

Deficit of CD47 Results in a Defect of Marginal Zone Dendritic Cells, Blunted Immune Response to Particulate Antigen and Impairment of Skin Dendritic Cell Migration¹

Sven Hagnerud,^{2*} Partha Pratim Manna,^{2†} Marina Cella,[‡] Åsa Stenberg,^{*} William A. Frazier,[†] Marco Colonna,[‡] and Per-Arne Oldenborg^{3*}

CD47 is a ubiquitously expressed cell surface glycoprotein that associates with integrins and regulates chemotaxis, migration, and activation of leukocytes. CD47 is also a ligand for signal regulatory protein α , a cell surface receptor expressed on monocytes, macrophages, granulocytes, and dendritic cell (DC) subsets that regulates cell activation, adhesion, and migration. Although the function of CD47 in macrophages and granulocytes has been studied in detail, little is known about the role of CD47 in DC biology in vivo. In this study we demonstrate that CD47^{-/-} mice exhibit a selective reduction of splenic CD11c^{high}CD11b^{high}CD8 α ⁻CD4⁺ DCs. These DCs correspond to marginal zone DCs and express signal regulatory protein α , possibly explaining their selective deficiency in CD47^{-/-} mice. Deficiency of marginal zone DCs resulted in impairment of IgG responses to corpusculate T cell-independent Ags. Although epidermal DCs were present in normal numbers in CD47^{-/-} mice, their migration to draining lymph nodes in response to contact sensitization was impaired, while their maturation was intact. In vitro, CD47^{-/-} mature DCs showed normal CCR7 expression but impaired migration to CCL-19, whereas immature DC response to CCL-5 was only slightly impaired. These results demonstrate a fundamental role of CD47 in DC migration in vivo and in vitro and in the function of marginal zone DCs. *The Journal of Immunology*, 2006, 176: 5772–5778.

The cell surface receptor CD47 (also called integrin-associated protein) is expressed by virtually all cells. It consists of an extracellular, highly glycosylated Ig variable type (IgV) domain, a five-segment membrane-spanning domain, and a short hydrophilic cytoplasmic tail (1). CD47 can interact in *cis* with $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, and $\alpha_2\beta_1$ integrins and can also bind in *trans* to the extracellular matrix protein thrombospondin (1). CD47 promotes activation of phagocytes by $\alpha_v\beta_3$ -binding extracellular matrix proteins (1). The importance of this function is underscored by the remarkable defects in antibacterial phagocytic defense observed in CD47^{-/-} mice (2). CD47 also mediates transendothelial migration of neutrophils (3) and costimulation of T cells (4). To mediate these functions, CD47 delivers intracellular signals by directly recruiting heterotrimeric G proteins (for review, see Ref. 1).

CD47 is also a ligand for the cell surface glycoprotein known as signal regulatory protein α (SIRP α)⁴ (5, 6), which is highly expressed by monocytes, macrophages, dendritic cell (DC) subsets,

granulocytes, and neuronal cells (7). SIRP α is an Ig family member with three extracellular Ig domains and an intracellular region with two ITIMs. Upon ligation by CD47, SIRP α ITIMs are tyrosine phosphorylated and bind the Src homology 2 domain-containing phosphatases SHP-1 and SHP-2 (7). The CD47/SIRP α interaction inhibits macrophage phagocytosis (8) but also promotes adhesion and migration of several cell types as well as fusion of macrophages into multinucleated giant cells (3, 9, 10).

The function of CD47 and its receptor SIRP α in DCs has recently become a focus of attention. DCs are professional APCs that acquire Ags in peripheral tissues and, upon maturation, transport them to draining lymph nodes through afferent lymphatics and present processed Ags to naive T cells (11, 12). In vivo, DCs include multiple subsets with distinct morphologies, localizations, and phenotypes that carry out different functions. These subsets include, among others, the following: Langerhans cells and dermal DCs in the epidermis and dermis of the skin, respectively; interdigitating DCs and germinal center DCs in the T cell and B cell areas of secondary lymphoid organs, respectively; and marginal zone DCs at the spleen red/white pulp junction (13–15). Although all DCs express CD47, SIRP α has been found only on some DC subsets such as murine Langerhans cells (16), pig lamina propria DC (17), sheep lymph DCs (18), and rat CD4⁺ lymph and spleen DCs (19). The function of CD47 and SIRP α has been mostly investigated in vitro in the human monocyte-derived DCs, which expresses both molecules. CD47 inhibits monocyte-derived DC maturation and cytokine production upon interaction with thrombospondin (20, 21). Similarly, engagement of SIRP α by a soluble CD47-Fc fusion protein inhibits maturation of human DCs and IL-12 production (22). Recent analysis of SIRP α -deficient mice demonstrated that SIRP α is essential in the migration of murine Langerhans cells from the skin to the draining lymph nodes (16).

In this work we studied the role of CD47 in the biology of DCs in vivo by using CD47-deficient mice. We found that CD47^{-/-} mice have a marked reduction in splenic CD11c⁺CD4⁺CD11b^{high}

*Department of Integrative Medical Biology, Section for Histology and Cell Biology, Umeå University, Umeå, Sweden; and †Department of Biochemistry and Molecular Biophysics and ‡Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110

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² These two authors contributed equally to this work.

