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IL-6 Controls the Innate Immune Response against *Listeria monocytogenes* via Classical IL-6 Signaling

Judith Hoge,*[,1] Isabell Yan,*[,1] Nathalie Jänner,[†] Valéa Schumacher,* Athena Chalaris,[†] Oliver M. Steinmetz,[‡] Daniel R. Engel,[§] Jürgen Scheller,[¶] Stefan Rose-John,[†] and Hans-Willi Mittrücker*

The cytokine IL-6 plays a protective role in immune responses against bacterial infections. However, the mechanisms of IL-6–mediated protection are only partially understood. IL-6 can signal via the IL-6R complex composed of membrane-bound IL-6Rα (mIL-6Rα) and gp130. Owing to the restricted expression of mIL-6Rα, classical IL-6 signaling occurs only in a limited number of cells such as hepatocytes and certain leukocyte subsets. IL-6 also interacts with soluble IL-6Rα proteins and these IL-6/soluble IL-6Rα complexes can subsequently bind to membrane-bound gp130 proteins and induce signaling. Because gp130 is ubiquitously expressed, this IL-6 trans-signaling substantially increases the spectrum of cells responding to IL-6. In this study, we analyze the role of classical IL-6 signaling and IL-6 trans-signaling in the innate immune response of mice against *Listeria monocytogenes* infection. We demonstrate that *L. monocytogenes* infection causes profound systemic IL-6 production and rapid loss of IL-6Rα surface expression on neutrophils, inflammatory monocytes, and different lymphocyte subsets. IL-6–deficient mice or mice treated with neutralizing anti–IL-6 mAb displayed impaired control of *L. monocytogenes* infection accompanied by alterations in the expression of inflammatory cytokines and chemokines, as well as in the recruitment of inflammatory cells. In contrast, restricted blockade of IL-6 trans-signaling by application or transgenic expression of a soluble gp130 protein did not restrain the control of infection. In summary, our results demonstrate that IL-6Rα surface expression is highly dynamic during the innate response against *L. monocytogenes* and that the protective IL-6 function is dependent on classical IL-6 signaling via mIL-6Rα. *The Journal of Immunology*, 2013, 190: 703–711.

The cytokine IL-6 is a central regulator of inflammatory processes. In response to infection but also during chronic inflammation, IL-6 is produced by variety of cells and has pro- as well as anti-inflammatory activities (1, 2). IL-6 is a major inductor of acute phase proteins and is involved in the control of neutrophil and monocyte responses following infection. IL-6 is also a survival factor for lymphocytes and it promotes Ab production by B cells (1–3). More recently, IL-6 has been identified as a decisive cytokine for the differentiation of $CD4^+$ T cells into Th17 cells, which are considered to be a major proinflammatory T cell population (4, 5). As a consequence, blockade of IL-6 or IL-6 signaling has a profound impact on innate and acquired immune responses. Impaired IL-6 function causes enhanced susceptibility of mice to infection with various pathogens (6–15) but also results in protection in several mouse models for chronic inflammatory diseases (16–18). Not surprisingly, IL-6 signaling is also an important target of intervention in chronic inflammatory human diseases such as rheumatoid arthritis and Crohn's disease (19).

The IL-6 receptor consists of membrane-bound IL-6Rα (mIL-6Rα, CD126) and the gp130 chain (CD130). mIL-6Rα binds IL-6 but has no intrinsic signaling capacity. Upon IL-6 binding, mIL-6Rα interacts with gp130, which subsequently initiates IL-6 signal transduction (2). In contrast to the ubiquitous expression of gp130, expression of mIL-6Rα is restricted to hepatocytes and certain subsets of leukocytes (20). Thus, IL-6 can only activate a limited number of cells via classical IL-6R signaling. However, a soluble form of IL-6Rα (sIL-6Rα) is constitutively found in the serum, and following inflammation mIL-6Rα is actively shed from inflammatory cells, resulting in enhanced local and systemic sIL-6Rα concentrations (21, 22). sIL-6Rα still binds IL-6, and the IL-6/sIL-6Rα complexes can interact with membrane-bound gp130 and thereby provoke signal transduction (23). This process, which is called IL-6 trans-signaling, allows activation of mIL-6Rα–negative cells by IL-6 and thereby substantially expands the number of IL-6–responsive cells (2, 3). Under normal conditions, IL-6 levels in human blood are in the range of 1–5 pg/ml. sIL-6Rα and soluble gp130 (sgp130) levels, however, are in the range of 50–100 and 100–200 ng/ml, respectively. Secreted IL-6 therefore will bind to sIL-6Rα and the complex of IL-6/sIL-6Rα will bind to sgp130 and thereby be neutralized. Hence, sIL-6Rα and sgp130 constitute a buffer for systemically active IL-6. Only when IL-6 concentrations exceed those of sIL-6Rα and sgp130 can IL-6 act systemically (24, 25).

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Abbreviations used in this article: HKL, heat-killed listeria; KO, knockout; mIL-6Rα, membrane-bound IL-6Rα; SAA, serum amyloid A; sgp130, soluble gp130; sgp130Fc, soluble gp130 Fc fusion protein; sIL-6Rα, soluble IL-6Rα; Tg, transgenic; wt, wild-type.

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IL-6/sIL-6Rα complexes also offer a target for therapeutic intervention. Soluble gp130 proteins have been designed that neutralize IL-6/sIL-6Rα complexes but do not interfere with signaling via mIL-6Rα (24, 25). These proteins have been used to dissect the role of classical IL-6 signaling and IL-6 trans-signaling in different mouse models for infection and inflammatory diseases (22, 26, 27). Because sgp130 proteins show beneficial effects in models of chronic inflammation (16, 28, 29), they might also offer a new option for the treatment of chronic inflammatory diseases in humans (30).

The Gram-positive bacterium *Listeria monocytogenes* can cause severe disease in immune-suppressed individuals, and in pregnant women, infection of the fetus can lead to abortion or to high fatality rates in neonates (31). In mice, infection with *L. monocytogenes* provokes a rapid activation of the innate immune system, which is essential for the restriction of bacteria replication. In particular, the production of proinflammatory cytokines such as TNF-α and IFN-γ and the recruitment of inflammatory monocytes to sites of infection are crucial for the early control of *L. monocytogenes* (32, 33). Infection also leads to robust production of IL-6, and IL-6 deficiency or IL-6 neutralization with Abs results in enhanced susceptibility to *L. monocytogenes* (6, 34–41); however, mechanisms of IL-6–mediated protection are so far unclear.

In this study, we characterize the role of different IL-6 signaling pathways for the innate control of *L. monocytogenes*. We demonstrate that following infection, mIL-6Rα is rapidly lost from the surface of all leukocytes analyzed. In parallel, high serum levels of IL-6 can be detected, providing the basis for classical IL-6 signaling and IL-6 trans-signaling. Using neutralizing anti–IL-6 mAbs, which block IL-6 classical and trans-signaling, and a sgp130 Fc fusion protein (sgp130Fc), which only blocks IL-6 trans-signaling, as well as IL-6–deficient mice and mice transgenic (Tg) for sgp130Fc, we can show that classical IL-6 signaling but not IL-6 trans-signaling is crucial for the IL-6–mediated control of *L. monocytogenes*.

Materials and Methods

Abs and reagents

Anti-CD16/CD32 mAb (clone 2.4G2) and anti–IL-6 mAb (MP5-20F3) were purified from hybridoma supernatants according to standard protocols. Fluorochrome-conjugated mAbs against CD4 (RM4-5), CD8α (53-6.7), CD11b (M1/70), CD19 (eBio1D3), CD45 (30-F11), Ly6C (AL-21), Gr-1 (RB6-8C5), F4/80 (BM8), TNF-α (MP6-XT22), IL-6 (MP5-20F3), and IL-6Rα (D7715A7) were purchased from BioLegend (San Diego, CA), eBioscience (San Diego, CA), or BD Pharmingen (San Diego, CA).

A soluble fusion protein of the extracellular domains of gp130 and the Fc part of human IgG1 (sgp130Fc) was constructed and purified as described in Jostock et al. (25). As demonstrated before, human sgp130Fc can bind and neutralize mouse IL-6/IL-6Rα complexes with similar efficacy as mouse sgp130Fc (25). Purified human IgG was purchased from Dianova (Hamburg, Germany).

Ligand binding capacity and biological activity of sgp130Fc were measured by a Ba/F3-gp130 cell proliferation assay. Ba/F3 cells (murine pre-B cells) stably transfected with human gp130 (Ba/F3-gp130 cells) have been described previously (25). Ba/F3-gp130 cells were seeded with 5×10^3 cells per well in 96-well plates. Proliferation was induced by adding 100 ng/ml IL-6 plus 50 ng/ml sIL-6Rα and different concentrations of sgp130Fc. Proliferation was measured by the cell titer colorimetric assay (Promega) as described previously (42). The activity assay for the batch of sgp130Fc used for the present study is shown in Supplemental Fig. 1.

