Published in final edited form as:

Clin Immunol. 2009 October ; 133(1): 138–144. doi:10.1016/j.clim.2009.06.002.

The Mer receptor tyrosine kinase is expressed on discrete macrophage subpopulations and mainly uses Gas6 as its ligand for uptake of apoptotic cells

Wen-Hai Shao^{*}, Yuxuan Zhen^{*}, Robert A. Eisenberg[†], and Philip L. Cohen^{*}

^{*}Department of Medicine, Division of Rheumatology, Temple University, Philadelphia, PA 19140

[†]Department of Medicine, Division of Rheumatology, University of Pennsylvania, Philadelphia, PA 19104

Abstract

The Mer receptor tyrosine kinase is both an important mediator of apoptotic cell phagocytosis and a regulator of macrophage and DC cytokine production. Since phenotypically distinguishable macrophages are known to have different functions, we have examined Mer expression of murine splenic macrophages. We also used serum deficient in the Mer ligand, growth arrest-specific protein 6 (Gas6) to define better the role of this Mer ligand in macrophage function. By immunofluorescence staining, we found Mer to be strongly expressed in splenic red pulp, largely on platelets. We also found Mer expression on marginal zone macrophages. Strikingly, all tingible body macrophages bore Mer. In functional phagocytosis assays of apoptotic cells, Gas6 appeared to be the sole ligand for Mer, and this system accounted for about 30% of splenic macrophage phagocytosis of apoptotic cells. Taken together, the expression pattern of mer on macrophage subpopulations in the spleen and its Gas6-dependent role in macrophage phagocytosis suggest an important role for Mer in the modulation of immune responses.

Keywords

Mer; apoptotic cell clearance; macrophage; phagocytosis; Gas6

Introduction

Clearance of apoptotic cells through phagocytosis plays an important role during embryogenesis and normal tissue homeostasis. The Mer receptor tyrosine kinase (RTK) is an important receptor for apoptotic cells and is essential for their normal clearance [1;2]. Mer belongs to the Tyro-3 subfamily of RTK, with a conserved KW(I/L)A(I/L)ES signature sequence in the kinase domain. Its expression is limited to certain hematopoietic lineages, namely monocytes/macrophages, dendritic cells (DC), NK cells, megakaryocytes, platelets, and, as we have shown recently, certain activated B cells [3;4;5]. The expression pattern of Mer in the immune system and its function in modulating inflammatory responses have

Address correspondence and reprint requests to Dr. Philip L. Cohen, Section of Rheumatology, Temple University, 3420 N. Broad St., Rm 709, Philadelphia, PA 19140. E.mail address: philco@temple.edu. Phone: 215-707-5660. Fax: 215-707-3508.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

received much attention lately [2;6;7;8], but its expression in intact organs of the immune system is not well understood.

The principal ligand for Mer is believed to be growth arrest-specific protein 6 (Gas6), a member of the vitamin K-dependent protein family. Gas6 binds and activates all three receptors in the Tyro-3 subfamily [9;10;11;12;13], but has a remarkably lower affinity for Mer (3–10-fold lower) [10;14]. Gas6 shares ~40% amino acid identity and has a similar domain organization with protein S, a negative regulator of blood coagulation [15]. Gas6 has been extensively studied and repeatedly reported to bind and activate Mer [16], yet the role of protein S as an additional ligand for Mer remains unclear [1;5]. Human protein S was initially reported to activate murine Tyro-3 [9;17]. More recently, Uehara et al found that human protein S oligomerization is required for human Mer autophosphorylation in a culture system using fetal serum [17].

To understand better the function of Mer in the regulation of immune system in lymphoid organs, we investigated the expression pattern of Mer in the mouse spleen and the contribution of Gas6 in mediating Mer-mediated macrophage phagocytosis. We found that Mer was present on all CD68⁺ macrophages and on a fraction of MZ (marginal zone) macrophages, but not on metallophilic macrophages. Interestingly, Mer mediated macrophage phagocytosis was mediated mainly through the soluble bridge ligand Gas6.

