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Role of CD47 and Signal Regulatory Protein Alpha (SIRPα) in Regulating the Clearance of Viable or Aged Blood Cells

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Keywords
Erythrocytes · Phagocytosis · Apoptosis

Summary
The ubiquitously expressed cell surface glycoprotein CD47 is expressed by virtually all cells in the host, where it can function to regulate integrin-mediated responses, or constitute an important part of the erythrocyte band 3/Rh multi-protein complex. In addition, CD47 can protect viable cells from being phagocytosed by macrophages or dendritic cells. The latter mechanism is dependent on the interaction between target cell CD47 and SIRPα on the phagocyte. In this context, SIRPα functions to inhibit prophagocytic signaling from Fcγ receptors, complement receptors, and LDL receptor-related protein-1 (LRP-1), but not scavenger receptors. The expression level and/or distribution of CD47 may be altered on the surface of apoptotic/senescent cells, rendering the phagocytosis inhibitory function of the CD47/SIRPα interaction reduced or eliminated. Instead, the interaction between these 2 proteins may serve to enhance the binding of apoptotic/senescent target cells to the phagocyte to promote phagocytosis.

Schlüsselwörter
Erythrozyten · Phagozytose · Apoptose

Zusamenfassung
Das ubiquitär exprimierte Oberflächenglykoprotein CD47 wird von praktisch allen Wirtszellen exprimiert, wo es integrinvermittelte Antworten reguliert bzw. einen wichtigen Teil des erythrozytären Band 3/Rh-Multiproteinkomplexes darstellt. Zusätzlich kann CD47 lebensfähige Zellen vor der Phagozytose durch Makrophagen oder dendritische Zellen schützen. Letzterer Mechanismus hängt von der Interaktion zwischen CD47 auf der Zielzelle und SIRPα auf dem Phagozyten ab. In diesem Zusammenhang fungiert SIRPα als Inhibitor der prophagozytären Signalvermittlung durch Fcγ-Rezeptoren, Komplement-Rezeptoren und LRP-1 (LDL receptor-related protein-1), nicht jedoch Scavenger-Rezeptoren. Auf der Oberfläche apoptotischer/alternder Zellen kann der Grad der Exprimierung und/oder Verteilung von CD47 verändert sein, was die Phagozytose hemmende Funktion der CD47/SIRPα-Interaktion vermindern oder beseitigen würde. Stattdessen könnte die Interaktion zwischen den beiden Proteinen dazu dienen, die Bindung apoptotischer/alternder Zielzellen an den Phagozyten zu verstärken und somit die Phagozytose zu fördern.

CD47 and SIRPα

CD47 is an ubiquitously expressed 50 kDa transmembrane glycoprotein that consists of a highly glycosylated extracellular IgV domain, a hydrophobic multiple membrane-spanning domain, and a short alternatively spliced cytoplasmic tail [1]. It was originally discovered as an integrin-associated protein associated with αvβ3 integrins in the placenta and in neutrophil granulocytes (hence its alternative name integrin-associated protein, IAP), with the capacity to regulate integrin-mediated responses to RGD-containing extracellular matrix proteins [2, 3]. IAP was found to be identical to the

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Rh-related protein CD47 [4] and the OA-3/OVTL3 antigen highly expressed on most ovarian carcinomas, and also to be homologous to a protein family of variola and vaccinia viruses [1]. Since CD47 is highly expressed by erythrocytes and mature erythrocytes do not express integrins, other important functions were expected for CD47 in these cells. A close relationship to Rh proteins was early suggested as erythrocytes from Rh-null individuals, expressing none of the Rh complex proteins, only express about 25% of the normal levels of CD47 [4, 5]. Rh polypeptides form a complex with many other proteins in the erythrocyte membrane (e.g. Rh-associated glycoprotein (RhAG), glycophorin B, LW, and CD47) [6]. The band 3 protein forms another complex with several proteins (e.g. glycophorin A, protein 4.2, and ankyrin) which are supposed to be involved in anchoring the spectrin cytoskeleton to the erythrocyte membrane [7, 8], and it was found that the Rh complex and the band 3 complex may be associated in the erythrocyte membrane [9]. Mutations in band 3 or complete band 3 deficiency in humans result in reduced expression of Rh polypeptides and RhAG [10], and almost complete absence of CD47 [9]. In addition, human erythrocytes lacking protein 4.2 have a marked deficiency in CD47 and altered glycosylation of RhAG [11]. Together, this suggest that CD47 of the Rh complex may form a link to the band 3 complex by binding to protein 4.2 [9]. Despite the established link between CD47, the Rh complex, and protein 4.2 in human erythrocytes, the interactions between CD47 and the same proteins in murine erythrocytes is not well understood since erythrocytes from CD47-deficient mice contain normal amounts of murine Rh and RhAG polypeptides [12], and erythrocytes from protein 4.2-deficient mice have normal amounts of CD47 [13]. In addition, erythrocytes from band 3-deficient mice have little or no Rh polypeptides whereas expression of CD47 is only slightly reduced [9].

