The Interaction Between Signal Regulatory Protein Alpha (SIRPα) and CD47: Structure, Function, and Therapeutic Target

A. Neil Barclay[1] and Timo K. van den Berg[2]

[1]Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK; email: neil.barclay@path.ox.ac.uk

[2]Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, 1066 CX Amsterdam, The Netherlands; email: t.k.vandenberg@sanquin.nl

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Abstract

CD47 is a broadly expressed membrane protein that interacts with the myeloid inhibitory immunoreceptor SIRPα (also termed CD172a or SHPS-1). SIRPα is the prototypic member of the SIRP paired receptor family of closely related SIRP proteins. Engagement of SIRPα by CD47 provides a downregulatory signal that inhibits host cell phagocytosis, and CD47 therefore functions as a "don't-eat-me" signal. Here, we discuss recent structural analysis of CD47-SIRPα interactions and implications of this for the function and evolution of SIRPα and paired receptors in general. Furthermore, we review the proposed roles of CD47-SIRPα interactions in phagocytosis, (auto)immunity, and host defense, as well as its potential significance as a therapeutic target in cancer and inflammation and for improving graft survival in xenotransplantation.

INTRODUCTION

SIRPα: signal regulatory protein alpha

SHPS-1, CD172a, BIT, p84, MYD-1, PTPNS1: all acronyms for the protein more commonly termed SIRPα

Paired receptor: term for a family of closely related membrane receptors that contain at least one member with inhibitory and one with activating potentials

Signal regulatory protein alpha, or SIRPα (also known as CD172a), was first identified as a membrane protein present mainly on macrophages and myeloid cells that was associated with the Src homology region 2 (SH2) domain–containing phosphatases—SHP-1 and SHP-2; initially it was also termed SHPS-1 (1). SIRPα contains three Ig-like domains, a single transmembrane region, and a cytoplasmic region that contains four Tyr residues (1) with ITIM motifs (see sidebar on Immunoreceptor Tyrosine-Based Inhibitory Motif), a structure that is consistent with SIRPα's role as an inhibitory receptor.

The ligand was identified independently as CD47 in mouse (2), human (3), and rat (4); it was also known as integrin-associated protein (IAP) because it is associated with integrins such as $\alpha_v\beta_3$. CD47 is expressed at high levels on cancer cells, from which it was first cloned (5). CD47 is an unusual protein in that it contains five transmembrane regions together with a single Ig-like domain that interacts with the NH_2-terminal domain of SIRPα. The interaction plays a role in controlling phagocytosis, and CD47 is often termed a "don't-eat-me" signal through its effect on SIRPα (6, 7). This review focuses on the interaction of CD47 and SIRPα in the immune system. We discuss structural aspects of the interaction, the SIRP paired receptor family, and the implications for the evolution of paired receptors. We also discuss functional analysis of the outcome of the interaction and strong recent interest in developing therapeutics to target this interaction in cancer and xenotransplantation. Both SIRPα and CD47 are also present in the nervous system, but this is discussed elsewhere (see, e.g., 2, 8, 9).

MOLECULAR ANALYSIS OF CD47 AND THE SIRP FAMILY

The SIRP Paired Receptor Family

The SIRP family consists of an inhibitory receptor, SIRPα (SHPS-1 or CD172a); an activating receptor, SIRPβ1 (CD172b); and a nonsignaling receptor, SIRPγ (CD172g) (**Figure 1**) (6, 10–13).[1] There are also some more distantly related proteins such as SIRPδ and SIRPβ2 that have not yet been characterized (**Figure 1**). There is considerable variability in the repertoire of SIRP genes in different species, although SIRPα and SIRPβ1 are present in mammals and chickens (10, 15).

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIF

ITIM is a tandem arrangement of two tyrosine-centered peptide motifs in the cytoplasmic tail of inhibitory receptors. Upon phosphorylation of the tyrosine residues, the ITIM mediates the recruitment and activation of SH2 domain–containing tyrosine phosphatases, such as SHP-1 and SHP-2, which may act to dephosphorylate phosphoprotein substrates and thereby affect intracellular signaling pathways, most often in a negative fashion. The spacing of the tyrosine residues, which is larger in ITIM than in ITAM (immunotyrosine tyrosine-based activating motif), is a major determinant of specificity (147). The ITIMs in some receptors, such as the inhibitory FcγRIIb, also have the capacity to bind and activate the inositol phosphatases SHIP-1 and SHIP-2.

[1] SIRPγ was originally called SIRPβ2, but is now called SIRPγ because SIRPβ is reserved for activating genes (14). Thus, herein, SIRPγ refers to the old term SIRPβ2 and SIRPβ1 refers to the old term SIRPβ.

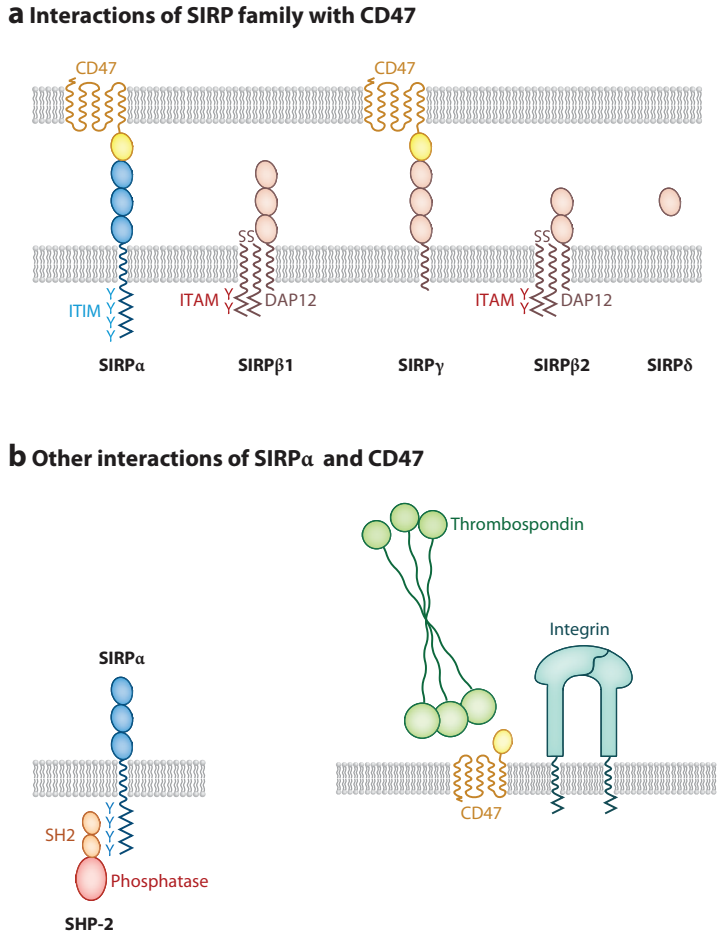

Figure 1

(*a*) SIRPα contains three Ig-like domains, an NH_2-terminal V-set domain, and two C1-set domains. SIRPβ1 and SIRPγ have similar topologies, and the poorly characterized SIRPβ2 and SIRPδ have two and one domains, respectively. SIRPα contains four Tyr residues in its cytoplasmic domain in ITIM motifs (*blue Y*). SIRPβ1 can associate with DAP12, which contains ITAM (activating) motifs (indicated by the *red Y*), and SIRPβ2 is also predicted to associate with DAP12 or a similar adaptor. SIRPδ has no obvious means of membrane attachment. (The Ig-like domains are shown as *ovals*, and for CD47 and SIRPα they are colored to correspond to the domains depicted in **Figure 2**: *blue* for SIRPα Ig-like domains and *yellow* for CD47 Ig-like domain. Other Ig-like domains are colored *brown*.) There are variations in the organization of the SIRP family members in other species as described in Reference 10. (*b*) SIRPα interacts with phosphatases through recruitment via ITIM motifs in its cytoplasmic region. CD47 can interact via its transmembrane region with integrins such as $\alpha_v\beta_3$ in the membrane, and with extracellular thrombospondin via its Ig-like domain (18, 60). The dimensions are approximate and taken from electron microscopy and X-ray crystallography data.