³ Address correspondence and reprint requests to Dr. Per-Arne Oldenborg, Department of Integrative Medical Biology, Section for Histology and Cell Biology, Umeå University, SE-901 87 Umeå, Sweden. E-mail address: per-arne.oldenborg@histocel.umu.se

⁴ Abbreviations used in this paper: SIRP α , signal regulatory protein α ; DC, dendritic cell; DNFB, 2,4-dinitro-1-fluorobenzene; DTH, delayed type hypersensitivity; KLH, keyhole limpet hemocyanin.

SIRP α ⁺ DCs, which correspond to marginal zone DCs. This defect resulted in impaired Ab responses to corpusculate Ags. Following contact sensitization, we also detected a strong reduction in the migration of epidermal DCs to the draining lymph nodes of CD47^{-/-} mice. In vitro, CD47^{-/-} mature DCs showed an impaired migration to the CCR7 ligand CCL-19, whereas CD47^{-/-} immature DCs responded to CCL-5. Thus, these data indicate that CD47 is required for the trafficking and function of specific DC subsets.

Materials and Methods

Mice

CD47^{-/-} C57BL/6J mice (2), backcrossed to C57BL/6J for 21 or more generations, and their homozygous littermates were from our own breeding colony. The mice were housed in a pathogen-free barrier facility according to local guidelines. All experiments were approved by the local animal ethics committee.

In vitro generation of bone marrow-derived DCs

Bone marrow-derived DCs were generated from wild-type or CD47-deficient mice by culture of bone marrow cells in 50 ng/ml GM-CSF (Amgen) and 1 ng/ml IL-4 (R&D Systems) for 9 days. Nonadherent CD11c⁺ cells were used for in vitro migration experiments. DC expression of CCR7 was determined by flow cytometry using the anti-mouse CCR7 mAb 4B12 (eBioscience).

Isolation of splenic or lymph node DCs

Following mechanical disruption, spleens or lymph nodes were treated with 0.1 mg/ml collagenase D (Roche) for 1 h at 37°C. DCs were enriched by CD11c⁺ magnetic adsorption cell sorting or by using a CD8⁺ DC isolation kit according to the manufacturer's instructions (MACS; Miltenyi Biotec).

Isolation of epidermal ear sheets and epidermal DCs

Preparation of epidermal ear sheets and isolation of epidermal DCs were performed essentially as previously described (16).

Antibodies and flow cytometry

Conjugated mAbs, streptavidin-PE, streptavidin-allophycocyanin, and avidin-FITC were from BD Pharmingen. Cells were labeled with Abs for 30 min on ice and analyzed using flow cytometry (FACScan or FACScalibur, BD Biosciences) and Cell Quest software (BD Biosciences).

In vivo uptake of particulate Ag

Washed SRBCs (Hatanaholm) were labeled with PKH26 (Sigma-Aldrich). Mice were injected i.v. with 2.5×10^8 PKH26-labeled SRBCs diluted in 200 μ l of 0.9% NaCl. After 4 h the mice were sacrificed, and their spleens were harvested for flow cytometric analysis or immunohistochemistry.

Immunizations and ELISA

All mice were bled on the day before immunization to get preimmune sera. On day 0, wild-type or CD47^{-/-} mice were immunized i.v. with 10^8 SRBCs or keyhole limpet hemocyanin (KLH) (1 μ g or 25 μ g), followed by a booster immunization on day 28. Sera of blood collected at times indicated were stored at -20°C until assayed for Ig content. Diluted serum samples were analyzed using ELISA as previously described (23).

Immunohistochemistry

Frozen sections of spleens were labeled with mAb N418 (anti-CD11c) and fluorescein tyramide amplification (PerkinElmer) as previously described (8). Sections were further incubated with rat anti-mouse CD19 or anti-CD8 α , followed by Cy5-conjugated goat anti-rat IgG (BD Pharmingen) and analyzed using laser confocal microscopy (Leica TSP-2). Staining of resident epidermal DCs was performed using en face staining of epidermal ear sheets with anti-SIRP α mAb P84 (rat IgG1) and FITC-conjugated goat anti-rat IgG (Sigma-Aldrich) as previously described (16).

Skin migration assay

CellTracker Green (Invitrogen Life Technologies) was dissolved 1:20 in a 50:50 (v/v) acetone dibutyl phthalate mixture and painted on the dorsal side of the ears. After 24, 36, or 48 h, single cell suspensions of collagenase-digested draining lymph nodes, with or without CD11c magnetic sorting, were labeled with Abs and analyzed by flow cytometry as described above.

Delayed-type hypersensitivity (DTH) response

Mice were sensitized epicutaneously on day 0 by applying 50 μ l of a 0.5% 2,4-dinitro-1-fluorobenzene (DNFB) solution in 4:1 acetone/olive oil (v/v) onto 2 cm² of shaved abdominal skin. On day 5 after sensitization the mice were challenged on the right ear by topical application of 0.2% DNFB, whereas the left ear received vehicle alone. Contact sensitivity was determined by measuring the increase in the thickness of the challenged ear compared with that of the control left ear and was expressed in micrometers.

