Retinoid X receptor α controls innate inflammatory responses through the up-regulation of chemokine expression

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The retinoid X receptor α (RXRα) plays a central role in the regulation of many intracellular receptor signaling pathways and can mediate ligand-dependent transcription by forming homodimers or heterodimers with other nuclear receptors. Although several members of the nuclear hormone receptor superfamily have emerged as important regulators of macrophage gene expression, the existence in vivo of an RXR signaling pathway in macrophages has not been established. Here, we provide evidence that RXRα regulates the transcription of the chemokines Ccl6 and Ccl9 in macrophages independently of heterodimeric partners. Mice lacking RXRα in myeloid cells exhibited reduced levels of CCL6 and CCL5, impaired recruitment of leukocytes to sites of inflammation, and lower susceptibility to sepsis. These studies demonstrate that macrophage RXRα plays key roles in the regulation of innate immunity and represents a potential target for immunotherapy of sepsis.

Nuclear hormone receptors | macrophages | innate immunity | sepsis

Nuclear receptors are ligand-dependent transcription factors that regulate diverse aspects of development and homeostasis (1, 2). Several members of the nuclear receptor superfamily have emerged recently as key regulators of inflammation and immune responses (2, 3). Retinoid X Receptors (RXRs) occupy a central position in the nuclear receptor superfamily because they form heterodimers with many other family members and hence are involved in the control of a variety of physiologic processes (4, 5). RXRs are also able to activate transcription from cognate reporter genes as homodimers (6, 7). There are three RXR isotypes, RXRα (NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3), which show tissue-specific differences in expression (4, 8). Previous studies suggest that the most abundant RXR in myeloid cells, or at least the most functionally important, is RXRα (9). RXRs are receptors for ligands such as 9-cis-retinoic acid and endogenous fatty acids (10, 11), and for a variety of synthetic agonists (called retinoids), such as LG100268 (12). Selective RXR ligands are being developed for cancer therapy and are promising agents for the treatment of metabolic diseases (13).

Innate immunity is an ancient form of host defense that is activated rapidly to enable, through a multiplicity of effector mechanisms, defense against a broad spectrum of foreign substances (14). Innflammation, one of the first responses of the immune system to infection, is mediated by immune system cells, whose accumulation in injured tissues triggers the removal of the foreign agent, prevents subsequent infections, and promotes tissue repair (15). Normally, inflammation is self-controlled in intensity and duration (16). However, when this process is dysregulated, as in sepsis, excessive proinflammatory mediators are released into the bloodstream, resulting in multiple organ failure. Sepsis-induced multiorgan failure has a high death rate in humans and is one of the leading causes of death in intensive care units (17).

Chemokines and their receptors have been implicated in the modulation of leukocyte trafficking, immune-inflammatory responses, sepsis, and multiorgan failure (18, 19). Clinical studies have also identified elevated levels of chemokines associated with human sepsis and acute lung injury (20).

We have examined the role of RXRα in the innate immune system by conditionally disrupting RXRα in myeloid cells. We show that chemokines Ccl6 and Ccl9 are novel target genes for RXRα in primary peritoneal macrophages. RXRα deletion also results in decreased levels of CCL6 and CCL9 in vivo, correlating with impaired leukocyte recruitment to inflammatory sites and prolonged survival in sepsis induced by cecal ligation and puncture (CLP) or lipopolysaccharide (LPS). These results establish that RXRα is involved in the regulation of the innate immune response and provide evidence for the existence of RXRα signaling in macrophages in vivo.