Both CD47 and SIRPα also engage in other interactions (**Figure 1**), and these have been discussed in more detail elsewhere. Briefly, CD47 interacts in *cis* with certain integrins (16, 17) and functions as a receptor for the extracellular matrix proteins thrombospondin (reviewed in References 18 and 19; see also References 16, 17). Investigators have suggested that the lung surfactant proteins SP-A and SP-D control inflammatory responses in the lung through interactions

with SIRPα (20). These interactions can be blocked by SIRPα antibodies that also block the interaction with CD47, which would indicate an interaction with the NH_2-terminal domain. However this idea is contradicted by another study showing that SP-D can bind in a carbohydrate-dependent manner to the membrane-proximal domain of both SIRPα and SIRPβ (21).

HSC: hematopoietic stem cell

HLH: hemophagocytic lymphohistiocytosis

Expression of CD47 and SIRPα

CD47 is expressed on virtually all cells, including erythrocytes and platelets, whereas SIRPα is expressed on monocytes, most subpopulations of tissue macrophages, granulocytes, subsets of dendritic cells (DCs) in (lymphoid) tissues, some bone marrow progenitor cells, and to varying levels on neurons, with a notably high expression in synapse-rich areas of the brain, such as the granular layer of the cerebellum and the hippocampus (3, 22–24; T.K. van den Berg, unpublished data). One caveat in determining the expression of paired receptors is that reagents—including monoclonal antibodies—may cross-react with other closely related members of the family (25–27) (see sidebar, Paired Receptor Families). A further complicating issue is that there may be major genetic strain differences, as demonstrated, for example, for the mouse CD200R, Nkpr, and Ly49 families (28, 29).

Whereas the levels of SIRPα on phagocytes appear quite stable and do not seem to be much affected by inflammatory conditions (22), the expression of CD47 varies according to immune status or disease. For instance, the levels of CD47 expression fluctuate on CD4 effector T cells during immune responses such that long-lived memory T cell progenitors are associated with high levels of CD47, which may support their survival by preventing clearance by macrophages (30). CD47 is present on hematopoietic stem cells (HSCs) and progenitors, and its expression increases when these cells are mobilized to the circulation following cytokine induction (31). This increased expression may be important in minimizing engulfment by phagocytes. The finding that leukemias express relatively high levels of CD47 suggests that it may be an important survival signal for cancer cells—this is discussed further below with regard to therapeutic possibilities of targeting CD47-SIRPα interactions (32–34). Finally, there is evidence that CD47 may be downregulated during disease. This apparently happens during hemophagocytic lymphohistiocytosis (HLH) syndromes,

PAIRED RECEPTOR FAMILIES

The term "paired receptor" is used for families of related membrane proteins that have closely related extracellular regions but differing transmembrane and cytoplasmic regions. One type gives inhibitory signals usually through ITIM motifs recruiting phosphatases; the other has activating potential and a shorter cytoplasmic region with no motifs, but it associates with an adapter such as DAP12 that contains activating ITAM motifs. Paired receptors are common on natural killer (NK) cells, and well-studied examples include Ly49, KIR, and Nkrp1, but there are also several in addition to SIRPs on myeloid cells such as CD200R, CD300, Siglecs, and ILTs (Ig-like transcripts) (reviewed in References 28, 148–150). The CD200-CD200R interaction, for instance, has many similarities with the CD47-SIRPα interaction. CD200 is a broadly distributed membrane protein that interacts with an inhibitory receptor, CD200R, present on myeloid cells (51). Blocking this interaction is also attracting attention as a possible cancer therapeutic, although there are fewer data available (151–153). One feature of paired receptor families is that they evolve quickly, as shown by their high levels of polymorphism (discussed below) and variable number of genes within a species, as shown for Nkrp1 and Ly49 families (29). The latter could be driven by infection with, e.g., DNA viruses, such as herpes and pox viruses, which are known to have hijacked host ligands for the purpose of evading host immunity (48, 154, 155).

when a selective downregulation of CD47 occurs on $CD34^+CD38^-$ HSCs, which makes them more susceptible to phagocytosis by macrophages (35).

Molecular Characterization of the CD47-SIRPα Interaction

Ig-like domains are the most common domain type at the leukocyte surface, but the organization of CD47 and SIRPα is unusual in that SIRPα has three domains and CD47 a single domain. However, the interaction is between the NH_2-terminal domain of SIRPα (i.e., domain 1) and the single domain of CD47, so the overall spanning distance is similar to that of the interactions of proteins with each of the two Ig-like domains (see the section below entitled "The CD47-SIRPα Interaction and the Immunological Synapse"). CD47 is predicted to have five transmembrane regions—this is a very rare topology. X-ray crystal structures have been determined for all three domains of human SIRPα (36) and human SIRPγ (37); for the single domain of CD47; for the NH_2-terminal domains of human SIRPβ1, SIRPγ (38, 39), and mouse SIRPα (40); and for a complex of SIRPα domain 1 with CD47 (38). A nuclear magnetic resonance structure of the NH_2-terminal domain of human SIRPβ1 has also been determined (PDB code 2D9C). The interaction of human SIRPα and CD47 is of low affinity (K_D on the order of 1 μM), which is typical of interactions between leukocyte surface proteins (41). The SIRPα interaction face is highly convoluted, involving the equivalent of the complementarity-determining regions (CDRs) in antigen receptors (38) (**Figure 2**). SIRPγ interacts with CD47 with an affinity about tenfold weaker than that of SIRPα

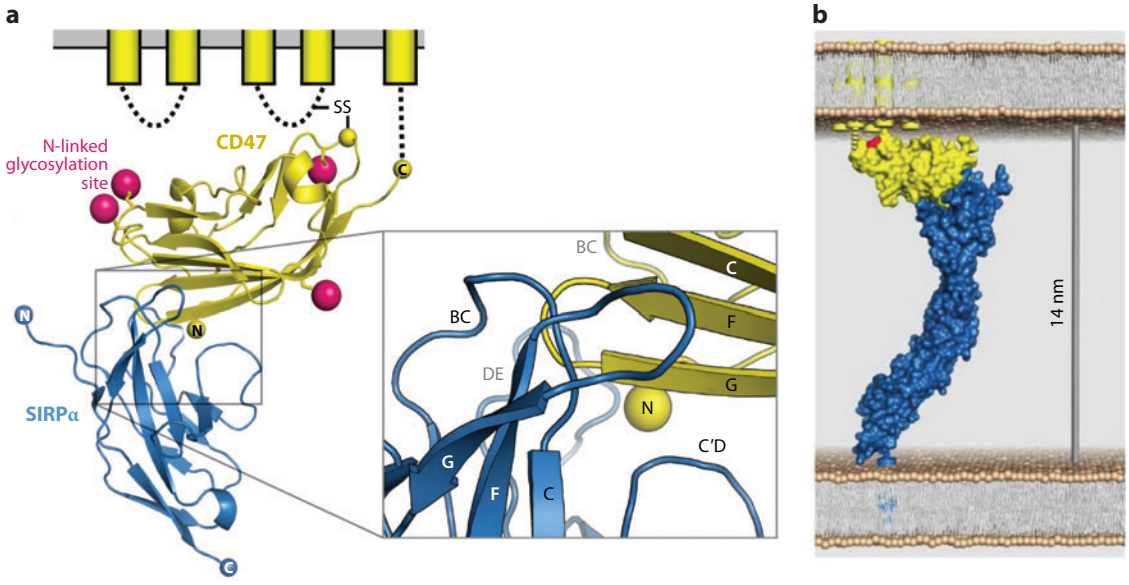

Figure 2

The structure of SIRPα. (*a*) SIRPα domain 1 (*blue ribbons*) complexed with the Ig-like domain of CD47 (*yellow ribbons*). A schematic representation of the 5-transmembrane helix C-terminal domain of CD47 is shown. This includes the proposed disulfide bond between Cys15 (Gly in the structure; *yellow sphere*) and Cys245 of CD47 (146). The interaction with SIRPα is mediated predominantly by loops at the NH_2-terminal ends of the two molecules that interdigitate to form a highly convoluted interaction surface (*see inset*). The NH_2 terminus of CD47 is designated with an "N." (*b*) Topology of the interaction between CD47 and SIRPα. X-ray crystallography shows that SIRPα has an extended structure. The overall distance between opposing cells is estimated to be around 14 nm from analysis of the CD47-SIRPα domain 1 structure and the SIRPα domain 1–3 structure. This distance is similar to that found in immunological synapses. (Adapted with permission from References 36, 38.)

