (FAIM3) as a novel IgM receptor (Fc\textsubscript{M}R).
**Methods**

**Animals**

C57BL/6J mice of 7–10 weeks old were obtained from CLEA Japan. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals in RIKEN.

**Complementary DNA cloning and plasmid vector construction**

Human and mouse TOSO/FAIM3 complementary DNAs (cDNAs) were obtained from Integrated Molecular Analysis of Genomes and their Expression consortium (cDNA clone ID: 4852791) and RIKEN FANTOM (clone accession number: AK007714), respectively. Mouse FcαR cDNA was cloned by PCR amplification of oligo-dT-primed spleen cDNA of C57BL/6J mice. Mammalian expression vectors for C-terminally hemagglutinin (HA)-tagged FAIM3 and FcαR were constructed into BamHI and XhoI sites of pcDNA3-HAC, as described previously (23).

To establish mAbs against FAIM3, a cDNA fragment corresponding to the extracellular domains of mouse FcαR was amplified by PCR using primers 5′-GCAGATCTAGAAGTCCTCCAGAAGTAC-3′ and 5′-CTACTCGAGAATTCTGGGATGTTCACTCCCAGAAGTAC-3′. The obtained cDNA fragment was inserted into the BamHI/XhoI cloning sites of a modified pcDNA3 expression vector containing an XhoI/XbaI fragment encoding the Fc segment of human IgG1 (pcDNA3-mFcαR-Fc).

**Antibodies**

The mFcαR–Fc fusion protein was purified with a protein A Sepharose column from culture supernatant of HEK293T cells transfected with pcDNA3-mFcαR-Fc. Anti-mouse FcαR mAbs were generated by fusing the P3U1 myeloma cell line with splenocytes from C57BL/6 mice immunized by injecting into their footpads the purified mFcαR–Fc protein as an antigen. The specificity of the FcαR mAb was confirmed by surface staining of control HeLa or HeLa cells transfected with an FcαR expression vector, as well as by staining of B cells from wild type and FcαR-knockout mice.

**Screening of a novel Fc receptor**

To establish mAbs against FAIM3, a cDNA fragment corresponding to the extracellular domains of mouse FcαR was amplified by PCR using primers 5′-GCAGATCTAGAAGTCCTCCAGAAGTAC-3′ and 5′-CTACTCGAGAATTCTGGGATGTTCACTCCCAGAAGTAC-3′. The obtained cDNA fragment was inserted into the BamHI/XhoI cloning sites of a modified pcDNA3 expression vector containing an XhoI/XbaI fragment encoding the Fc segment of human IgG1 (pcDNA3-mFcαR-Fc).

**Ig uptake assay**

Mouse Igα, IgA, IgM and IgG, were covalently conjugated with yellow–green fluorescent 0.2-μm microsphere beads (F-8811, Molecular Probe) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide as a catalyst. The detailed protocol is available on the manufacturer’s website: http://www.probes.invitrogen.com/media/pis/mp05001.pdf. HeLa cells were transfected with the expression vectors for human and mouse FcαR tagged with the C-terminal HA-epitope, using FuGene6 transfection reagent. As a control, mouse FcαR was also transfected. The transfected cells were cultured for 24 h and then incubated for 1 h at 37°C with mouse Ig-conjugated microbeads. The cells were washed twice with PBS and fixed with 4% paraformaldehyde/PBS for 30 min at room temperature and then permeabilized with 0.1% Triton X-100/ PBS and incubated for 1 h at room temperature with anti-HA (3F10) antibody. The cells were washed twice with PBS and stained with Cy3-conjugated anti-rat IgG secondary antibody. The stained cells were observed with a DM-IRE2 confocal laser scanning microscope and Leica confocal software (Leica Microsystems).

**Flow cytometric analysis**

Mouse splenocytes were incubated in FACS buffer (2% FCS in PBS) with anti-CD 16/32 (clone 94) for 15 min and then the cells were stained with anti-mouse FcαR mAb (clone #4B5) in combination with FITC- or PE-conjugated antibodies in combination with PECy7-conjugated streptavidin. The stained cells were analyzed using a FACS Calibur with CellQuest software (BD Biosciences).

