Two murine homologues of human MCP-1 chemokine are differently expressed during intracellular infection with *Listeria* strains

BOŻENA DZIADEK[1], JAROSŁAW DZIADEK[2], TOMASZ POPŁAWSKI[2], TERESA GOŚCICKA[1]

[1]Department of Immunoparasitology, University of Lodz, Lodz, Poland; [2]Center for Microbiology and Virology Polish Academy of Sciences, Lodz, Poland

Abstract

The murine cells express two homologues of human MCP-1 β-chemokine, which plays a crucial protective role in the early phase of immunological response against intracellular pathogens. We have found that the expression of mRNA for murine MCP-1/JE and MCP-5 CC-chemokines is upregulated in peritoneal exudate cells during Listeria infection. The MCP-5 mRNA expression was observed exclusively in the early stage of infection with both pathogenic and nonpathogenic Listeria strains. The upregulation of MCP-1/JE mRNA production depended on strain pathogenicity and increased gradually during four hours postinfection.

Key words: *cytokines, chemokines, monocyte chemotactic protein-1/JE (MCP-1/JE), monocyte chemotactic protein-5 (MCP-5), listeriosis*

(Centr Eur J Immunol 2004; 29 (1): 23-28)

Introduction

Chemotactic cytokines (chemokines) are a superfamily of proinflammatory proteins, which activate leukocyte extravasation to the sites of their production and therefore play an important role during inflammatory and pathological processes including infectious diseases and cancer. The common future of almost all chemokines is a presence within their aminoacid sequences of four conserved cysteine residues that form disulfide bonds. Based on the arrangement of the conserved cysteines, chemokines are categorized into four subfamilies named: CXC, CC, C and CX3C [1-7].

Monocyte Chemotactic Proteins (MCPs) belong to one of the most important families of chemotactic cytokines, CC (β)-chemokines, with the first two cysteine residues lying adjacent. The parental molecule of cytokines belonging to this family is human MCP-1 protein. At present there are known four human (MCP-1, MCP-2, MCP-3, MCP-4) and three murine (MCP-1/JE, MCP-5, MARC) monocyte chemotactic proteins. Murine MCP-1/JE and MCP-5 chemokines are homologues of human MCP-1 protein, with 55% and 66% aminoacid sequence identity, respectively. Murine MARC cytokine is a homologue of human MCP-3 protein and shares 59% aminoacid identity with this human β-chemokine [1, 4, 8-13].

Monocyte Chemotacic Proteins, similarly to other CC-chemokines are produced by different types of leukocytes: monocytes, macrophages, granulocytes, lymphocytes and other cell types such as fibroblasts, endothelial cells, epithelial cells, smooth muscle cells, keratinocytes, chondrocytes, astrocytes and malignant cells. There is no specific cellular source of each MCP protein but a particular type of cells can produce several different chemotactic cytokines depending on a stimulator used [1, 4, 8].

The inductors of MCPs proteins production are endogenic (cytokines) and exogenic (bacteria, bacterial antigens, viruses and plant lectins) factors. Murine MCP-1/JE chemokine is produced in the early phase of immunological response to LPS and plays a protective immunoregulatory role in the experimental model of endotoxemia by inhibiting the synthesis of IL-12 and TNF-α and inducing IL-10

Correspondence: Bożena Dziadek, University of Lodz, Department of Immunoparasitology, Banacha 12/16 St., 90-237 Lodz, Poland. Phone: +48 42 635 43 55, fax +48 42 665 58 18, e-mail: bodzia@biol.uni.lodz.pl

production, which was shown to be protective in LPS-treated animals [14-17]. The presence of MCP-1/JE protein was also described in infections caused by intracellular pathogens such as *L. monocytogenes* and *Mycobacterium tuberculosis* [18-23]. Flesch et al. found that spleen cells isolated from mice injected with *L. monocytogenes* showed the increased level of MCP-1/JE protein in the early nonspecific phase of immunological response against this pathogen. Thus MCP-1/JE chemokine seems to be responsible for triggering movement and accumulation of macrophages to the site of infection which suggests that MCP-1/JE can participate in nonspecific resistance to *L. monocytogenes* [18].