Besides its interaction in cis with integrins, the CD47 IgV domain also binds the cell-binding domain of the extracellular matrix protein thrombospondin [1]. The receptor signals via the β3 integrin cytoplasmic tail, and in a pertussis toxin-sensitive manner via heterotrimeric G proteins and adenylate cyclase [1]. At present, it is unclear how much these 2 signaling pathways overlap. CD47 has the ability to induce a caspase-independent form of apoptosis in activated T or B cells following cross-linking of the receptor [14–16]. It also augments Fas-induced apoptosis, making CD47-deficient Jurkat T cells or primary murine T cells resistant to Fas-induced killing [17]. This is not a general apoptosis defect, since CD47-deficient cells can undergo apoptosis in response to other apoptosis-inducing stimuli.

Besides being a receptor for thrombospondin, CD47 is also a ligand for SIRPα, an interaction originally identified in neurons [18]. SIRPα (also known as SHPS-1, MyD-1, BIT, MFR, CD172a, P84, or PTPNS1 [19]) has 3 extracellular Ig domains and an intracellular tail with 2 immuno-receptor tyrosine-based inhibitory motifs (ITIMs) [20], and has been shown to be expressed in myeloid cells, neurons, endothelial cells and fibroblasts, but not by T cells or B cells [21]. However, SIRPα gene expression was detected following B cell receptor cross-linking in the Burkitt's lymphoma cell line BL2 [22], and a subset of human CD34– CD19+ bone marrow B cells were found to express SIRPα [23], suggesting expression of the receptor in specific B cell subsets. SIRPα plays important roles in regulating cell migration in several cell types [24], and migration of cultured fibroblasts from mice that lack the intracellular domain of SIRPα is markedly reduced [25]. Integrin engagement by extracellular matrix proteins induces tyrosine phosphorylation of the SIRPα cytoplasmic domain [26–29], which is suggested to be mediated by the Src-family kinase Lyn [30]. Such integrin-mediated phosphorylation is CD47-independent in myeloid cells, but dependent on CD47 in endothelial cells [27].

The interaction between CD47 and SIRPα during cell-cell contact can generate intracellular signaling from both proteins, and has been found to regulate several important biological systems (one of these, inhibitory regulation of host cell phagocytosis, is further described in subsequent sections). CD47 and SIRPα are both expressed in the brain, in particular in synapse-rich areas of the hippocampus, cerebellum, and retina [31]. In addition, these proteins are involved in regulating osteoclast formation [32–34], osteoblast function [33], lymphocyte homeostasis [35, 36], dendritic cell (DC) maturation [37], proper localization of certain DC subsets in secondary lymphoid organs [38–40], and cellular transmigration [41, 42].

Phagocytosis of Viable Host Cells Is Negatively Regulated by Target Cell CD47

Macrophages or DCs can discriminate between self and non-self. 'Self' is represented by CD47, recognized by SIRPα on macrophages or DCs where it functions as an inhibitory receptor [43, 44]. This is a regulatory system which turned out to be analogous to that described for natural killer cells [45], where activation is regulated by a large number of activating receptors and different inhibitory receptors. In general, immune inhibitory receptors have cytoplasmic domains containing ITIMs [45] which, when tyrosine phosphorylated, can bind the tyrosine phosphatases SHP-1 and SHP-2 [20, 26] to inhibit tyrosine kinase-dependent signaling pathways [46]. CD47–/– blood cells are rapidly cleared from the circulation of wild-type (Wt) but not CD47–/– mice [43, 44, 47]. For erythrocytes, this clearance mechanism is independent of complement and antibody, but virtually entirely based on clearance by splenic F4/80+ red pulp macrophages [44]. By blocking SIRPα on isolated splenic macrophages, the level of phagocytosis of Wt erythrocytes can be increased to that seen with CD47–/– erythrocytes [44]. These findings suggested that all erythro-