Materials and Methods

Materials

Biotin conjugated anti-mouse Mer antibody and recombinant mouse Gas6 (rmGas6) were purchased from R&D Systems (Minneapolis, MN). Anti-mouse FITC-CD11b, PE-CD11b, FITC-B220, PE-CD4, FITC-CD61, and FITC-CD11c were from BD Biosciences (San Jose, CA). Macrophage specific antibodies, PE-MARCO, FITC-MOMA-1, and PE-CD68 were obtained from Serotec Inc. (Raleigh, NC).

Mice

C57BL/6J (B6) mice (Jackson Laboratories, Bar Harbor, ME) were used as WT controls. Mer-KO mice were generated as described previously [8] and were 10 generations backcrossed to B6 mice. The Gas6-KO mice were generated by Dr. Carmeliet [18] and were subsequently bred and maintained in our mouse colony at the University of Pennsylvania or at Temple University. Recipient and donor mice were sex and age matched within each independent experiment. All of the experimental procedures performed on these animals were conducted according to the guidelines of the Institutional Animal Care and Use Committee.

Immunofluorescent staining

Mouse spleen was snap-frozen in liquid nitrogen. Sections (5 μ m) were air-dried for 10 minutes, followed by 30 minutes incubation with 1% BSA in PBS. Sample sections were then blocked with 3% BSA in PBS. Mer expression was detected by overnight incubation with biotin-labeled anti-Mer at 4°C and visualized with PE-streptavidin or FITC-streptavidin. All other antibodies were incubated with tissue sections for 4–6 hrs at 4°C. Slides were mounted in Anti-fading Aqueous Mounting Medium (Biomedica Corp., Foster City, CA), and images were acquired using an Olympus BX60 fluorescence microscope equipped with camera (Center Valley, PA).

Preparation of peritoneal macrophages

B6 and Mer-KO mice were injected i.p. with 1 ml of 3% thioglycolate (in 1 $\times$ PBS, 3 months aged in the dark). Peritoneal macrophages were collected at day 5 with wash buffer (RPMI1640 with 2% FBS and 0.2 mM EDTA). 500 μ l cell suspensions were placed in 24-well plates at a

concentration of 2×10^6 cells/ml in complete medium (RPMI with 10% FBS, 1% penicillin/streptomycin, 1% sodium pyruvate, and 0.1% 2-ME). After 2hrs, nonadherent cells were washed off with PBS and the remaining cells used for phagocytosis assay.

Mouse sera preparation and heat-inactivation

Mouse blood samples from Gas6-KO and WT mice were collected by intracardiac puncture terminal bleeding under anesthesia. Mouse sera were collected after overnight clotting at 4°C. Sera were then stored at -20°C until further assay. Heat-inactivation was performed by incubating serum samples at 56°C for 30 minutes.

Macrophage in vitro phagocytosis

Single-cell thymocyte suspensions were made by pushing thymi from 4–6 weeks old B6 mice through cell strainers (50µm). After washing twice with PBS, cells were labeled with CFSE (Invitrogen, Carlsbad, CA), and then rendered apoptotic by exposure to 500 Rads of γ -irradiation followed by 4 hrs culture in complete RPMI medium. Apoptosis was verified using an Annexin-V/7-AAD Apoptosis detection kit (BD Pharmingen, San Diego, CA) [3]. Apoptotic thymocytes were co-cultured for 4 hrs with phagocytes at various ratios as indicated. The phagocytes were extensively washed with PBS containing EDTA to remove physically bound but not internalized apoptotic cells. Percentages of macrophages that had ingested labeled thymocytes were determined by FACS analysis, with gating on CD11b-PE and CFSE.

FACS analysis

Cell surface staining was routinely performed as previously described [19]. Cells were blocked with 2.4G2 (anti-Fc receptor monoclonal), followed by direct incubation with labeled Abs for 40-min and washing. An additional 30-min incubation with streptavidin-PE was performed to detect biotinylated Abs. Cells were fixed in PBS containing 1% paraformaldehyde and were then analyzed on a BD Biosciences FACScan (Mountain View, CA). Relative fluorescence intensity was plotted on a logarithmic scale using CellQuest software.