Mice and animal experiments

C57BL/6 mice, IL-6 knockout (IL-6^{KO}) mice (6), sgp130Fc^{Tg} mice (24), and $CCR2^{KO}$ mice (43) were bred at the Central Animal Facilities of the University Medical Center Hamburg–Eppendorf, at the House for Experimental Therapy in Bonn, or at the Victor Hensen Facility of the University of Kiel. IL-6^{KO} and sgp130^{Tg} mice were on the C57BL/6N background. In experiments with these mouse strains, matched C57BL/6N mice were used as controls. All other experiments were done with C57BL/6J mice. Animal experiments were conducted according to the German Animal Protection Law.

Mice were i.v. infected via the lateral tail vein with 2×10^4 *L. monocytogenes* strain EGD in 200 μl PBS. Bacterial inoculi were always controlled by plating serial dilutions on tryptic soy broth agar plates. For determination of bacterial burdens in spleens and livers, mice were killed, organs were homogenized in PBS containing 0.5% Triton X-100, serial dilutions of homogenates were plated on tryptic soy broth agar, and colonies were counted after 48 h incubation at 37°C.

For blocking of IL-6 signaling or trans-signaling, mice were treated with 500 μg neutralizing anti–IL-6 mAb (clone MP5-20F3) (44) or 250 μg sgp130Fc. Concentrations of anti–IL-6 mAb and sgp130Fc have been shown to be effective in blocking global IL-6 signaling and trans-signaling (26). Control mice received 250 μg purified human IgG. Abs and fusion proteins were injected i.p. in a volume of 200 μl PBS 1 d prior to *L. monocytogenes* infection. Abs and sgp130Fc proteins were controlled for low endotoxin content using a commercial *Limulus* amebocyte lysate assay (Lonza, Basel, Switzerland).

Purification and staining of cells

For characterization of different leukocyte subsets, spleens and livers were cut into small pieces and digested for 45 min with each 0.25 mg/ml collagenase D (Roche, Penzberg, Germany) and collagenase VIII (Sigma-Aldrich, Steinheim, Germany), and 15 μg/ml DNAse I (Sigma-Aldrich) in RPMI 1640 medium supplemented with glutamine, gentamicin, 2-ME, and 10% heat-inactivated FCS (complete RPMI 1640 medium). The tissue was gently meshed through a 70-μm cell strainer and the suspension was centrifuged to pellet the cells. Liver cells were resuspended and centrifuged through a 40/70% Percoll gradient (Biochrom, Berlin, Germany) for 30 min at $600 \times g$. Cells were collected from the interface of the gradient and washed in complete RPMI 1640 medium. Erythrocytes were lysed and cells were washed and stored on ice. After collagen digestion, spleen cells were pelleted and erythrocytes were lysed. Cells were washed and stored on ice. For analysis of surface IL-6Rα and induction of IL-6, spleen cells were purified without collagen digestion.

For surface staining, cells were washed and incubated for 5 min with rat serum and anti-CD16/CD32 mAb to block unspecific Ab binding. Subsequently, cells were incubated on ice with Abs as indicated. To mark dead cells, 2 μg/ml Pacific Orange succinimidyl ester (Invitrogen, Darmstadt, Germany) was added. After 20 min, cells were washed in PBS and fixed with PBS/1% paraformaldehyde. Cells were analyzed using a FACSCanto II and the FACSDiva software (Becton Dickinson, Mountain View, CA).

Induction of cytokines and intracellular cytokine staining

Cells were cultured in a volume of 1 ml complete RPMI 1640 medium. Cells were stimulated for 6 h with 10^7 heat-killed listeria (HKL)/ml. During the final 4 h of culture, 10 μg/ml brefeldin A (Sigma-Aldrich) was added. Cultured cells were washed and incubated for 5 min with rat serum and anti-CD16/CD32 mAb to block unspecific Ab binding. Subsequently, cells were stained with Abs against surface proteins, and after 30 min on ice, cells were washed with PBS and fixed for 20 min at room temperature with PBS/4% paraformaldehyde. Cells were washed with PBS/0.2% BSA, permeabilized with PBS/0.1% BSA/0.3% saponin, and incubated in this buffer with rat serum and anti-CD16/CD32 mAbs. After 5 min, fluorochrome-conjugated anti–IL-6 mAb and anti–TNF-α mAb was added. After a further 20 min on ice, cells were washed with PBS and fixed with PBS/1% paraformaldehyde.

For the preparation of HKL, bacteria from an overnight culture were washed twice in PBS and inactivated by incubation for 30 min at 80°C.

IL-6 and sIL-6Rα determination

Serum of naive and infected mice was appropriately diluted and analyzed for IL-6 and sIL-6Rα by ELISA according to the manufacturer's protocols (R&D Systems, Wiesbaden, Germany). The addition of sIL-6Rα did not interfere with the detection of IL-6 in this ELISA (Supplemental Fig. 2). Spleen and liver tissues of mice were mixed with lysis buffer containing tissue protein extraction reagent (Thermo Scientific, Dreieich, Germany) and protease inhibitors (complete protease inhibitor mixture tablets; Roche). Lysates were stored at −80°C. After thawing, lysates were homogenized with a TissueLyser (Qiagen, Hilden, Germany). Lysates were centrifuged, protein content in the supernatant was determined with a BCA protein assay kit (Thermo Scientific), and the concentrations of IL-6 and serum amyloid A (SAA) were determined by ELISA. The murine SAA ELISA was conducted according to the manufacturer's protocol (Tridelta Development, Maynooth, Ireland).

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Real-time RT-PCR analysis

Total RNA of spleen and liver was prepared using TRIzol reagent (Invitrogen, Karlsruhe, Germany) and a TissueLyser (Qiagen) according to the manufacturers' protocols. cDNA was generated with random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR was performed for 40 cycles (initial denaturation at 95°C for 10 min, denaturation at 95°C for 20 s, primer annealing and elongation at 60°C for 1 min) with 1.5 μl cDNA samples in the presence of 2.5 μl (0.9 μM) specific murine primers and 6.25 μl SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich) in a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA). All samples were run in duplicate and normalized to 18S rRNA to account for small RNA and cDNA variability (45).

The following primers were used: 18S, forward, 5′-CAG GGC CGG TAC AGT GAA AC-3′, reverse, 5′-AGA GGA GCG AGC GAC CAA A-3′; CXCL1, forward, 5′-GCA CCC AAA CCG AAG TCA TAG-3′, reverse, 5′-CAA GGG AGC TTC AGG GTC AA-3′; CXCL2, forward, 5′-CAC TGC GCC CAG ACA GAA-3′, reverse, 5′-CAG GGT CAA GGC AAA CTT TTT G-3′; CCL2, forward, 5′-GGC TCA GCC AGA TGC AGT TAA-3′, reverse, 5′-CCT ACT CAT TGG GAT CAT CTT GCT-3′; IL-6, forward, 5′-TGG GAA ATC GTG GAA ATG AGA-3′, reverse, 5′-AAG TGC ATC ATC GTT GTT CAT ACA-3′, TNF-α, forward, 5′-AAA TGG CCT CCC TCT CAT CAG T-3′, reverse, 5′-GCT TGT ACA AAT TTT GAG AAG-3′; SAA, forward 5′-AGA GGA CAT GAG GAC ACC ATT GCT-3′, reverse, 5′-AGG ACG CTC AGT ATT TGT CAG GCA-3′.

Statistical analysis

All mice per group were independently analyzed. Statistical analysis was performed with GraphPad Prism 4 (GraphPad Software, La Jolla, CA). Quantifiable data were transformed into logarithmic values. Afterward, a Student *t* test or one-way ANOVA and the Bonferroni multiple posttest was performed to define differences among differently treated mice. All results are expressed as arithmetic means ± SD (bar graphs) or median (dot plots). Differences were considered significant with $p < 0.05$.

Results

IL-6 and IL-6Rα expression during early L. monocytogenes *infection*

IL-6 signals via membrane-bound IL-6Rα and gp130 (classical signaling). Alternatively, IL-6 can form complexes with sIL-6Rα, which subsequently interact with gp130 on the cell surface and thereby allow activation of cells lacking surface expression of IL-6Rα. IL-6Rα can be actively shed from the surface of cells under inflammatory conditions. Thus, the mode of IL-6 signaling could switch from classical to trans-signaling following infection. In a first set of experiments, we therefore determined the concentrations of IL-6 and sIL-6Rα in serum and tissues as well as the surface expression level of IL-6Rα on different leukocyte populations following infection of mice with *L. monocytogenes*.