cytes are recognized by splenic macrophages when SIRPα is blocked, and that these macrophages must have a receptor for erythrocytes. Indeed, the endocytic receptor LDL receptor-related protein (LRP-1) was found to mediate uptake of CD47–/– murine erythrocytes by binding to calreticulin on the erythrocytes [48, 49]. However, it still needs to be proven to what extent LRP-1-mediated uptake of erythrocytes is involved in the clearance of murine or human erythrocytes in vivo. The inhibitory signals generated by macrophage SIRPα upon ligation of CD47 also affects prophagocytic signaling via Fcγ and complement receptors [50, 51]. As a result, CD47–/– mice are more sensitive to experimentally induced autoimmune cytopenias, such as autoimmune hemolytic anemia (AIHA) [52] and immune thrombocytopenic purpura (ITP) [47], when antibodies against erythrocytes or platelets are injected into recipient mice. Autoimmune-prone non-obese diabetic (NOD) mice are mostly known for their high spontaneous incidence of type 1 diabetes, but NOD mice not developing diabetes may instead develop a milder form of AIHA at an older age [53]. Interestingly, CD47–/– NOD mice succumb from an accelerated form of AIHA at a fairly young age [52]. The exact explanation behind this is not known, but it is most likely closely associated with the autoimmune phenotype of NOD mice, since CD47–/– mice on non-autoimmune-prone genetic backgrounds (e.g. C57BL/6 or Balb/c) do not spontaneously develop AIHA. However, since CD47–/– erythrocytes are phagocytosed at a much higher rate than equally opsonized Wt erythrocytes both in vitro and in vivo, it is possible that the accelerated development of AIHA in CD47–/– NOD mice is due to a higher rate of antigen uptake, antigen presentation, and autoantibody production [54]. The fact that lack of CD47 on erythrocytes does not per se result in AIHA development in mice, suggests that AIHA should not be expected in the rare examples of complete CD47 deficiency in humans, unless the CD47 deficiency is combined with enhanced sensitivity to develop AIHA. In macrophages, SHP-1 is predominantly recruited to SIRPα upon CD47 binding [50], and SHP-1-deficient motheaten viable (mev/mev) mice have severely reduced CD47/SIRPα signaling [51]. However, in other cell types, SIRPα associated with SHP-2 has been implicated in positive regulation of cell migration [55]. It has been suggested that SHP-1-mediated dephosphorylation of non-muscular myosin IIA at the phagocytic synapse between the phagocyte and a host cell is responsible for phagocytosis inhibition by the CD47/SIRPα interaction [56].

Activation of phagocytosis in a macrophage or DC in contact with a viable target host cell is a balance between signals from activating prophagocytic receptors and the inhibitory signal from SIRPα ligated by target cell CD47. Neither signal appears to be dominant, but rather the decision to phagocytose is based on an integration of positive prophagocytic signals and inhibitory CD47-SIRPα signals [51]. Thus, when an opsonized cell also expresses CD47, a larger amount of opsonin (e.g. IgG) is required for phagocytosis to occur, as compared with that when the opsonized cell lacks CD47. Although the interaction between CD47 and SIRPα regulates phagocytosis in a similar way in all species investigated, the interaction is very species-specific [57], which is one of the explanations for the rapid phagocytosis seen when xenogenic cells are transplanted [58]. However, expression of CD47 from the host in xenogenic cells results in marked tolerance by host macrophages following transplantation [59]. Recently, a number of studies have also shown that overexpression of CD47 is a way for human tumor cells to avoid phagocytosis by macrophages, and antibodies against CD47 were found to be effective in mediating enhanced phagocytosis and killing of tumor cells [60].

Phagocytosis of Apoptotic Cells Is Not Inhibited But Rather Promoted by CD47

Apoptosis is an important process where aged, damaged, or potentially destructive host cells are removed by a programmed and physiological form of cell death [61]. Apoptotic cells are preferentially cleared by macrophages or DCs to prevent release of toxic or immunogenic intracellular components as a result of cell lysis [62–64]. Uptake of pathogens normally stimulates macrophages or DCs to release proinflammatory substances, whereas uptake of apoptotic cells will rather stimulate resolution of inflammation [65]. Several studies have indicated that recognition and ingestion of apoptotic cells is a complex process where many prophagocytic receptors, bridging molecules, and several 'eat-me' markers on apoptotic cells are involved, together controlling phagocyte behavior during the phagocytosis of these cells [61, 66]. Phosphatidylserine (PS) is normally present in the inner plasma membrane leaflet, but flips to the outer leaflet early in the apoptotic process, which has been found to stimulate phagocytosis [67]. Other cell surface changes are alterations in the pattern of glycosylation of glycoproteins and glycolipids [67, 68], changed expression levels of specific molecules, and non-specific changes such as surface charge [69, 70]. Alterations in sugar chains, surface charge, and oxidation result in the generation of sites resembling oxidized lipoprotein particles, thrombospondin (TSP) binding sites, sites capable of binding lectins or the complement proteins C1q and C3b, as well as various collectin-binding sites. These surface alterations, resulting in new binding sites for receptors, have important implications for the removal of the apoptotic cell [71–73].

The fact that apoptotic cells are efficiently taken up by phagocytes, whereas viable cells are not, suggested that reduced inhibition through the CD47/SIRPα interaction could be one mechanism contributing to facilitated phagocytosis. Indeed, several changes occur in CD47 on apoptotic cells in both mice and humans. First, the cell surface levels of CD47 was found to be reduced on apoptotic fibroblasts and neu-

trophils, but not on apoptotic Jurkat T cells [48]. Second, CD47, which is evenly distributed on the cell surface of viable cells, becomes clustered in distinct domains of the plasma membrane of apoptotic fibroblasts, neutrophils, and lymphocytes [48, 74]. Domains containing clustered CD47 are different from those containing some ligands for prophagocytic receptors, suggesting a segregation mechanisms where the CD47/SIRPα interaction would not come into play during phagocytosis [48]. However, it was also found that macrophage phagocytosis of murine CD47-deficient apoptotic cells was reduced as compared with that of apoptotic CD47-expressing cells, showing that the CD47/SIRPα interaction could serve to mediate binding of the apoptotic cells to the phagocytes without inhibiting phagocytosis [74, 75].