Statistical analysis

Data are presented as means $\pm$ SD. Statistical significance was determined using Student's *t* test.

Results

Mer is expressed on splenic platelets, DCs, and macrophages

We have previously reported the expression pattern of Mer in the hematopoietic lineages [3]. We confirmed that Mer-KO mice (with the tyrosine kinase domain replaced by a *neo* cassette) fully disrupted surface expression of Mer on activated peritoneal macrophages [8] (Figure 1). We, therefore, used Mer-KO mice as negative controls to study the expression pattern of Mer in the spleen. As shown in Figure 2, the most intense expression of Mer was found in red pulp. This expression was colocalized to platelets (Figure 2C). Consistent with previous findings [3;4], Mer was not expressed on T- or B- cells (Figure 2A and B). Dendritic cells (DC) and macrophages (M ϕ) expressed Mer on their surfaces (Figure 2D and E).

Mer is expressed on tingible body macrophages and on some but not all MZ macrophages

Within the hematopoietic lineage, the highest expression of Mer was shown on macrophages [4]. Macrophages play an essential role in apoptotic cell clearance and antigen capture and presentation. We identified different subpopulations of macrophages using unique markers, namely MARCO for MZ macrophages, MOMA for metallophilic macrophages, and CD68 for tingible body macrophages. Strikingly, all CD68⁺ macrophages also expressed Mer (Figure

3A). We found a small portion of MZ macrophages expressed Mer (Figure 3B). No overlap was shown on MOMA positive macrophages (Figure 3C).

Mouse serum dependent phagocytosis is heat sensitive

Because mouse serum is generally not used to evaluate the efficiency of phagocytosis of mouse macrophages, it was necessary to test the phagocytosis efficiency using different concentrations of mouse serum. We found that 10% mouse serum in the assay was needed for efficient macrophage phagocytosis, as in other assay systems [7] (Figure 4A). Therefore, we use 10% mouse serum for the rest of assays. This concentration also makes our results comparable to published data. On the other hand, heat inactivation led to a ~20% reduction in overall macrophage phagocytosis (Figure 4B left), presumably due to denaturation of complement components in the fresh serum. The relative decrease in phagocytosis that resulted from heat-inactivation of serum was not significantly different for WT and Mer-KO macrophages. FBS-mediated macrophage phagocytosis was unchanged when heat-inactivated serum was used (Figure 4B right), probably reflecting the absence of active complement in the stored FBS.

Mer-mediated macrophage phagocytosis is Gas6-dependent

Protein S was reported as a ligand for Mer mediated signaling [9]. We set out to test whether mouse protein S might also facilitate macrophage phagocytosis through Mer. We collected peritoneal macrophages 5-days after i.p. thioglycolate. Macrophages (CD11b⁺) that ingested apoptotic cells were analyzed by FACS after 4-hr co-culture with apoptotic cells. As shown in Figure 5A, macrophage phagocytosis was reduced to a similar degree (compared to wild type cells in normal serum), when either Gas6 was absent or when cells lacked the receptor Mer. The reduction of macrophage phagocytosis was apparent at all ratios tested of macrophages to thymocytes. We further confirmed our findings using the combination of Mer-KO macrophages and Gas6-KO serum in the assay with the 4M of apoptotic cells (Figure 5B). Data are thus consistent with the notion that Gas6 is the major ligand for Mer-mediated phagocytosis.

Mer mediated macrophage phagocytosis can be blocked by anti-Mer antibody

We tested the blocking function of goat anti-mouse polyclonal antibody (R&D system) in mouse serum dependent phagocytosis, in order to explore the potential therapeutic application of anti-Mer antibody in Mer-mediated mouse disease model. Figure 6 shows this antibody could block phagocytosis by WT macrophages down to the level seen with Mer-KO macrophages.