Results

RXRs Control Chemokine Gene Expression in Macrophages. To investigate the role of RXRα in inflammation and in macrophage function, we generated mice lacking RXRα specifically in myeloid cells (RXRα KO) (Fig. S1 A–D). Gene expression profiling of WT and RXRα KO peritoneal macrophages showed that Ccl6 (C10. Mrp-1, Scy6) and Ccl9 (Mip-1γ, Mrp-2, Scy9) are down-regulated in the KO mice. Reduction in Ccl6 and Ccl9 gene expression in RXRα KO macrophages was confirmed by real-time quantitative PCR (Q-PCR) (Fig. 1 A). Q-PCR further showed that the RXR ligands 9-cis-retinoic acid (RA) and LG100268 induced Ccl6 and Ccl9 gene expression in WT macrophages, but expression was not induced by LG100754 (Fig. 1B). LG100268 and 9-cis-RA are RXR pan-agonists, whereas LG100754 is an agonist of PPAR/RXR and RAR/RXR heterodimers but an antagonist of RXR homodimers (12). Ccl6 and Ccl9 mRNA levels were maximally induced after 24 h stimulation with 9-cis-RA (Fig. S2A), and the induction of Ccl6 and Ccl9 by LG100268 and 9-cis-RA was inhibited by LG100754 (Fig. S2B). The effect of RXR ligands on protein expression was examined by ELISA. Treatment of macrophages with 9-cis-RA or LG100268 significantly increased CCL6 and CCL9.


The authors declare no conflict of interest.

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accumulation in the culture medium (Fig. 1C). The ligand-induced mRNA expression of these chemokines was substantially lower in RXRα KO macrophages (Fig. 1D); the residual effect of the ligands is consistent with the presence of RXRβ.

*Ccl6 and Ccl9 Are Transcriptionally Regulated by RXRα.* The induction of *Ccl6* and *Ccl9* mRNA by treatment with RXRα ligands raised the possibility that these genes might be direct targets of RXRα. To test this, we generated luciferase reporter constructs (pGL3 vector) driven by the proximal 1.1 kb of the *Ccl6* or *Ccl9* promoter. These constructs were separately cotransfected into the mouse macrophage cell line RAW 264.7 together with empty (pcMX) or pcMX-RXRα expression vectors. After transfection, cells were treated with vehicle or the RXR-specific ligands 9-cis-RA (1 μM), LG268 (50 nM), or LG754 (1 μM). *P* ≤ 0.05 compared with control (Ctr). (C) ELISA of CCL6 and CCL9 protein in macrophages treated as in B for 48 h. *P* ≤ 0.05 compared with control (Ctr). (D) Q-PCR analysis of *Ccl6* and *Ccl9* mRNA expression in WT and RXRα KO macrophages treated with LG268 or 9-cis-RA for 24 h. *P* ≤ 0.05 compared with WT. Data are means ± SEM of at least three independent experiments.

Of the 49 nuclear receptors encoded by the mouse genome, systematic quantitative PCR analysis has demonstrated that 28 are expressed in macrophages (21). Of these, PPARγ, PPARδ, and Nur1 have been demonstrated to form heterodimers with RXRs on DR-1 elements. RXR also forms heterodimers with LXRα, TRs, RARs, and VDR on DR-4, DR-2/5, and DR-3 elements, respectively (4). To investigate whether RXR induces *Ccl6* and *Ccl9* transcription as a homodimer or by forming a heterodimer, we tested the ability of potential heterodimeric partners to influence expression of these genes in macrophages. Expression of *Ccl6* and *Ccl9* was up-regulated in cells treated with the RXR ligand LG100268 but not the PPAR ligands rosiglitazone, GW327647, or GW617042; moreover, we observed no additive or synergistic effect when PPAR and RXR ligands were both present (Fig. 2H). PPAR ligands were, however, able to induce the expression of the PPAR- and RXR-target genes *Abeh1* and *Adyp* (Fig. S2D). Furthermore, no effect was observed upon treatment with the Nur1/RXRα selective ligand XCT0135980, the LXR ligand T1317, the RAR ligand TTPNB, the VDR ligand Vit D3, or the TR ligand T3 (Fig. S2E). Collectively, these findings provide evidence for an RXRα signaling pathway in macrophages that is independent of RXR heterodimeric partners.

