Differential chemokine response of murine macrophages stimulated with cytokines and infected with \textit{Listeria monocytogenes}

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\section*{Abstract}

During inflammatory processes the infected macrophage is a rich source of chemokines which induce infiltration of leukocytes to the site of infection. We investigated the regulation of chemokine production by murine macrophages in response to infection with the intracellular bacterial pathogen, \textit{Listeria monocytogenes}. As a source of quiescent macrophages, murine bone marrow-derived macrophages (BMM) cultured under serum-free conditions were used. With RT-PCR, we detected induction of RNA message for the chemokines macrophage inflammatory protein (MIP)-2, KC, MIP-1\textsubscript{α}, MIP-1\textsubscript{β}, IFN-γ-inducible protein-10 and RANTES in \textit{L. monocytogenes}-infected macrophages. Accordingly, ELISA-detectable MIP-1\textsubscript{α}, MIP-2 and KC protein was induced by infection with \textit{L. monocytogenes}. In contrast, \textit{L. monocytogenes} infection of BMM alone failed to induce considerable expression of monocyte chemoattractant protein (MCP)-1 at the mRNA or protein level, but co-treatment with IFN-γ was necessary. Release of infection-triggered MIP-2, MIP-1\textsubscript{α} and KC was negatively regulated by IFN-γ. Similarly, IL-4 stimulated MCP-1 release by infected macrophages but reduced production of MIP-1\textsubscript{α}, MIP-2 and KC. IL-10 turned out to be a general deactivator in terms of macrophage chemokine production. IL-13 had no effect on MIP-1\textsubscript{α}, MIP-2 and KC production by infected BMM, but slightly reduced MCP-1 release. By using IFN-γ and IL-4 gene deletion mutant mice, in vivo regulation of these chemokines by IL-4 and IFN-γ in listeriosis was studied. In summary, our results show that chemokines are produced by macrophages infected with \textit{L. monocytogenes}, and that chemokine release is differentially regulated by the macrophage modulators IFN-γ, IL-4, IL-10 and IL-13.

\section*{Introduction}

Chemokines are small (6–14 kDa), inducible, proinflammatory mediators which selectively attract and activate distinct types of leukocytes (1,2). They have been divided into different families depending on the arrangement of the four conserved cysteine residues that form disulfide bonds. In CXC or \textalpha-chemokines, the first two cysteines are separated by a single amino acid, whereas in CC or \textbeta-chemokines the cysteines are adjacent (3,4). Most of the CXC chemokines recruit neutrophilic and basophilic granulocytes, while the CC chemokines mainly act on monocytes and lymphocytes. Lymphotactin and single cysteine motif-1 are the only members of the third family, the C or \textgamma-chemokines, with only one N-terminal cysteine residue (3–5).

Chemokines are produced by several cell types and are considered important mediators of inflammation. They are induced during viral, protozoal and bacterial infections (3,4), and a number of studies in human and murine systems have shown that chemokine expression is tightly controlled by cytokines: IL-10 elicits monocyte chemoattractant protein (MCP)-1 release by unstimulated human blood mononuclear cells (6,7). IL-4 increases expression of MCP-1 mRNA and protein in human endothelial cells (7,8). Tumor necrosis factor (TNF)-\textalpha, IL-1 and IFN-γ stimulate release of MCP-1, MCP-4 and eotaxin in human endothelial cells (9,10). TNF-\textalpha and IL-1\textbeta also stimulate accumulation of eotaxin mRNA and protein in bronchial epithelial cell lines (11). In addition, human NK cells are potent producers of macrophage inflammatory protein (MIP)-1\textalpha after treatment with IL-12, TNF-\textalpha, IL-1β or IL-10, and after co-stimulation with IL-12 and IL-15 (12). In murine bone marrow-derived macrophages (BMM) and
resident peritoneal macrophages, IL-4, IL-3 and granulocyte macrophage colony stimulating factor induce the CC chemokine C10 (13). In a recent paper, Romano et al. (14) elegantly demonstrated the crucial role of IL-6 in leukocyte recruitment to sites of inflammation by using IL-6 gene disruption mutant mice. Treatment of these mice with IL-6 restored production of the CC chemokine MCP-1 and reversed impaired leukocyte recruitment.