Discussion

Identifying the expression of Mer among macrophage subpopulations in lymphatic organs will aid in understanding the role of Mer in the clearance of apoptotic cells, including lymphocytes. We have shown that Mer-KO mice develop a lupus-like autoimmune syndrome and splenomegaly. In addition, excessive apoptotic cells and cell debris were observed in Mer-KO mice spleens after exogenous infusion of apoptotic cells [8;20]. Therefore, the anatomical localization and expression pattern of Mer in spleen are of particular interest. Mer seems to be universally expressed among CD68-bearing macrophages — so-called tingible body macrophages. These cells have been reported to be of critical importance in engulfing apoptotic debris, and, in lymph nodes from SLE patients, these cells have a marked deficiency in uptake of apoptotic cells [21]. CD68⁺ tingible body macrophages are found in splenic germinal centers, where they engulf newly generated apoptotic B cells. Our data suggest that Mer may be of special importance in the elimination of apoptotic B cells generated in the processes of negative selection and affinity maturation. In the absence of Mer, the build-up of these apoptotic B cells, which express nuclear antigens and other self proteins, may spur the development of

autoimmunity by providing an antigenic stimulus for nearby autoreactive B cells. Together with the overactivated macrophages found in Mer-deficient mice, the potentially immunogenic apoptotic B cells may stimulate the spontaneous development of autoimmunity. Another important receptor/ligand system that facilitates uptake of apoptotic debris, milk fat globule-EGF factor 8 (MFG-E8)/lactadherin, is also centered on these tingible macrophages [22], reinforcing the notion that they are central in apoptotic debris clearance.

The splenic marginal zone is the first area of the spleen encountered by circulating antigens and is believed to be of central importance in capturing antigens. Further, MZ B cells have been reported to contribute disproportionately to the production of autoantibodies, supporting a crucial role for the MZ in the genesis of autoimmunity. Given the importance of Mer in phagocytosis of apoptotic debris and in regulating macrophages activation, we were surprised to observe that Mer was not present on the metallophilic macrophages that are so important in the MZ. Instead, Mer was expressed on a small portion of MZ macrophages, which may represent a previously unrecognized MZ macrophage population. A proportion of these Mer⁺ MZ macrophages expresses MARCO, and may be functionally distinguishable from other MZ macrophages. The exact function of MARCO is unknown, but it has been suggested that it facilitates cell adhesion and shape change [23]. Recently, a role for MARCO in the clearance of apoptotic cell was reported [24]. Co-expression of Mer with MARCO might be necessary for the engulfment and antigen presentation of specific pathogens.

We addressed another important issue concerning how Mer functions in macrophages in vivo. This concerned the physiological ligand for Mer. While Gas6 was first identified as a ligand for Axl and subsequently a common ligand for all three members (Tyro-3, Axl, and Mer) within the same subfamily [5], it is unclear whether this molecule is the only ligand and a strong case has been made for human protein S as an alternative ligand [17]. A difficulty has been that all studies were done in a culture system involving bovine serum. Considering the cross-species activation of receptor by protein S [9], care must be taken when interpreting data. Our data, using murine serum from wild type and from Gas6-KO mice, indicate that Gas6 is indeed the major ligand for Mer-mediated phagocytosis. Gas6-KO mice do not develop autoimmune disease (our unpublished data), despite their apparent inability to phagocytose apoptotic cells through Mer. This may indicate that Mer signaling for regulation of macrophage cytokine production may proceed through alternative pathways, viz., protein S, while phagocytosis of apoptotic cells through Mer largely depends on Gas6. These studies will require confirmation in other systems, but underline the complexity of the signaling through Mer and other related molecules in regulation of innate and adaptive immunity.

Acknowledgments

We thank Dr. Peter Carmeliet at Katholieke University Leuven, Belgium, for providing Gas6 knockout mice. This work was supported by grants from the NIDCR and the US Department of Veterans Affairs.

