Differential expression of chemokines in synovial cells exposed to different *Borrelia burgdorferi* isolates

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Abstract

Objective

Lyme borreliosis is characterized by strong inflammatory reactions probably due to the presence of Borrelia burgdorferi *in the joint. It has been suggested that* Borrelia *induces the immunological mechanisms that either can amplify the inflammatory response or can suppress it. To reveal the underlying mechanisms of chemoattraction and activation of responding leukocytes, we investigated the induction of chemokines in human synoviocytes exposed to two different* B. burgdorferi *sensu stricto isolates (strain Geho and B31).*

Methods

Synoviocytes were exposed in vitro *up to 5 days. Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was used to assess the relative chemokine mRNAexpression of RANTES/CCL5, SDF-1alpha/CXCL12 alpha, SDF-1beta/CXCL12 beta, MCP-1/CCL2, MCP-2/CCL8, IL-8/CXCL8 and MIP-1alpha/CCL3, and enzyme-linked immunosorbant assay (ELISA) was used to assess the protein expression of RANTES, SDF-1, MCP-1, and MIP-1alpha in the culture supernatant.*

Results

MCP-1 gene expression was not changed by strain B31 but MCP-1 gene expression along with protein concentration was suppressed by strain Geho. Both strains induced RANTES mRNA and protein concentration. SDF-1 gene expression was suppressed, whereas protein concentrations were unchanged by both strains. IL-8 gene expression was unchanged by using strain Geho but significantly upregulated by strain B31. Both strains induced MCP-2 mRNA expression. MIP-1alpha mRNA expression was induced, but chemokine concentration was suppressed by both strains.

Conclusion

This study suggests that the orchestra of chemokines plays an important role in the immunopathogenesis of early Lyme arthritis.

Key words

Borrelia burgdorferi, chemokine, Lyme arthritis, pathogen host interaction, synovial cells.

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Introduction

Lyme borreliosis is a multi-system disease caused by infection of the spirochete *Borrelia burgdorferi* (1-4). Spirochetes can be isolated from various affected tissues, suggesting that the clinical symptoms and histopathological signs of inflammation are associated with the presence of Borrelia at lesional sites (5). We and others have shown that *in vitro B. burgdorferi* can persist in close association to human fibroblasts, endothelial cells and synovial cells (6-9). *B. burgdorferi* can be detected in the inflamed joint by different methods including electron microscopy and PCR techniques even after antibiotic treatment (10-13). Production of proinflammatory cytokines by synovial cells in response to *B. burgdorferi* (14, 15) and presumably other activated components of the local immune system induce the typical joint effusions and arthritis (16). In Lyme arthritis a vigorous synovial mononuclear infiltration and hypertrophy similar to rheumatoid arthritis has been observed (2). Through a co-ordinated series of signals generated within the tissue lesions, the local vascular endothelial barrier becomes activated and is directing the leukocyte trafficking from the circulation into the inflamed tissue (17, 18). In addition, predominantly granulocytes are found in joint fluid at least at initial stages of disease (19). Thus, *B. burgdorferi* infection induces an effective chemotactic response, which augments the local inflammation. Synovial cells are capable to express a variety of molecules including cell adhesion molecules ICAM-1 (10,20) and VCAM-1 (21), metalloproteinases (22, 23), chemokines (24, 25) cyclooxygenases (16).

Chemokines appear to be the main factors responsible for the recruitment of distinct effector cells during inflammatory diseases. Members of the C-C chemokine subfamily, such as MIP-1α (macrophage inflammatory protein 1α)/CCL3, MCP-1 (monocyte chemotactic protein 1)/CCL2, SDF-1α (stromal derived factor 1α)/CXCL12α, SDF-1β (stromal derived factor 1β)/CXCL12β and RANTES (regulated upon activation, normal T cell expressed and secreted)/CCL5 preferentially attract lymphocytes. Expression and release of proinflammatory cytokines and chemokines was strongly enhanced by lipopolysaccharide, which is known to be a powerful producer of a wide variety of immunological mediators (26). More specifically, *Treponema pallidum* and *B. burgdorferi* derived lipoproteins have been shown to be potent macrophage activators and inducers of proinflammatory cytokines (27). This observation indicates that the interaction of bacterial molecules with host cells, presumably via Toll like receptors (28), induces the production and release of chemotactic factors such as chemokines.