In this study we have shown that both murine homologues of human MCP-1 protein are upregulated during *Listeria* infection, however, the upregulation of MCP-1/JE mRNA and not that of MCP-5 mRNA is specific for pathogenic *Listeria* strains, exclusively. Moreover, the MCP-5 mRNA expression was observed in the very early stage of infection whereas the mRNA was detectable only for a short period of time.

Materials and methods

Experimental animals

Male mice of BALB/c strain were raised under conventional conditions and were used for the experiments at 8-12 weeks of age.

Bacterial strains

A virulent, hemolytic strain of *L. monocytogenes* CIP 8738 and nonpathogenic strain of *L. innocua* CIP 8011 were obtained from the Department of Infectious Biology University of Lodz and were maintained in our laboratory. Both *Listeria* strains were passaged by BALB/c mice, and after isolation followed by microbiological and biochemical identification they were grown in tryptose agar, washed three times with PBS and resuspended in PBS with 25% of sterile glycerol. The aliquots of bacteria suspension were stored at -75°C. Before each experiment listeriae were freshly thawed, grown in tryptose agar supplemented with B_1 vitamin for 18-20 hours at 37°C and then washed twice with PBS. The concentration of bacteria suspensions was determined spectrophotometrically, based on the prepared standard curve. For all experiments the suspensions were adjusted to a concentration of $6x10^8$ viable bacteria/ml (absorbance value A=0.35 at λ=430 nm) and then diluted to appropriate working concentrations immediately prior to stimulation or injection. The number of viable bacterial cells was counted also by determination of CFU with the pour plate method after serial dilutions.

Isolation of peritoneal exudate cells

Peritoneal exudate cells were isolated from BALB/c mice 96 hours after intraperitoneal injection with 1 ml of 10% thioglicollate broth (Difco). PEC cells were washed and suspended in RPMI 1640 culture medium (Institute of Experimental Immunology and Therapy, Polish Academy of Sciences, Wroclaw) supplemented with 10% heat-inactivated NCS (Sigma), 2 mM L-glutamine (Sigma), 0.05 mM 2-β-mercaptoethanol (SERVA), 100 μg/ml streptomycin (POLFA), 100 μg/ml gentamicin (POLFA) and 2.4 μg/ml amphotericin (Sigma). One day prior to infection $5x10^6$/well PEC cells were seeded in 24-well tissue culture plates and after overnight incubation in a humidified 5% CO_2 atmosphere at 37°C the cells were washed twice using prewarmed RPMI 1640 medium supplemented only with 5% heat-inactivated NCS, and finally the same medium was added to each well.

Induction of *in vitro* expression of MCP-1/JE and MCP-5 mRNA

To stimulate the MCP-1/JE and MCP-5 mRNA production, the PEC cells were infected with $5x10^7$ and $5x10^5$ viable *L. monocytogenes* rods and incubated for 1, 2, 3 and 4 hours in a 5% CO_2 atmosphere at 37°C. Then cells were washed twice with RPMI 1640 medium and one time with cold PBS and were used for RNA extraction. To determine the ability of IL-4 to influence the MCP-1/JE/mRNA and MCP-5/mRNA expression, the PEC cells were stimulated for 2 hours with 10 ng/ml of recombinant mouse IL-4. The cells cultured without bacteria or IL-4 served as a control.

Stimulation of *in vivo* expression of MCP-1/JE and MCP-5 mRNA

Ninety six hours after intraperitoneal injection of 10% thioglicollate medium mice were infected intraperitoneally with $5x10^5$ and $5x10^3$ viable *L. monocytogenes* or with $5x10^5$ viable *L. innocua* cells resuspended in 0.5 ml of PBS or with 0.5 ml of PBS as a control. At 1, 2, 3 and 4 hours postinfection PEC cells were isolated from experimental and control animals and the total RNA was extracted.

Total mRNA extraction

Total mRNA was extracted using Promega Total RNA Isolation Kit according to the manufacturer's indications. The amount and purity of the isolated total RNA were measured spectrophotometrically at λ=260 nm and λ=300 nm, respectively.