**Results**

**Screening of a novel Fc receptor**

We used a bioinformatics approach to identify novel Fc receptors for IgA and/or IgM. We performed EST database searches to identify genes containing Ig domains with homology to the Ig domain of plgR, FcαR and CD300 antigen-like family member G (CD300LG), another Ig receptor-like small intestine, testis, spleen, thymus, lymph node and bone marrow (BM), mouse spleen subpopulation (CD3+, B220+), CD11b+ or CD11c+ isolated using a MACS column (Miltenyi Biotech) and mouse B cell lines (pro-B cells, Ba/F3; pre-B cells, 18.81 and 70Z/13; immature B cells, CH31; mature B cells, A20/2J and WEHI279). The first-strand cDNA was synthesized with ReverTra Ace-α (TOYOBO) using oligo-dT30 primer. The pro-B, pre-B and immature B cells were sorted from BM as described previously (24). The germinal center B, follicular B, marginal zone B and the newly formed B cells were sorted as described (25).

All procedures were performed in accordance with the according to the manufacturer's instructions. Primers used for amplification of mouse FcβR cDNA fragments were as follows: 5′-CTCGGATCCATTATCATCGAATGCC-3′ and 5′-ATACTCGA GCTGGGAGTTCACTCC-3′. glyceraldehyde-3-phosphate dehydrogenase primers were described elsewhere (26).

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protein exclusively expressed on capillary endothelium (23). As a result, TOSO/FAIM3 was found to possess an Ig domain that shared ~36, 36 and 29% amino acid identities with those of pIgR, FcμR and CD300LG proteins, respectively (Fig. 1A and Table 1). In addition, the mouse TOSO/FAIM3 protein possessed 57.7% homology with human TOSO/FAIM3 protein (Fig. 1B), whereas the coding region of mouse Toso/FAIM3 gene shared 70.2% homology with that of human TOSO/FAIM3 gene (data not shown). Multiple serine and tyrosine residues are contained in the cytoplasmic region and may play a role in signal transduction by the FcμR. Several asparagine (N) residues are also present but do not match the consensus N-X-S/T motif known to be the preferred substrate for N-glycosylation (27) and it remains to be determined whether these residues actually undergo glycosylation. Furthermore, we found that the TOSO/FAIM3 gene is clustered with PIGR and FCAMR on chromosome 1 both in human and mouse (Fig. 1C), in support of the possibility that Toso encodes an Fc receptor. In addition, Toso encodes a 60-kDa type I integral membrane protein with an Ig-like domain. Taken

![Fig. 1. Molecular characterization of TOSO/FcμR. (A) Multiple alignment of the amino acid sequences of Ig domains from mouse pIgR, FcμR, CD300LG and TOSO/FcμR. (B) Comparison of amino acid sequence of human and mouse FcμR. Signal peptide, Ig domain and transmembrane domain are depicted with blue dotted line, red line and box, respectively. Conserved residues are shaded blue. (C) FCAMR is clustered with PIGR and FCAMR in the same locus on chromosome 1 in both mouse and human.](https://academic.oup.com/intimm/article-abstract/22/3/149/790983/visuals)
Identification of TOSO as an IgM receptor

Table 1. Homologies among each Ig domain from mouse TOSO/Fc\(\mu\)R, plgR, Fc\(\alpha/\mu\)R and CD300LG

<table>
<thead>
<tr>
<th>mTOSO/Fc(\mu)R</th>
<th>mplgR</th>
<th>mFc(\alpha/\mu)R</th>
<th>mCD300LG</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTOSO/Fc(\mu)R</td>
<td>100</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>mplgR</td>
<td>100</td>
<td>43</td>
<td>36</td>
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<tr>
<td>mFc(\alpha/\mu)R</td>
<td>100</td>
<td></td>
<td>36</td>
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<tr>
<td>mCD300LG</td>
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Discussion