Infection of mice with *L. monocytogenes* caused a profound increase in serum levels of IL-6, which reached a maximum at day 2 postinfection (Fig. 1A). Enhanced IL-6 concentrations were also measured in tissue extracts from spleens but not from liver (Fig. 1B, 1C), although both organs are main target sites for listeria replication. A similar pattern was observed for IL-6 mRNA (Fig. 1F). IL-6 mRNA induction was largely confined to the spleen (100- to 1000-fold induction) and only marginally in the liver (<10-fold induction). Most likely as a consequence of the high serum concentration of IL-6, a strong induction of the mRNA for the IL-6 target gene *saa* followed by enhanced SAA protein concentration was observed in livers of infected mice (Fig. 1E, 1G). In contrast to the strong increase in IL-6, there was only a marginal change in the serum concentrations of sIL-6Rα during the first days of *L. monocytogenes* infection (Fig. 1D).

In a next set of experiments, we determined the expression of IL-6Rα on innate immune cells following *L. monocytogenes* infection. As shown before (46, 47), infection of mice with *L. monocytogenes* caused a rapid accumulation of neutrophils and

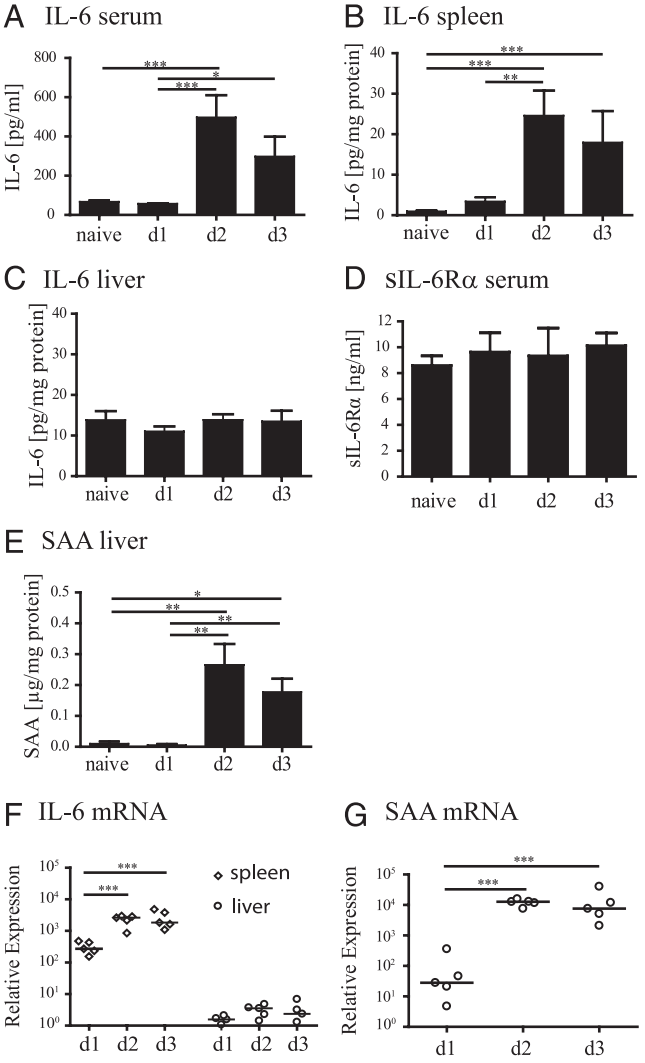

FIGURE 1. IL-6 and sIL-6Rα during the early phase of *L. monocytogenes* infection. C57BL/6 mice were i.v. infected with 2×10^4 *L. monocytogenes*. On days 1, 2, and 3 postinfection, mice were killed and IL-6 concentrations were determined in serum (**A**) as well as spleen (**B**) and liver extracts (**C**). IL-6Rα concentrations were measured in the serum (**D**) and SAA in liver extracts (**E**). mRNA levels for IL-6 were determined in the spleen and liver (**F**) and for SAA in the liver (**G**) by quantitative RT-PCR. mRNA levels were normalized to 18S RNA and are given as fold change compared with the mean values of naive mice. Bars in (A)–(E) represent means ± SD from five to seven individually analyzed mice per time point. Graphs in (F) and (G) give values for individual mice and the median. Figures are representative of at least two independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

inflammatory monocytes in spleen and liver (Supplemental Figs. 3, 4). Flow cytometric analysis revealed IL-6Rα surface expression on inflammatory monocytes as well as on $CD4^+$ and $CD8^+$ T cells of naive mice. In contrast, neutrophils and B cells displayed only relatively low mIL-6Rα expression (Figs. 2, 3). Closer examination of $CD4^+$ and $CD8^+$ T cells showed that in particular naive $CD4^+$ T cells ($CD44^{low}CD62L^+$) were IL-6Rα$^+$. Activated and memory $CD4^+$ T cells ($CD44^{high}CD62L^-$ or $CD44^{high}CD62L^+$) contained both IL-6Rα$^+$ and IL-6Rα$^-$ populations. Following infection, we observed a significant loss of IL-6Rα surface expression on all cells analyzed (Figs. 2, 3). Loss of IL-6Rα expression was particular strong on $CD4^+$ T cells and included the naive $CD44^{low}CD62L^+$ $CD4^+$ T cell population (Fig. 3). These results indicate that following *L. monocytogenes* infection, all analyzed cell populations

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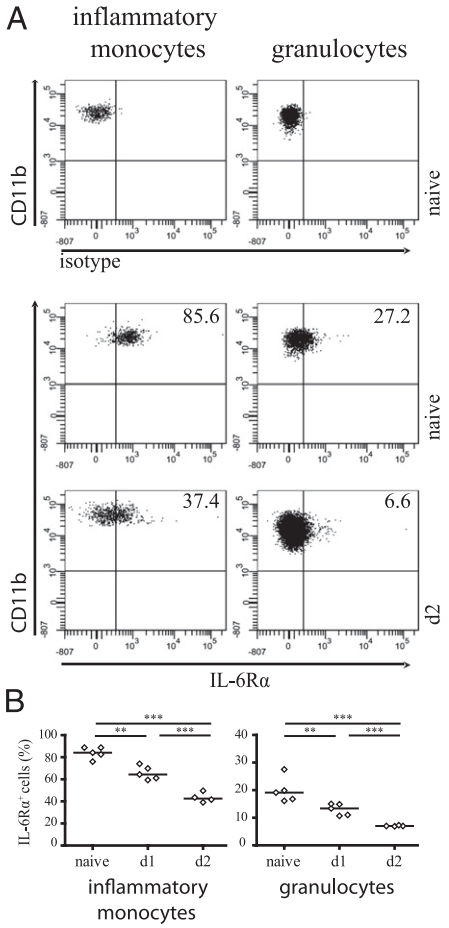

FIGURE 2. IL-6Rα on inflammatory monocytes and granulocytes during *L. monocytogenes* infection. C57BL/6 mice were i.v. infected with 2×10^4 *L. monocytogenes*. On the indicated time points postinfection, spleen cells were analyzed for the expression of IL-6Rα. (**A**) Representative results for the surface expression of IL-6Rα on gated cell populations. Numbers give percentage values of mIL-6Rα$^+$ cells among the gated cell population. (**B**) Combined results from groups of individually analyzed mice. Results are representative of two independent experiments. Bars in (B) represent median values. $^{**}p < 0.01$, $^{***}p < 0.001$.