Abbreviations

Gas6	growth arrest-specific protein 6
rmGas6	recombinant mouse Gas6
B6	C57BL/6

References

1. Lemke G, Rothlin CV. Immunobiology of the TAM receptors. *Nat Rev Immunol* 2008;8:327–36. [PubMed: 18421305]
2. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 2001;411:207–11. [PubMed: 11346799]
3. Behrens EM, Gadue P, Gong SY, Garrett S, Stein PL, Cohen PL. The mer receptor tyrosine kinase: expression and function suggest a role in innate immunity. *Eur J Immunol* 2003;33:2160–7. [PubMed: 12884290]
4. Graham DK, Bowman GW, Dawson TL, Stanford WL, Earp HS, Snodgrass HR. Cloning and developmental expression analysis of the murine c-mer tyrosine kinase. *Oncogene* 1995;10:2349–59. [PubMed: 7784083]
5. Linger RM, Keating AK, Earp HS, Graham DK. TAM receptor tyrosine kinases: biologic functions, signaling, and potential therapeutic targeting in human cancer. *Adv Cancer Res* 2008;100:35–83. [PubMed: 18620092]
6. Wallet MA, Sen P, Flores RR, Wang Y, Yi Z, Huang Y, Mathews CE, Earp HS, Matsushima G, Wang B, Tisch R. MerTK is required for apoptotic cell-induced T cell tolerance. *J Exp Med* 2008;205:219–32. [PubMed: 18195070]
7. Seitz HM, Camenisch TD, Lemke G, Earp HS, Matsushima GK. Macrophages and dendritic cells use different Axl/Mertk/Tyro3 receptors in clearance of apoptotic cells. *J Immunol* 2007;178:5635–42. [PubMed: 17442946]
8. Camenisch TD, Koller BH, Earp HS, Matsushima GK. A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock. *J Immunol* 1999;162:3498–503. [PubMed: 10092806]
9. Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Mattsson K, Fisher J, Gies DR, Jones PF, et al. The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. *Cell* 1995;80:661–70. [PubMed: 7867073]
10. Chen J, Carey K, Godowski PJ. Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation. *Oncogene* 1997;14:2033–9. [PubMed: 9160883]
11. Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG. Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. *Cell* 1995;82:355–8. [PubMed: 7634325]
12. Mark MR, Chen J, Hammonds RG, Sadick M, Godowsk PJ. Characterization of Gas6, a member of the superfamily of G domain-containing proteins, as a ligand for Rse and Axl. *J Biol Chem* 1996;271:9785–9. [PubMed: 8621659]
13. Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H, Mizuno K. Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. *J Biol Chem* 1996;271:30022–7. [PubMed: 8939948]
14. Fisher PW, Brigham-Burke M, Wu SJ, Luo J, Carton J, Staquet K, Gao W, Jackson S, Bethea D, Chen C, Hu B, Giles-Komar J, Yang J. A novel site contributing to growth-arrest-specific gene 6 binding to its receptors as revealed by a human monoclonal antibody. *Biochem J* 2005;387:727–35. [PubMed: 15579134]
15. Dahlback B, Villoutreix BO. Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. *Arterioscler Thromb Vasc Biol* 2005;25:1311–20. [PubMed: 15860736]
16. Sasaki T, Knyazev PG, Clout NJ, Cheburkin Y, Gohring W, Ullrich A, Timpl R, Hohenester E. Structural basis for Gas6-Axl signalling. *EMBO J* 2006;25:80–7. [PubMed: 16362042]
17. Uehara H, Shacter E. Auto-oxidation and oligomerization of protein S on the apoptotic cell surface is required for Mer tyrosine kinase-mediated phagocytosis of apoptotic cells. *J Immunol* 2008;180:2522–30. [PubMed: 18250462]
18. Angelillo-Scherrer A, de Frutos P, Aparicio C, Melis E, Savi P, Lupu F, Arnout J, Dewerchin M, Hoylaerts M, Herbert J, Collen D, Dahlback B, Carmeliet P. Deficiency or inhibition of Gas6 causes