The macrophage is a major source of chemokines during acute infections (3,4). The activity of macrophages is mainly controlled by IFN-γ, the classical macrophage-activating factor, and by IL-4, IL-10 and IL-13, which have been described as cytokines with macrophage-deactivating capacity (15–18). In murine listeriosis, an important model system for investigating cytokine responses to infectious agents, IFN-γ plays a central role in protection (19,20). Accordingly, mutant mice which lack the receptor for the macrophage activator IFN-γ suffer from increased susceptibility to listeriosis (21,22).

On the other hand, administration of anti-IL-4 mAb enhanced resistance of mice to Listeria monocytogenes (23). IL-10 gene disruption mutant mice were less susceptible to infection with L. monocytogenes than their wild-type littermates (24), so that endogenous IL-4 and IL-10 both turned out to antagonize an efficient antilisterial host defence. However, treatment of mice with IL-13, a cytokine with strong similarities to IL-4 and with putative macrophage-deactivating properties, yet increased resistance to L. monocytogenes (25).

The assumed importance of chemokines in infections and the obvious discrepancies in the in vivo actions of the different macrophage deactivators IL-4, IL-10 and IL-13 on listeriosis prompted us to investigate the chemokine response of infected macrophages. Using RT-PCR and ELISA we observed that the rapid chemokine response of BMM to infection with L. monocytogenes is differentially regulated by IFN-γ, IL-4, IL-10 and IL-13. To correlate these in vitro findings with the in vivo setting, C57BL/6, IFN-γ and IL-4 gene deletion mutant mice were infected with L. monocytogenes and chemokine responses were analyzed.

Methods

Mice and microorganisms

C57BL/6 mice were raised in our own breeding colonies under specific pathogen-free conditions. IFN-γ gene deletion mutant mice (IFN-γ–/–) on C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, ME). IL-4 gene deletion mutant mice (IL-4–/–) on C57BL/6 background were kindly provided by Drs F. Brombacher and M. Kopf, (MPI for Immunobiology, Freiburg, Germany). L. monocytogenes EGD were routinely passaged in C57BL/6 mice. Bacteria were grown in Trypticase-soy broth (Life Technologies, Paisley, UK), and aliquots were frozen and stored at –70°C. Numbers of viable bacteria were determined by plating 1:10 dilutions on Trypticase-soy agar plates (Life Technologies).

Cytokines

Human CHO cell-derived rIL-13, which is also active on mouse cells, was a kind gift from Drs A. Minty and P. Ferrara (Sanofi Recherche, Labege, France). The sp. act. was 1×10⁷ U/mg protein. Murine Escherichia coli-derived rIFN-γ, sp. act. 1.5×10⁹ U/mg protein, was kindly provided by Dr G. Adolf (Ernst Bohringer Institut fur Arzneimittelforshung, Vienna, Austria). Murine E. coli-derived rIL-4, sp. act. 1×10⁷ U/mg protein, E. coli-derived rIL-10, sp. act. 5×10⁷ U/mg protein and rTNF-α, sp. act. 5×10⁷ U/mg protein, were obtained from Genzyme (Cambridge, MA). Murine MCP-1 from Baculovirus-infected Sf9 cells, sp. act. 1×10⁷ U/mg protein, was purchased from Pharmingen (Hamburg, Germany). Mouse E. coli-derived rMIP-1α, sp. act. 1.6–2.5×10⁶ U/mg protein, E. coli-derived rMIP-2, sp. act. 3–6×10⁵ U/mg protein, and E. coli-derived KC, sp. act. 1–3×10⁵ U/mg protein, were obtained from R & D Systems (Wiesbaden, Germany). Recombinant cytokines were routinely tested for endotoxin content by the Limulus amoebocyte assay (Sigma, München, Germany). They were found to contain <6 pg/ml endotoxin.