CD47 and Erythrocyte Senescence

Since CD47 can protect viable cells from phagocytosis, and damaged or senescent erythrocytes are efficiently phagocytosed by macrophages, possible changes to CD47 have been investigated in relation to erythrocyte storage or senescence. Indeed, CD47 was reported to be lost from human erythrocytes during storage at +4 °C [76, 77], and it was found that older circulating erythrocytes in mice had less CD47 on their surface [78, 79], raising the possibility that reduced levels of CD47 could facilitate uptake of senescent or damaged erythrocytes. In light of the suggested importance of natural antibodies in the recognition and clearance of senescent erythrocytes [80], it is interesting to note that the amount of CD47 on viable IgG-opsonized murine erythrocytes does indeed determine the rate of phagocytosis by macrophages [81]. Further data indicating that the CD47/SIRPα system may regulate the normal clearance rate and lifespan of platelets and erythrocytes, comes from studies in mice lacking the signaling SIRPα cytoplasmic domain. These mice have spontaneous thrombocytopenia and anemia due to accelerated clearance of platelets and erythrocytes [82, 83]. Although senescent erythrocytes may display some features similar to apoptotic cells, such as vesiculation, cell shrinkage, PS exposure, and binding of natural antibodies [84], the mechanisms behind physiological erythrocyte senescence in vivo are not completely understood. This, in combination with the fact that senescent erythrocytes are very difficult to isolate from blood, makes it challenging to investigate mechanisms behind macrophage phagocytosis of senescent erythrocytes in detail. However, since scavenger receptors are likely involved (in concert with Fcγ receptors, complement receptors, and others) in mediating macrophage uptake of senescent or apoptotic cells [85], it is interesting to note that scavenger receptor-mediated uptake of oxidatively damaged murine erythrocytes was found not to be regulated by the CD47/SIRPα interaction, whereas Fcγ receptor-mediated uptake of the same cells was still negatively regulated [86]. Thus, depending on the combination of prophagocytic receptors involved in the phagocytosis of senescent erythrocytes, it is possible that CD47 may or may not have an inhibitory effect in regulating this process. Based on the observation that CD47 on apoptotic cells becomes clustered and does rather promote phagocytosis of these cells [48, 74, 75], it is interesting to note that the anti-human CD47 monoclonal antibody 2D3 can cluster CD47 on erythrocytes in a way that results in enhanced binding of SIRPα [57]. Of marked interest, clustering of CD47 may occur during erythrocyte senescence, where the CD47/SIRPα interaction will in turn result in enhanced binding of erythrocytes to macrophages and rather promote erythrophagocytosis [87]. Similar to nucleated cells, an accelerated form of cell death (eryptosis) can also be induced in erythrocytes [88, 89]. Although eryptosis may or may not share similarities with natural senescence, phagocytosis of eryptotic erythrocytes could serve as a starting point to further investigate uptake mechanisms that may also be involved in the uptake of senescent erythrocytes in vivo. Interestingly, CD47 ligands can induce eryptosis in human erythrocytes in vitro [90], although the physiological relevance of this in vivo has still to be determined.

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Disclosure Statement

The author does not have any competing financial interest.

References

1 Brown EJ, Frazier WA: Integrin-associated protein (CD47) and its ligands. Trends Cell Biol 2001;11:130–135.
2 Lindberg FP, Gresham HD, Schwarz E, Brown EJ: Molecular cloning of integrin-associated protein: an immunoglobulin family member with multiple membrane spanning domains implicated in alpha-v, beta-3-dependent ligand binding. J Cell Biol 1993;123:485–496.
3 Brown EJ, Hooper L, Ho T, Gresham HD: Integrin-associated protein: a 50-kD plasma membrane antigen physically and functionally associated with integrins. J Cell Biol 1990;111:2785–2794.
4 Lindberg FP, Lublin DM, Telen MJ, Veile RA, Miller YE, Donis-Keller H, Brown EJ: Rh-related antigen CD47 is the signal-transducer integrin associated protein. J Biol Chem 1994;269:1567–1570.
5 Avent N, Judson PA, Parsons SF, Mallinson G, Anstee DJ, Tanner MJA, Evans PR, Hodges E, MacIver AG, Holmes C: Monoclonal antibodies that recognize different membrane proteins that are deficient in Rh_{null} human erythrocytes. Biochem J 1988;251:499–505.
6 Cartron JP: Rh blood group system and molecular basis of Rh-deficiency. Balliéres Best Pract Res Clin Haematol 1999;12:655–689.