**Decreased CCL6 and CCL9 Expression Impairs Leukocyte Recruitment in Myeloid RXRα Knockout Mice.** We next investigated the functional consequences of RXRα-mediated chemokine regulation in an in vivo model of acute inflammation. We induced peritonitis by injecting thioglycollate or zymosan into the mouse peritoneal cavity and monitored the accumulation of CCL6 and CCL9 in cell-free peritoneal lavage fluid. Levels of CCL6 and CCL9 peaked at 24 h and remained high 48 h after injection (Fig. S3A). Peritoneal exudates collected from RXRα KO mice 24 or 48 h after the initiation of peritonitis contained significantly less CCL6 and CCL9 than did time-matched WT exudates (Fig. 3A). Similarly, the exudate levels of IL-6 and MCP-1 in response to thioglycollate- or zymosan-induced peritonitis, respectively, were lower in RXRα KO mice (Fig. S3B).

Because chemokines are chemotaxants that direct leukocytes to inflammation sites, we next investigated whether decreased CCL6 and CCL9 levels in peritoneal exudates had an impact on leukocyte recruitment. Thioglycollate or zymosan injection induced strong recruitment of monocytes/macrophages and granulocytes from 24 h up to 48 h into the abdominal cavity of WT mice (Fig. S3 C and D). In contrast, the influx induced by these agents in RXRα KO mice was significantly lower (Fig. 3 B and C). The number of resident monocytes/macrophages and granulocytes was, however, unaffected by deletion of RXRα (Fig. S4A). The impaired ability of RXRα KO mice to mount a proper leukocyte response was not due to any overt leukocyte defect, as shown by the similar sizes and composition of leukocyte sub-populations in blood and bone marrow from WT and RXRα KO mice (Table S1 and Fig. S4 B and C). In addition, RXRα KO mice challenged with i.p. thioglycollate showed normal leukocyte population profiles in bone marrow and peripheral blood (Fig. S4D), strongly indicating that the low number of leukocytes infiltrating the inflamed peritoneum in the absence of RXRα is the result of impaired leukocyte migration.

To explore the mechanistic link between decreased peritoneal exudate levels of CCL6 and CCL9 and impaired leukocyte migration, we conducted a series of in vitro migration assays. Peritoneal exudates from WT mice were as effective as recombinant CCL6 and CCL9 in inducing monocyte/macrophage migration; in contrast, exudates from RXRα KO mice were significantly less chemotaxuant (Fig. 3D). We next tested the chemotaxant potential of the conditioned media obtained from peritoneal macrophages stimulated in culture with the RXRα ligands LG100268 or 9-cis-RA for 72 h. These conditioned media significantly induced the migration of bone marrow mononuclear cells across activated endothelial cells.

**Fig. 1.** Loss of RXRα in macrophages reduces *Ccl6* and *Ccl9* chemokine expression. (A) Q-PCR analysis of *Ccl6* and *Ccl9* mRNA expression in RXRα KO peritoneal macrophages. *P* ≤ 0.05 compared with WT. (B) Q-PCR analysis of *Ccl6* and *Ccl9* gene expression in peritoneal macrophages treated for 24 h with the RXR ligands 9-cis-RA (1 μM), LG268 (50 nM), or LG754 (1 μM). *P* ≤ 0.05 compared with control (Ctr). (C) ELISA of CCL6 and CCL9 protein in macrophages treated as in B for 48 h. *P* ≤ 0.05 compared with control (Ctr). (D) Q-PCR analysis of *Ccl6* and *Ccl9* mRNA expression in WT and RXRα KO macrophages treated with LG268 or 9-cis-RA for 24 h. *P* ≤ 0.05 compared with WT. Data are means ± SEM of at least three independent experiments.
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KO macrophages showed signiﬁcant differences in the expression levels of CCL6, CCL9, MCP-1, or IL-6 in peritoneal exudates after thioglycollate injection (Fig. S6C), and no changes in Ccl6 and Ccl9 gene expression in peritoneal macrophages in vitro (Fig. S6D). These results suggest that the regulation of chemokine production and leukocyte migration by RXRα is independent of PPARγ.