Infection of mice and preparation of splenocytes

Mice were infected i.v. with 1×10⁶ L. monocytogenes organisms in 0.2 ml of PBS via the lateral tail vein. At the time points post infection (p.i.) indicated in Results, splenocytes were prepared by teasing spleens through stainless steel meshes. After lysis of erythrocytes with ammonium chloride buffer, spleen cells were resuspended in Clicks/RPMI medium (Seromed, Berlin, Germany) supplemented with 10% FCS, 2 mM glutamine (Gibco, Paisley, UK), 100 U/ml penicillin-streptomycin (Gibco) and 5×10⁻⁵ M 2-mercaptoethanol (Gibco). Splenocytes were seeded into round-bottom microdilution plates (Nunc, Wiesbaden, Germany) with 1×10⁵ cells/well and re-stimulated with heat-killed listeriae (HKL), 1×10⁴/μl. Supernatants were harvested after 24 h and the chemokine content was measured by ELISA.

Culture and stimulation of macrophages

BMM were obtained in a serum-free culture medium as described previously (26). BMM were harvested on day 9 and seeded in Iscove’s modified Dulbecco’s medium (Seromed, Berlin, Germany) supplemented with 10% FCS, 2 mM glutamine (Gibco, Paisley, UK), 100 U/ml penicillin-streptomycin (Gibco) and 5×10⁻⁵ M 2-mercaptoethanol (Gibco). BMM were infected with 5×10⁴ wells viable L. monocytogenes and cytokines were added simultaneously with bacteria. Gentamycin, 10 μg/ml, was added 1 h p.i. to kill extracellular bacteria. Supernatants were harvested 24 h p.i. For RT-PCR, BMM were seeded into 24-well plates (Nunc) with 5×10⁵ cells/well. To stimulate chemokine release, BMM were infected with 5×10⁵/well viable L. monocytogenes and cytokines were added simultaneously with bacteria. Gentamycin, 10 μg/ml, was added 1 h p.i. to kill extracellular bacteria. Supernatants were harvested 24 h p.i. For RT-PCR, BMM were seeded into 24-well plates (Nunc) with 5×10⁵ cells/well. BMM were infected with 5×10⁵/well L. monocytogenes in the presence or absence of cytokines. Gentamycin was added 1 h p.i. and cells were harvested 5 h p.i. Non-infected control BMM cultures were treated with cytokines and gentamycin.

RT-PCR for detection of chemokine mRNA in BMM

Total cellular RNA was obtained from BMM by the acid guanidinium thiocyanate–phenol–chloroform extraction method (27). cDNA strands were generated using Moloney murine leukemia virus reverse transcriptase purchased from Gibco. PCR primer sequences for the chemokines and GAPDH were taken from (28). Primers were synthesized by Interactiva (Ulm, Germany). RNA from 5×10⁵ BMM was reverse transcribed and 1/10 (= 3 μl) of the resulting volume after transcription was used for one PCR reaction. PCR reagents, including Taq polymerase (Gibco) and primers
specific for each mouse chemokine transcript of interest, were added to each set of tubes. Mouse GAPDH served as an internal control to confirm that each sample had an equal amount of RNA. The total reaction volume was 50 µl. The cDNA was amplified for 26 (GAPDH) or 35 (all chemokines) thermocycles of 30 s at 95°C, 30 s at 65°C and 2 min at 72°C in a PTC-100 thermocycler (MJ Research, Watertown, MA). Afterwards, 20 µl of each PCR tube were fractionated on a 1.5% agarose gel, containing ethidium bromide. On an UV transilluminator, the densities of bands of the correct predicted length were assessed using the optical system and EASY plus software from Herolab (Wiesloch, Germany).