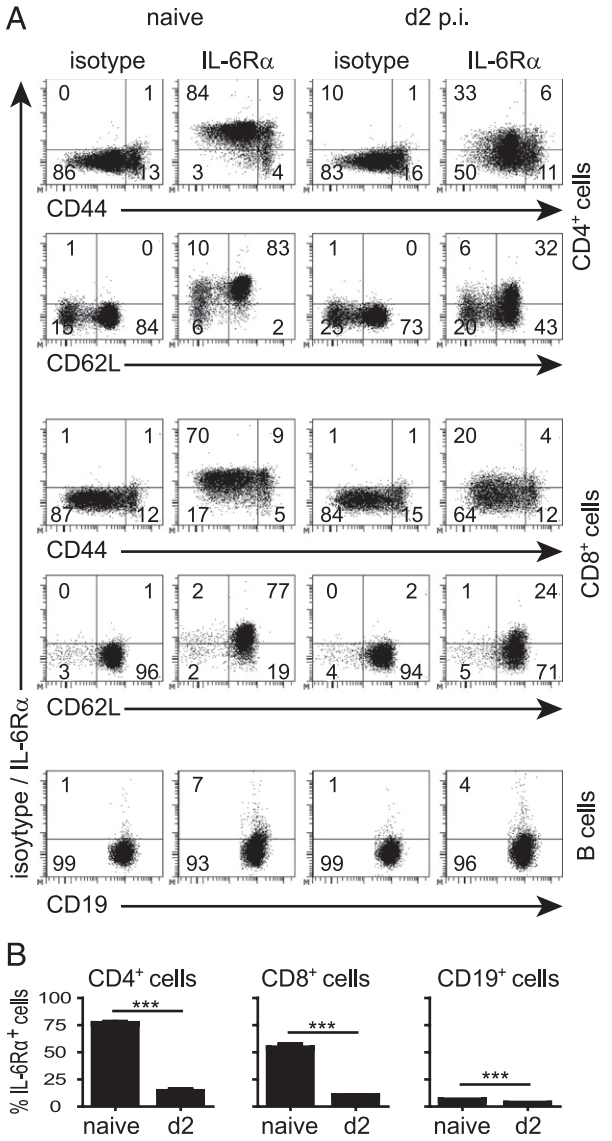

FIGURE 3. IL-6Rα on T and B lymphocytes during *L. monocytogenes* infection. C57BL/6 mice were i.v. infected with 2×10^4 *L. monocytogenes*. On day 2 postinfection, spleen cells were analyzed for the expression of IL-6Rα. (**A**) Graphs show representative results for surface expression profiles for IL-6Rα or isotype control and CD44 or CD62L for CD4$^+$ and CD8$^+$ T cells, and for IL-6Rα or isotype control on CD19$^+$ B cells. (**B**) Combined results from groups of individually analyzed mice. Bars represent means ± SD for three or four individually analyzed mice per time point and are representative of three independent experiments. $^{***}p < 0.001$.

rapidly lose the ability to respond to IL-6 via classical IL-6 signaling and become dependent on IL-6 trans-signaling.

Loss of surface IL-6Rα expression could be due to proteolytic shedding (22). However, it is also possible that the receptor is internalized in response to IL-6 signaling. Finally, IL-6 could interfere with the binding of the anti–IL-6Rα mAb to mIL-6Rα. To distinguish between these processes, IL-6^{KO} mice were infected with *L. monocytogenes* and the expression of mIL-6Rα was analyzed. On all leukocyte subsets including CD4$^+$ T cells, CD8$^+$ T cells, granulocytes, and inflammatory monocytes, we observed a similar loss of mIL-6Rα expression in wild-type (wt) and IL-6^{KO} mice (Fig. 4 and data shown). Therefore, loss of mIL-6Rα expression is most likely due to shedding and is not a consequence of receptor internalization or epitope masking.

Inflammatory monocytes are not an essential source of IL-6 and sIL-6Rα

Different cell types have been identified as sources of IL-6 during inflammation and infection (1). Because we observed a strong accumulation of granulocytes and inflammatory monocytes, we tested whether these cells were potential sources of IL-6 during early *L. monocytogenes* infection. Upon stimulation of spleen cells with HKL, a large fraction of inflammatory monocytes were indeed able to produce IL-6 as determined by intracellular cytokine staining (Fig. 5). Frequencies of inflammatory monocytes producing IL-6 in response to HKL increased until day 2 of infection and then declined again. In these cells, IL-6 production was accompanied by a strong expression of TNF-α. Without stimulation, neutrophils showed already low expression levels of IL-6, but expression did not increase after addition of HKL. Frequencies of granulocytes producing IL-6 declined postinfection.

Because inflammatory monocytes expressed IL-6Rα on their surface and were able to secrete IL-6 upon stimulation with listeria, we determined the impact of this cell population on serum levels of IL-6 and IL-6Rα during the early phase of *L. monocytogenes* infection. Serbina and Pamer (48) recently demonstrated that following *L. monocytogenes* infection, mobilization of

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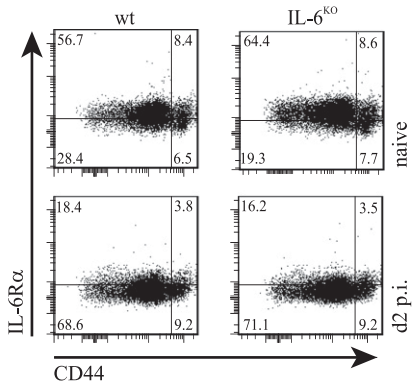

FIGURE 4. Loss of IL-6Rα surface expression occurs in the absence of IL-6. C57BL/6 mice and IL-6^{KO} mice were i.v. infected with 2×10^4 *L. monocytogenes*. On day 2 postinfection, spleen cells were analyzed for the expression of IL-6Rα. Graphs show representative results for surface expression profiles of IL-6Rα and CD44 on $CD4^+$ T cells. Results are representative of two independent experiments with three mice per group.

inflammatory monocytes from the bone marrow and accumulation of these cells in infected tissues were strictly dependent on the chemokine receptor CCR2. Therefore, wt and $CCR2^{KO}$ mice were infected with *L. monocytogenes* and analyzed 3 d postinfection. Determination of *L. monocytogenes* burden in spleen and livers revealed 100-fold higher titers in $CCR2^{KO}$ mice (Fig. 6A).

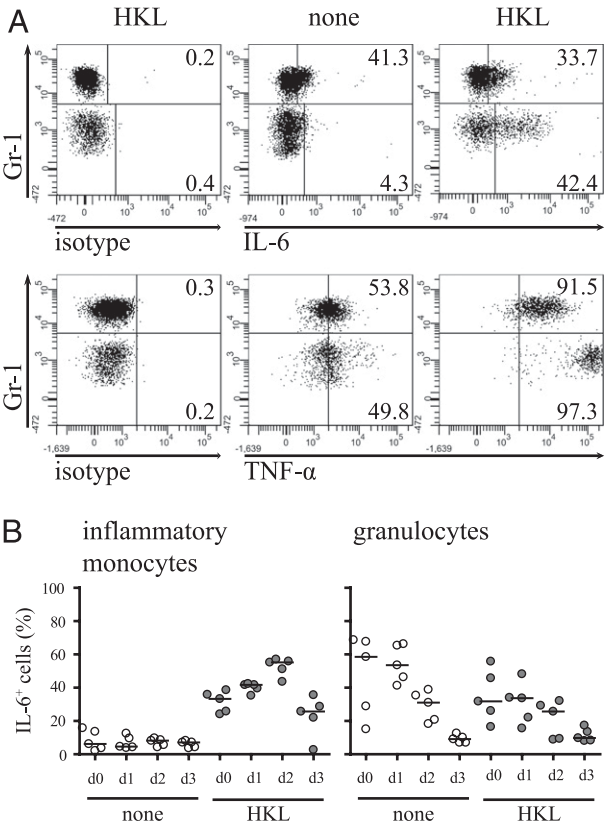

FIGURE 5. Induction of IL-6 in inflammatory monocytes and granulocytes. C57BL/6 mice were i.v. infected with 2×10^4 *L. monocytogenes*. On the indicated time points postinfection, spleen cells were cultured for 6 h with or without HKL. Production of IL-6 was determined by intracellular IL-6 staining. (**A**) Representative results for gated inflammatory monocytes ($Gr\text{-}1^{low}$) and granulocytes ($Gr\text{-}1^{high}$). Numbers give the percentage values for cytokine-positive granulocyes and inflammatory monocytes, respectively. (**B**) Combined results from individually analyzed mice. Results are representative of two independent experiments. Bars represent median values.

Compared to infected wt mice, infected $CCR2^{KO}$ mice completely failed to recruit inflammatory monocytes to the spleen and had significantly reduced frequencies of these cells in the liver (Fig. 6B). In contrast, $CCR2^{KO}$ mice displayed high frequencies of granulocytes in spleen and liver following infection (data not shown). Determination of IL-6 revealed increased serum IL-6 levels in infected $CCR2^{KO}$ mice when compared with infected wt mice (Fig. 6C). Serum concentrations of sIL-6Rα were reduced in naive and infected $CCR2^{KO}$ mice when compared with wt controls (Fig. 6C), but reduction did not reach a significant level in infected animals (naive, wt 16.73 ± 0.86 ng/ml versus $CCR2^{KO}$ 12.21 ± 1.13 ng/ml, $p = 0.018$; infected, wt 15.68 ± 1.28 ng/ml versus $CCR2^{KO}$ 12.25 ± 1.57 ng/ml, $p = 0.054$). In summary, our results indicate that although inflammatory monocytes are fundamental in control of *L. monocytogenes* they are not an essential source for IL-6 and they only partially contribute to the generation of sIL-6Rα.