RT-PCR procedure

RT-PCR reaction was performed using Promega Access RT-PCR System. RT-PCR primers pairs specific for murine MCP-1/JE and MCP-5 were designed in our laboratory (MCP-1/JE: 1-5'AGTGCTTGAGGTGGTTGTGG3'; 2-5'CATTCACCAGCAAGATGATC3'; MCP-5: 1-5'AGT CCTCAGGTATTGGCTGG3'; 2-5'GAGACGTCTTATC CAAGTGG3') and primers for murine β-actin were described by Nishibori et al. [24]. All primers were purchased from BIONOVO Company. Complementary cDNA was synthesized using 0.5 μg of total RNA and AMV reverse

transcriptase for 45 min at 48°C and was directly used for *in vitro* amplification. The amplification step was carried out in the presence of 50 pM each of specific primers, 2.25 mM dNTPs, 2.5 U thermostable Tfl polymerase and 1 mM $MgSO_4$ in a final volume of 30 µl of 1x AMV/Tfl Reaction Buffer. After the initial denaturation step performed for 2 min at 94°C, the 35 cycles for MCP-1/JE and MCP-5 mRNA and 30 cycles for β-actin mRNA were run as follows: 30 sec of denaturation at 94°C, 60 sec of renaturation at 56°C for MCP-1/JE and β-actin or at 60°C for MCP-5 and 90 sec of amplification at 68°C. The final amplification step was carried out for 7 min at 68°C. The RT-PCR reaction products were visualized by electrophoresis of the postreaction mixture in a 7% acrylamide gel in the presence of DNA ladder (pUC19 DNA digested with HaeIII endonuclease). Electrophoretically separated DNA fragments were stained with 0.5 µg/ml of ethidium bromide and were densytometrically analyzed with ScanPack 3.0 computer program (BIOMETRA).

Results

Induction of MCP-1/JE and MCP-5 mRNA in peritoneal exudate cells by *in vitro* *L. monocytogenes* infection

The human MCP-1 chemokine plays an important role in the defense against intracellular pathogens. The murine macrophages produce two counterparts of this protein, MCP-1/JE and MCP-5 chemotactic cytokines. In our study we asked the question which of these MCP-1 homologues plays a role in the inflammation due to *Listeria* infections. We have investigated the level of MCP-1/JE and MCP-5 mRNAs in mouse peritoneal exudate cells after *in vitro* infection with live *L. monocytogenes*. The mouse peritoneal exudate cells were isolated and stimulated with 1 and 10 listeriae per peritoneal cell. At 1, 2, 3 and 4 hours postinfection the total cellular RNA was extracted and the levels of MCP-1/JE and MCP-5 mRNAs were analyzed with standardized RT-PCR reaction.

The significant induction of MCP-1/JE mRNA expression has been detected in PEC cells infected with *L. monocytogenes* at the ratio 10:1 bacteria per eukaryotic cell. The increased amount of cellular MCP-1/JE mRNA in comparison with the control cells was noted at 2 hours postinfection but the highest level of MCP-1/JE mRNA was detected after 3 or 4 hours. On the other hand, the level of MCP-1/JE mRNA in PEC cells stimulated with tenfold times lower number of *L. monocytogenes* organisms was comparable to the control cells (data not shown). To confirm the obtained results, the level of mRNA for MCP-1/JE was compared to the level of mRNA for constitutively expressing gene encoding β-actin, simultaneously amplified in the same reaction. This method allows a more adequate, quantitative determination of mRNA levels in the examined samples by the comparison of the amount of RT-PCR reaction product amplified for cytokine mRNA-derived cDNA with the amount of RT-PCR product coamplified for β-actin mRNA-derived cDNA. The detected levels of β-actin mRNA were identical in the experimental and control samples (Fig. 1).

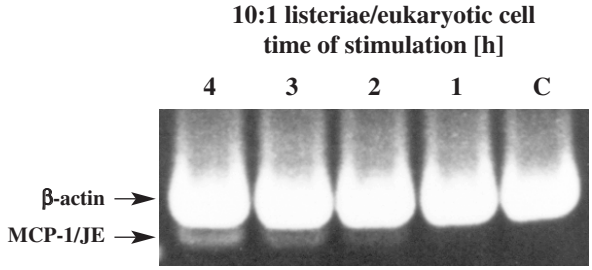

Fig. 1. Expression level of MCP-1/JE/mRNA and β-actin/mRNA in murine peritoneal exudate cells stimulated *in vitro* with viable *L. monocytogenes* at ratio: 10: 1 bacteria per eukaryotic cell detected with competitive RT-PCR; c – control (expression of MCP-1/JE/mRNA and β-actin/mRNA in unstimulated cells); one of five experiments is shown