In the present study, we have identified TOSO/FAIM3 as an Fc\(\mu\)R. Fc\(\mu\)R is a type 1 integral membrane protein with a single Ig domain in its extracellular region. Similar homologies of 36–43% at the protein level are conserved among the Ig domains of mouse Fc\(\mu\)R and other known mouse Fc receptors for IgM: plgR and Fc\(\alpha/\mu\)R. Interestingly, homology is slightly lower between mouse Fc\(\mu\)R and mouse CD300LG, which was also identified by homology searches with plgR and Fc\(\alpha/\mu\)R as probes and also exhibits IgA/IgM binding (23). Exogenously expressed mouse and human Fc\(\mu\)R can mediate binding to soluble IgM, but show only a marginal or no binding to IgA or IgG, respectively. Furthermore, Fc\(\mu\)R-expressing HeLa cells specifically internalize IgM-conjugated microbeads. These observations demonstrate that Fc\(\mu\)R is an Fc receptor specific for IgM and further implicate its role as an uptake receptor for IgM-opsonized pathogens, similar to Fc\(\alpha/\mu\)R (4). Indeed, Kubagawa et al. (28), in collaboration with us, has recently shown that human TOSO/FAIM3 is a bona fide Fc\(\mu\)R. Although Fc\(\mu\)R did not mediate uptake of IgA-conjugated microbeads, it appeared to show marginal...
binding to soluble IgA by FACS analysis. Further studies are required to resolve this discrepancy.

Mouse FcμR is predominantly expressed on B cells with no apparent expression on T cells freshly isolated from mouse spleen. Furthermore, mouse *FcμR* transcripts were barely detectable in pro-B cells, the earliest stage in B-cell differentiation, and appear at the pre-B-cell stage with an apparently gradual up-regulation during B-cell maturation.
These expression patterns of mouse FcR during B-cell development and differentiation implicate a potential role for FcR in B-cell maturation and/or function. It is also known that chronic lymphocytic leukemia cells express increased levels of FcR compared with healthy B cells (29) and that its expression in the leukemia cells is controlled by B-cell receptor signaling (30). These observations may suggest a role for FcR in B-cell receptor signaling and/or B-cell activation as well.

FcR was initially identified as an inhibitor for Fas-mediated apoptosis when over-expressed in T cells (31). Subsequently, inhibition of Fas-mediated apoptosis was shown to involve binding of the cytoplasmic region of FcR with Fas-associated death domain (32). FcR was also shown to be involved in IL-2-mediated activation-induced cell death in human T cells (33). These observations suggest that FcR could protect human T cells from Fas-mediated apoptosis under certain conditions. However, we have recently found that human FcR itself has no inhibitory activity in Fas-mediated apoptosis when an anti-Fas antibody of an IgG isotype is used (28). The inhibition is only achieved with IgM isotype anti-Fas antibody, strongly suggesting that the simultaneous binding of the anti-Fas antibody of IgM isotype to both Fas and FcR affected the apoptosis-inducing activity. It is of note that human FcR is expressed on T cells in addition to B cells (28). Therefore, the expression pattern of FcR is not identical between human and mice, which may suggest species-specific functions. However, it is possible that mouse T cells may express FcR under certain conditions, for example, upon activation. Further studies are required to clarify the differential regulation of FcR expression in B and T cells.

It has also been reported that enzymatically modified low-density lipoprotein up-regulates FAIM3 (FCMR) transcript levels in human macrophages and enhances the survival of these cells (34). Consistent with this observation, we detected Fcmr transcripts in macrophages and dendritic cells derived from mouse spleen, although no surface expression of FcR on these cells could be detected (data not shown). It is possible that FcR expression in these cells is regulated both at the transcriptional and posttranscriptional levels and that certain activation signals may induce cell surface FcR expression. A future issue to be resolved is whether IgM binding to FcR modulates the survival of these cells upon activation. Recently, we have successfully established FcR-knockout mice, which are fertile and show no grossly apparent abnormalities. This is in contrast to the finding by Song et al. (32) who indicated in the discussion of their paper that FcR deficiency is embryonically lethal. Detailed analyses of these mice are now in progress and will
uncover the physiological roles of FcμR in B cells as well as other hematopoietic cells.

Supplementary data
Supplementary Figures 1 and 2 are available at *International Immunology* Online.

Funding
Grant-in-Aid for Scientific Research on Priority Areas ‘Membrane Traffic’ (15079203 to H.O.), Young Scientists (B) (17790335 to H.T.), Scientific Research (B) (19390143 to H.O.) and Scientific Research on Innovative Areas ‘Intracellular Logistics’ (20113003 to H.O.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Acknowledgements
We would like to thank Drs T. Murakami, A. Yoshino and K. Kawano for critical discussions; Dr S. Seki for technical support; Y. Yamada for secretarial assistance. The authors have no conflicting financial interests.
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