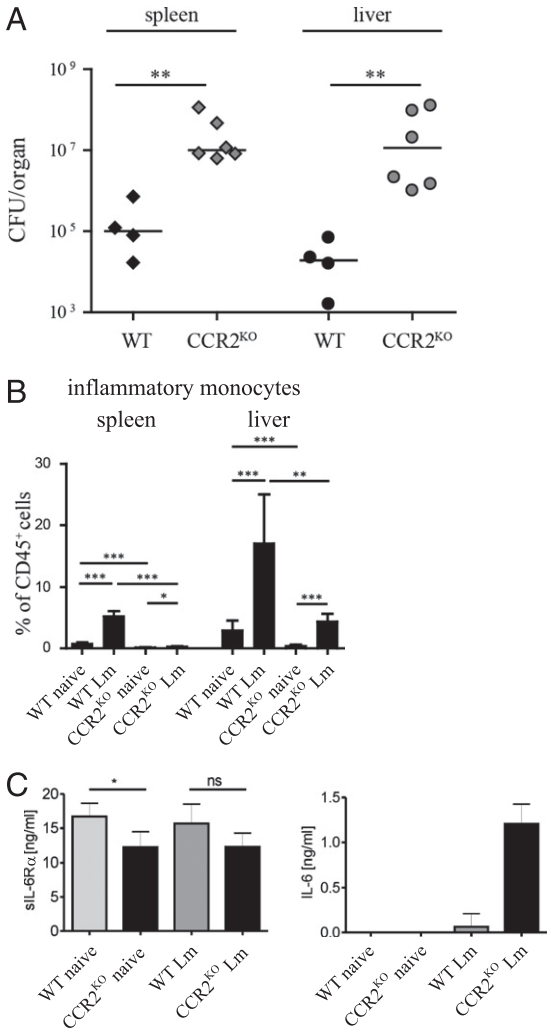

FIGURE 6. Inflammatory monocytes are not an essential source of IL-6 and sIL-6Rα. C57BL/6 wt and $CCR2^{KO}$ mice were i.v. infected with 2×10^4 *L. monocytogenes*. On day 3 postinfection, spleen and liver were analyzed. (**A**) Listeria titers in spleen and liver of infected animal. (**B**) Accumulation of inflammatory monocytes in spleen and liver. (**C**) Serum concentration of sIL-6Rα and IL-6. Individual values and the median are shown in (A). Bars in (B) and (C) give the means ± SD for four to five individually analyzed mice per group and time point. Figures are representative of two independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

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Blocking of conventional IL-6 signaling but not IL-6 trans-signaling caused impaired control of L. monocytogenes *infection*

Our results demonstrated the presence of IL-6 and sIL-6Rα during the early phase of *L. monocytogenes* infection. Thus, IL-6 could interact with target cells both via classical and trans-signaling. To determine the impact of classical and trans-signaling on the control of *L. monocytogenes*, mice were treated with a neutralizing anti–IL-6 mAb, which blocks all IL-6 signaling (44), or with a sgp130Fc fusion protein to selectively block IL-6 trans-signaling (26–29). Treatment of mice with a neutralizing anti–IL-6 mAb caused an increase in the listeria burden in the spleen and particularly in the liver of infected mice (Fig. 7A). In contrast, mice treated with sgp130Fc showed listeria titers in both organs, which were comparable to infected control animals. Thus, classical IL-6 signaling but not IL-6 trans-signaling was important for the control of *L. monocytogenes* infection.

Following infection, we observed pronounced accumulation of inflammatory monocytes in spleen and liver, which was not further affected by the treatment with anti–IL-6 mAb or sgp130Fc (Fig. 7B). At day 3 postinfection, there was still an enlarged neutrophil population in spleens of infected mice. Pretreatment of mice with sgp130Fc but not with anti–IL-6 mAb resulted in a reduction of this neutrophil population. In the liver, neither treatment with anti–IL-6 mAb or with sgp130Fc caused an alteration of neutrophil frequencies.

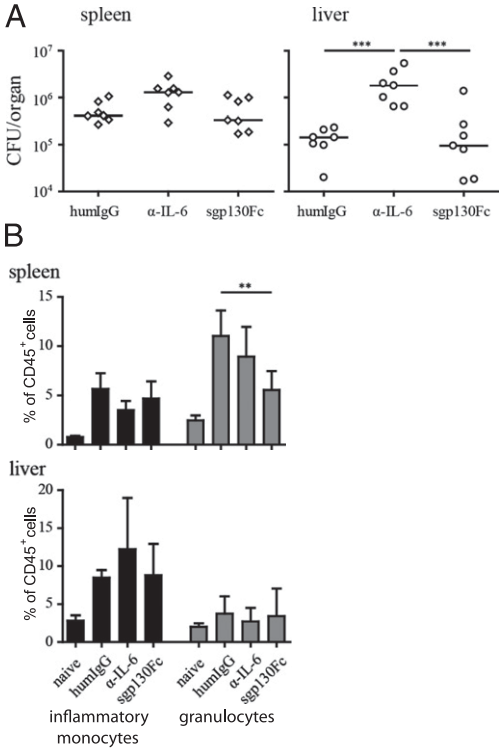

FIGURE 7. Role of classical IL-6 signaling and IL-6 trans-signaling in the innate immune response against *L. monocytogenes*. C57BL/6 mice were treated i.p. with anti-IL-6 mAb, sgp130Fc or control IgG (humIgG). One day later, mice were i.v. infected with 2×10^4 *L. monocytogenes*. After a further 3 d, mice were analyzed. (**A**) Listeria burden in spleen and liver. (**B**) Accumulation of inflammatory monocytes and neutrophils in spleen and liver. Values of individual mice and the median are shown in (A). Bars in (B) give the means ± SD of seven individually analyzed mice per group. Results in (A) are representative of four independent experiments and in (B) of two independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$.

To determine changes in the degree of inflammation following different treatments, the mRNA levels of SAA, IL-6, TNF-α, and of different chemokines were measured. *L. monocytogenes* infection caused a strong induction of SAA mRNA in livers of mice, which was reduced by anti–IL-6 mAb but not by sgp130Fc treatment (Fig. 8A). The mRNA for IL-6 was enhanced in spleens of infected mice but was not further affected by anti–IL-6 mAb or sgp130Fc treatment (Fig. 8B). There was limited induction of IL-6 mRNA in the livers of mice. Treatment with anti–IL-6 mAb caused enhanced IL-6 mRNA expression, but the difference did not reach a significant level. The mRNA for TNF-α was increased in spleens and livers of infected mice, but neither anti–IL-6 mAb nor sgp130Fc caused a change in expression (Fig. 8C). We detected enhanced levels of the neutrophil-attracting chemokines CXCL1 and CXCL2 and of the monocyte-attracting chemokine CCL2 (Fig. 8D–F). All three chemokines were more strongly induced in the spleen than in the liver. For all three chemokines, we observed

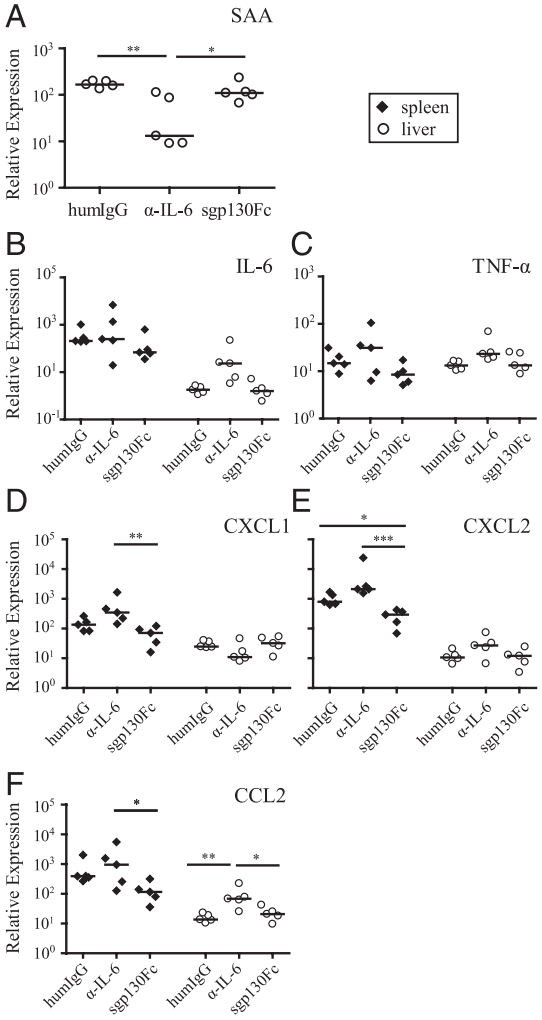

FIGURE 8. Role of classical IL-6 signaling and IL-6 trans-signaling for induction of cytokines and chemokines during the early phase of infection with *L. monocytogenes*. C57BL/6 mice were treated i.p. with anti–IL-6 mAb, sgp130Fc, or control IgG. One day later, mice were i.v. infected with 2×10^4 *L. monocytogenes*. After a further 3 d, mRNA expression of SAA (**A**), IL-6 (**B**), TNF-α (**C**), and of the chemokines CXCL1 (**D**), CXCL2 (**E**), and CCL2 (**F**) was determined. mRNA levels were normalized to 18S RNA and are given as fold change compared with the mean values of naive mice. Graphs give the values for individual mice and the median. Results are representative of four independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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the strongest expression levels in spleens of anti–IL-6 mAb–treated mice, which were significantly higher than the expression levels in spleens of sgp130Fc-treated mice.