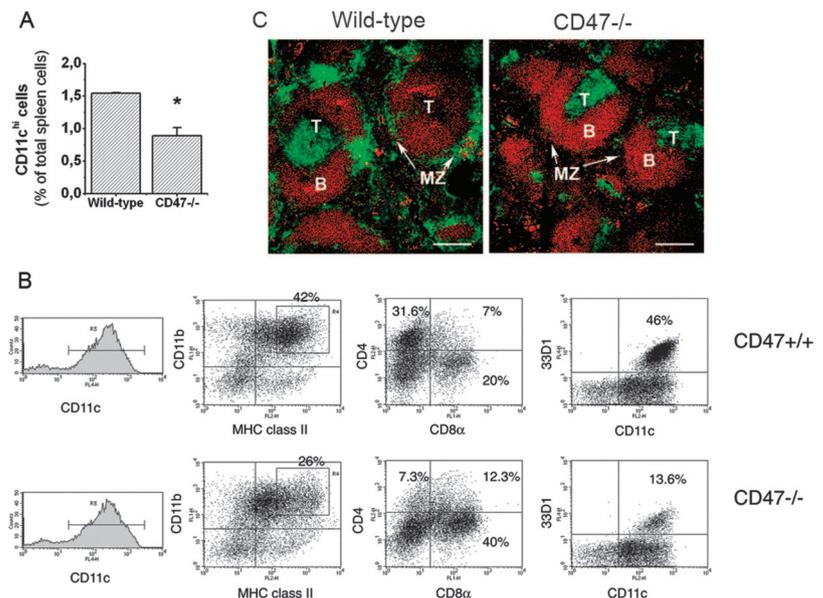
In vitro chemotaxis assay

Chemotaxis of bone marrow-derived DCs to CCL-5 or CCL-19 (Pepro-Tech) was measured in a Transwell migration assay using 24-well Costar Transwell chambers (5- μ m pore size; Corning). For migration to CCL-19, the bone marrow-derived DCs were cultured with LPS (1 μ g/ml) for 24 h before the experiment.

Statistics

Statistical analysis was performed by using the two-tailed Student's *t* test for paired or unpaired samples (see legends to the figures).

FIGURE 1. Reduced numbers of marginal zone DCs in CD47-deficient spleens. **A**, The fraction of CD11c^{high} cells from total spleen cells was reduced in CD47^{-/-} mice as compared with that in wild-type controls. Values are mean \pm SD for three mice in each group. *, *p* < 0.05 using Student's *t* test for paired comparisons. **B**, Phenotype of CD11c magnetic bead-isolated DCs in wild-type or CD47^{-/-} spleens. CD11c⁺ cells were labeled with Abs as indicated and analyzed by flow cytometry. **C**, Absence of marginal zone (MZ) DCs in spleens of CD47^{-/-} mice. Frozen sections of spleens were stained with anti-CD11c mAb N418 (green) and anti-CD19 (red) and analyzed using laser confocal microscopy. Representative images are shown; scale bar represents 200 μ m. B, B cell area; T, T cell area.



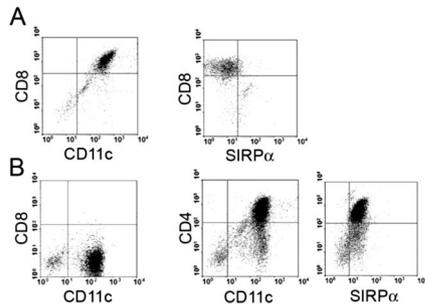


FIGURE 2. SIRP α is expressed by splenic CD8 α ⁻CD11c^{high} DCs, but not by CD8 α ⁺CD11c^{high} DCs. CD8 α ⁺CD11c⁺DCs isolated by magnetic adsorption cell sorting from collagenase-digested spleens of wild-type mice (A) or CD11b⁺CD4⁺CD8 α ⁻CD11c⁺ DCs collected from the negative fraction (B) were labeled with Abs and analyzed by flow cytometry. The CD8 α ⁺ DC subpopulation lack SIRP α expression, whereas DCs positive for CD4 and CD11b (not shown) but negative for CD8 α readily express SIRP α . Data from one representative experiment of four are shown.

Results

Reduced numbers of marginal zone DCs in spleens of CD47-deficient mice

We examined splenocytes in wild-type and CD47-deficient mice by flow cytometry and found that CD11c^{high} DCs were reduced in CD47^{-/-} mice, as compared with their wild-type controls ($0.89 \pm 0.13\%$ vs $1.55 \pm 0.1\%$ of total spleen cells; Fig. 1A). Following magnetic bead purification of CD11c⁺ splenocytes, we recovered a reduced number of CD11c^{high} cells (data not shown). Further

investigation of the phenotype of purified splenic CD11c⁺ DCs in CD47^{-/-} compared with that of wild-type mice revealed a reduced proportion of CD11b^{high} DCs (Fig. 1B), whereas the proportion of CD8 α ⁺ DCs was increased (Fig. 1B). Notably, we also found a strong reduction of CD4⁺ and 33D1⁺ DCs in CD47^{-/-} spleens (Fig. 1B). Because 33D1 is a marker of marginal zone DCs (15), these data suggested that the spleens of CD47^{-/-} mice were deficient in marginal zone DCs. Indeed, immunohistochemical staining of CD47^{-/-} and wild-type spleens showed a virtual absence of CD11c⁺ DCs in the splenic marginal zones of CD47^{-/-} mice, whereas CD11c⁺ DCs were maintained in the T cell area surrounding the central artery of the white pulp (Fig. 1C). We did not find any difference in the expression levels of the activation markers CD40, CD80, or CD86 (data not shown). Moreover, B220⁺Gr1⁺CD11b⁻ plasmacytoid DCs were equally represented in wild-type or CD47^{-/-} mice (data not shown). Thus, these findings demonstrate that CD47^{-/-} mice exhibit a selective defect of CD11c^{high}CD11b^{high}CD8 α ⁻CD4⁺33D1⁺ DCs, which correspond to marginal zone DCs.