Interestingly, in our *in vitro* study the MCP-5 mRNA in peritoneal exudate cells infected with viable *L. monocytogenes* was undetectable. The PEC cells were also stimulated with 10 ng/ml of recombinant mouse IL-4 for 2 hours to test if the well known inducer of MCP-1/JE chemokine could upregulate MCP-5 production. We found that IL-4 can increase the expression of MCP-1/JE but not the expression of MCP-5 mRNA separately or simultaneously with *L. monocytogenes* cells (Fig. 2).

An *in vivo* MCP-1/JE and MCP-5 mRNA expression in mice infected with pathogenic and nonpathogenic *Listeria strains*

Since our in vitro study of mouse MCP-1/JE and MCP-5 mRNAs expression can suggest that production of these two β-chemokines may be differently regulated during listeriosis, we further investigated and compared the *in vivo* expression level of MCP-1/JE and MCP-5 mRNA in PEC cells isolated from mice injected intraperitonealy with $5x10^3$ and $5x10^5$ viable *L. monocytogenes* or $5x10^5$ viable *L. innocua* per peritoneum. To estimate the time course of mRNA induction, PEC cells were isolated 1, 2, 3 and 4 hours after *Listeria* administration and then a total cellular RNA was extracted from the same number of peritoneal cells for each experiment. The obtained results indicate that expression of mouse MCP-1/JE and MCP-5 mRNAs in PEC cells isolated from mice infected by *Listeria* rods depends on the number of injected bacteria, their pathogenicity and genetic features of infected animals. Contrary to the *in vitro* study described above we observed a very strong induction of both MCP-1/JE and MCP-5 mRNA expression in PEC cells of mice infected with $5x10^5$ viable *L. monocytogenes*, but the kinetics of their production was completely different (Fig. 3). The highest expression of MCP-1/JE mRNA was

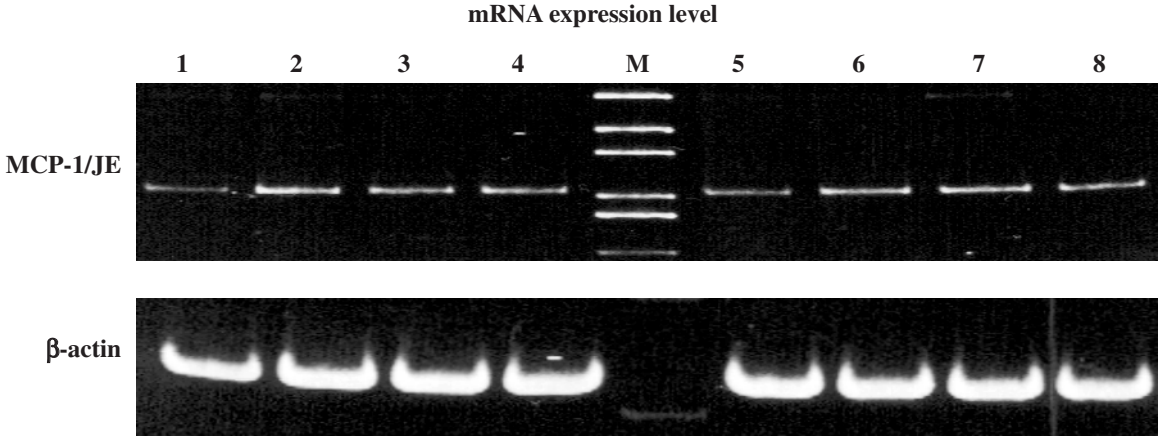

Fig. 2. Expression of MCP-1/JE/mRNA and β-actin/mRNA in murine peritoneal exudate cells stimulated *in vitro* with exogenous IL-4, viable *L. monocytogenes* and simultaneously with IL-4 and *L. monocytogenes* organisms; lines 1,5 – control (unstimulated cells), lines 2,6 – stimulation with *L. monocytogenes*, lines 3,7 – stimulation with exogenous IL-4 (10 ng/ml) and line 4, 8 – simultaneous stimulation with *L. monocytogenes* and exogenous IL-4, M – size marker (DNA of pUC19 digested with restriction endonuclease HaeIII); two of three experiments are shown