7 Rybicki AC, Schwartz RS, Hustedt EJ, Cobb CE: Increased rotational mobility and extractability of band 3 from protein 4.2-deficient erythrocyte membranes: evidence of a role for protein 4.2 in strengthening the band 3-cytoskeleton linkage. Blood 1996;88:2745–2753.
8 Tanner MJ: Band 3 anion exchanger and its involvement in erythrocyte and kidney disorders. Curr Opin Hematol 2002;9:133–139.
9 Bruce LJ, Beckmann R, Ribeiro ML, Peters LL, Chasis JA, Delaunay J, Mohandas N, Anstee DJ, Tanner MJA: A band 3-based macrocomplex of integral and peripheral proteins in the RBC membrane. Blood 2003;101:4180–4188.
10 Booth PB, Serjeantson S, Woodfield DG, Amato D: Selective depression of blood group antigens associated with hereditary ovalocytosis among melanesians. Vox Sang 1977;32:99–110.
11 Bruce LJ, Ghosh S, King MJ, Layton DM, Mawby WJ, Stewart GW, Oldenborg P-A, Delaunay J, Tanner MJ: Absence of CD47 in protein 4.2-deficient hereditary spherocytosis in man: an interaction between the Rh complex and the band 3 complex. Blood 2002;100:1878–1885.
12 Mouro-Chanteloup I, Johansen M, Brown EJ, Cartron JPCY: Normal red cell membrane expression of Rh and RhAG polypeptides in CD47-deficient mice (abstract). Vox Sang 2000;78:P030.
13 Mouro-Chanteloup I, Delaunay J, Gane P, Nicolas V, Johansen M, Brown EJ, Peters LL, Le Van Kim C, Cartron JP, Colin Y: Evidence that the red cell skeleton protein 4.2 interacts with the Rh membrane complex member CD47. Blood 2003;101:338–344.
14 Manna PP, Frazier WA: The mechanism of CD47-dependent killing of T cells: heterotrimeric Gi-dependent inhibition of protein kinase A. J Immunol 2003;170:3544–3553.
15 Mateo V, Lagneaux L, Bron D, Biron G, Armant M, Delespesse G, Sarfati M: CD47 ligation induces caspase-independent cell death in chronic lymphocytic leukemia. Nat Med 1999;5:1277.
16 Pettersen RD, Hestdal K, Olafsen K, Lie SO, Lindberg FP: CD47 signals T cell death. J Immunol 1999;162:7031.
17 Manna PP, Dimitry JM, Oldenborg P-A, Frazier WA: CD47 augments Fas/CD95-mediated apoptosis. J Biol Chem 2005;280:29637–29644.
18 Jiang P, Lagenaur CF, Narayanan V: Integrin-associated protein is a ligand for the P84 neural adhesion molecule. J Biol Chem 1999;274:559–562.
19 Barclay AN, Brown MH: The SIRP family of receptors and immune regulation. Nat Rev Immunol 2006;6:457–464.
20 Kharitonenkov A, Chen Z, Sures I, Wang H, Schilling J, Ullrich A: A family of proteins that inhibit signalling through tyrosine kinase receptors. Nature 1997;386:181–186.
21 Van Beek EM, Cochrane F, Barclay AN, van den Berg TK: Signal regulatory proteins in the immune system. J Immunol 2005;175:7781–7787.
22 Nakayama Y, Iwamoto Y, Maher SE, Tanaka Y, Bothwell ALM: Altered gene expression upon BCR cross-linking in Burkitt's lymphoma B cell line. Biochem Biophys Res Commun 2000;277:124–127.
23 Seiffert M, Cant C, Chen Z, Rappold I, Brugger W, Kanz L, Brown EJ, Ullrich A, Bühring H-J: Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterrecteptor CD47. Blood 1999;94:3633–3643.
24 Oshima K, Ruhul Amin AR, Suzuki A, Hamaguchi M, Matsuda S: SHPS-1, a multifunctional transmembrane glycoprotein. FEBS Lett 2002;519:1–7.
25 Inagaki K, Yamao T, Noguchi T, Matozaki T, Fukanaga K, Takada T, Hosooka T, Akira S, Kasuga M: SHPS-1 regulates integrin-mediated cytoskeletal reorganization and cell motility. EMBO J 2000;19:6721–6731.
26 Fujioka Y, Matozaki T, Noguchi T, Iwamatsu A, Yamao T, Takahashi N, Tsuda M, Takada T, Kasuga M: A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. Mol Cell Biol 1996;16:6887–6899.
27 Johansen ML, Brown EJ: Dual regulation of SIRPα phosphorylation by integrins and CD47. J Biol Chem 2007;282:24219–24230.
28 Oh ES, Gu H, Saxton TM, Timms JF, Hausdorff S, Frevert EU, Kahn BB, Pawson T, Neel BG, Thomas SM: Regulation of early events in integrin signaling by protein tyrosine phosphatase SHP-2. Mol Cell Biol 1999;19:3205–3215.
29 Tsuda M, Matozaki T, Fukunaga K, Fujioka Y, Imamoto A, Noguchi T, Takada T, Yamao T, Takeda H, Ochi F, Yamamoto T, Kasuga M: Integrin-mediated tyrosine phosphorylation of SHPS-1 and its association with SHP-2. Roles of Fak and Src family kinases. J Biol Chem 1998;273:13223–13229.
30 Pereira S, Lowell C: The Lyn tyrosine kinase negatively regulates neutrophil integrin signaling. J Immunol 2003;171:1319–1327.
31 Matozaki T, Murata Y, Okazawa H, Ohnishi H: Functions and molecular mechanisms of the CD47-SIRPα signalling pathway. Trends Cell Biol 2009;19:72–80.
32 Lundberg P, Koskinen C, Baldock PA, Löthgren H, Stenberg Å, Lerner UH, Oldenborg P-A: Osteoclast formation is strongly reduced both in vivo and in vitro in the absence of CD47/SIRPα-interaction. Biochem Biophys Res Commun 2007;352:444–448.