Chemokine expression by synovial cells might play an important role in the onset and progression of Lyme arthritis. So far, especially the initial steps of inflammation in Lyme arthritis have not been delineated. It is not clear, whether such a direct interaction of *B. burgdorferi* with synoviocytes can be a first step. Therefore, the present study was designed to examine the interaction of synovial cells with two different *B. burgdorferi* isolates Geho and B31. We examined the expression of chemokines MIP-1α/CCL3, MCP-1/CCL2, MCP-2/CCL8, SDF-1α/CXCL12α, SDF-1β/CXCL12β, IL-8/CXCL8, and RANTES/CCL5 in human synovial cells after exposure to *B. burgdorferi* isolates Geho and B31 *in vitro.*

Material and methods

Cell culture

Human synovial cells were prepared from normal human joint tissue by trypsin enzymatic digestion, maintained in culture and passaged as described (29,30). These synovial cells have been characterized previously as synovial cells type B (9). Synovial cells were cultured in 10 cm^2 culture plates (Nalge, Nunc international, Denmark) using RPMI-1640 medium (GIBCO, Invitrogen corporation, UK) supplemented with 10% fetal calf serum (FCS) (Biochrom KG, Berlin, Germany) at 37°C in a 5% CO_2 humidified incubator. Synovial cells were used between passages 10 and 12. For all experiments synoviocytes (1 x 10^5) were seeded in

different culture plates and used at a confluent growth state.

Spirochetal strains and their growth conditions

In this study two different *B. burgdor - feri* sensu stricto strains Geho and B31 have been used. Strains B31 and Geho were received as a kind gift from Prof. M. Frosch, Institute of Hygiene and Microbiology, Wuerzburg, Germany. *B. burgdorferi* isolate B31 had been isolated from a joint of a patient with Lyme arthritis. Strain Geho was isolated from skin biopsy of a patient (31). Strains Geho and B31 were cultured at 35°C in Barbour-Stoenner-Kelly (BSK-H) (Sigma-Aldrich, Taufkirchen, Germany) medium supplemented with 6% rabbit serum (Sigma-Aldrich). Both isolates have been used in the 6-8th passage.

Co-culture of synovial cells with Borrelia

Only those culture plates of synovial cells were used for the experiment, which contained morphologically a uniform confluent monolayer of synovial cells. To infect the synovial cells, spirochetes were used at a logarithmic phase of growth and counted by dark field microscopy. Cells were cocultured with a multiplicity of infection (MoI) of 10 *B. burgdorferi* per one synovial cell for different durations ranging from 12 hrs to 5 days. Synovial cell number had been assessed by counting 20 plates of confluent synovial cells and determining the mean. Controls, lacking *B. burgdorferi* have also been used in the experiment for the same time points. The same constant volume of pH-matched BSK-H medium lacking spirochetes has been added to the controls in each series of experiments. Spirochetes remained viable in coculture up to 90 days tested (9).

Isolation of RNA and synthesis of first strand complementary DNA

Total cellular RNA was isolated from synovial cells of each individual experimental set by using RNeasy mini kit (Qiagen, Hilden, Germany) according to Qiagen's protocol. Contaminating genomic DNA was removed using RNase-free DNase (Qiagen). Aliquots of RNA from each experimental set were reverse transcribed into cDNAby using Superscript RNase H reverse transcriptase (RT) (Invitrogen Life Technologies, Karlsruhe, Germany). Two negative samples were prepared, one only by adding RT mix (RT+) without RNA and another lacking RT (RT-). Positive controls were run using total RNA isolated from tonsil tissue. The following reagents were added to each tube for reverse transcription reaction mix, 8 µl 5X First buffer (Invitrogen Life Technologies), 4 µl dTT (0.1M) (Invitrogen Life technologies), 2 µl dNTP (Sigma, Karlsruhe, Germany), 2 µl Superscript RNase H reverse transcriptase (Invitrogen Life Technologies) and 1 µl Rnasin (Promega, Madison, USA)

The reaction mix along with RNA was initially incubated for 15 minutes at 70°C, followed by incubation at 42°C for 50 minutes and 70°C for 15 minutes, finally the tubes were kept on ice for 10 minutes. Tubes containing cDNA were stored at –70°C. Polymerase chain reaction (PCR) was performed to amplify cDNA of expressed genes of interest (Table I).