(25). SIRPβ1 shows no significant binding to CD47, nor has any other ligand been identified yet (38, 42); the possibility that it has evolved for interaction with pathogens is discussed below (28, 43). Mutagenesis and analysis of the X-ray crystal structures indicate that the failure of SIRPβ1 to bind to CD47 is due to subtle differences in the binding site region compared with SIRPα. The two variants of SIRPβ1 utilize different residue changes to avoid binding to CD47 (38).

Evolution of SIRPα and CD47

The two membrane-proximal domains of SIRPα show considerable amino acid sequence similarity and structural similarity to the C1-set of Ig-like domains that are restricted to proteins involved in vertebrate antigen recognition: immunoglobulins, T cell antigen receptors, major histocompatibility complex (MHC) antigens, β_2 microglobulin, and tapasin (36, 44). It is intriguing that this common feature—the presence of C1-set domains—has not evolved outside the group of antigen receptors apart from the SIRPs. In addition, the SIRP V-set Ig-like domains have a sequence similar to the J-segments of T and B cell antigen receptors (44), and as indicated above, they also share with antigen receptors ligand recognition involving the CDR loops. In fact, the immediate precursor of rearranging antigen receptors, which formed the presumed target for the initial transposition event that segmented the V-set Ig domain of the primordial antigen receptors (45), may well have been a SIRPβ-like protein (44).

Another feature of the SIRP family is that it is rapidly evolving, with different repertoires of genes present across species (10). The general principles for the evolution of paired receptors are that the inhibitory receptor is primordial and involved in homeostasis and that the activating genes arise by gene duplication, mutation, and gene loss so that the latter show greater variation than the inhibitory receptors (46). SIRPα domain 1 is particularly polymorphic (47). This is not uncommon for paired receptors on natural killer (NK) cells, but in many cases these receptors bind highly polymorphic MHC antigens, such as KIR and Ly49 receptors. In contrast, the ligand-binding domain of CD47 is essentially nonpolymorphic. Interestingly, most of the polymorphisms in SIRPα result in changes in surface residues that are distant from but adjacent to the binding site. Thus, they are unlikely to affect ligand (CD47) binding (38, 43). The pressure to select for polymorphisms may be induced by pathogens targeting the inhibitory receptors, and this may be an important mechanism in the generation of genetic diversity in paired receptors, including the generation of activating members (discussed in 28, 43). CD47 has been acquired by poxviruses, but the extracellular viral region is rather divergent, and there is no reported evidence that it binds SIRPα. Instead, an immune modulatory role for the multipass transmembrane region seems more likely, perhaps involving integrins. Nevertheless, it is interesting that the myxoma CD47 appears essential for establishing a productive infection in rabbits and also seems to act to suppress activation of myeloid cells (48).

The CD47-SIRPα Interaction and the Immunological Synapse

The topology of the CD47-SIRPα interaction can be estimated from the structure of the complex of CD47 with the SIRPα domain 1 and the structure of the complete SIRPα extracellular region (**Figure 2**). The distance spanned by four Ig-like domains is similar to that of many interactions formed when leukocytes interact to form an immunological synapse (49, 50). Although most studies on immunological synapses have involved NK or T cells, synapses are likely also formed by myeloid membrane receptors such as CD200R and SIRPα interacting with their ligands on endothelium or other cells (51). Evidence suggests that CD47-SIRPα is present in synapses at the contacts between human macrophages and human red blood cells, where the accumulation of SIRPα

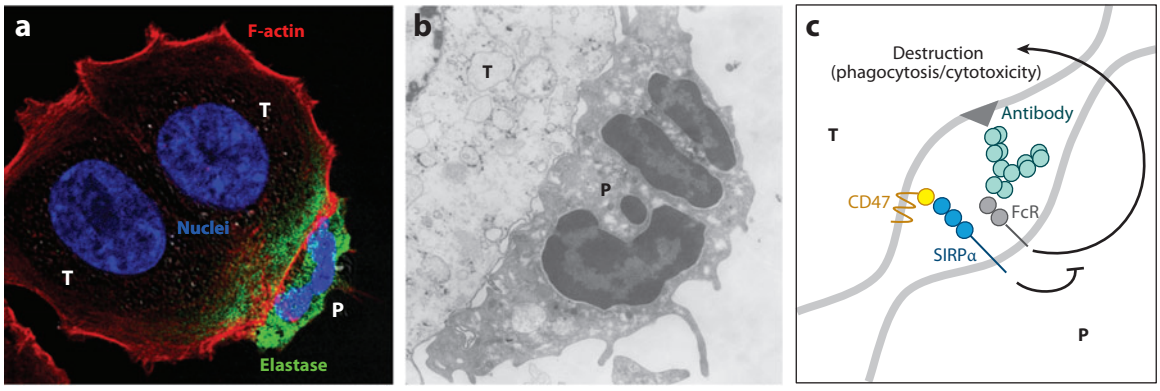

Figure 3

CD47-SIRPα interactions in the immunological synapse. Confocal (*a*) and electron microscopic (*b*) images of cytotoxic synapse formation between a human phagocyte (P)—in this case a neutrophilic granulocyte—and SKBR-3 breast cancer tumor cells opsonized with the therapeutic antibody Trastuzumab (T). (*c*) Schematic representation of the regulation of antibody-mediated destruction of cancer cells by CD47-SIRPα interactions. Antibody on the surface of tumor cells triggers Fc receptor–dependent phagocytosis or cytotoxicity by phagocytes (P), such as neutrophils or macrophages, and this is counteracted by CD47-SIRPα interactions and subsequent SIRPα signaling in phagocytes. Therefore, targeting of CD47-SIRPα interactions can potentiate the clinical efficacy of cancer therapeutic antibodies.

within these synapses is dependent on human CD47 on the red blood cell (52). Furthermore, cytotoxic synapses are found at the interface between human neutrophils and target cancer cells during antibody-dependent cellular cytotoxicity (ADCC) (see **Figure 3** and References 27, 53), where CD47-SIRPα interactions actually seem to restrict integrin-dependent synapse formation (X.W. Zhao & T.K. van den Berg, unpublished data). Improved cytotoxic synapse formation by interference with CD47-SIRPα interactions seems to be at least one way to promote killing of target cells (27) (see also below in the "Cancer Therapy" section).

FUNCTIONAL ROLE OF CD47-SIRPα INTERACTIONS

Signaling Through CD47 and SIRPα

The cytoplasmic domain of SIRPα contains four tyrosine residues, which form two typical ITIM motifs. Upon phosphorylation, these can recruit and activate the cytosolic tyrosine phosphatases SHP-1 or SHP-2 (but not the SH2-domain containing inositol phosphatases SHIP-1 or SHIP-2), which can in turn dephosphorylate a variety of substrates and regulate downstream signaling pathways, most commonly in an inhibitory fashion. Two Tyr residues (449 and 473) were identified as being essential for SHP-2 binding (54). When present in the cytosol, SHP-1 and SHP-2 are largely inactive because of an autoinhibitory mechanism involving the SH2 domains. However, recruitment of the SH2 domains to the phosphorylated ITIMs of SIRPα or other inhibitory receptors causes a conformational change that unmasks the phosphatase activity (55). SHP-2 has a ubiquitous expression pattern and could therefore be relevant for SIRPα signaling in either myeloid or neuronal cells, whereas SHP-1 is primarily expressed in hematopoietic and epithelial cells. SHP-1 can therefore act exclusively in the context of myeloid cells. Although it seems reasonable to assume that SHP-1 and/or SHP-2 mediate many of the inhibitory functions of SIRPα, direct evidence for this, such as by mutagenesis of the ITIM tyrosines, is not always available (56). Therefore, some of the signaling functions of SIRPα could be mediated by some

ADCC: antibody-dependent cellular cytotoxicity

SH2: Src homology 2 domains bind to phosphotyrosine residues within a cytoplasmic peptide motif

of the other signaling molecules that can bind to SIRPα, as indicated by biochemical experiments (12, 57, 58), although there are not strong direct binding or functional data for these interactions. It is possible that the two proline-rich regions that are found between the tandem tyrosines of the ITIMs in the cytoplasmic tail of SIRPα interact with SH3 domains in certain signaling proteins.