detected at 3 or 4 hours postinfection but the increased amount of investigated mRNA was noted just 1 hour after bacteria administration. In contrast, the production of MCP-5 mRNA in peritoneal cells isolated from mice infected with $5x10^5$ *L. monocytogenes* was more rapid in comparison to the production of MCP-1/JE mRNA. The maximum of MCP-5 mRNA upregulation was found at 1 or 2 hours postinfection, however, 3 or 4 hours after bacteria injection the MCP-5 mRNA was not detectable. The infection of mice with a lower number of *L. monocytogenes* organisms ($5x10^3$) did not result in the induction of MCP-1/JE and MCP-5 mRNAs synthesis (data not shown).

Interestingly, the nonpathogenic strain of *L. innocua* stimulated the MCP-5 mRNA expression but did not affect the production of MCP-1/JE mRNA. The induced level of MCP-5 mRNA by *L. innocua* was detected 2 hours postinfection but the amount of MCP-5 mRNA measured in PEC cells isolated from mice treated with nonpathogenic *L. innocua* was lower than the amount in the cells isolated from animals infected with pathogenic *L. monocytogenes* organisms (Fig. 4).

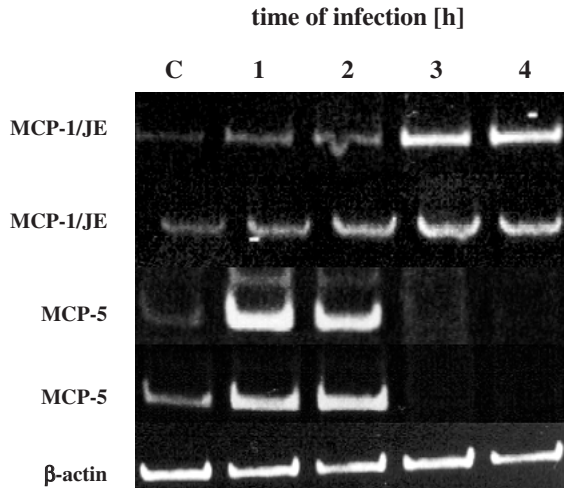

Fig. 3. Production of MCP-1/JE/mRNA and MCP-5/mRNA in murine peritoneal exudate cells after *in vivo* infection with $5x10^5$ viable *L. monocytogenes per peritoneum*; c – control (expression of MCP-1/JE/mRNA and MCP-5/mRNA in PEC cells obtained from uninfected animals); two of four experiments are shown

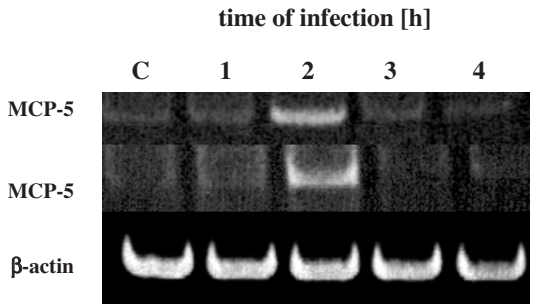

Fig. 4. Expression level of MCP-5/mRNA in peritoneal exudate cells after *in vivo* administration 5x105 viable *L. innocua per peritoneum*; c – control (expression of MCP-5/mRNA in PEC cells obtained from uninfected animals); two of four experiments are shown

Discussion

The broad spectrum of biological activity of β-chemokines indicates that they can play an important role during infections caused by intracellular microbial pathogens, like *L. monocytogenes* and *M. tuberculosis* [18, 20-22]. Actually, it is known that active movement and accumulation of macrophages into infection sites observed during the early nonspecific phase of immunological response to experimental listeriosis in mice are induced by MCP-1/JE chemokine, the homologue of human MCP-1 protein [18]. The study presented in this paper investigated and compared the mRNA synthesis of murine MCP-1/JE and MCP-5 proteins in peritoneal exudate cells after *in vitro* and *in vivo* infection with *L. monocytogenes* or *L. innocua* rods. Our findings indicate that mRNA expression for these two murine β-chemokines, both of which are homologues of human MCP-1 protein, can be differently regulated and that murine MCP-5 protein, similarly to MCP-1/JE, can be involved in the nonspecific, immunological response to *Listeria* infection.