Quantification of cDNA in samples

For quantification of cDNA in the different samples, PCR amplification for the housekeeping gene β-actin was performed in triplicate at 25, 30 and 32 cycles each. RT-PCR product was analyzed on 1.8% agarose gel. The gel image was acquired by using a gel documentation unit (Bio-Rad, Muenchen, Germany). The intensities of PCR product bands were analyzed by quantity one software (Bio-Rad). The mean counts of the intensities of these triplicates in the linear range of PCR amplification were calculated and were used to calculate an amount of cDNA(in µl) of each experimental sample that contained the same relative content of housekeeping gene cDNA as has been described previously (32). The calculated volume of cDNA was subjected to further PCR amplification of expressed genes. The amplified PCR product was again analyzed by agarose gel electrophoresis.

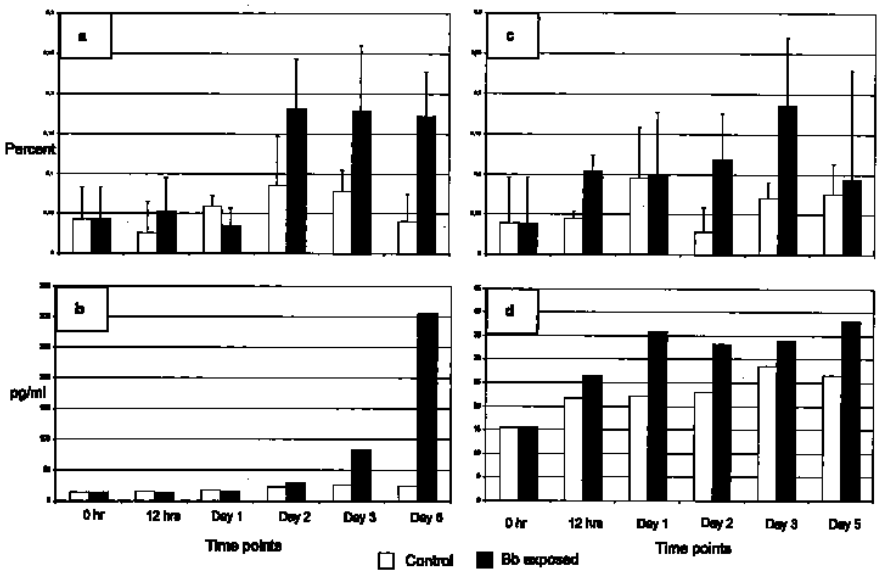
Polymerase chain reaction (PCR)

The PCR was performed in a mastercycler (Eppendorf AG, Hamburg, Germany). The β-actin adjusted amount of cDNA from the respective experimental sets was mixed with 20 µl molecular biology grade water (Eppendorf AG), 2.5 µl thermophilic DNA poly 10X buffer (Promega, Madison, WI, USA), 1.5 µl, 25 mM MgCl2 (Promega), 0.5 µl 10mM dNTP (Sigma, Karlsruhe, Germany) and 0.25 µl of 50 pmol 3' and 5' primers (MWG, Ebersberg,

Table 1. List of primer sequences.

Primer	Sequence
SDF-1alpha sense	5'AGAGCCAACGTCAAGCATCT3'
SDF-1alpha anti sense	5'GGTACAGGGCATGGATGAAT3'
SDF-1beta sense	5'GCATTGACCCGAAGCTAAAG 3'
SDF-1beta anti sense	5'AGAATCCAAAACCCAGGAGC 3'
RANTES sense	5'CTGTCATCCTCATTGCTACT3'
RANTES anti sense	5'TGATGTACTCCCGAACCCAT3'
IL-8 sense	5'AACATGACTTCCAAGCTGGC 3'
IL-8 anti sense	5'ACTTCTCCACAACCCTCTGC 3'
MIP-1 alpha sense	5'GGCAGATTCCACAGAATTTCA3'
MIP-1 alpha anti sense	5'TCCATAGAAGAGGTAGCTGTGGA3'
MCP-1 sense	5'TGGCTGTGTTTGCTTCTGTC 3'
MCP-1 anti sense	5'CCAGTTGACTGGTGCTTTCA3'
MCP-2 sense	5'ATGCTGAAGCTCACACCCTT3'
MCP-2 anti sense	5'ATGGAATCCCTGACCCATCT3'