Absence of RXRα from Macrophages Prolongs Mouse Survival in CLP- and LPS-Induced Sepsis. To investigate the role of RXRα in the innate immune response to experimental sepsis, we performed cecal ligation and puncture (CLP) in WT and RXRα KO mice. After 24 h, plasma levels of CCL6 and CCL9 and peritoneal levels of CCL6 in RXRα KO mice were signiﬁcantly lower than in WT animals (Fig. 4A). In addition, levels of MCP-1 were lower in RXRα KO peritoneal exudate, whereas they were unaltered in plasma, and there was also no signiﬁcant difference in peritoneal GRO-α, MIP-1α, or Rantes, or in the peritoneal and plasma concentrations of IL-6, IL-1α, IL-12, and TNFα (Fig. S7A and B). We also found that RXRα KO mice survived CLP-induced sepsis for signiﬁcantly longer than WT (Fig. 4B). Consistently, the severity of CLP, scored by quantiﬁcation of liver histological parameters (hemorrhage, ﬁbrin accumulation, inﬂammatory inﬁltrate, and degenerated tissue), was lower in RXRα KO mice (Fig. 4C and D).

RXRα KO mice were also less susceptible than WT mice to septic shock induced by LPS (Fig. 4E), and the increase in plasma CCL9 after LPS challenge (90 min) was signiﬁcantly lower in RXRα KO mice (Fig. 4F). The absence of RXRα did not, however, affect the plasma concentrations of IL-6, IL-1α, IL-12, TNFα, and MCP-1 (Fig. S7C). We did not ﬁnd signiﬁcant differences in the plasma levels of CCL9, MCP-1, and IL-6 after LPS injection in PPARγ KO mice (Fig. S7D). Next, we studied the effect of RXR signaling on LPS-induced CCL9 production by peritoneal macrophages in vitro. In WT macrophages, LPS induced the expression of CCL9 mRNA, peaking at 4 h, and a strong accumulation of CCL9 protein in culture supernatants; in contrast, RXRα KO macrophages showed signiﬁcantly lower CCL9 mRNA expression and CCL9 protein secretion (Fig. S8A and B). No changes in LPS-induced Ccl9 expression were found in PPARγ KO mice.

Fig. 2. Transcription of Ccl6 and Ccl9 is regulated by RXRα via DR-1 rexinoid response elements. (A and B) RAW 264.7 macrophages were cotransfected with a luciferase reporter plasmid under the transcriptional control of either the Ccl6 (pGL3-CCL6-luc) or the Ccl9 (pGL3-CCL9-luc) promoters together with an RXRα expression plasmid (pCMX-RXRα) or empty vector, as indicated. Cells were treated with LG268 (50 nM) or 9-cis-RA (1 μM) and analyzed for reporter activity 24 h later. (C) Mutations introduced into the Ccl6 and Ccl9 DR-1 motifs. (D and E) RAW 264.7 cells were transfected with 1 kb, 200 bp, or DR-1-mutated 200 bp (MUT) proximal sequences from the Ccl6 or Ccl9 promoters. Cells were treated with LG268 (50 nM) or 9-cis-RA (1 μM) and analyzed for reporter activity 24 h later. Values are means ± SEM from at least three experiments. (F) ChIP analysis of the binding of RXRα to Ccl6 and Ccl9 proximal promoter regions in macrophages treated for the indicated times with LG268 (50 nM). ChIP assays were performed with antibodies against RXRα and control IgG. Immunoprecipitated DNA was analyzed by Q-PCR. Values are means ± SEM. (G) Recombinant GST-labeled RXRα homodimers were bound to 32P-labeled oligonucleotides corresponding to the RXRE from the Ccl6 and Ccl9 promoter or a version mutated as in C (mut). Competition assays were conducted with unlabeled WT and mutant RXRα oligonucleotides. (H) Q-PCR analysis of mRNA expression in peritoneal macrophages treated with ligands for PPARα (GW7647, 0.1 μM), PPARγ (GW742, 0.1 μM), PPARα (Rosiglitazone, 1 μM), or RXRα (LG268, 50 nM), alone or in the indicated combinations. Values are means ± SEM. **, P ≤ 0.01; ***, P ≤ 0.001.
Mice lacking RXRα in myeloid cells show impaired recruitment of leukocytes to sites of inflammation and are less susceptible than control mice to sepsis. These defects are associated with decreased levels in vivo of the chemokines CCL6 and CCL9, which are shown to be transcriptional targets of RXRα.