IL-6 deficiency but not Tg expression of sgp130Fc resulted in impaired control of L. monocytogenes

To confirm results from studies with anti–IL-6 mAb and sgp130Fc treatment, IL-6^{KO} mice and sgp130Fc^{Tg} mice were infected with *L. monocytogenes*. spg130Fc^{Tg} mice express a gp130Fc fusion protein under the control of the liver-specific phosphoenolpyruvate carboxykinase promoter, resulting in constitutively high serum levels of sgp130Fc protein and selective blockade of IL-6 trans-signaling (24). Deficiency in IL-6 caused enhanced listeria titers in spleen and liver (Fig. 9A). In contrast, Tg expression of sgp130Fc did not impair the control of *L. monocytogenes* infection. Listeria titers were even slightly lower than titers of infected wt mice. Similar to mice treated with sgp130Fc, sgp130Fc^{Tg} mice demonstrated reduced accumulation of granuloytes in spleens of infected mice (Fig. 9B). However, sgp130Fc^{Tg} mice in addition displayed diminished frequencies of inflammatory monocytes in spleen and liver.

Discussion

Following *L. monocytogenes* infection, we measured high serum levels of IL-6 at days 2 and 3 postinfection, which is in agreement with published results (39–41). Inflammatory monocytes are essential for the protective innate immune response against *L. monocytogenes* (33, 48), and we could demonstrate that these cells produced IL-6 as well as TNF-α following stimulation with HKL. However, despite their fundamental role in *L. monocytogenes* control and their IL-6 response to listeria Ag, inflammatory monocytes were not an essential source for systemic IL-6. Infection of CCR2^{KO} mice, which failed to mobilize inflammatory monocytes from the bone marrow (48), resulted in even enhanced IL-6 serum concentration when compared with infected control mice. Alternative IL-6 sources could include other myeloid cells such as tissue-resident macrophages, dendritic cells, and granulocytes or T and B cells, but also nonhematopoietic cells such as endothelial or epithelial cells (49). Owing to the more severe infection, some of these cells might respond even stronger in CCR2^{KO} mice.

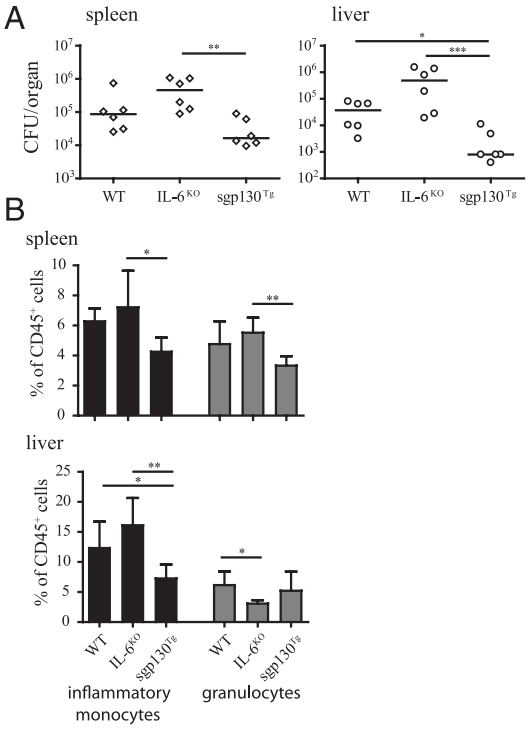

FIGURE 9. Role of classical IL-6 signaling and IL-6 trans-signaling in the immune response against *L. monocytogenes*. C57BL/6 wt, IL-6^{KO}, and sgp130Fc^{Tg} mice were i.v. infected with 2×10^4 *L. monocytogenes*. After a further 3 d, mice were analyzed. (**A**) Listeria burden in spleen and liver. (**B**) Accumulation of inflammatory monocytes and neutrophils in spleen and liver. Values of individual mice and the median are shown in (A). Bars in (B) give the means ± SD of six individually analyzed mice per group. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

In naive mice, we detected mIL-6Rα expression on T cells and inflammatory monocytes. In particular, CD4$^+$ T cells expressed high levels of mIL-6Rα. Granulocytes expressed only low mIL-6Rα levels and B cells were largely negative. Naive T cells (CD62L^{high}CD44^{low}) were uniformly mIL-6Rα$^+$, and effector as well as memory T cells (CD62L^{low}CD44^{high} or CD62L^{high} CD44^{high}) contained mIL-6Rα$^+$ and mIL-6Rα$^-$ subpopulations. Following *L. monocytogenes* infection, all analyzed leukocyte subsets lost mIL-6Rα expression. On T cells, reduction of mIL-6Rα was observed on naive as well as preactivated subpopulations. A similar reduction of mIL-6Rα expression was also observed in infected IL-6^{KO} mice, which excludes IL-6–induced receptor internalization or interference of IL-6 with anti-IL6Rα mAb binding to IL-6Rα as a cause for low IL-6Rα surface staining (50). Shedding of mIL-6Rα by ADAM10 and ADAM17 has been identified as a general mechanism of sIL-6Rα generation in mice (51), and it is very likely that shedding is responsible for the loss of IL-6Rα surface expression following *L. monocytogenes* infection. TCR triggering has been described as a signal for IL-6Rα shedding (52, 53); however, the global loss of IL-6Rα on naive and effector cells argues against a specific TCR signal at this early stage of infection. It is more likely that loss of IL-6Rα expression is due to shedding induced by inflammatory mediators such as proinflammatory cytokines, chemokines, or acute phase proteins (52, 53). Our present work aims at defining these mediators in our infection model.

Despite the profound loss of mIL-6Rα on all analyzed leukocyte subsets, we observed only a marginal increase in serum concentration of sIL-6Rα following *L. monocytogenes* infection. This result would argue that leukocytes are not a major source of sIL-6Rα or that concentrations of sIL-6Rα are tightly controlled and excessive sIL-6Rα is rapidly eliminated from the circulation. Interestingly, naive and infected CCR2^{KO} mice showed a reduction of sIL-6Rα concentrations. Thus, inflammatory monocytes or other CCR2-dependent cells represent a source for sIL-6Rα already under homeostatic conditions. Recently, it was demonstrated that IL-6Rα deficiency in hepatocytes (AlbCre$^+$*Il6rα*$^{fl/fl}$ mice) results in a 30% reduction of sIL-6Rα serum levels, and deficiency in granulocytes and macrophages (LysMCre$^+$*Il6rα*$^{fl/fl}$ mice) results in a 60% reduction of these levels (54). Because lysozyme M is most likely also expressed in inflammatory monocytes, our results are in accordance with this conclusion and add inflammatory monocytes to the list of cells involved in constitutive sIL-6Rα production.

Differential blockade of global IL-6 signaling by neutralizing mAb and IL-6 trans-signaling by sgp130Fc indicated that classical signaling is sufficient for early control of *L. monocytogenes* infection. Infection of IL-6^{KO} and sgp130Fc^{Tg} mice confirmed this observation. Compared to wt mice or mice treated with sgp130Fc proteins, sgp130Fc^{Tg} mice demonstrated even better control of *L. monocytogenes* infection. It is currently unclear why sgp130^{Tg} mice show this improved control. The constant Tg expression of sgp130Fc and the constant inhibition of IL-6 trans-signaling could

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cause alterations in development and cellular composition of the immune system, which might be responsible for the enhanced control of infection. In mice with a global blockade of IL-6, the increase in listeria titers was more pronounced in the liver, and thus IL-6 could be either particularly important for the innate response in the liver (e.g., through the hepatic induction of acute phase proteins) or there might be compensatory mechanisms in the spleen. Listeria infection induced a strong upregulation of the mRNA for several proinflammatory cytokines and chemokines. We observed a slight increase in chemokine mRNA expression following anti–IL-6 mAb treatment; however, it is unclear whether this was due to defective IL-6 signaling or due to a higher bacterial load. In contrast, treatment of mice with sgp130Fc did not change the mRNA expression levels of any of the analyzed factors. Infection with *L. monocytogenes* induced accumulation of neutrophil granulocytes in spleen and liver. Interestingly, sgp130Fc treatment caused a reduction of neutrophil accumulation in the spleen. Recent studies from Shi et al. (46) and Carr et al. (47) analyzed the role of neutrophils for the control of *L. monocytogenes*. In those studies, depletion of neutrophils had either no consequences (46) or only affected control of infection when relatively high doses of bacteria where applied (47). Under the latter conditions, depletion of neutrophils mainly impaired control of infection in the liver (47). Thus, it is not surprising that reduction in neutrophil numbers in the spleen was not accompanied by a more severe infection.