33 Maile LA, DeMambro VE, Wai C, Aday AW, Capps BE, Beamer WG, Rosen CJ, Clemmons DR: An essential role for the association of CD47 to SHPS-1 in skeletal remodeling. J Bone Miner Res 2011;26:2068–2081.
34 Uluckan O, Becker SN, Deng H, Zou W, Prior JL, Piwnica-Worms D, Frazier WA, Weilbaecher KN: CD47 regulates bone mass and tumor metastasis to bone. Cancer Res 2009;69:3196–3204.
35 Legrand N, Huntington ND, Nagasawa M, Bakker AQ, Schotte R, Strick-Marchand H, de Geus SJ, Pouw SM, Böhne M, Voordouw A, Weijer K, Di Santo JP, Spits H: Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer-(NK) cell homeostasis in vivo. Proc Nat Acad Sci U S A 2011;108:13224–13229.
36 Sato-Hashimoto M, Saito Y, Ohnishi H, Iwamura H, Kanazawa Y, Kaneko T, Kusakari S, Kotani T, Mori M, Murata Y, Okazawa H, Ware CF, Oldenborg PA, Nojima Y, Matozaki T: Signal regulatory protein alpha regulates the homeostasis of T lymphocytes in the spleen. J Immunol 2011;187:291–297.
37 Latour S, Tanaka H, Demeure C, Mateo V, Rubio M, Brown EJ, Maliszewski C, Lindberg FP, Oldenborg A, Ullrich A, Delespesse G, Sarfati M: Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal-regulator protein-alpha: down-regulation of IL-12 responsiveness and inhibition of dendritic cell activation. J Immunol 2001;167:2547–2554.
38 Hagnerud S, Manna PP, Cella M, Stenberg Å, Frazier WA, Colonna M, Oldenborg P-A: Deficit of CD47 results in a defect of marginal zone dendritic cells, blunted immune response to particulate antigen and impairment of skin dendritic cell migration. J Immunol 2006;176:5772–5778.
39 Saito Y, Iwamura H, Kaneko T, Ohnishi H, Murata Y, Okazawa H, Kanazawa Y, Sato-Hashimoto M, Kobayashi H, Oldenborg P-A, Naito M, Kaneko Y, Nojima Y, Matozaki T: Regulation by SIRPα of dendritic cell homeostasis in lymphoid tissues. Blood 2010;116:3517–3525.
40 Van VQ, Bouguermouh S, Gautier P, Rubio M, Levesque M, Nguyen S, Galibert L, Sarfati M: Expression of the self-marker CD47 on dendritic cells governs their trafficking to secondary lymphoid organs. EMBO J 2006;25:5560–5568.
41 De Vries HE, Hendriks JJ, Honing H, De Lavalette CR, van der Pol SM, Hoijberg E, Dijkstra CD, van den Berg TK: Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. J Immunol 2002;168:5832–5839.
42 Liu Y, Bühring H-J, Zen K, Burst SL, Schnell FJ, Williams IR, Parkos CA: Signal regulatory protein (SIRPα), a cellular ligand for CD47, regulates neutrophil transmigration. J Biol Chem 2002;277:10028–10036.
43 Blazar BR, Lindberg FP, Ingulli E, Panoskaltsis-Mortari A, Oldenborg P-A, Iizuka K, Yokoyama WM, Taylor PA: CD47 (integrin-associated protein) engagement of dendritic cell and macrophage counterreceptors is required to prevent clearance of donor lymphohematopoietic cells. J Exp Med 2001;194:541–549.
44 Oldenborg P-A, Zheleznyak A, Fang Y-F, Lagenaur CF, Gresham HD, Lindberg FP: Role of CD47 as a marker of self on red blood cells. Science 2000;288:2051–2054.
45 Ravaetch JV, Lanier LL: Immune inhibitory receptors. Science 2000;290:84–89.
46 Lienard H, Bruhns P, Malbec O, Fridman WH, Daëron M: Signal regulatory proteins negatively regulate immunoreceptor-dependent cell activation. J Biol Chem 1999;274:32493–32499.
47 Olsson M, Bruhns P, Frazier WA, Ravetch JV, Oldenborg P-A: Platelet homeostasis is regulated by platelet expression of CD47 under normal conditions and in passive immune thrombocytopenia. Blood 2005;105:3577–3582.
48 Gardai S, McPhillips K, Frasch SC, Starefeldt A, Bratton DL, Oldenborg P-A, Michalak M, Henson PM: Cell surface calreticulin initiates clearance of viable or apoptotic cells through trans activation of LRP on the phagocyte. Cell 2005;123:321–334.
49 Nilsson A, Vesterlund L, Oldenborg PA: Macrophage expression of LRP1, a receptor for apoptotic cells and unopsonized erythrocytes, can be regulated by glucocorticoids. Biochem Biophys Res Commun 2012;417:1304–1309.
50 Okazawa H, Motegi S, Ohyama N, Ohnishi H, Tomizawa T, Kaneko Y, Oldenborg P-A, Ishikawa O, Matozaki T: Negative regulation of phagocytosis in macrophages by the CD47-SHPS-1 system. J Immunol 2005;174:2004–2011.
51 Oldenborg P-A, Gresham HD, Lindberg FP: CD47-SIRPα regulates Fcγ and complement receptor-mediated phagocytosis. J Exp Med 2001;193:855–862.
52 Oldenborg P-A, Gresham HD, Chen Y, Izui S, Lindberg FP: Lethal autoimmune hemolytic anemia in CD47-deficient NOD mice. Blood 2002;99:3500–3504.
53 Baxter AG, Mandel TE: Hemolytic anemia in non-obese diabetic mice. Eur J Immunol 1991;21:2051–2055.
54 Oldenborg P-A: Role of CD47 in erythroid cells and in autoimmunity. Leuk Lymphoma 2004;45:1319–1327.