Chemokines are required for leukocyte trafficking during inflammatory responses and for host responses during sepsis (18–20). CCL6 and CCL9 are CC chemokines that serve as chemotaxins for macrophages, CD11b+ cells, B cells, CD4+ and CD8+ T lymphocytes, and eosinophils (28–30). These chemokines are present in a variety of inflammatory and remodeling disorders, but their transcriptional regulation is not well characterized (29, 31, 32). Ccl6 and Ccl9 are paralogous genes, separated by only 9.4 kb on chromosome 11, and show a high degree of sequence similarity (28). Few reports have shown chemokines to be direct target genes for nuclear receptors. Retinoic acid (RA) induces Ccl2 (Mcp-1) expression in human monocyteic cells lines and peripheral blood monocytes in vitro, but the relevance of this finding in vivo is unclear (33).

A few genes have been reported to be regulated by RXR homodimers signaling through specific RXREs (DR-1 type elements): rat cellular retinol-binding protein type II (CRBPII), human apo A-I, human Apo A-II, and rat growth hormone (rGH) (34). However, many of these gene regulations were identified by in vivo chemical transfection assays (35), and the possibility that these genes might be activated by binding of RXR heterodimers to DR-1 elements in vivo was not explored. More recently, Ijpenberg et al. (7) have shown that RXR homodimers are able to regulate metabolic pathways in vivo. By excluding roles of other nuclear receptors that are expressed in macrophages and have the ability to heterodimerize with RXRα on the Ccl6 and Ccl9 RXREs, we provide evidence for an RXR-selective signaling pathway in primary macrophages. We have identified the RXRE in the Ccl6 and Ccl9 proximal promoters as a DR-1 cis-element, consisting of two imperfect AGGTC sequences separated by a single nucleotide. RXRα deletion thus results in a significant reduction of Ccl6 and Ccl9 gene expression in primary mouse macrophages, although the residual induction of Ccl6 and Ccl9 in KO macrophages indicates a possible compensatory effect of RXRβ.

The role of RXRα in inflammation was investigated in the present study using four mouse models of peritonitis, all characterized by leukocyte influx into the peritoneal cavity and the release of inflammatory mediators (cytokines/chemokines): i.p. administration of zymosan or thioglycollate produces a sterile peritonitis, LPS-endotoxemia mimics bacterially induced sepsis, and CLP produces a polymicrobial bacterial peritonitis that closely mimics human sepsis. Using these models, we have shown that RXRα signaling occurs in vivo. Our results show that the impaired leukocyte migration in response to inflammatory stimuli in RXRα KO mice is associated with the lower secretion of CCL6 and CCL9 in the peritoneal cavity in these animals. Reduced levels of CCL6 and CCL9 might also regulate the secretion of other cytokines/chemokines such as IL-6 and MCP-1, contributing to the phenotype. In contrast, although PPARγ also binds a DR-1 element, myeloid PPARγ null mice showed no defects in leukocyte recruitment to inflammatory sites and alterations in the levels of CCL6, CCL9, IL-6, or MCP-1. Although PPARγ has been shown to negatively regulate cytokines and chemokines in chronic inflammation (2, 3, 36), we did not observe any role of PPARγ in the in vivo models of acute inflammation. This unexpected result might be due to the lack of PPARγ expression in monocytes and resting peritoneal macrophages, which play key roles in the earlier phases of acute inflammatory responses (36, 37), or it may be that repression of proinflammatory genes by PPARγ does not require RXRα. Our data demonstrate the existence of an RXR signaling pathway independent of PPARγ. Moreover, given the known macrophage expression profiles of nuclear receptors able to interact