SIRP α is selectively expressed by marginal zone DCs

Because the interaction between CD47 and its receptor SIRP α has been found to be important for normal migration of both human and rat leukocytes (3, 9), we compared the SIRP α expression levels on purified CD11c⁺CD8 α ⁺ and CD11c⁺CD8 α ⁻ DCs from the spleens of CD47^{+/+} mice. This analysis showed that CD8 α ⁺CD11c⁺ DCs expressed marginal, if any, amounts of SIRP α (Fig. 2A). In contrast, CD11c⁺CD8 α ⁻ DCs, which include CD4⁺ marginal zone DCs, all expressed SIRP α (Fig. 2B).

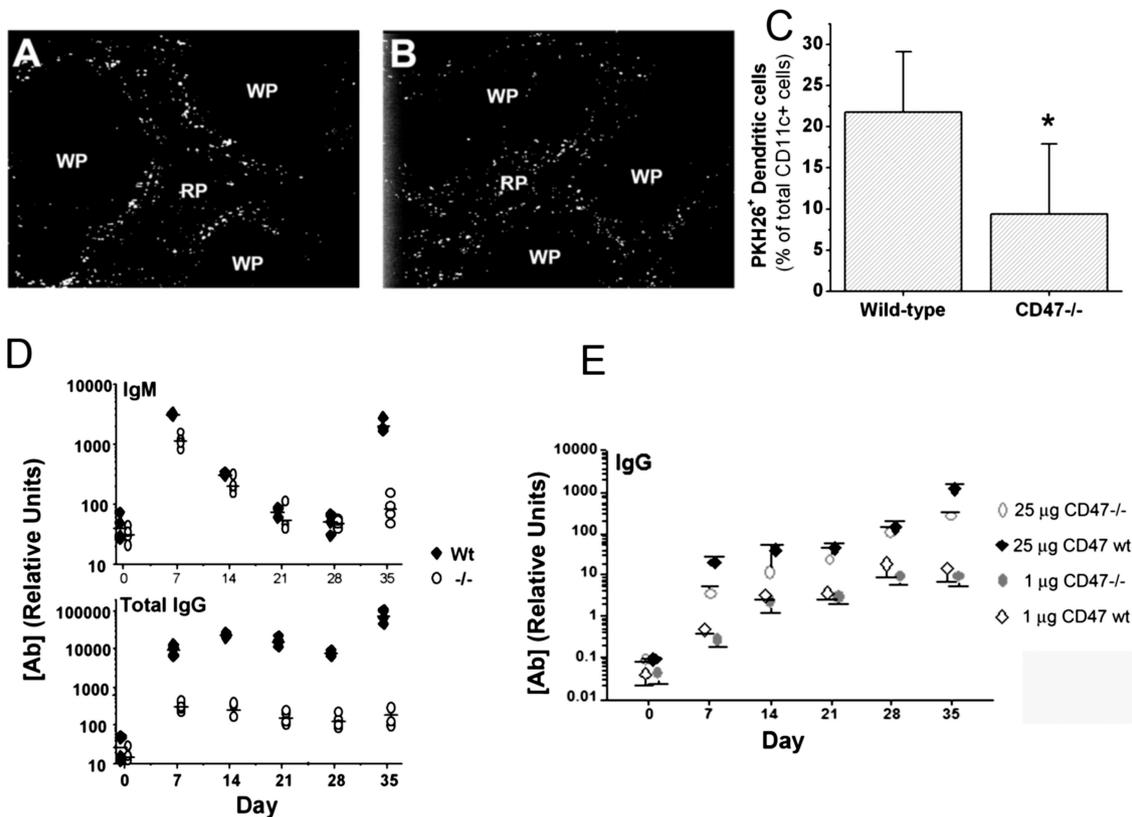


FIGURE 3. Blunted immune response due to impaired marginal zone uptake of particulate Ags in CD47-deficient mice. A and B, Wild-type or CD47^{-/-} mice were injected i.v. with 2.5×10^8 PKH26-labeled SRBCs, and spleens were harvested after 4 h. Injected PKH26-labeled SRBCs accumulated in the marginal zone of wild-type mice (A), whereas the SRBCs were distributed into the red pulp (RP) of CD47^{-/-} mice (B). WP, white pulp. C, Magnetic bead-purified CD11c^{high} DCs were labeled with FITC-conjugated anti-CD11c and analyzed for the uptake of SRBCs by flow cytometry. Values are mean \pm SD for three mice in each group. *, $p < 0.05$, using Student's *t* test for paired comparisons. D and E, Mice were immunized i.v. with 10^8 SRBC (D) or KLH (E) on day 0, and a booster injection was given on day 28. Serum Ab titers were measured by ELISA at the days indicated. wt, wild type.