SH3: Src homology 3 domains generally bind to proline-rich cytoplasmic peptide motifs

A key feature in SIRPα signaling is that the tyrosines in the SIRPα cytoplasmic domain need to be phosphorylated before they can recruit phosphatases. The phosphorylation status may be generally low at least in cultured resting phagocytes (23), but it may also be enhanced by integrin-mediated adhesion (59), and the levels of SIRPα phosphorylation on the various populations of cells that express the receptor in vivo remain unknown. Nevertheless, engagement of SIRPα by CD47 promotes SIRPα ITIM phosphorylation, most likely by the activity of Src family kinases, enhancing phosphatase recruitment and activity in the phagocytic synapse and thereby limiting phagocytosis. At least one potentially important intermediate in this context is the motor protein myosin IIA that is dephosphorylated upon SIRPα triggering in macrophages (52).

Many functional data can be interpreted in terms of SIRPα signaling, but engagement of CD47 can also lead to signaling. Although we do not discuss CD47 signaling in detail, it may involve both heterotrimeric Gi protein–dependent and –independent pathways, and signaling may also occur through the integrin cytoplasmic tail (18, 60).

Adhesion and Migration

Several studies have suggested a role for CD47-SIRPα interactions and signaling in cellular adhesion and migration, but this has not yielded a clear and universal picture. Evidence suggests that interactions between SIRPα expressed on neutrophils or monocytes and CD47 expressed on endothelial or epithelial cells play a role during transendothelial or transepithelial migration in vitro (61–64). In particular, the diapedesis step appears to require CD47-SIRPα interactions and signaling via CD47 to open the junctions between the endothelial cells (61). Although these findings initially appeared to be supported by the reduced neutrophil infiltration observed during bacterial peritonitis in CD47-deficient mice (65), more recent studies in sterile inflammation models, such as zymosan-induced peritonitis, do not show a substantial requirement for CD47 in phagocyte extravasation (66), arguing against a generalized nonredundant role for CD47-SIRPα interactions in this process.

Could there nevertheless be a role for SIRPα signaling independent of CD47 and CD47-SIRPα interactions? Indeed, early studies by the Kasuga group (1) and others (67, 68) with immortalized nonhematopoietic cells, such as fibroblasts or epithelial cells, with (forced) overexpression of SIRPα showed that integrin-mediated adhesion promoted phosphorylation of the SIRPα ITIM tyrosines and SHP-2 recruitment and activation, which in turn stimulated mitogen-activated protein kinase activation and cell migration. SIRPα can also be phosphorylated following β_2 integrin–mediated adhesion in macrophages and neutrophils (59), and although SIRPα seems to be involved in integrin-induced cytoskeletal reorganization in macrophages (12), the functional relevance of these findings in the context of myeloid cell migration remains in doubt. In fact, our own recent thioglycollate-induced peritonitis studies with mice that lack the SIRPα cytoplasmic tail and, as a result, lack its signaling capacity have revealed only a minor delay in the kinetics of neutrophil and macrophage infiltration and no differences in its magnitude (J. Alvarez-Zarate, H.L. Matlung, I. Maridonneau-Parini, T.K. van den Berg, manuscript submitted) (see sidebar on SIRPα Mutant Mice). Therefore, CD47 and SIRPα appear not to play a prominent or generalized physiological role in neutrophil and monocyte extravasation. However, as is discussed in more detail in the "Dendritic Cell Function and T Cell Activation" section below, there is

SIRPα MUTANT MICE

Whereas a completely CD47-deficient mouse cell line has commonly been used to study the role of CD47 (65), a mutant strain of mice has been used to study the function of SIRPα and, in particular, the contribution of SIRPα signaling. These SIRPα mutant mice express a truncated SIRPα receptor that lacks the cytoplasmic tail and is therefore unable to provide intrinsic intracellular signals (75, 156).

growing evidence that CD47 plays a role in DC migration and function, although the underlying mechanism is unclear.

Host Cell Phagocytosis

One of the best-characterized physiological functions of CD47-SIRPα interactions is their role in the homeostasis of hematopoietic cells, in particular red blood cells and platelets. Initial evidence for this role came from pioneering in vivo experiments in which CD47-deficient erythrocytes were infused into wild-type mice and were found to be cleared within hours (70). In contrast, normal red blood cells survive for 60–80 days in mice. CD47 was thus identified as a marker of self, or as a don't-eat-me signal, that acts to prevent the homeostatic clearance of red blood cells. Subsequent studies confirmed that this was related to the interaction of CD47 with SIRPα on macrophages (71), which in vivo are most likely those found within the red pulp of the spleen, the major site of normal red blood cell clearance. Furthermore, the prevention of macrophage phagocytosis involves inhibitory signaling by SIRPα, again at least in part via SHP-1 (72), to inhibit macrophage phagocytosis, as shown by in vitro and in vivo experiments with the SIRPα mutant mice (reviewed in 73). Although the actual receptors that mediate homeostatic red blood cell clearance by macrophages are not understood, CD47-SIRPα interactions also suppress the clearance of antibody- or complement-opsonized red blood cells through, respectively, Fcγ and complement receptors on macrophages, and this could be relevant in the context of autoimmune hemolytic anemia (AIHA) (reviewed in 18). A recent study has shown that the role of CD47-SIRPα interactions in red blood cell clearance may be more complicated than earlier thought. In particular, human red blood cell aging appears to be associated with a conformational change in CD47, possibly dependent on oxidative modification, which turns the molecule from a don't-eat-me into an eat-me configuration (74). Therefore, CD47 may not only act as a don't-eat-me signal; rather, it may also function as a molecular switch that regulates erythrocyte clearance.

An intriguing finding in the original study that established the role of CD47-SIRPα interactions in red blood cell clearance is that, when CD47-deficient erythrocytes are transfused into CD47-deficient recipients, the kinetics of clearance are virtually normal compared with the clearance in wild-type controls. This is consistent with the only mild anemia observed in CD47-deficient or SIRPα mutant mice (75; J. Alvarez-Zarate & T.K. van den Berg, unpublished data). Systematic analysis of this phenomenon using bone marrow chimeric mice demonstrated that it was the lack of CD47 on nonhematopoietic cells that was tolerizing macrophages to clear CD47-deficient red blood cells (76). It seems, therefore, that macrophages need to be educated by the presence of CD47 on stromal cells to acquire the inhibitory capacity of the SIRPα interaction (7).

Of interest, a comparable mechanism, termed "licensing," involves signaling via the ITIMs of NK cell inhibitory receptors for MHC class I molecules, as has been described in the context of NK cell killing (77). Notably, the stromal CD47-mediated licensing of the phagocytic activity of macrophages was more efficient toward nucleated hematopoietic cells, including both

AIHA: autoimmune hemolytic anemia

NOD: nonobese diabetic

SCID: severe combined immunodeficiency

myeloid and lymphoid cells, than toward red blood cells (76), indicating a certain level of cell-type specificity, termed "split tolerance." One interesting possibility is that additional inhibitory receptors on macrophages participate in the recognition and prevention of clearance of hematopoietic (progenitor) cells other than red blood cells, which suggests that licensing the phagocytosis of some cells depends on interactions in addition to those between CD47 and SIRPα. This observation is consistent with the normal white blood cell counts observed in CD47$^{-/-}$ and SIRPα mutant mice (70, 75).