Detection of chemokines in culture supernatants
MCP-1, MIP-1α, MIP-2 and KC were determined by ELISA. To detect MCP-1, hamster anti-mouse MCP-1 mAb 2H5 (PharMingen) (5 µg/ml) was used for coating and biotinylated hamster anti-murine MCP-1 mAb 4E2 (PharMingen) (2 µg/ml) for detection. The detection limit was 300 pg/ml. For determination of MIP-1α, goat anti-murine MIP-1α IgG (R & D Systems) (4 µg/ml) was employed for coating and biotinylated goat anti-murine MIP-1α IgG (R & D Systems) (1 µg/ml) for detection. The detection limit was 150 pg/ml. For MIP-2, goat anti-murine MIP-2 IgG (R & D Systems) (0.5 µg/ml) was used for coating and biotinylated goat anti-mouse MIP-2 IgG (R & D Systems) (0.25 µg/ml) was employed for detection. The detection limit was 10 pg/ml. For determination of KC, goat anti-murine KC IgG (R & D Systems) (1 µg/ml) was employed for coating and biotinylated goat anti-mouse KC IgG (R & D Systems) (0.5 µg/ml) for detection. The detection limit was 100 pg/ml.

TNF-α determination
For quantitation of TNF-α in culture supernatants a cytotoxicity assay using TNF-sensitive L929 cells was employed (29). The detection limit was 10 pg/ml.

Statistics
Data are given as means ± SD and differences were tested using the two-sided Student’s t-test. Means were considered significantly different from each other with \( P \leq 0.05 \).

Results

Induction of chemokine mRNA in BMM after infection with L. monocytogenes
The mRNAs of the neutrophil-attracting CXC chemokine KC and the CC chemokines MIP-1β and RANTES were constitutively expressed at low levels in non-infected control BMM under serum-free conditions, as measured by RT-PCR. In contrast, messages for MIP-1α, MIP-2, IFN-γ-inducible protein (IP)-10 and MCP-1 were not detectable. In BMM infected with L. monocytogenes for 5 h, mRNAs for the CXC chemokines MIP-2, KC and IP-10, and for the CC chemokines MIP-1α and MIP-1β were up-regulated. Expression of MCP-1 and RANTES mRNA was not influenced by infection alone (Fig. 1). In these experiments, equal total numbers of naive and infected cells were subjected to mRNA extraction.

Time course of chemokine release from BMM infected with L. monocytogenes
Frequently, mRNA levels do not accurately provide information about the quantity of the respective protein. We, therefore, used ELISA for determining MCP-1, MIP-1α, MIP-2 and KC concentrations in BMM cultures in the following experiments. Kinetic analysis of chemokine production revealed that—in good correlation with the RT-PCR data—considerable amounts of MIP-1α, MIP-2 and KC were released by BMM within 3–12 h after infection, whereas only trace amounts of MCP-1 were produced by listeria-infected cells (Fig. 2). Thus, certain chemokines are released by BMM shortly after infection with the intracellular pathogen L. monocytogenes.

Differential modulation by IFN-γ, IL-4, IL-10 and IL-13 of chemokine release by BMM
Next we examined whether and how the macrophage-activating factor IFN-γ, and the putative macrophage-deactivating
cytokines IL-4, IL-10 and IL-13 influenced the release of chemokines by BMM. None of these cytokines directly induced chemokine release by non-infected macrophages (data not shown). Rather, infection with *L. monocytogenes* was necessary. As mentioned above, MCP-1 was the only chemokine tested which was not induced at appreciable levels by infection of BMM with *L. monocytogenes* alone. However, high quantities of MCP-1 were released by infected BMM after treatment with IFN-γ (Fig. 3A). MIP-1α production was down-regulated by higher concentrations of IFN-γ (Fig. 3B). MIP-2 as well as KC release was reduced by IFN-γ (Fig. 3C, D). Another cytokine which induced MCP-1 secretion by infected BMM was IL-4 (Fig. 4A), but it diminished MIP-1α, MIP-2 and KC release (Fig. 4B, C and D). IL-10 acted as a general and potent down-modulator of infection-induced chemokine release, only MCP-1 was not significantly influenced by this cytokine. IL-13 exerted no effect on chemokine production, except the slight reduction in the low levels of listeria-induced MCP-1. In summary, these data show that chemokine production by BMM is differentially regulated by IFN-γ, IL-4, IL-10 and IL-13.