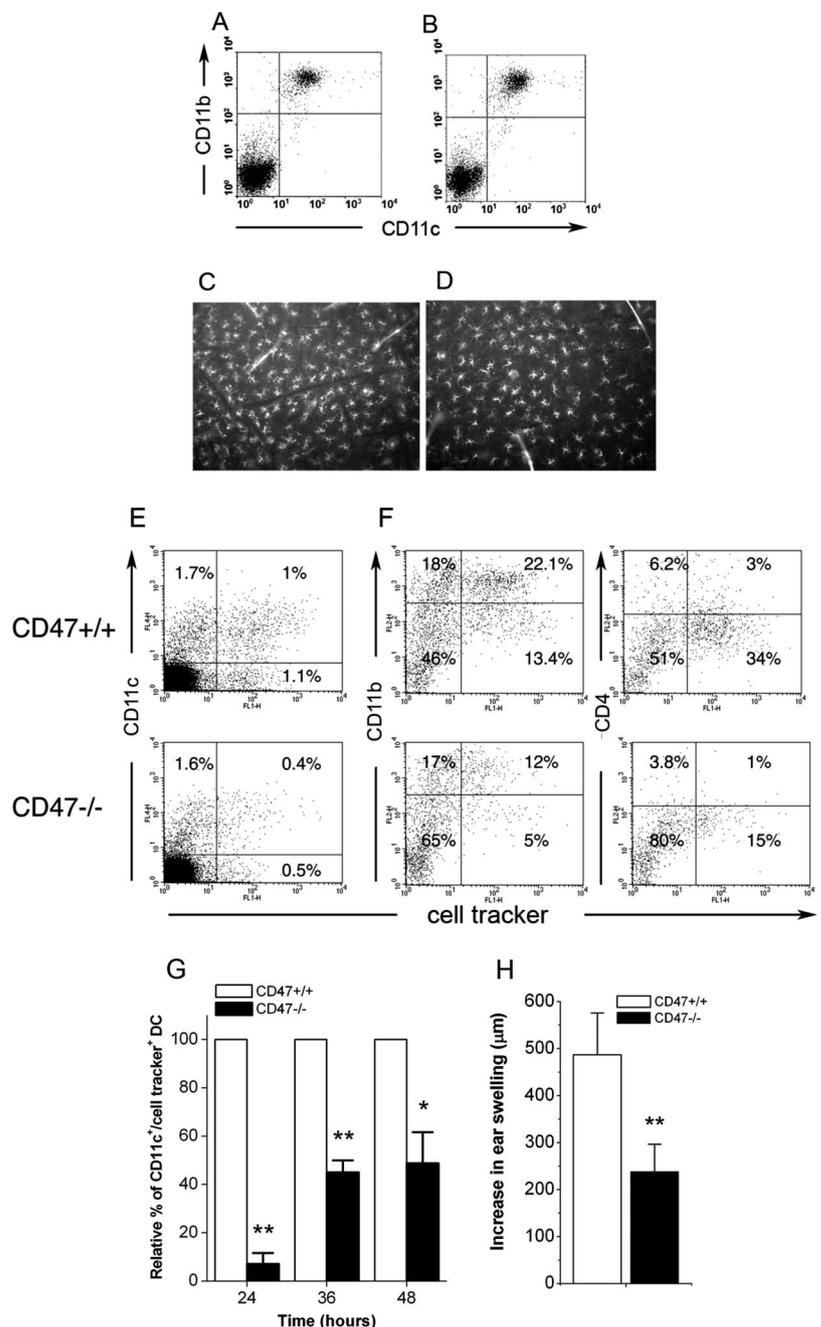
Reduction of marginal zone DC in CD47^{-/-} mice impairs uptake of corpusculate Ags and Ab responses

Because marginal zone DCs are important for the uptake of blood-borne particulate Ags (24), we studied DC-mediated uptake of PKH26-labeled SRBCs in CD47^{-/-} and wild-type mice. In wild-type mice, the majority of SRBCs were retained in the marginal zone of wild-type spleens 4 h after injection (Fig. 3A), whereas the distribution of SRBCs in the spleens of CD47^{-/-} mice was diffuse throughout the red pulp and not distinctly localized to the marginal zone (Fig. 3B). Further labeling of the spleen sections with anti-red pulp macrophage Abs (mAb F4/80) revealed a substantial interaction between SRBC and F4/80⁺ macrophages in the spleens of CD47^{-/-} mice, but not in wild-type spleens (not shown). These data were confirmed by flow cytometric analysis of purified splenic DCs from wild-type or CD47^{-/-} mice 4 h after the injection of PKH26-labeled SRBCs, showing a substantial uptake of

SRBCs by CD11c⁺ CD8 α ⁻ DCs in wild-type mice ($21.8 \pm 7.3\%$; Fig. 3C). In contrast, we found a significantly reduced uptake of SRBC by CD11c⁺ DCs in CD47^{-/-} mice ($9.4 \pm 8.5\%$, $p < 0.05$, Fig. 3C).