In addition to the anemia observed in mice mutant for CD47 or SIRPα, these animals are also mildly thrombocytopenic (75; J. Alvarez-Zarate & T.K. van den Berg, unpublished data). Evidence suggests that the lower platelet numbers are also caused by enhanced clearance rather than by abnormal platelet formation (75). Although the numbers of the other blood cell populations under steady-state conditions are not affected, there is a clear role for CD47-SIRPα interactions in HSC transplantation and in cancer cell elimination (which are discussed in detail below in the next section, "Transplantation and Xenotransplantation," and the subsection below under "CD47-SIRPα Interactions in Disease" on cancer). This indicates that the function of CD47-SIRPα in host cell clearance (and/or killing) is a more generalized one that extends beyond that of erythrocytes and platelets.

Transplantation and Xenotransplantation

Several studies show that the CD47-SIRPα interaction is less effective across species than within a given species (78, 79), and this may have important implications for xenotransplantation. Essentially, two areas in xenotransplantation are of particular interest for human disease, and both have been explored with respect to the role of CD47-SIRPα interactions. The first is the generation of optimal humanized mouse models for studying various types of disease in an in vivo human immunologic-hematologic context. The second relates to the use of animal-derived xenotransplants for the replacement of damaged tissues and organs in human patients (see the section on "Xenotransplantation" below). Takenaka et al. (47) used an unbiased positional cloning approach to study why mouse strains of the NOD/SCID background represent superior models for human hematopoietic cell engraftment and identified SIRPα as the causative genetic factor (see the sidebar on NOD Immunodeficient Mouse Strains for Human Cell Engraftment). Murine SIRPα

NOD IMMUNODEFICIENT MOUSE STRAINS FOR HUMAN CELL ENGRAFTMENT

Immunodeficient mouse strains with complex names have been developed that allow human cell engraftment. These are deficient in either of two enzymes involved in the recombination event in the generation of B and T cell receptors, which perturbs further lymphocyte development and thereby facilitates graft survival. Two mutants are commonly used: One has a mutation in the catalytic subunit of the DNA-dependent protein kinase (DNA-PK, or PRKDC) and is known as a severe combined immunodeficiency (SCID) mouse; the second has a mutation in one of the two recombination activating genes (*Rag1* or *Rag2*). Most often the mice employed are also deficient in NK cells owing to the deletion of the gene encoding CD132 (common gamma chain of several cytokine receptors). These mutations are mostly used in the genetic context of the nonobese diabetic (NOD) mouse because of its superior performance in terms of human cell or tissue engraftment compared with other commonly used strains such as BALB/c or C57Bl/6. The immunodeficient NOD strains typically used today are known as NSG (NOD/SCID gamma)—for the NOD strain that has the SCID and IL-2Rγ^{null} chain mutations—and NRG (NOD Rag2 gamma)—NOD mice that harbor the *Rag2*^{null} mutation together with the *IL2rγ*^{null} mutation.

is highly polymorphic, and the SIRPα polymorphic variant of NOD mice (in contrast to SIRPα in other commonly used mouse strains) appears to have a particularly high affinity for human CD47, which is even higher than that between mouse CD47 and mouse SIRPα or between human CD47 and human SIRPα (L.-S. Kwong, M.H. Brown, A.N. Barclay & D. Hatherley, unpublished data; 80). This promotes acceptance of the graft, probably by preventing phagocytosis of the human cells by the mouse macrophages. A subsequent study demonstrated that the engraftment of human acute myeloid leukemic stem cells in NOD mice was also dependent on this superior interaction of human CD47 with the NOD SIRPα (80). Breeding of the NOD *SIRPA* gene onto the Rag2^{null}IL-2Rγ^{null}/BALB/c or Rag2^{null}IL-2Rγ^{null}/C57Bl/6 background has provided formal proof that NOD mouse SIRPα was sufficient to enhance human hematopoietic cell engraftment (81, 82). An additional advantage is that such mice lack other abnormalities of the NOD background, such as the known C5 deficiency, DC maturation defects, and late-onset lymphoma development (82). In any case, the restoration of interactions between donor CD47 and recipient SIRPα seem to substantially improve the transplantation of human hematopoietic cells into mice. This was also confirmed by two other approaches in which either the host cells or the donor cells were manipulated to provide a compatible CD47-SIRPα interaction. Strowig et al. (83) introduced a BAC clone encoding a human SIRPα into Rag2^{null}IL-2Rγ^{null} mice of a mixed 129/BALB/c background. These investigators confirmed that the human transgenic SIRPα was expressed on myeloid cells and demonstrated significantly improved multilineage engraftment after introduction of human HSCs. The level of engraftment, which was ~3–5 times higher in absolute cell numbers than in control mice, was comparable to that found in Rag2^{null}IL-2Rγ^{null}/NOD mice. In particular, the numbers of T cells were relatively high and the mice had higher levels of both basal and inducible antibody responses.

Legrand et al. (81) showed that the introduction of mouse CD47 into human hematopoietic progenitor cells considerably enhanced their potential to provide long-term multilineage engraftment in Rag2^{null}IL-2Rγ^{null}BALB/c mice and even in Rag2^{null}IL-2Rγ^{null}C57Bl/6 mice, which are otherwise refractory to human hematopoietic cell engraftment. In particular, investigators observed not only enhanced thymopoiesis and considerably higher peripheral T and NK cell numbers, but also improved T cell homeostasis, with a lower level of basal CD4$^+$ and CD8$^+$ T cell activation, a more physiological organization of the cells in peripheral lymphoid tissues, and an apparently better support of B cell antibody responses. The numbers of peripheral B cells and myeloid cells were also enhanced. The multilineage increase in engraftment observed in these models can likewise be attributed to a combination of effects, including a sustained increase in HSCs in the bone marrow as well as a survival benefit of the various types of hematopoietic cells. However, findings from the Legrand et al. (81) study suggest that the presence of mouse CD47-expressing human hematopoietic cells also partially enhanced the survival of the mouse CD47-negative human cells present in this system, which points to some kind of bystander mechanism of induced macrophage tolerance for recognition of CD47-deficient cells. Although this may act in a way similar to that described above for CD47-deficient murine cells (76), this situation clearly involves CD47 on hematopoietic instead of nonhematopoietic cells. Taken together, these findings provide compelling evidence that functional CD47-SIRPα interactions improve the engraftment of human hematopoietic cells in immunocompromised mouse models.

Dendritic Cell Function and T Cell Activation

Given that SIRPα is expressed on defined subsets of DCs in rats, mice, and humans and that CD47 is expressed on both T cells and DCs, DC and T cell responses may be affected by CD47-SIRPα

interactions at multiple levels. For example, various in vitro and in vivo studies in which the CD47-SIRPα interaction is perturbed make clear that DC function is compromised, particularly in the triggering of Th1, Th2, Th17, and NKT cell responses (12, 84–98). This impairment could be due to a requirement for CD47-SIRPα interactions at different levels, including DC maturation, migration, and antigen presentation. However, the interpretation of some of these studies in mechanistic terms is not always straightforward: For example, it is not clear whether the reagents (antibodies, recombinant proteins) used for manipulation were acting as agonists or antagonists or whether potential interactions between CD47 and SIRPα in *cis* on DC were involved as well. Furthermore, evidence supports a role for SIRPα signaling that occurs independently of CD47 in the induction of delayed-type hypersensitivity responses (91). We are unaware of evidence that CD47-SIRPα interactions play a direct role in triggering CD8 T cell responses, although an enhanced level of uptake of target cells by opsonization and/or blocking of CD47-SIRPα interactions does promote such responses and may also contribute to cancer cell elimination (99). Clearly, a generalized defect in CD4 T cell priming may affect the composition of the T cell compartment in mice with defects in CD47 or SIRPα, and indeed SIRPα mutant animals have selectively decreased numbers of CD4 T cells and reduced T cell areas in peripheral lymphoid tissues (100).