**Differential modulation by IFN-γ, IL-4, IL-10 and IL-13 of TNF-α release by BMM**

To date, regulation of the chemokine response of macrophages by cytokines has not been examined in detail. For comparison, we also measured TNF-α in our cultures, a macrophage product, the modulation of which by other cytokines has extensively been studied. We found that IFN-γ (500 U/ml) massively up-regulated infection-triggered production of TNF-α from 85 ± 6 pg/ml to 2797 ± 158 pg/ml (*P* ≤ 0.01). Non-infected BMM did not release TNF-α (< 10 pg/ml), while IL-4 (10 ng/ml) and IL-13 (10 ng/ml) slightly reduced release of the mediator from infected BMM (50 ± 13 and 43 ± 3 pg/ml respectively, *P* ≤ 0.05). IL-10 diminished TNF-α production from *L. monocytogenes*-infected BMM to undetectable levels. These results underscore the macrophage-activating properties of IFN-γ, and the deactivating actions of IL-4, IL-10 and IL-13 in our system of listeria-infected BMM.

**Chemokine production by spleen cells from *L. monocytogenes*-infected C57BL/6, IFN-γ−/− and IL-4−/− mice**

As described above, IFN-γ and IL-4 increased MCP-1 release, and diminished production of MIP-1α, MIP-2 and KC by *L. monocytogenes*-infected BMM. To correlate these *in vitro* data with the *in vivo* setting, C57BL/6, IFN-γ−/− and IL-4−/− mice were infected with *L. monocytogenes*, and splenocytes were tested for chemokine production at 2 h and 24 h p.i. At 2 h p.i., no differences in chemokine release were measured (data not shown). At 24 h p.i., MIP-1α, MIP-2 and KC release were increased in spleens of infected IFN-γ−/− mice compared to C57BL/6 mice. MIP-1α production was also enhanced in spleens of IL-4−/− mice; however, MIP-2 and KC release were similar in spleens of IL-4−/− and control C57BL/6 mice (Fig. 5).
Fig. 3. Effect of IFN-γ on chemokine production by listeria-infected BMM. BMM (5×10⁴/well) were infected with L. monocytogenes (5×10⁵/well) and simultaneously stimulated with increasing concentrations of IFN-γ. At 24 h p.i., supernatants were harvested and tested for chemokines by ELISA. Data are means ± SD (n = 3) from one out of three experiments with similar results. *P ≤ 0.05 versus L. monocytogenes alone.

Surprisingly, MCP-1 secretion by splenocytes of L. monocytogenes-infected C57BL/6, IFN-γ−/− and IL-4−/− mice was inconsistent. In one out of five experiments, MCP-1 production was decreased in L. monocytogenes-infected IFN-γ−/− and IL-4−/− mice compared to C57BL/6 mice (data not shown). We assume that MCP-1 secretion might be highly sensitive to other stimuli in addition to infection, e.g. stress or age of mice.

Discussion

During the early non-specific immune response to L. monocytogenes, infected macrophages release IL-12 and TNF-α which stimulate NK cells to produce IFN-γ. This cytokine, in turn, activates the antimicrobial potential of macrophages and, together with IL-12, promotes differentiation of IFN-γ-producing Tn1 cells which finally leads to the resolution of infection (30–32). We recently described prompt IL-4 production by spleen cells after L. monocytogenes infection of mice and we identified this early IL-4 as inducer of the CC chemokine MCP-1 (33). This finding led us to investigate in more detail the regulation of chemokine production by murine macrophages in response to L. monocytogenes infection in a more defined in vitro model. Three CC chemokines (RANTES, MCP-1 and MIP-1α) and three CXC chemokines (IP-10, KC and MIP-2) were selected for our studies. Two members of each group (MCP-1 and MIP-1α, KC and MIP-2) were studied in more detail using ELISA. Murine MCP-1 is a potent chemoattractant for monocytes and activated T cells (34). Murine MIP-1α mainly attracts and activates neutrophils but also acts on monocytes (35–37). Murine KC and MIP-2 recruit neutrophils and they are considered putative homologues of human growth-related gene chemokines (GRO) α/β/γ. MIP-2 and KC are dominant chemokine species in inflammatory responses in the mouse (38,39).