Because marginal zone DCs have been shown to promote T cell-independent Ab responses (24), we next studied the Ab response to SRBCs in wild-type or CD47^{-/-} mice. As shown in Fig. 3D, the primary IgM response was not different between CD47^{-/-} or wild-type mice. However, the total IgG response (Fig. 3D) was markedly reduced. Interestingly, we found no difference in the immune response to either low (1 μ g) or high (25 μ g) concentrations of KLH (Fig. 3E). These data further emphasize that CD47^{-/-} mice have a specific defect in their splenic IgG response to particulate Ag but not to soluble Ag, and the normal response to soluble Ags suggests that T and B cell responses are normal in CD47^{-/-} mice.

FIGURE 4. Normal epidermal DC numbers but impaired skin DC migration in vivo in CD47-deficient mice. *A* and *B*, DCs were isolated from epidermal sheets of the ears from naive wild-type (*A*) or CD47^{-/-} (*B*) mice and labeled with anti-CD11c-FITC and anti-CD11b-PE, followed by flow cytometric analysis. Shown is one representative experiment of four with similar results. *C* and *D*, Epidermal sheets were prepared from the ears of naive wild-type (*C*) or CD47^{-/-} (*D*) mice and stained for SIRP α using mAb P84 and FITC-conjugated goat anti-rat IgG. Cell counting of eight microscopic fields per ear of four mice per genotype showed no significant differences in cell numbers (114 ± 28 vs 125 ± 19 cells per field in wild-type and CD47^{-/-} mice, respectively). *E* and *F*, CellTracker Green/acetone/dibutyl phthalate was painted on the dorsal side of the ears of wild-type or CD47^{-/-} mice. After 36 h, draining lymph nodes were collected, and collagenase-digested cells were stained with CD11c-APC and PE-conjugated mAbs to either CD4 or CD11b, followed by flow cytometric analysis. *G*, Percentage of cell tracker-positive CD11c⁺ cells in draining lymph nodes of wild-type or CD47^{-/-} mice at 24, 36, or 48 h following skin painting. Values are mean \pm SD for three separate experiments. *H*, DTH response in wild-type or CD47^{-/-} mice. Mice were sensitized by abdominal application of 0.5% DNFB on day 0, followed by application of 0.2% DNFB to the right ear on day 5. Increase in ear thickness was determined 24 h later and is shown relative to the thickness of the vehicle-treated left ear of the same mouse. Data are mean \pm SD for data pooled from 11 mice in each group. *, $p < 0.05$; **, $p < 0.01$; as compared with wild-type mice using Student's *t* test for unpaired comparisons.



Impaired migration of CD47^{-/-} DCs from the skin to draining lymph nodes in vivo

A recent study showed that epidermal DCs express SIRP α (16). Therefore, we investigated skin DCs in CD47^{-/-} mice. We initially compared the numbers of epidermal DCs in the ears of wild-type or CD47^{-/-} mice. No significant difference was detected by either flow cytometry of isolated epidermal DCs (Fig. 4, A and B) or immunohistochemistry of epidermal sheets of naive CD47 wild-type or CD47^{-/-} mice stained for SIRP α (Fig. 4, C and D; 114 ± 28 vs 125 ± 19 cells/field in wild-type and CD47^{-/-} mice, respectively; $n = 4$). We then studied the impact of CD47 deficiency on skin DC mobilization and maturation in vivo induced by skin painting with a fluorescent contact sensitizer and analysis of CD11c⁺ DCs that had migrated to the draining lymph nodes 24, 36, or 48 h later. In wild-type mice we found a clear accumulation of CellTracker Green-positive cells that expressed CD11c and CD11b (Fig. 4, E and F). In contrast, lymph nodes of CD47^{-/-} mice showed a strongly reduced accumulation of green fluorescent cells (Fig. 4, E and F). At 24 h, lymph nodes of CD47^{-/-} mice showed an accumulation of green fluorescent cells, which was only 15% of that in wild-type mice (Fig. 4G). Even at 36 or 48 h, the migration of DCs from the skin to the lymph nodes in the CD47^{-/-} mice was only 45 and 49%, respectively, of that in the wild-type mice (Fig. 4G). To understand the physiological significance of the reduced skin DC migration in CD47^{-/-} mice, we also investigated the DTH reaction to the chemical Ag DNFB. These experiments revealed a 51% inhibition of the DTH response in CD47^{-/-} mice ($p < 0.01$; Fig. 4H). Thus, despite a strong impairment of skin DC migration in CD47^{-/-} mice, a DTH response was still present although strongly inhibited. Wild-type and CD47^{-/-} DCs that migrated from the skin showed similar up-regulation of the activation markers CD40 (Fig. 5), CD80, and CD86 (data not shown), indicating that impaired migration of epidermal DCs observed in CD47^{-/-} mice is not the result of a maturation defect.