It has been demonstrated that IL-6 trans-signaling is responsible for a switch from a granulocyte to a monocyte/macrophage response (55). We observed reduction of monocyte frequencies in sgp130Fc^{Tg} mice, but there was no change in monocyte frequencies in sgp130Fc- or anti-IL-6–treated animals. Thus, blockade of classical IL-6 signaling or IL-6 trans-signaling does not generally result in a reduced recruitment of monocytes to sites of *L. monocytogenes* infection. The failure to observe profound changes in the monocyte responses is most likely due to the infection model. Control of *L. monocytogenes* infection is highly dependent on recruitment of monocytes to the site of infection. Chemokines such as CCR2 ligands have been shown to release monocytes from the bone marrow (48). *L. monocytogenes* or material from these bacteria can induce production of CCR2 ligands in infected sites but also within the bone marrow, and this increase in local chemokines might be sufficient for releasing monocytes into the circulation (56). Alternatively, CCR2 ligands and chemokines in general appear to be dispensable for the accumulation of monocytes in the infected spleen and liver (48, 57). Thus, monocyte recruitment following *L. monocytogenes* infection could be largely independent of IL-6 and IL-6–mediated chemokine production at the sites of infection.

In summary, our results indicate that the protective function of IL-6 occurs in the absence of IL-6 trans-signaling, which also implies that protection is mainly due to mIL-6Rα^+ cells. The identity of these cells is currently unclear. Although recruitment of inflammatory monocytes is not impaired by IL-6 deficiency, these cells might be less functional without IL-6 stimulation. IL-6 could also operate in the activation and differentiation of NK cells, of unconventional "innate" T cells, or of conventional T cells. Finally, IL-6 might induce protective mechanisms in hepatocytes, for example, via the induction of the acute phase proteins. The response of these different cell types is currently under investigation by our group. Our results also suggest that IL-6 trans-signaling is not essential for protection against certain bacterial infections. Owing to this residual antibacterial immune response, blockade of IL-6 trans-signaling could therefore be of advantage to a global blockade of IL-6 for the treatment of chronic inflammatory diseases.

Acknowledgments

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Disclosures

Dr. Rose-John is an inventor on patents describing the function of sgp130Fc. He is also a shareholder of the CONARIS Research Institute (Kiel, Germany), which is commercially developing sgp130Fc proteins as therapeutics for inflammatory diseases. The other authors have no financial conflicts of interest.

References

1. Scheller, J., A. Chalaris, D. Schmidt-Arras, and S. Rose-John. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim. Biophys. Acta* 1813: 878–888.
2. Chalaris, A., C. Garbers, B. Rabe, S. Rose-John, and J. Scheller. 2011. The soluble interleukin 6 receptor: generation and role in inflammation and cancer. *Eur. J. Cell Biol.* 90: 484–494.
3. Kishimoto, T., S. Akira, M. Narazaki, and T. Taga. 1995. Interleukin-6 family of cytokines and gp130. *Blood* 86: 1243–1254.
4. Bettelli, E., Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, and V. K. Kuchroo. 2006. Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. *Nature* 441: 235–238.
5. Weaver, C. T., R. D. Hatton, P. R. Mangan, and L. E. Harrington. 2007. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.* 25: 821–852.
6. Kopf, M., H. Baumann, G. Freer, M. Freudenberg, M. Lamers, T. Kishimoto, R. Zinkernagel, H. Bluethmann, and G. Köhler. 1994. Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* 368: 339–342.
7. Dube, P. H., S. A. Handley, J. Lewis, and V. L. Miller. 2004. Protective role of interleukin-6 during *Yersinia enterocolitica* infection is mediated through the modulation of inflammatory cytokines. *Infect. Immun.* 72: 3561–3570.
8. Dann, S. M., M. E. Spehlmann, D. C. Hammond, M. Iimura, K. Hase, L. J. Choi, E. Hanson, and L. Eckmann. 2008. IL-6-dependent mucosal protection prevents establishment of a microbial niche for attaching/effacing lesion-forming enteric bacterial pathogens. *J. Immunol.* 180: 6816–6826.
9. Bienz, M., W. J. Dai, M. Welle, B. Gottstein, and N. Müller. 2003. Interleukin-6-deficient mice are highly susceptible to *Giardia lamblia* infection but exhibit normal intestinal immunoglobulin A responses against the parasite. *Infect. Immun.* 71: 1569–1573.
10. LeBlanc, R. A., L. Pesnicak, E. S. Cabral, M. Godleski, and S. E. Straus. 1999. Lack of interleukin-6 (IL-6) enhances susceptibility to infection but does not alter latency or reactivation of herpes simplex virus type 1 in IL-6 knockout mice. *J. Virol.* 73: 8145–8151.
11. Williams, D. M., B. G. Grubbs, T. Darville, K. Kelly, and R. G. Rank. 1998. A role for interleukin-6 in host defense against murine *Chlamydia trachomatis* infection. *Infect. Immun.* 66: 4564–4567.
12. Ladel, C. H., C. Blum, A. Dreher, K. Reifenberg, M. Kopf, and S. H. Kaufmann. 1997. Lethal tuberculosis in interleukin-6-deficient mutant mice. *Infect. Immun.* 65: 4843–4849.
13. Suzuki, Y., S. Rani, O. Liesenfeld, T. Kojima, S. Lim, T. A. Nguyen, S. A. Dalrymple, R. Murray, and J. S. Remington. 1997. Impaired resistance to the development of toxoplasmic encephalitis in interleukin-6-deficient mice. *Infect. Immun.* 65: 2339–2345.
14. Dalrymple, S. A., R. Slattery, D. M. Aud, M. Krishna, L. A. Lucian, and R. Murray. 1996. Interleukin-6 is required for a protective immune response to systemic *Escherichia coli* infection. *Infect. Immun.* 64: 3231–3235.
15. Romani, L., A. Mencacci, E. Cenci, R. Spaccapelo, C. Toniatti, P. Puccetti, F. Bistoni, and V. Poli. 1996. Impaired neutrophil response and $CD4^+$ T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*. *J. Exp. Med.* 183: 1345–1355.
16. Nowell, M. A., P. J. Richards, S. Horiuchi, N. Yamamoto, S. Rose-John, N. Topley, A. S. Williams, and S. A. Jones. 2003. Soluble IL-6 receptor governs IL-6 activity in experimental arthritis: blockade of arthritis severity by soluble glycoprotein 130. *J. Immunol.* 171: 3202–3209.
17. Ohshima, S., Y. Saeki, T. Mima, M. Sasai, K. Nishioka, S. Nomura, M. Kopf, Y. Katada, T. Tanaka, M. Suemura, and T. Kishimoto. 1998. Interleukin 6 plays a key role in the development of antigen-induced arthritis. *Proc. Natl. Acad. Sci. USA* 95: 8222–8226.
18. Okuda, Y., S. Sakoda, C. C. Bernard, H. Fujimura, Y. Saeki, T. Kishimoto, and T. Yanagihara. 1998. IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein. *Int. Immunol.* 10: 703–708.
19. Nishimoto, N., and T. Kishimoto. 2006. Interleukin 6: from bench to bedside. *Nat. Clin. Pract. Rheumatol.* 2: 619–626.
20. Rose-John, S., J. Scheller, G. Elson, and S. A. Jones. 2006. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J. Leukoc. Biol.* 80: 227–236.
21. Honda, M., S. Yamamoto, M. Cheng, K. Yasukawa, H. Suzuki, T. Saito, Y. Osugi, T. Tokunaga, and T. Kishimoto. 1992. Human soluble IL-6 receptor: its detection and enhanced release by HIV infection. *J. Immunol.* 148: 2175–2180.