Germany) in a total volume of 25 µl. The primer pairs used in the experiment are listed in Table I. All primer pairs for chemokines were designed from the published human cDNA sequence data (NCBI genbank) by using primer designing software (Primer 3' Whitehead Institute of Biomedical Research, USA). In addition, primer designs from previous analyses were used (32,33). The PCR of the target genes was done at the following settings: 5 minutes initial denaturation at 95°C in the first cycle, followed by 1 min denaturation at 94°C, 2 min annealing at 60°C and 3 minutes elongation at 72°C for all the primer sets except SDF-1beta and MIP-1 alpha. The annealing temperature in SDF-1beta and MIP-1 alpha were 58°C and 59°C respectively. To assess the appropriate number of cycles for the amplification of the genes of interest, PCR of beta-actin adjusted cDNA samples was run at 30, 38 and 40 cycles and the linear range of PCR was determined: 38 cycles have finally been used to run the PCR of the target genes (32). Experiments were repeated three times each. Intensities of the PCR product bands were calculated in a way that allowed to compare different experiments. Intensities of the individual experiments (Borrelia exposed and controls) were added and the sum normalized to 100%. Fractions of cDNA expression related to the total of a 100% were calculated for the individual time points. Percentages of relative gene expression of the consecutive experiments at a given time point were added up, and the mean of relative cDNA expression was calculated, as described previously (32).



	B. burgdorferi strain Geho		B. burgdorferi strain B31	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
Control	5.6 *	2.3	5.9 *	2.6
Exposed	11.1 *	7.4	10.7 *	4.7

Fig. 1. RANTES mRNA and protein expression by synovial cells exposed to *B. burgdorferi* strain Geho (**a**, **b**, respectively) and strain B31 (**c**, **d**, respectively).
RANTES mRNAexpression was assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**c**) after 12 hrs, 1 day, 2 days, 3 days and 5 days. In parallel, protein concentration of RANTES was measured by ELISAin the culture supernatant collected from *B. burgdorferi* strain Geho (**b**) and strain B31 (**d**) exposed samples. Results of the mRNAexpression represent the means of three different consecutive experiments. ELISAdata is shown from one representative experimental series.
(**e**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative RANTES gene expression measured in synovial cells exposed to *B. burgdorferi* strains Geho and B31 over 5 days. Using serial t-test the differences of RANTES mRNAexpression in Geho and B31 exposed cultures compared to their controls were significant (*p = 0.0032, **p = 0.0035, respectively).

Enzyme-linked immunosorbant assay (ELISA)
To assess the concentration of secreted chemokine, culture supernatants were assayed with ELISAfor RANTES (Biosource International, Camarillo, CA), MIP-1alpha (R&D Systems, Minneapolis, MN), MCP-1 (Biosource International) and SDF-1 (R&D Systems) according to the protocols provided by manufacturers. Chemokine concentrations were assayed in the culture supernatants collected from the experimental sets at given time points exposed to both the strains *B. burgdorferi* B31 and Geho.

Statistical analysis
Descriptive statistics were used to express the mean of gene expression at a given time point in a particular experimental series. In addition to overall mean of all time points together and the standard deviation in one particular series were calculated. To compare control cultures with Borrelia exposed ones the serial t-test was used, following probit analysis for normal distribution (34). Results are shown in the figure legends and tables.

Results

Chemokine mRNA expression in synovial cells exposed to B. burgdorferi
Semiquantitatively calibrated reverse transcription polymerase chain reaction analysis was used to assess the rel-

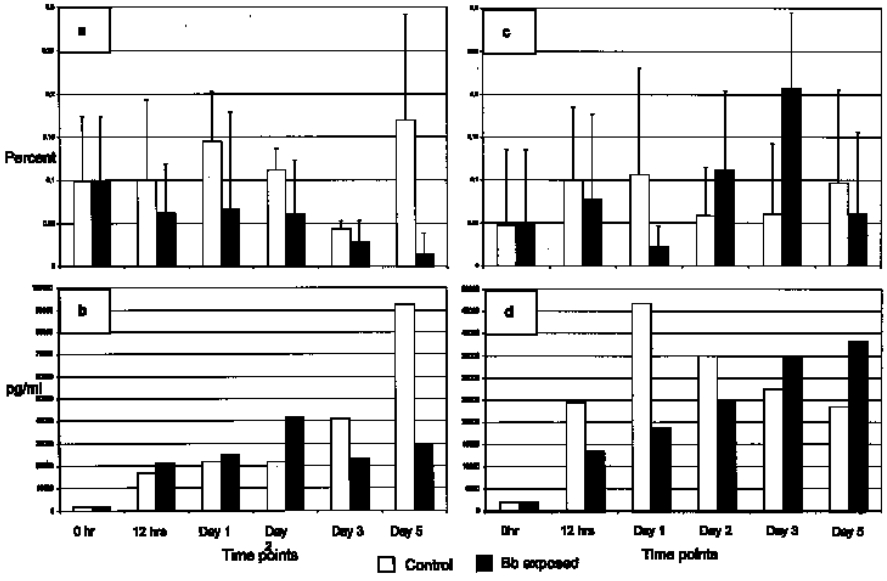
ative chemokine gene expression for RANTES, SDF-1α, SDF-1β, MCP-1, MCP-2, MIP-1α, IL8 by synovial cells exposed to *B. burgdorferi* strains Geho and B31. Experiments were done in triplicate and revealed reproducible and comparable results each. Synovial cells incubated with *B. burgdorferi* strain Geho showed increased mRNAexpression of the chemokine RANTES (Fig. 1a, 1e) (p = 0.0032, serial t-test) and MCP-2 (Fig. 6a, 6c) (p = 0.0032, serial t-test) from day 2 to day 5 and MIP-1α (Fig. 3a, 3e) (p = 0.0044, serial t-test) from 12 hrs to day 5, when compared to controls. MCP-1 mRNA(Fig. 2a, 2e) (p = 0.0007, serial t-test) expression was strongly downregulated from 12 hrs to day 5 and SDF-1β (Fig. 4b, 4d) (p= 0.036, serial t-test) gene expression was shown to be mildly downregulated from 12 hrs to day 5. SDF-1α (Fig. 4a, 4d) (p = 0.21, serial t-test) expression showed no consistent change. IL8 (Fig. 7a, 7c) (p=0.34, serial t-test) mRNA has shown fluctuating expression compared to controls using *B. burgdorferi* strain Geho.