CD47 deficiency impairs the migration of bone marrow-derived mature DCs to CCL-19, whereas immature DCs respond to CCL-5 in vitro

CCR7 has been identified as a key regulator of migration of mature DCs into lymph nodes (25–27). To see whether CD47 deficiency affects DC responsiveness to CCR7 ligands, we studied the mi-

gration of LPS-activated wild-type or CD47^{-/-} bone marrow-derived DCs toward the CCR7 ligand CCL-19. These experiments revealed a significant reduction in the dose-dependent migration of CD47^{-/-} DCs to CCL-19 as compared with that of the wild-type DCs (Fig. 6A). However, we observed no differences in the expression of CCR7 (Fig. 6B) or the maturation markers CD40, CD80, and CD86 (data not shown) in response to LPS between wild-type and CD47^{-/-} DCs. The migration of immature DCs to CCL-5 was similar in wild type and CD47^{-/-} DCs except at 250 ng/ml CCL-5, where the migration of CD47^{-/-} DCs was significantly reduced (Fig. 6A). These results demonstrate that the deficit of CD47 mostly affects the chemokine responsiveness of mature DCs.

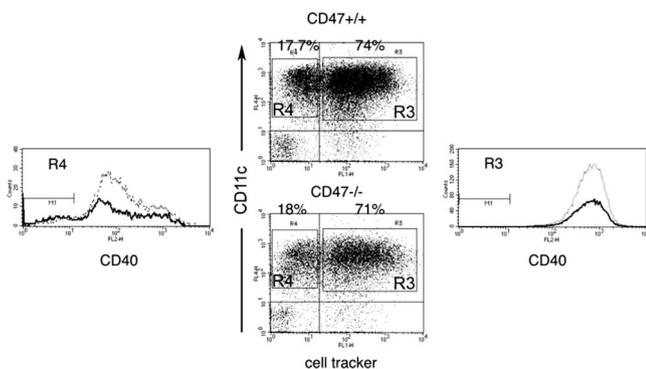


FIGURE 5. Normal maturation of skin-derived DCs in lymph nodes of CD47-deficient mice. At 36 h following FITC skin painting, CD11c⁺ DCs were isolated from lymph nodes of wild-type or CD47^{-/-} mice using CD11c magnetic beads, labeled with anti-CD11c-allophycocyanin and anti-CD40-PE, and analyzed by flow cytometry. Albeit reduced numbers of CD11c⁺ DCs were recovered from the lymph nodes of CD47^{-/-} mice, resident DCs of both wild-type (dotted line) and CD47^{-/-} mice (bold line) expressed low levels of CD40 (gate R4), whereas migrated cell tracker-positive DCs of both genotypes up-regulated CD40 (gate R3).

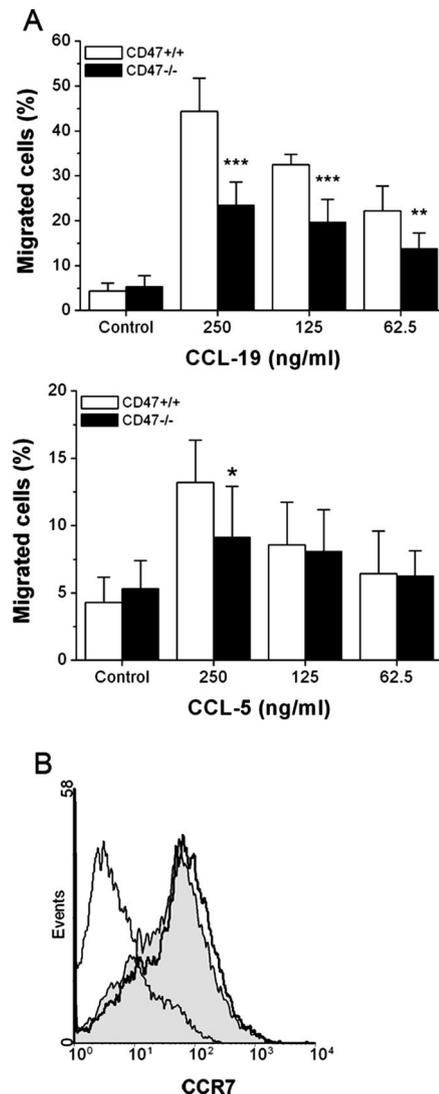


FIGURE 6. Bone marrow-derived DCs from CD47^{-/-} mice show reduced migration to CCL-19 but not to CCL-5 in vitro. **A**, Bone marrow-derived immature DCs (for CCL-5) or mature DCs (for CCL-19; cultured with 1 μ g/ml LPS for 24 h) were incubated with the respective chemokine for 2 h in 24-well Transwell chambers, after which the number of migrated cells was counted. Values are mean \pm SD for three independent experiments. *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$; as compared with CD47^{+/+} mice at the same chemokine concentration using Student's t test for paired comparisons. **B**, Following the culture of CD47^{+/+} (gray histogram) or CD47^{-/-} (bold line) bone marrow-derived DCs in the presence of 1 μ g/ml LPS for 24 h, CCR7 expression levels were determined by flow cytometry. Dotted line, isotype control. Data are representative of three independent experiments with identical results.