Resident peritoneal macrophages are frequently employed to study macrophage activation and deactivation by cytokines. However, these cells gave inconsistent results probably due to unknown in vivo stimuli (data not shown). Therefore, we used BMM raised and stimulated in serum-free medium. These cells represent a homogeneous population of quiescent macrophages which are responsive to activating and deactivating stimuli (26). In parallel, experiments were performed with BMM cultured in FCS-containing medium. Upon infection, this cell population showed a similar chemokine response compared with BMM cultured under serum-free conditions. However, it turned out that the stimulating effect of IFN-γ on listeria-induced MCP-1 production could be replaced by FCS (J. Barsig et al., in press). Obviously, FCS components can activate certain functions of murine macrophages and the serum-free culture system provides a means to avoid undefined activation signals.

In BMM infected with L. monocytogenes, MIP-2, KC, MIP-1α, MIP-1β, IP-10 and RANTES mRNAs were expressed, and the products MIP-1α, MIP-2 and KC could be detected in culture supernatants within 3 h, reaching a maximum at 12 h p.i. MCP-1 was the only chemokine tested which was induced...
Chemokine response of murine macrophages

**Fig. 4.** Influence of IL-4, IL-10 and IL-13 on listeria-induced chemokine production by BMM. BMM (5×10^4/well) were infected with *L. monocytogenes* (5×10^5/well) and simultaneously treated with increasing concentrations of IL-4, IL-10 and IL-13. At 24 h p.i., supernatants were harvested and tested for chemokines by ELISA. Data are means ± SD (n = 3) from one out of three experiments with similar results. *P* < 0.05 versus *L. monocytogenes* alone.

only in trace amounts by *L. monocytogenes* infection alone. In the next set of experiments, the effect of IFN-γ, IL-4, IL-10 and IL-13 on chemokine release by *L. monocytogenes*-infected BMM was investigated. Both, IFN-γ and IL-4 induced MCP-1 release by *L. monocytogenes*-infected BMM, while IL-10 had no effect. In contrast, IFN-γ, IL-4 and IL-10 diminished production of MIP-1α, MIP-2 and KC. Treatment of infected BMM with IL-13 had no effect on MIP-1α, MIP-2 and KC release but slightly reduced the low level of listeria-induced MCP-1. Together, these results show that murine BMM produce a variety of chemokines upon infection with *L. monocytogenes* and that chemokine release is not only under the control of IFN-γ but also of the putative macrophage deactivating cytokines IL-4, IL-10 and IL-13. It is particularly noteworthy that IFN-γ, generally considered as macrophage-activating factor, down-regulated release of MIP-1α, MIP-2 and KC. Hence, not only IL-10 production (40) but also release of certain chemokines seem to be under negative control of IFN-γ. This finding in part is in line with data from Ohmori et al. (41), who found reduction of LPS-induced KC mRNA in peritoneal macrophages by IFN-γ.

By semiquantitative RT-PCR and ELISA, Rhoades et al. (42) investigated chemokine production by murine BMM in response to *M. tuberculosis*, another intracellular pathogen. MCP-1, MIP-1α, MIP-2 and IP-10 mRNA could be detected at 2 h p.i., with maximal levels at 12–30 h p.i. In line with our results, IFN-γ was not a general activator of chemokine expression by BMM, except that of IP-10; and using RT-PCR, we also observed induction of IP-10 message in IFN-γ-treated BMM (data not shown). MCP-1 production was induced directly by *Mycobacterium tuberculosis* in BMM and was not affected by IFN-γ treatment, which might be explained by the use of serum-containing medium in that study. IL-4 and IL-13 have been described as T \( \text{h} \) \( \text{2} \) cytokines with overlapping activities on macrophages (18). Both cytokines down-regulate the cytotoxic and inflammatory activities of murine and human macrophages (17,43,44), and, in fact, they both down-regulated listeria-induced TNF-α release from BMM in this study. However, this overlapping activity is not evident concerning modulation of infection-induced chemokine release. We did not detect stimulation of MCP-1 release by IL-13 from infected BMM as was observed with IL-4. IL-10, originally termed cytokine synthesis inhibitory factor, was shown to suppress the production of a wide range of cytokines in different cell types (45). IL-4 directly stimulates MCP-1 production by macrophages and endothelial cells (8,46). The \( \beta \)-chemokine C10 is strongly induced in both BMM and resident peritoneal macrophages after IL-4 treatment (13). In contrast, MCP-1 production by human peripheral blood monocytes and alveolar macrophages is enhanced by IL-10 but reduced by IL-4 and IL-13 (7). In human blood mononuclear cells, IL-13 suppresses release of IL-8 and MCP-1 (47),
and IL-10 was described as potent stimulator of MCP-1 production and inhibitor of IL-8 release (6). In our experimental system, MIP-1α, MIP-2 and KC were down-regulated by IL-4 and IL-10, and MCP-1 release was enhanced by IL-4. In contrast to IL-4, IL-13 had no significant effect on MIP-1α, MIP-2 and KC production but reduced the low level of L. monocytogenes-induced MCP-1 release by BMM. Our findings are in contrast to published reports (6,7,47) pointing to possible differences between the models investigated.