55 Motegi S, Okazawa.H., Ohnishi H, Sato R, Kaneko Y, Kobayashi H, Tomizawa K, Ito T, Honma N, Buhring HJ, Ishikawa O, Matozaki T: Role of the CD47-SHPS-1 system in regulation of cell migration. EMBO J 2003;22:2634–2644.
56 Tsai RK, Discher DE: Inhibition of 'self' engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. J Cell Biol 2008;180:989–1003.
57 Subramanian S, Parthasarathy R, Sen S, Boder ET, Discher DE: Species- and cell-type specific interactions between CD47 and human SIRPα. Blood 2006;107:2548–2556.
58 Ide K, Wang H, Tahara H, Liu J, Wang X, Asahara T, Sykes M, Yang YG, Ohdan H: Role for CD47-SIRPα signaling in xenograft rejection by macrophages. Proc Natl Acad Sci U S A 2007;104:5062–5066.
59 Wang H, VerHalen J, Madariaga ML, Xiang S, Wang S, Lan P, Oldenborg PA, Sykes M, Yang YG: Attenuation of phagocytosis of xenogeneic cells by manipulating CD47. Blood 2006;109:836–842.
60 Chao MP, Weissman IL, Majeti R: The CD47–SIRPα pathway in cancer immune evasion and potential therapeutic implications. Curr Opin Immunol 2012;24:225–232.
61 Savill J, Dransfield I, Gregory C, Haslett C: A blast from the past: clearance of apoptotic cells regulates immune responses. Nat Rev Immunol 2002;2:965–975.
62 Savill J, Whylie AH, Henson JE, Walport MJ, Henson PM, Haslett C: Macrophage phagocytosis of ageing neutrophils in inflammation: programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 1989;83:865–875.
63 Savill J: Apoptosis in resolution of inflammation. J Leukoc Biol 1997;61:375–380.
64 Henson PM, Bratton DL, Fadok VA: The phosphatidylserine receptor: a crucial molecular switch? Nat Rev Mol Cell Biol 2001;2:627–633.
65 Fadok VA, Bratton DL, Henson PM: Phagocyte receptors for apoptotic cells: recognition, uptake, and consequences. J Clin Invest 2001;180:957–962.
66 Devitt A, Marshall LJ: The innate immune system and the clearance of apoptotic cells. J Leukoc Biol 2011;90:447–457.
67 Azuma Y, Inami Y, Matsumoto K: Alterations in cell surface phosphatidylserine and sugar chains during apoptosis and their time-dependent role in phagocytosis by macrophages. Biol Pharm Bull 2002;25:1277–1281.
68 Messmer UK, Pfeilschifter J: New insights into the mechanism for clearance of apoptotic cells. Bioassays 2000;22:878–881.
69 Gardai SJ, Bratton DL, Ogden CA, Henson PM: Recognition ligands on apoptotic cells: a perspective. J Leukoc Biol 2006;79:896–903.
70 Savill JS, Henson PM, Haslett C: Phagocytosis of aged human neutrophils by macrophages is mediated by a novel 'charge-sensitive' recognition mechanism. J Clin Invest 1989;84:1518–1527.
71 Chang MK, Bergmark C, Laurila A, Horkko S, Han KH, Friedman P, Dennis EA, Witztum JL: Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. Proc Natl Acad Sci U S A 1999;96:6353–6358.
72 Takizawa F, Tsuji S, Nagasawa S: Enhancement of macrophage phagocytosis upon iC3b deposition on apoptotic cells. FEBS Lett 1996;397:269–272.
73 Vandivier RW, Ogden CA, Fadok VA, Hoffmann PR, Brown KK, Botto M, Walport MJ, Fisher JH, Henson PM, Greene KE: Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells in vivo and in vitro: calreticulin and CD91 as a common collectin receptor complex. J Immunol 2002;169:3978–3986.