- platelet dysfunction and protects mice against thrombosis. *Nat Med* 2001;7:215–21. [PubMed: 11175853]
19. Reap EA, Felix NJ, Wolthuisen PA, Kotzin BL, Cohen PL, Eisenberg RA. bcl-2 transgenic Lpr mice show profound enhancement of lymphadenopathy. *J Immunol* 1995;155:5455–62. [PubMed: 7594564]
 20. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, Earp HS, Matsushima G, Reap EA. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-met membrane tyrosine kinase. *J Exp Med* 2002;196:135–40. [PubMed: 12093878]
 21. Baumann I, Kolowos W, Voll RE, Manger B, Gaip U, Neuhuber WL, Kirchner T, Kalden JR, Herrmann M. Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum* 2002;46:191–201. [PubMed: 11817590]
 22. Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* 2004;304:1147–50. [PubMed: 15155946]
 23. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* 2005;23:901–44. [PubMed: 15771589]
 24. Rogers NJ, Lees MJ, Gabriel L, Maniati E, Rose SJ, Potter PK, Morley BJ. A defect in Marco expression contributes to systemic lupus erythematosus development via failure to clear apoptotic cells. *J Immunol* 2009;182:1982–90. [PubMed: 19201851]

Figure 1. Mer expressed on peritoneal macrophages

Macrophages were prepared from Mer-KO (right) and WT (left) mice and stained with FITC conjugated CD11b and biotin conjugated anti-Mer antibody followed by streptavidin-PE. The cells were then fixed in 1% paraformaldehyde. Data were acquired using a BD Biosciences FACSscan. Expression of Mer was analysed using a FlowJo software (Ashland, OR).

Figure 2. Mer expression in mouse spleen

Snap-frozen spleen samples from B6 mice were sectioned (3 μ m) onto slides, followed by 3% BSA in PBS blocking. Sections were then incubated with biotin-conjugated anti-mouse Mer antibody. Mer expression was visualized with a 1:100 dilution of PE-biotin (A, C, D, and E) or FITC-biotin (B). Cell populations were identified by incubating with specific antibodies for 4 hours as indicated in the figure. Data represent a repeat of three individual staining.

Figure 3. Mer expression in macrophage subpopulations

B6 mice spleen sections were prepared, and Mer staining was done as described for Figure 2. Macrophage subpopulations were stained with antibodies against unique surface antigens, as follows: MOMA-1 (1:10 dilution), MARCO (1:10), and CD68 (1:20). Images were taken under fluorescence microscopy at a magnification of 400 $\times$.

Figure 4. Phagocytic efficiency with mouse serum

Macrophages were prepared as described in the methods and co-cultured with a 4× excess of CFSE-labeled apoptotic thymocytes for 4hrs.. Cells were then washed twice with 1 × PBS containing EDTA. Phagocytic uptake of apoptotic thymocytes by peritoneal macrophages was evaluated by FACS analysis gated on CD11b positivity. Phagocytic efficiency was evaluated with different percentages of mouse serum (A) or under the condition of heat-inactivation, as compared with phagocytosis in the presence of FBS (B).

Figure 5. Mer-mediated phagocytosis is Gas6 dependent

Peritoneal macrophages were collected from WT or Mer-KO mice 5 days after thioglycolate injection. Cells were washed and incubated with CFSE-labeled apoptotic thymocytes for 4hrs. The percentage of phagocytic macrophages (CFSE positive) was determined by FACS analysis of CD11b-positive cells (A). Increasing numbers of apoptotic thymocytes were added to wells in which 1 million macrophages had been plated 2 hours before (B). At a fixed input of apoptotic thymocytes (4 million), Mer-KO and WT macrophages were tested for phagocytosis in the presence of WT or Gas6-KO serum. Phagocytosis efficiency was presented as percentage of phagocytic macrophage over total macrophages. Data are representative of three experiments.

Figure 6. Blockage of Mer-mediated phagocytosis

Biotin-conjugated goat anti-Mer antibody was added together with the apoptotic cells at concentrations indicated in the figure. Goat biotin-IgG was used as isotype control. Phagocytic efficiency was examined as previously described. Data shown here represent three repeat experiments.