### Discussion

Nuclear hormone receptors are important modulators of the immune response (2), and nuclear receptors such as PPARγ and LXRXα play important roles in macrophage biology, affecting innate and adaptive immunity (3, 22). These receptors heterodimerize with RXRα to regulate gene transcription (4). Previous studies have suggested that activation of RXRα promotes Th2 (Th2) differentiation, establishing a role for RXRα in adaptive immunity through its interaction with VDR and RAR (23). In addition, various stimuli, including IL-1β and LPS, have been shown to regulate RXRα gene expression or subcellular localization in liver (24–26), and several reports have indicated possible roles of RXRα in skin and liver inflammation and viral immune responses (24, 25, 27). However, the role of macrophage RXRα in innate immunity and inflammation is not established. Our results show that RXRα is important for the proper control of genes involved in the innate immune responses to inflammatory stimuli.
with RXRs on DR-1 elements (4, 21), our findings provide evidence supporting an action of RXR homodimers. However, the variety of potential heterodimers formed by RXRs means that the possibility that a different heterodimer contributes to the observed effects cannot be completely ruled out at present. Whether other nuclear receptors are also involved in the control of acute inflammatory responses in vivo will require conditional macrophage-specific deletion of each candidate RXR partner.

Sepsis is a very complex syndrome in which the underlying inflammatory response involves the interplay of several biological systems (the complement, coagulation, and fibrinolytic cascade and the autonomic nervous system) and cell types, resulting in an imbalance of the inflammatory network (17, 38). Recent strategies to identify potential therapeutic targets have focused on these systems but with little clinical success. The finding that survival after LPS- or CLP-induced sepsis is prolonged in RXR KO mice is therefore of possible clinical interest. Interestingly, mice deficient for CCR1, the receptor for CCL6 and CCL9, are also significantly protected against CLP-induced lethality (39). However, immunoneutralization of CCL6 enhances sepsis-related mortality (31). This discrepancy might arise because the immunoneutralization only interferes with CCL6, potentially allowing a compensatory effect of CCL9. It is also possible that the decreased MCP-1 levels in the peritoneum of RXR KO mice after CLP-induced sepsis might contribute to the phenotype.

RXR is activated in vitro by the vitamin A metabolite 9-cis-RRA, which binds with high affinity to the RXR ligand binding domain; however, 9-cis-RRA has been difficult to detect in vivo (40). Recently, it has been shown that RXR can be activated by polyunsaturated long chain fatty acids (PUFA) (11), although the nature of the physiologically relevant agonists remains to be established. Interestingly, in response to LPS, RAW 264.7 cells and thioglycollate-elicited or BM-derived macrophages produce PUFA (www.lipidmaps.org), which might be important for the regulation of RXR-mediated inflammatory responses. Ccl9 is also induced by LPS and this response is lost in RXR KO macrophages, suggesting that natural ligands for RXR are produced in response to LPS treatment. Further studies are required to determine whether endogenous ligands for RXR are produced during inflammatory processes.

Our data support a model in which peritoneal macrophages respond to inflammatory stimuli by secreting cytokines and endogenous RXR ligands. The activation of the RXRα-transcriptional program in macrophages will result in the production of chemokines such as CCL6 and CCL9, leading to increased leukocyte recruitment and an inflammatory response; excessive activation of this program will have deleterious consequences, as in the case of sepsis. This study outlines a previously unrecognized role for RXRα in the regulation of leukocyte migration and sepsis, and supports the existence of an RXR signaling pathway in vivo. Our data suggest that RXRs are potential targets for immunotherapy in sepsis patients and in chronic inflammatory diseases.