Previous study performed by Barsig et al., demonstrated that murine bone marrow-derived macrophages and murine hepatocytic cells of TIB 75 cell line infected *in vitro* with *L. monocytogenes*, secreted a wide pattern of different chemokines, including MCP-1/JE protein [19]. Additionally we found that also *in vitro* treatment of murine peritoneal exudate cells with live *L. monocytogenes* resulted in an increased production of MCP-1/JE mRNA, but not of MCP-5 mRNA. The observed MCP-1/JE mRNA expression was dependent on the number of bacteria used for stimulation as well as the time after infection, and was elevated very early within the first hour after bacteria administration with the maximum peak at 3 or 4 hours postinfection. The similar effect occurred after *in vitro* stimulation of peritoneal exudate cells with exogenous IL-4, which also elicited the expression of MCP-1/JE mRNA only. It suggests that the expression of MCP-5 mRNA in peritoneal exudate cells may not be influenced by *in vitro* stimulation with *L. monocytogenes* or exogenous IL-4 and that these factors could not be responsible for *in vitro* induction of MCP-5 mRNA expression in this cell type. On the other hand, we cannot exclude a possibility that other factors, like various cytokines, absent or produced at lower concentrations during *in vitro* stimulation may be essential for upregulation of mRNA for this chemokine.

In the next part of our study we determined the expression of mRNA of MCP-1/JE and MCP-5 chemotactic cytokines in peritoneal exudate cells after *in vivo Listeria* administration. We found that the synthesis of MCP-1/JE mRNA depends on bacterial virulence, however, the MCP-5 mRNA expression is upregulated either by pathogenic *L. monocytogenes* or nonpathogenic *L. innocu*a strain. It could indicate that listerial virulence factors are essential for upregulation of MCP-1/JE but not MCP-5 mRNA expression. Barsig et al. found that listeriolysin is required for induction of MCP-1/JE chemokine in murine bone marrow-derived macrophages and hepatocytic cells but not for a number of other chemokines [19]. These and our findings suggest that although the production of most chemotactic cytokines may not be dependent on the virulence of *Listeria* strains, the expression of MCP-1/JE mRNA could be determined by the presence of *Listeria* pathogenicity factors. The induction mechanism is not the only difference in the regulation of MCP-1/JE and MCP-5 mRNA expression. The synthesis of MCP-5 mRNA was increasing rapidly with the maximum in the second hour postinfection and soon after, at 3 and 4 hours postinfection, MCP-5 mRNA was not detectable. On the other hand, the maximum level of MCP-1/JE mRNA expression occurred at 3 and 4 hours postinfection. We conclude from our results that both counterparts of human MCP-1 protein may play an important role in nonspecific protection against *L. monocytogenes*. However, the expression of MCP-1/JE and MCP-5 mRNA is regulated in a different way. It is likely, that MCP-5 protein whose mRNA expression can be induced by both pathogenic and nonpathogenic *Listeria* works at the earlier stage of inflammation and its upregulation is less specific than that of MCP-1/JE mRNA. Moreover, the infection caused by pathogenic *Listeria* induces rapidly MCP-5 mRNA production and next increases the synthesis of MCP-1/JE mRNA which may be a key signal for accumulation of phagocytic cells, playing an important role during the early nonspecific protection against intracellular pathogens. However, more experiments would be required to understand the relation between both chemokines, if any exists.