Taken together, these findings show a prominent role for CD47-SIRPα interactions in the induction of CD4 T cell responses. They also provide at least a partial explanation for why such interactions are required in autoimmunity and certain infections (see below).

CD47-SIRPα Interactions in Disease

Blood disorders. Based on the involvement of CD47-SIRPα interactions in phagocytosis, deregulation of CD47 and/or SIRPα expression in disease could disturb homeostasis and cause altered host blood cell clearance and thereby contribute to pathogenesis of blood disorders. A recent study by Kuriyama et al. (35) suggests that this occurs in HLH syndromes. These syndromes, which are characterized by hemophagocytosis (i.e., phagocytosis of hematopoietic cells) and pancytopenia, can be caused by mutations in genes that encode for proteins that are part of the cellular machinery mediating the biosynthesis or exocytosis of cytotoxic granule contents. When confronted with viral infections such as cytomegalovirus or Epstein-Barr virus, the cytotoxic T cells and NK cells are unable to clear virus-infected cells. As a result, they continuously produce proinflammatory cytokines, such as IFN-γ and others, that then activate macrophages to engulf hematopoietic cells. CD47 is selectively downregulated on $CD34^{+}CD38^{-}$ HSCs during exacerbations of HLH, which renders the cells prone to phagocytosis by macrophages (35). Moreover, the proinflammatory cytokines that are produced during HLH can mimic this selective decrease in CD47 expression on normal HSCs. This decrease in HSC CD47 expression during HLH may be primarily responsible for, or at least contribute to, the dramatic reduction in numbers of HSCs on the bone marrow of HLH patients and the associated pancytopenia. In the mouse, CD47 is highly expressed on HSCs, with a particularly high expression on circulating mobilized HSCs, and CD47 constitutes an important in vivo determinant for clearance of circulating HSCs by macrophages (31).

Cancer. Because CD47 serves as a don't-eat-me signal and, as such, is an important determinant of host cell phagocytosis by macrophages, the potential contribution of CD47-SIRPα interactions in cancer cell clearance has been intensely investigated in recent years. In a series of studies pioneered by the Weissman group (34), various cancer cells, including those from both hematologic and nonhematologic origin, expressed relatively high levels of CD47, with the highest levels generally found on the cancer stem cells. In fact, high levels of CD47 were an adverse prognostic factor in a

human context, with cancers with a relatively high CD47 expression level having a worse prognosis compared to those with a relatively low CD47 expression level (33, 34). Indeed, consistent with CD47 being a don't-eat-me signal, CD47 on cancer cells apparently acts to inhibit macrophage phagocytosis, given that overexpression of CD47 on myeloid leukemia cells prevented clearance of tumor cells by macrophages and enhanced survival of tumor cells in vivo (31). Studies with syngeneic solid tumors in SIRPα mutant mice suggested that SIRPα signaling itself is not important for cancer metastasis and outgrowth but that CD47-SIRPα interactions and subsequent SIRPα signaling do restrict the efficacy of cancer therapeutic antibodies (27). Collectively, these findings have created a rational basis for targeting CD47-SIRPα interactions in cancer and, in particular, for enhancing the efficacy of antibody therapy in cancer (see the "Cancer Therapy" section below).

EAE: experimental autoimmune encephalomyelitis

APC: antigen-presenting cell

Autoimmunity. If CD47-SIRPα interactions play a critical homeostatic role in regulating the effector functions of phagocytes, do they protect against autoimmunity? In other words, does deficiency of CD47 or SIRPα or interference with CD47-SIRPα interactions enhance the susceptibility to autoimmunity? In general, the answer seems to be no. For instance, neither $CD47^{null}$ nor SIRPα mutant mice show any signs of overt autoimmunity (65, 75; J. Alvarez-Zarate & T.K. van den Berg, unpublished data); furthermore, the treatment of mice (33), rats (A. van der Goes & T.K. van den Berg, unpublished data), and cynomolgus monkeys (101) with CD47-SIRPα antagonists does not result in signs of autoimmunity. No toxic effects of genetic or pharmacological interference have been observed other than a mild degree of anemia that could be expected based on the homeostatic role of CD47-SIRPα interactions in erythrocyte clearance (see "Host Cell Phagocytosis" subsection above). Evidence from several studies with different models suggests that CD47-SIRPα interactions are instead required for the induction of T cell–mediated autoimmunity. This includes results from studies with in vivo models for experimental autoimmune encephalomyelitis (EAE) (97, 102), bacteria- or collagen-induced arthritis (103–105), colitis (95, 96), and Crohn's disease patients (106), in which either mutant mice, antagonistic antibodies, or recombinant proteins were used. Mechanistic studies indicate that CD47-SIRPα interactions are required for DC functions, in particular migration and/or antigen presentation, that are necessary to generate autoreactive Th1 and/or Th17 cells (95–97). In certain situations, such as arthritis, colitis, and Crohn's disease, antagonistic interference with CD47-SIRPα interactions initiated at a time of established disease can suppress the clinical symptoms (96, 104, 106), suggesting that such antagonists may be considered as therapeutics. However, during EAE this may actually worsen autoimmunity and the associated disease, possibly by an enhanced uptake and presentation of myelin by APCs (102, 107). In diabetes the situation may be more complicated because CD47-SIRPα interactions may also regulate insulin secretion and insulin responsiveness (108, 109). Finally, as might be expected, the clearance of red blood cells and platelets in passive mouse models of AIHA and idiopathic thrombocytopenic purpura (ITP) is exaggerated in the absence of CD47 (72, 110, 111). However, no evidence exists for dysregulation of CD47 on blood cells in patients with AIHA or ITP (112–114).

Infection and Toll-Like Receptor–Induced Inflammatory Mediator Production

Surprisingly few reports address the role of CD47-SIRPα interactions in host defense against infection, and those that do are essentially restricted to bacterial infection. The simplest explanation is that there is no prominent role, at least in innate host defense, yet a role cannot be excluded, particularly given the function of CD47-SIRPα interactions in the induction of T cell responses. Indeed, supporting this idea is a study by Li et al. (98) showing that SIRPα mutant mice have an

increased susceptibility to infection with *Salmonella typhimurium*. Whereas control mice clear the infection by T cell– and antibody-mediated immunity and survive, the SIRPα mutant mice show impaired antigen presentation by DCs, reduced *Salmonella*-specific T cell and antibody responses, and increasingly higher loads of bacteria, until they finally succumb to the infection. An additional (or alternative) explanation might be that the SIRPα mutant mice, probably as a consequence of their mild chronic anemia, have an increased level of extramedullary erythropoiesis in the spleen, which is known to confer resistance to *Salmonella* infection (115, 116).

Several studies have reported downregulation of SIRPα in macrophages upon stimulation with Toll-like receptor (TLR) ligands (117–119). Interference studies with siRNA- or shRNA-mediated knock-down of SIRPα in macrophages (118, 119) suggest that this downregulation of SIRPα facilitates TLR signaling and downstream responses, including production of cytokines and other inflammatory mediators as well as bacterial killing. On the basis of these observations, investigators proposed that inhibitory signaling downstream of SIRPα would restrict TLR signaling (118, 119). However, introduction of foreign nucleic acids such as siRNA or shRNA into macrophages is tricky because it may activate endogenous danger pathways (120), and studies with SIRPα mutant macrophages have not revealed any abnormalities in the responsiveness of these cells to TLR ligands or IFN-γ (56). The only inflammatory mediators shown to be moderately downregulated by CD47-SIRPα interactions and SIRPα signaling are the reactive oxygen species generated by the phagocyte NADPH oxidase, and this appeared to be due to a selective downregulation of $gp91^{phox}$, the catalytic component of this oxidase (56).

Finally, the findings in the original studies performed with the $CD47^{null}$ mice—which show that the mice have defective neutrophil recruitment during *Escherichia coli* peritonitis (65)—may not necessarily be explained by a disruption of interactions between SIRPα on neutrophils and CD47 on other cells. As already discussed above (in the section on "Adhesion and Migration"), CD47-SIRPα interactions apparently do not play a prominent or generalized role in phagocyte extravasation. Furthermore, more recent *E. coli* pneumonia experiments show that it is neutrophil CD47, rather than stromal CD47, that is important for lung inflammation (121). Whether neutrophil CD47 acts to prevent local or systemic neutrophil clearance or to cooperate with neutrophil integrins to optimize pathogen recognition or other adhesive events is not clear.