Since IFN-γ as well as IL-4 and IL-10 are detectable in the early stage of listeriosis in mice (33,48,49), the in vitro findings presented here also point to an in vivo relevance. Consistent with our in vitro findings, we found enhanced production of MIP-1α, MIP-2 and KC in spleens of L. monocytogenes-infected IFN-γ⁻/⁻ mice. MIP-1α release was also enhanced in spleens of infected IL-4⁻/⁻ mice; however, MIP-2 and KC production were comparable in spleens of C57BL/6 and IL-4⁻/⁻ mice. Apparently, IFN-γ had more profound effects on chemokine release in L. monocytogenes infection. Analysis of MCP-1 production by splenocytes of infected C57BL/6,
IFN-γ−/− and IL-4−/− mice gave inconsistent results, although mice were bred under specific pathogen-free conditions. We assume that other factors than infection might influence MCP-1 synthesis and hesitate to draw any decisive conclusions. A correlation between CC chemokines and a Tγ1 response has been proposed by Schrum et al. (50). In contrast, Chenue et al. (51) showed that MCP-1 contributes to Tγ2- rather than to Tγ1-mediated inflammation. Stimulation of T cells in the presence of MIP-1α enhanced IFN-γ production while stimulation of T cells in the presence of MCP-1 led to increased IL-4 release (52). In murine listeriosis, which is dominated by a Tγ1-type immune response, CC chemokines (MCP-1 and MIP-4 release (52). In murine listeriosis, which is dominated by a Tγ1-type immune response, CC chemokines (MCP-1 and MIP-1α) as well as CXC chemokines (MIP-2, KC and IP-10) were detected in the early stage of infection, and MCP-1 release was enhanced by triggering of macrophages with IFN-γ or IL-4. We have previously reported that IL-4 is produced in the initial stage of listeriosis and induces MCP-1 release in vivo (33). However, at later stages, IFN-γ production dominates and IL-4 production is switched off (53). Hence, rapid but transient IL-4 induction during listeriosis is apparently insufficient for promotion of Tγ1,2 cells. It is thus suggested that a sequence of early IL-4 and subsequent IFN-γ favours a sustained MCP-1 release at sites of infection. In cooperation with other chemokines MCP-1 can then support influx of inflammatory monocytes and lymphocytes which are necessary for granuloma formation and sterile eradication of the pathogen (19). Recently, Kurihara et al. (54) showed that mice deficient for the CC chemokine receptor CCR2, which mediates responses to MCP-1, failed to clear infection by L. monocytogenes. These results emphasize the importance of MCP-1 in host resistance against L. monocytogenes. In conclusion, our data provide further evidence for regulation of chemokines by cytokines such as IFN-γ and IL-4 in early stages of listeriosis.

Acknowledgements
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Abbreviations

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<tr>
<td>BMM</td>
<td>bone marrow-derived macrophages</td>
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<td>HKL</td>
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<td>IFN-γ−/−</td>
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<td>MIP</td>
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<td>p.i.</td>
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References