Materials and Methods

Cell Culture. Peritoneal macrophages were harvested from WT and RXR KO mice as previously described (37). Further information is provided in SI Materials and Methods.

RNA Analysis and Chemokine Quantification. Total RNA isolation and Q-PCR were performed as described in ref. 9. Further information is provided in SI Materials and Methods. CCL9 (R&D Systems) and CCL6 (Antigenix) were quantified with ELISA kits.

ChIP Assays. Macrophages were cross-linked with 1% formaldehyde at room temperature for 10 min. 100-bp regions of the Ccl6 and Ccl9 proximal promoters were amplified by Q-PCR. Primer sequences are available upon request. Anti-RXRα (D-20, sc-553) and anti-RXRD1-197 (sc-774) (Santa Cruz Biotechnology) antibodies were used in combination. IgG was used as a control of nonspecific binding.

Gel Shift and Transient Transfection Assays. Oligonucleotides were annealed and labeled using Klenow enzyme (Roche). Purified bacterially expressed RXRα protein was incubated with labeled DNA, and protein–DNA complexes were electrophoresed and visualized by autoradiography. For competition studies, a 5- to 100-fold excess of unlabeled oligonucleotide was added. Oligonucleotides corresponding to RXR binding sites in Ccl6 and Ccl9 were used.
promoters and their mutants are available upon request. Transient transfections of RAW 264.7 cells (ATCC) using Lipofectamine 2000 (Invitrogen) were as described (36). Promoter constructs for Ccl9 and Ccfl9 were cloned in the pGL3-luc vector (Promega). A β-galactosidase expression vector was cotransfected as an internal control. Point mutations in Ccfl6-luc and Ccfl9 luc were made with the QuikChange site-directed mutagenesis kit (Stratagene).

**Peritonitis Models.** Eight- to 9-week-old mice were i.p. administered with 2.5 mL of 3% thioglycollate broth (Difco) or 1 mg of type A zymosan (Sigma) in 0.5 mL of sterile PBS. In other experiments, 12-week-old mice of each genotype were i.p. injected with 40 mg/kg LPS (E. coli 0111:B4, Sigma). Sepsis induction by cecal ligation and puncture (CLP) was performed as described elsewhere (41). Severity of CLP was quantified by histological analysis. Liver sections were H&E-stained, and three blinded observers scored six pictures per mouse for distinct parameters: hemorrhage, fibrin deposition, hepatocyte degeneration, and leukocyte infiltrate. Mice were considered severely affected if ≥3 parameters were scored higher than the group average for each time point.

**Cell Migration Assay.** Cell migration assays were conducted using 8.0-μm-pore transwell filters (Costar). Peritoneal monocytes/macrophages (5 × 10^5) were resuspended in 150 μL of 0.5% BSA in RPMI medium and added to the upper chamber of transwell filters. The lower chamber contained rCCL6 (50 ng/mL), rCCL9 (5 ng/mL), or peritoneal exudate from WT or RXRKO mice previously injected with thioglycollate (48 h) or zymosan (24 h). After a 2-h incubation at 37 °C, the inserts were processed for fluorescence microscopy.

**Endothelial Transmigration Assay.** Cell transmigration assays were performed in 5-μm-pore Transwell chambers (Costar) as described in ref. 42. Further information is provided in SI Materials and Methods.

**Statistical Analysis.** The normal distribution of data was checked with the Kolmogorov-Smirnov test. Data were analyzed by unpaired Student’s t test and nonparametric Mann–Whitney U test. Survival rates were represented as a Kaplan–Meier curve, and the results were analyzed with a log-rank (Mantel–Cox) t test.

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