References

1. Baggiolini M, Dewalad B, Moser B (1997): Human chemokines: An update. Annu Rev Immunol 15: 675-705.
2. Haelens A, Wuyts A, Proost P, Struyf S, Opdenakker G, Van Damme J (1996): Leukocyte migration and activation by murine chemokines. Immunobiol 195: 499-521.
3. Oppenheim JJ, Zachariae CO, Nukaida N, Matsushima K (1991): Properties of the novel proinflammatory supergene intercrine cytokine family. Annu Rev Immunol 9: 617-648.
4. Sozzani S, Locati M, Allavena P, Van Damme J, Mantovani A (1996): Chemokines: a superfamily of chemotactic cytokines. Int J Clin Lab Res 26: 69-82.
5. Loetscher P, Moser B (2002): Homing chemokines in rheumatoid arthritis. Arthritis Res 4: 233-236.
6. Coussens LM, Werb Z (2002): Inflamation and cancer. Nature 420: 860-867.
7. Segerer S (2003): The role of chemokines and chemokine receptors in progress renal diseases. Am J Kidney Dis 41: 15-18.
8. Rollins BJ (1997): Chemokines. Blood 90: 909-928.
9. Sarafi MN, Garcia-Zepeda EA, MacLean JA, Charo IF, Luster AD (1997): Murine monocyte chemoattractant protein (MCP) -5: a novel CC chemokine that is a structural and functional homologue of human MCP-1. J Exp Med 185: 99-109.
10. Opdenakker G, Froyen G, Fiten P, Proost P, Van Damme J (1993): Human monocyte chemotactic protein-3 (MCP-3): molecular cloning of the cDNA and comparison with other chemokines. Biochem Biophys Res Commun 191: 535-542.

11. Van Coillie E, Van Damme J, Opdenakker G (1999): The MCP eotaxin subfamily of CC chemokines. Cytokine Growth Factor Rev 10: 61-86.
12. Zlotnik A, Morales J, Hedrick JA (1999): Recent advances in chemokines and chemokine receptors. Crit Rev Immunol 19: 1-47.
13. Menten P, Wuyts A, van Damme J (2001): Monocyte chemotactic protein-3. Eur Cytokine Netw 12: 554-560.
14. Zisman DA, Kunkel SL, Strieter RM, Tsai WC, Bucknell K, Wilkowski J, Standiford TJ (1997): MCP-1 protects mice in lethal endotoxemia. J Clin Invest 99: 2832-2836.
15. Howard M, Muchamuel T, Andrade S, Menon S (1993): Interleukin 10 protects mice from lethal endotoxemia. J Exp Med 177: 1205-1208.
16. Smith SR, Terminelli C, Kenworthy-Bott L, Calzetta A, Donkin J (1994): The cooperative effects of THF-alpha and IFN-gamma are determining factors in the ability of IL-10 to protect mice from lethal endotoxemia. J Leukoc Biol 55: 711-718.
17. Standiford TJ, Strieter RM, Lukacs NW, Kunkel SL (1995): Neutralization of IL-10 increases lethality in endotoxemia. Cooperative effects of macrophage inflammatory protein-2 and tumor necrosis factor. J Immunol 155: 2222-2229.
18. Flesh IEA, Wandersee A, Kaufmann SHE (1997): IL-4 secretion by CD4 (+) NK1 (+) T cells induces monocyte chemoattractant protein-1 in early listeriosis. J Immunol 159: 7-10.
19. Barsig J, Flesch IEA, Kaufmann SHE (1998): Macrophages and hepatocytic cells as chemokine producers in murine listeriosis. Immunobiol 199: 87-104.
20. Kasahara K, Tobe T, Tomita M, Mukaida N, Shao-Bo S, Matsushima K, Yoshida T, Sugihara S, Kobayashi K (1994): Selective expression of monocyte chemotactic and activating factor/monocyte chemoattractant protein 1 in human blood monocytes by Mycobacterium tuberculosis. J Infect Dis 170: 1238-1247.
21. Lin YG, Gong JH, Zhang M, Xue WF, Barnes PF (1998): Production of monocyte chemoattractant protein 1 in tuberculosis patients. Infect Immun 66: 2319-2322.
22. Lin YG, Zhang M, Barnes PF (1998): Chemokine production by a human alveolar epithelial cell line in response to Mycobacterium tuberculosis. Infect Immun 66: 1121-1126.
23. Rutledge BJ, Rayburn H, Rosenberg R, North RJ, Gladue RP, Corless CL, Rollins BJ (1995): High level monocyte chemoattractant protein-1 expression in transgenic mice increases their susceptibility to intracellular pathogens. J Immunol 155: 4838-4843.
24. Nishibori T, Xiong HB, Kawamura I, Arakawa M, Mitsuyama M (1996): Induction of cytokine gene expression by listeriolysin O and roles of macrophages and NK cells. Infect Immun 64: 3188-3195.