Thus, CD47-SIRPα interactions do not seem to play a dominant, nonredundant role in either positively or negatively controlling the innate host defense mechanisms of phagocytes. This limited role may be an advantage in the context of therapeutic intervention in cancer and other conditions.

CD47-SIRPα INTERACTION AS A THERAPEUTIC TARGET

Xenotransplantation

Pigs are generally considered to be a particularly suitable source of donor organs for xenotransplantation into humans. The initial in vitro studies by Ide et al. (122) suggest that a potential incompatibility between porcine CD47 and human SIRPα could form a barrier for xenotransplantation from pigs into humans. In particular, these investigators showed that porcine lymphoblastoid cells were readily phagocytosed by human macrophages but that this could be prevented by the forced expression of human CD47 in the pig cells. Other studies with mice as a recipient model for transplantation of pig erythrocytes or lymphoblastoid cells have suggested that porcine CD47 would not effectively engage with mouse SIRPα but that this could be overcome with the introduction of mouse CD47 into the porcine cells (123). In line with this observation, the transgenic expression of human CD47 into porcine cells enhances their survival in the NOD/SCID mouse model, suggesting that this may indeed be a feasible method for inhibiting the macrophage-mediated rejection of

xenografts in the clinic (124). Notably, measurements of CD47-SIRPα interactions were missing in these and many other studies in this field, rendering interpretation less straightforward. Other hurdles must also be overcome, such as the xenoresponse to the pig Galα1,3Galβ1,4GlcNAc epitope that occurs because humans lack the α1,3galactosyl transferase (GalT) (125), which may be tackled by knocking out the porcine GalT gene. However, manipulation of pigs to overexpress human CD47 may prove helpful.

Although hematopoietic donor cells have been used in most studies dealing with the role of CD47-SIRPα in xenotransplantation, CD47 may also be involved in and exploited for preventing transplant rejection of nonhematopoietic cells. This potential function has been shown for hepatocytes in a syngeneic setting with CD47-deficient donor cells (126) and in a xenotransplantation model with human hepatocytes overexpressing murine CD47 grafted into immunodeficient mice (127). However, we do not know if this is a universal principle. In a syngeneic thymus transplant model, for instance, the engraftment of thymic epithelial cells was not affected by the absence of CD47 on donor cells (128).

Again, in this context, it should be noted that despite the presence of several polymorphic variants of SIRPα documented in both mouse and human and the existence of interspecies incompatibility in CD47-SIRPα interactions, there is not much intraspecies variation in this interaction. Actually, relatively little variation appears in the affinities of the CD47-SIRPα interaction between mouse, rat, and human. Nevertheless, even a moderate (30%) reduction in surface CD47 expression in HSCs results in a level of macrophage phagocytosis five times greater than it would be without the reduced surface expression (35); thus, an enhancement of CD47 expression levels on donor cells could significantly improve allogeneic or autologous transplant survival.

Apart from the direct contribution of CD47-SIRPα interactions in preventing (xeno)transplant rejection by inhibiting phagocytosis of donor cells by macrophages, interactions of CD47 with SIRPα for tolerizing DCs may also play a role in the context of alloimmunization. Wang et al. (129) showed that the tolerizing activity of antigen-specific donor splenocytes toward skin grafting over an MHC class I barrier—seen upon transfusion of these splenocytes prior to grafting—depends on donor cell CD47, which probably acts to suppress DC activation.

Cancer Therapy

For reasons outlined above, the targeting of CD47-SIRPα interactions in cancer might be beneficial from a therapeutic perspective (for a schematic overview of targeting options, see **Figure 4**). Studies with various human leukemic cells in xenogeneic mouse models (with NSG and NRG mice—see the sidebar above entitled "NOD Immunodeficient Mouse Strains for Human Cell Engraftment") have indeed demonstrated that targeting human CD47 with a CD47 antibody that blocks CD47-SIRPα interactions inhibits phagocytosis and engraftment of the cancer cells and also that the antibody against human CD47 can eliminate preestablished leukemia (33, 34, 130–133). The contributions of CD47-SIRPα interactions may be somewhat exaggerated in these NOD-based mouse models, however, because of the superior interaction of human CD47 with NOD mouse SIRPα and perhaps also because other similar species-specific compatibility signals may be missing that could act redundantly with CD47-SIRPα interactions. Nevertheless, studies in syngeneic models have demonstrated a tumor-eliminating effect of antimouse CD47 in nonhematopoietic solid tumors (134), although the mechanism of action of the antibodies used in these experiments remains somewhat ambiguous (135, 136; see also below). Furthermore, the human CD47 antibody synergized with the cancer therapeutic antibody Rituximab in the clearance of human non-Hodgkin lymphoma cells (33). These effects of anti-CD47 were attributed

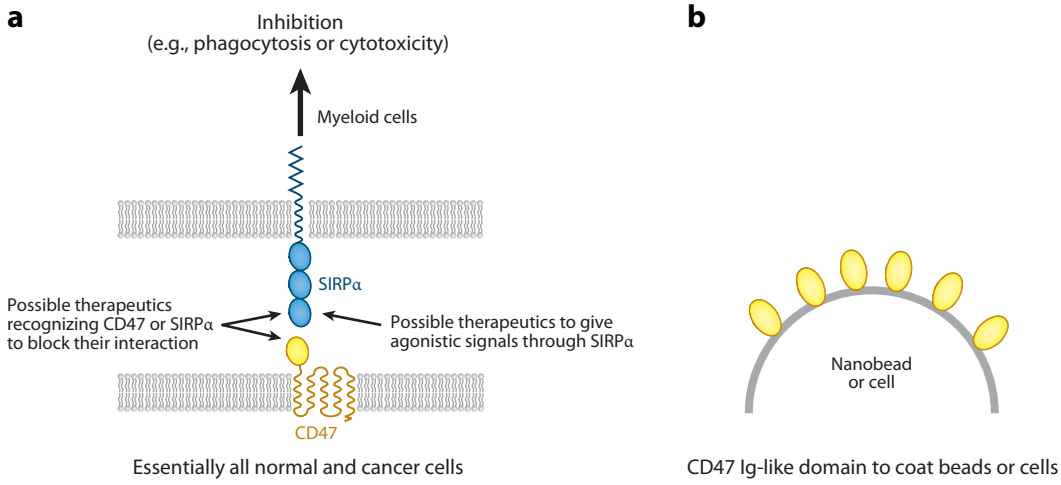

Figure 4

Therapeutic manipulation of the CD47-SIRPα interaction. (*a*) Both SIRPα and CD47 are possible therapeutic targets in which antibodies or other reagents react with the CD47-SIRPα interaction site and neutralize the subsequent inhibitory signals. (*b*) An alternative is to use CD47 protein as an inhibitor on, for instance, particles containing drugs to minimize phagocytosis of the particles or to limit phagocytosis of cells.

to the blocking of CD47-SIRPα interactions, resulting in an enhanced level of phagocytosis by macrophages in vivo (32, 137).

Although a direct growth inhibitory or proapoptotic effect of anti-CD47 triggering on the cancer cells could be excluded in these experiments (see also below), a major problem in interpretation is that intact CD47 antibodies were employed, which may not only have antagonized CD47-SIRPα interactions but also have resulted in direct antibody opsonization of the cancer cells, thereby rendering them susceptible to FcγR-mediated phagocytosis and/or ADCC. Indeed, evidence suggests that the B6H12 CD47 (mouse IgG1) antibody used in all these studies can trigger direct FcγR-dependent effector functions by macrophages or neutrophils (27, 101, 138). In vivo studies with SIRPα mutant mice injected with syngeneic tumor cells did not show detectable tumor-eliminating effects but were very effective in doing so when therapeutic antibodies against the tumor cells were applied as well (27). Furthermore, in vitro ADCC experiments with both human effector and target cells have demonstrated that the blocking of CD47-SIRPα interactions is insufficient to trigger cytotoxicity but that interference with CD47-SIRPα interactions potently synergizes with cancer therapeutic antibodies used to opsonize the tumor cells (27, 139). This view is supported by a recent study in which a synthetic, affinity-enhanced, single domain SIRPα recombinant protein was used to target human CD47 and antagonize CD47-SIRPα interactions (101).