Discussion

In the present study we demonstrate that CD47^{-/-} mice have a nearly total loss of marginal zone DCs, whereas DCs in the T cell zone are conserved. Consistent with previous studies, marginal zone DCs expressed CD11c, CD11b, CD4, and 33D1 and lacked CD8 (13, 15). Moreover, we found that marginal zone DCs expressed SIRP α , thus indicating that CD8⁻ SIRP α ⁺ murine splenic DCs may be the counterparts of rat CD4⁺ SIRP α ⁺ DCs (19). In contrast to marginal zone CD8⁻ SIRP α ⁺ DCs, we found that splenic T cell zone CD8 α ⁺CD11c⁺ DCs did not express SIRP α . Because SIRP α has been shown to regulate DC migration (16), it is possible that CD47 deficiency selectively impairs the migration and/or proper distribution of splenic marginal zone CD8 α ⁻ DCs expressing SIRP α , whereas splenic CD8 α ⁺ DCs that do not express SIRP α were normally distributed in the T cell area of the spleen of CD47^{-/-} mice. However, because CD47^{-/-} mice had normal numbers of epidermal DCs, which also express SIRP α , it is possible that the CD47/SIRP α interaction could have different effects on these different DC subsets. There is also a large difference in the turnover rate of skin DCs and splenic marginal zone DCs. Skin DCs persist in their tissue far longer than splenic DCs (28, 29). This rapid turnover could amplify a difference in migration or development of the marginal zone DC subset and thus play a role in the selective deficiency of splenic marginal zone DCs. Whatever the mechanism, the defect of marginal zone DCs resulted in a dramatic impairment of Ab (IgG) responses against corpuscular T cell-independent Ags. This observation is corroborated by recent studies showing that CD11c^{high} DCs in the marginal zone and the bridging channels are essential to support the expansion of marginal zone and B1 cells in response to particulate Ags such as bacteria, promoting early immune responses against T cell-independent particulate Ags (24).

Although epidermal DCs, like marginal zone DCs, express SIRP α , we found similar numbers of epidermal DCs in wild-type and CD47^{-/-} mice. However, migration of DCs from the skin to draining lymph nodes was strongly reduced in CD47^{-/-} mice following contact sensitization. This observation is consistent with previous studies showing that epidermal DC migration induced by contact sensitization is impaired in SIRP α mutant mice or by Ab-mediated blockade of SIRP α (16). The same inhibition of epidermal DC migration was found in the presence of soluble CD47 (16), and soluble CD47 or SIRP α Abs also inhibited neutrophil trans-epithelial migration (30). This finding supports the hypothesis that interaction between epithelial CD47 and leukocyte SIRP α may result in enhanced transepithelial migration (3). Such a mechanism could also explain the defects in DC migration shown in the present study, supporting the possibility that SIRP α -CD47 interaction is essential in regulating epidermal DC migration.

Because CD47 associates *in cis* with integrins and *in trans* with thrombospondin, these interactions may also be critical in regulating epidermal DC migration. It has been shown that the integrin $\alpha_L\beta_2$ mediates DC migration to lymph nodes (31), which is consistent with findings showing that β_2 integrin deficiency impaired DC migration to lymph nodes (32). CD47 has been shown to interact with both β_1 and β_3 -integrins, but the functional interaction between CD47 and the β_3 integrin is the interaction best understood to date (1). The fact that RGD peptides, which mediate the binding of β_3 integrins to extracellular matrix proteins, did not inhibit DC migration from skin argues against involvement of the $\alpha_V\beta_3$ integrin in skin DC migration to the lymph nodes (33). We have recently found that CD47 associates with $\alpha_L\beta_2$ integrin on Jurkat T cells and with $\alpha_M\beta_2$ on monocytes (A. Zheleznyak and W. A. Frazier, manuscript in preparation). Thus, the possibility

that the impaired migration of skin DCs observed in CD47-deficient mice is due to defects in β_2 integrin-dependent cell migration must be entertained. In contrast, the homing of DCs from the circulation to the skin was not impaired in CD18^{-/-} mice (34), which would also agree with our findings of normal numbers of epidermal DCs in CD47^{-/-} mice.

Previous studies on the human DC showed an inhibitory role of CD47 and SIRP α in DC maturation (20–22). Because migration of DCs to lymph nodes is driven by CCR7 (25–27), which is expressed on mature DCs, the defective migration of skin DCs in CD47^{-/-} mice could be explained by an altered DC maturation. However, we did not find any differences in expression of maturation markers (CD40, CD80, or CD86) between the CD47^{-/-} and wild-type DCs that migrated from the skin to the lymph nodes of mice painted with a fluorescent contact sensitizer. Moreover, we did not see differences in the maturation and expression of the CCR7 of bone marrow-derived DCs in response to LPS. Thus, the reduced responsiveness of CD47^{-/-} DC to a CCR7 ligand is not due to a maturation defect but most likely to the impairment of activation of cell migration following CCR7 engagement. Interestingly, migration of immature CD47^{-/-} DCs toward CCL-5 was only significantly impaired at the highest dose (250 ng/ml) of CCL-5 tested. Thus, CD47 may be critical in regulating the migration of distinct subsets of DCs and/or DCs in a specific state of activation.

In conclusion, our data demonstrate a fundamental role of CD47 in the biology of marginal zone DCs and the migration of skin DCs. These cells express SIRP α and rely on β_2 integrins for migration. CD47 is a ligand for SIRP α and associates with β_2 integrins. Thus, our results highlight two potentially critical functions of CD47, one acting *in trans* as a counter-receptor for SIRP α and the other acting *in cis* as a regulator of β_2 integrins on DC. These two functions of CD47 may be expressed differently in marginal zone vs skin DCs, yet both may be important in regulating trafficking of DC subsets.

Disclosures

The authors have no financial conflict of interest.

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