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22. Rose-John, S., G. H. Waetzig, J. Scheller, J. Grötzinger, and D. Seegert. 2007. The IL-6/sIL-6R complex as a novel target for therapeutic approaches. *Expert Opin. Ther. Targets* 11: 613–624.
23. Rose-John, S., and P. C. Heinrich. 1994. Soluble receptors for cytokines and growth factors: generation and biological function. *Biochem. J.* 300: 281–290.
24. Rabe, B., A. Chalaris, U. May, G. H. Waetzig, D. Seegert, A. S. Williams, S. A. Jones, S. Rose-John, and J. Scheller. 2008. Transgenic blockade of interleukin 6 transsignaling abrogates inflammation. *Blood* 111: 1021–1028.
25. Jostock, T., J. Müllberg, S. Ozbek, R. Atreya, G. Blinn, N. Voltz, M. Fischer, M. F. Neurath, and S. Rose-John. 2001. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur. J. Biochem.* 268: 160–167.
26. Barkhausen, T., T. Tschernig, P. Rosenstiel, M. van Griensven, R. P. Vonberg, M. Dorsch, A. Mueller-Heine, A. Chalaris, J. Scheller, S. Rose-John, et al. 2011. Selective blockade of interleukin-6 trans-signaling improves survival in a murine polymicrobial sepsis model. *Crit. Care Med.* 39: 1407–1413.
27. Sodenkamp, J., G. H. Waetzig, J. Scheller, D. Seegert, J. Grötzinger, S. Rose-John, S. Ehlers, and C. Hölscher. 2012. Therapeutic targeting of interleukin-6 trans-signaling does not affect the outcome of experimental tuberculosis. *Immunobiology* 217: 996–1004.
28. Nowell, M. A., A. S. Williams, S. A. Carty, J. Scheller, A. J. Hayes, G. W. Jones, P. J. Richards, S. Slinn, M. Ernst, B. J. Jenkins, et al. 2009. Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. *J. Immunol.* 182: 613–622.
29. Atreya, R., J. Mudter, S. Finotto, J. Müllberg, T. Jostock, S. Wirtz, M. Schütz, B. Bartsch, M. Holtmann, C. Becker, et al. 2000. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn disease and experimental colitis in vivo. *Nat. Med.* 6: 583–588.
30. Melton, L., and A. Coombs. 2008. Actemra poised to launch IL-6 inhibitors. *Nat. Biotechnol.* 26: 957–959.
31. Gellin, B. G., and C. V. Broome. 1989. Listeriosis. *JAMA* 261: 1313–1320.
32. Pamer, E. G. 2004. Immune responses to *Listeria monocytogenes*. *Nat. Rev. Immunol.* 4: 812–823.
33. Shi, C., and E. G. Pamer. 2011. Monocyte recruitment during infection and inflammation. *Nat. Rev. Immunol.* 11: 762–774.
34. Dalrymple, S. A., L. A. Lucian, R. Slattery, T. McNeil, D. M. Aud, S. Fuchino, F. Lee, and R. Murray. 1995. Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia. *Infect. Immun.* 63: 2262–2268.
35. Betz, U. A., W. Bloch, M. van den Broek, K. Yoshida, T. Taga, T. Kishimoto, K. Addicks, K. Rajewsky, and W. Müller. 1998. Postnatally induced inactivation of gp130 in mice results in neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects. *J. Exp. Med.* 188: 1955–1965.
36. Liu, Z., R. J. Simpson, and C. Cheers. 1995. Interaction of interleukin-6, tumour necrosis factor and interleukin-1 during *Listeria* infection. *Immunology* 85: 562–567.
37. Liu, Z., R. J. Simpson, and C. Cheers. 1994. Role of IL-6 in activation of T cells for acquired cellular resistance to *Listeria monocytogenes*. *J. Immunol.* 152: 5375–5380.
38. Miura, T., S. Nishikawa, S. Sasaki, K. Yamada, S. Hasegawa, D. Mizuki, M. Mizuki, I. Hatayama, K. Sekikawa, Y. Tagawa, et al. 2000. Roles of endogenous cytokines in liver apoptosis of mice in lethal *Listeria monocytogenes* infection. *FEMS Immunol. Med. Microbiol.* 28: 335–341.
39. Nakane, A., A. Numata, and T. Minagawa. 1992. Endogenous tumor necrosis factor, interleukin-6, and γ interferon levels during *Listeria monocytogenes* infection in mice. *Infect. Immun.* 60: 523–528.
40. Abram, M., D. Vu ković, B. Wraber, and M. Dorić. 2000. Plasma cytokine response in mice with bacterial infection. *Mediators Inflamm.* 9: 229–234.
41. Ehlers, S., M. E. Mielke, T. Blankenstein, and H. Hahn. 1992. Kinetic analysis of cytokine gene expression in the livers of naive and immune mice infected with *Listeria monocytogenes*: the immediate early phase in innate resistance and acquired immunity. *J. Immunol.* 149: 3016–3022.
42. Tenhumberg, S., G. H. Waetzig, A. Chalaris, B. Rabe, D. Seegert, J. Scheller, S. Rose-John, and J. Grötzinger. 2008. Structure-guided optimization of the interleukin-6 trans-signaling antagonist sgp130. *J. Biol. Chem.* 283: 27200–27207.
43. Kuziel, W. A., S. J. Morgan, T. C. Dawson, S. Griffin, O. Smithies, K. Ley, and N. Maeda. 1997. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc. Natl. Acad. Sci. USA* 94: 12053–12058.
44. Kim, K. J., J. Abrams, M. Alphonso, M. Pearce, G. J. Thorbecke, and M. A. Palladino. 1990. Role of endogenously produced interleukin-6 as a second signal in murine thymocyte proliferation induced by multiple cytokines: regulatory effects of transforming growth factor-beta. *Cell. Immunol,* 131: 261–271.
45. Panzer, U., O. M. Steinmetz, H. J. Paust, C. Meyer-Schwesinger, A. Peters, J.-E. Turner, G. Zahner, F. Heymann, C. Kurts, H. Hopfer, et al. 2007. Chemokine receptor CXCR3 mediates T cell recruitment and tissue injury in nephrotoxic nephritis in mice. *J. Am. Soc. Nephrol.* 18: 2071–2084.
46. Shi, C., T. M. Hohl, I. Leiner, M. J. Equinda, X. Fan, and E. G. Pamer. 2011. Ly6G$^+$ neutrophils are dispensable for defense against systemic *Listeria monocytogenes* infection. *J. Immunol.* 187: 5293–5298.
47. Carr, K. D., A. N. Sieve, M. Indramohan, T. J. Break, S. Lee, and R. E. Berg. 2011. Specific depletion reveals a novel role for neutrophil-mediated protection in the liver during *Listeria monocytogenes* infection. *Eur. J. Immunol.* 41: 2666–2676.
48. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat. Immunol.* 7: 311–317.
49. Klouche, M., S. Bhakdi, M. Hemmes, and S. Rose-John. 1999. Novel path to activation of vascular smooth muscle cells: up-regulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. *J. Immunol.* 163: 4583–4589.
50. Hotson, A. N., J. W. Hardy, M. B. Hale, C. H. Contag, and G. P. Nolan. 2009. The T cell STAT signaling network is reprogrammed within hours of bacteremia via secondary signals. *J. Immunol.* 182: 7558–7568.
51. Garbers, C., W. Thaiss, G. W. Jones, G. H. Waetzig, I. Lorenzen, F. Guilhot, R. Lissilaa, W. G. Ferlin, J. Grötzinger, S. A. Jones, et al. 2011. Inhibition of classic signaling is a novel function of soluble glycoprotein 130 (sgp130), which is controlled by the ratio of interleukin 6 and soluble interleukin 6 receptor. *J. Biol. Chem.* 286: 42959–42970.
52. Jones, G. W., R. M. McLoughlin, V. J. Hammond, C. R. Parker, J. D. Williams, R. Malhotra, J. Scheller, A. S. Williams, S. Rose-John, N. Topley, and S. A. Jones. 2010. Loss of CD4$^+$ T cell IL-6R expression during inflammation underlines a role for IL-6 trans signaling in the local maintenance of Th17 cells. *J. Immunol.* 184: 2130–2139.
53. Briso, E. M., O. Dienz, and M. Rincon. 2008. Cutting edge: soluble IL-6R is produced by IL-6R ectodomain shedding in activated CD4 T cells. *J. Immunol.* 180: 7102–7106.
54. McFarland-Mancini, M. M., H. M. Funk, A. M. Paluch, M. Zhou, P. V. Giridhar, C. A. Mercer, S. C. Kozma, and A. F. Drew. 2010. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. *J. Immunol.* 184: 7219–7228.
55. Chalaris, A., B. Rabe, K. Paliga, H. Lange, T. Laskay, C. A. Fielding, S. A. Jones, S. Rose-John, and J. Scheller. 2007. Apoptosis is a natural stimulus of IL6R shedding and contributes to the proinflammatory trans-signaling function of neutrophils. *Blood* 110: 1748–1755.
56. Shi, C., T. Jia, S. Mendez-Ferrer, T. M. Hohl, N. V. Serbina, L. Lipuma, I. Leiner, M. O. Li, P. S. Frenette, and E. G. Pamer. 2011. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating Toll-like receptor ligands. *Immunity* 34: 590–601.
57. Shi, C., P. Velázquez, T. M. Hohl, I. Leiner, M. L. Dustin, and E. G. Pamer. 2010. Monocyte trafficking to hepatic sites of bacterial infection is chemokine independent and directed by focal intercellular adhesion molecule-1 expression. *J. Immunol.* 184: 6266–6274.

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