Importantly for potential clinical applications of CD47-SIRPα antagonists, no or only minimal signs of cytotoxicity have been noted, at least in rodents and cynomolgus monkeys, apart from a mild anemia probably caused by an enhanced clearance of red blood cells (27, 33, 34, 101, 134). Thus, although controversy persists about whether the targeting of CD47-SIRPα interactions alone can have antitumor effects, preclinical experiments provide compelling evidence that interference with CD47-SIRPα interactions can enhance the efficacy of various cancer therapeutic antibodies, rendering it a promising generic strategy to potentiate the clinical effects of cancer therapeutic antibodies in patients. The latter may help to reduce, or perhaps at some point even eliminate, the use of nonspecific treatment regimens with, for example, chemotherapeutics that

have adverse effects and are even mutagenic. In fact, by causing leukopenia and thereby strongly reducing the number of available immune effector cells required for the antibody-mediated cellular effector functions, these chemotherapeutics may actually compromise the beneficial effects of therapeutic antibodies. Therefore, methods that could increase the efficacy of cancer therapeutic antibodies would be strong multipliers for cancer treatment.

Nevertheless, in addition to the effects that can be achieved through the targeting of CD47-SIRPα interactions, cross-linking of either CD47 or SIRPα can also exert direct effects on certain tumor cells in vitro. In various hematopoietic malignancies, such as B-chronic lymphatic leukemia, bivalent anti-CD47 antibodies or other cross-linking agents such as thrombospondin-1 induce apoptosis (reviewed in 18, 140). Whether CD47 cross-linking in vivo by SIRPα or other CD47 ligands has a physiological role in controlling the growth of these malignant cells is unknown. However, antibody-mediated cross-linking of CD47 can be used to deliver cell death–inducing signals to cancer cells in vivo (140, 141). The effects also seem to occur independently of CD47-SIRPα interactions because at least the lymphocytic malignant cells studied do not express SIRPα. Yet such interactions may be relevant for limiting the survival of certain types of tumor cells in vivo. Finally, it seems that in malignant myeloid cells SIRPα triggering can also induce apoptosis (142).

In addition to the use of antibodies and recombinant CD47- or SIRPα-based proteins in targeting the CD47-SIRPα interaction, investigators have also explored siRNA approaches. As shown in a recent report using a syngeneic mouse model, nanoparticles carrying siRNA that strongly reduces CD47 expression in B16F10 melanoma cells—both in vitro and in vivo—can significantly reduce cancer growth and metastasis (143). However, the antitumor effects in this study could well be attributed to mechanisms other than the lack of CD47-SIRPα interactions because no difference in B16F10 melanoma metastasis and outgrowth is observed in the SIRPα mutant mice (27).

Enhancing Drug Delivery

The capacity of CD47 to inhibit phagocytosis has been exploited for improving drug delivery in the context of cancer. In particular, Rodriguez et al. (144) used CD47 recombinant protein coupled to nanoparticles. This enhanced the half-life of IgG-opsonized nanoparticles in the circulation and improved the delivery of dye and cytostatic drugs to tumors in vivo (see **Figure 4**). CD47's role as a don't-eat-me signal—by inhibiting clearance of IgG-decorated particles via FcγR—is clearly consistent with the role of CD47-SIRPα interactions in the destruction of antibody-opsonized tumor cells, as discussed above. Nevertheless, we do not know definitively whether CD47 also inhibits the clearance through other phagocytic pathways. Rodriguez et al. also used a peptide mimic from the interaction site of CD47 as well as the whole domain. Using a peptide to mimic such an interaction is unprecedented, but we have found no binding of this peptide to SIRPα (D. Hatherley & A.N. Barclay, unpublished data). In a different approach, Hu et al. (145) coated particles with red blood cell membranes and showed that this coating inhibited phagocytosis of the beads in a CD47-dependent manner, suggesting that targeting CD47-SIRPα interactions may be useful for aiding drug delivery on particles.

Targeting Dendritic Cells

The studies described here concentrate on interfering with the CD47-SIRPα interactions that inhibit phagocytosis, but the interference can affect other phenomena. Migration of DCs in inflammation is influenced by CD47-SIRPα interactions, making these interactions potential targets for

manipulation. Thus, the administration of SIRPα-Fc or CD47-Fc fusion proteins protect BALB/c mice from allergic airway inflammation (85, 86). This likely involves changes in DC migration rather than direct effects, given that CD47-Fc fusion protein does not impair antigen presentation by DCs, T cell priming, or effector function (86).

CONCLUDING REMARKS

The CD47-SIRPα interaction is clearly important to the homeostatic regulation of myeloid cell function, particularly these cells' phagocytic activity. Because high levels of CD47 are found on various types of cancer cells—which is often associated with poor prognosis—investigators have shown considerable interest in targeting this interaction by, for example, antibodies or recombinant proteins. Indeed, convincing evidence shows that the interference with CD47-SIRPα interactions and the resultant suppression of inhibitory signaling via SIRPα potentiates the efficacy of cancer therapeutic antibodies. Genetic manipulation to achieve an overexpression of CD47 in donor cells or tissues may also be of value in improving xenotransplantation. The observed education of macrophages by CD47-expressing stromal cells with respect to their capacity to eat cells lacking CD47 raises interesting questions as to the molecular mechanism involved and how common such regulation is among innate immunoreceptors.

SIRPα is the prototypic member of the SIRP family of paired receptors, and many findings in the context of CD47 and SIRPα may have more general implications for paired receptor families. One attractive idea is that the homeostatic inhibitory receptors are targets for pathogens or their products to downregulate innate immune cell effector functions and therefore to evade host defense. This would also explain the extreme genetic diversity that exists among paired receptors in terms of both numbers of families and their genes and polymorphic variants. Inhibitory members across different paired receptor families may act in concert and in a (partly) redundant fashion to maintain the appropriate degree of insurance for controlling innate immune cell activity. Furthermore, the activating receptors may provide the host with a germline encoded repertoire of receptors for pathogen recognition.

Taken together, the CD47-SIRPα interaction acts as a central homeostatic mechanism to regulate myeloid cell function. Further characterization of SIRPα and its homologs should shed light on the evolution of the SIRP family and the potential for exploiting CD47-SIRPα interactions as a therapeutic target.

SUMMARY POINTS

1. SIRPα is an inhibitory receptor present on myeloid cells that interacts with CD47 present on most cells including erythrocytes and platelets.
2. SIRPα is the prototypic member of a paired receptor family where the closely related SIRPβ1 gives an activating signal.
3. Additional members of the SIRP paired receptor family are less well characterized.
4. CD47 gives a don't-eat-me signal to prevent phagocytosis by macrophages.
5. Reagents that block the CD47-SIRPα interaction are the subject of intense interest as possible therapeutics for cancer treatment.
6. Manipulation of the CD47-SIRPα interaction aids models for the engraftment of human hematopoietic stem cells in mice and may be important in xenotransplantation.

FUTURE ISSUES

1. Will reagents that block the CD47-SIRPα interaction be useful therapeutics for cancer and/or inflammatory conditions?
2. Will these have major side effects on platelets and erythrocytes?
3. If the evolution of the SIRP paired receptor family is being driven by pathogen pressure, what are the pathogens and how are they interacting?
4. Does the CD47-SIRPα axis signal in ways in addition to the recruitment of phosphatases?
5. When and where does CD47 signal and how does that affect possible therapeutics targeting CD47?
6. To what extent is there redundancy of the CD47-SIRPα axis with other inhibitory receptor pathways in myeloid cells?

DISCLOSURE STATEMENT

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