74 Nilsson A, Oldenborg P-A: CD47 promotes both phosphatidylserine-independent and phosphatidylserine-dependent phagocytosis of apoptotic murine thymocytes by non-activated macrophages. Biochem Biophys Res Commun 2009;387:58–63.
75 Tada K, Tanaka M, Hanayama R, Miwa K, Shinohara A, Iwamatsu A, Nagata S: Tethering of apoptotic cells to phagocytes through binding of CD47 to Src homology 2 domain-bearing protein tyrosine phosphatase substrate-1. J Immunol 2003;171:5718–5726.
76 Anniss AM, Sparrow RL: Expression of CD47 (integrin-associated protein) decreases on red blood cells during storage. Transfus Apheresis Sci 2002;27:233–238.
77 Stewart A, Urbaniak S, Turner M, Bessos H: The application of a new quantitative assay for the monitoring of integrin-associated protein CD47 on red blood cells during storage and comparison with the expression of CD47 and phosphatidylserine with flow cytometry. Transfusion 2005;45:1496–1503.
78 Fossati-Jimack L, Azeredo da Silvera S, Moll T, Kina T, Kuypers FA, Oldenborg P-A, reininger L, Izui S: Selective increase of autoimmune epitope expression on aged erythrocytes in mice: implications in anti-erythrocyte autoimmune responses. J Autoimmun 2002;18:17–25.
79 Khandelwal S, van Rooijen N, Saxena RK: Reduced expression of CD47 during murine red blood cell (RBC) senescence and its role in RBC clearance from the circulation. Transfusion 2007;47:1725–1732.
80 Lutz HU: Innate immune and non-immune mediators of erythrocyte clearance. Cell Mol Biol (Noisy-le-grand) 2004;50:107–116.
81 Olsson M, Nilsson A, Oldenborg PA: Dose-dependent inhibitory effect of CD47 in macrophage uptake of IgG-opsonized murine erythrocytes. Biochem Biophys Res Commun 2007;352:193–197.
82 Ishikawa-Sekigami T, Kaneko Y, Okazawa H, Tomizawa T, Okajo J, Saito Y, Okuzawa C, Sugawara-Yokoo M, Nishiyama U, Ohnishi H, Matozaki T, Nojima Y: SHPS-1 promotes the survival of circulating erythrocytes through inhibition of phagocytosis by splenic macrophages. Blood 2006;107:341–348.
83 Yamao T, Noguchi T, Takeuchi O, Nishiyama U, Morita H, Hagiwara T, Akahori H, Kato T, Inagaki K, Okazawa.H., Hayashi Y, Matozaki T, Takeda K, Akira S, Kasuga M: Negative regulation of platelet clearance and of the macrophage phagocytic response by the transmembrane glycoprotein SHPS-1. J Biol Chem 2002;277:39833–39839.
84 Bosman GJ, Willekens FL, Werre JM: Erythrocyte aging: a more than superficial resemblance to apoptosis? Cell Physiol Biochem 2005;16:1–8.
85 Erwig LP, Henson PM: Clearance of apoptotic cells by phagocytes. Cell Death Differ 2008;15:243–250.
86 Olsson M, Oldenborg P-A: CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcγ receptor-mediated but not scavenger receptor-mediated recognition by macrophages. Blood 2008;112:4259–4267.
87 Burger P, Hilarius-Stokman P, de Korte D, van den Berg TK, van Bruggen R: CD47 functions as a molecular switch for erythrocyte phagocytosis. Blood 2012;119:5512–5521.
88 Lang F, Lang KS, Lang PA, Huber SM, Wieder T: Mechanisms and significance of eryptosis. Antioxid Redox Signal 2006;8:1183–1192.
89 Lang F, Qadri SM: Mechanisms and significance of eryptosis, the suicidal death of erythrocytes. Blood Purif 2012;33:125–130.
90 Head DJ, Lee ZE, Swallah MM, Avent ND: Ligation of CD47 mediates phosphatidylserine expression on erythrocytes and a concomitant loss of viability in vitro. Br J Haematol 2005;130:788–790.