In contrast to strain Geho, *B. burgdorferi* strain B31 induced increased mRNA expression of the chemokines RANTES (Fig. 1c, 1e) (p = 0.0035, serial t-test), IL-8 (Fig. 7b, 7c) (p = 0.0073, serial t-test) from 12 hrs to day 5 and MIP-1α mRNA(Fig. 3c, 3e) (p < 0.00005, serial t-test) from 12 hrs to day 3, when compared to controls. MCP-2 mRNA (Fig. 6b, 6c) (p < 0.00005, serial t-test) expression was upregulated from day 1 to day 5 whereas MCP-1 mRNA (Fig. 2c, 2e) (p = 0.90, serial t-test) expression was not changed. SDF-1β mRNA (Fig. 5b, 5d) (p = 0.018, serial t-test) was strongly downregulated from 12 hrs to day 5, whereas the mRNA expression of chemokine SDF-1α (Fig. 5a, 5d) (p = 0.93, serial t-test) was not changed in synovial cells challenged with Borrelia compared to controls.

Chemokine concentration in supernatants of human synovial cell culture after exposure to B. burgdorferi

RANTES, MCP-1, MIP-1α and SDF-1 protein levels were measured by ELISA in culture supernatants of synovial cells exposed to the *B. burgdorferi* strain Geho or B31. In the culture supernatants collected from the samples, exposed to *B. burgdorferi* strain Geho, the concentration of RANTES protein increased from day 2 to day 5 (Fig. 1b). MCP-1 protein concentration has not shown much difference till day 2 and then started decreasing from day 2 to day 5 (Fig. 2b), MIP-1alpha protein concentration is lower (Fig. 3b) in exposed samples compared to controls. SDF-1 protein concentration has not shown any significant difference in the exposed samples compared to controls (Fig. 4c).

Using *B. burgdorferi* strain B31 the concentration of RANTES protein increases from 12 hrs to day 5 (Fig.1d), MCP-1 protein concentration decreases for most of the time except at day 3 and day 5 (Fig. 2d), MIP-1α protein concentration showed a decrease from 12 hrs to day 5 (Fig. 3d) in the exposed samples compared to controls. SDF-1 protein concentration has not shown any significant difference in the ex-



	B. burgdorferi strain Geho		B. burgdorferi strain B31	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
Control	11.2 *	4.3	7.8	2.6
Exposed	5.5 *	3.0	6.8	6.6

Fig. 2. MCP-1 mRNAand protein expression by synovial cells exposed to *B. burgdorferi* strain Geho (**a**, **b**, respectively) and strain B31 (**c**, **d**, respectively).
MCP-1 mRNA expression was assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**c**) after 12 hrs, 1 day, 2 days, 3 days and 5 days. In parallel, protein concentration of MCP-1 was measured by ELISA in the culture supernatant. Results of the mRNAexpression represent the means of three different consecutive experiments. ELISA data is shown from one representative experimental series. (**e**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative MCP-1 gene expression measured in synovial cells exposed to *B. burgdorferi* strains Geho and B31 over 5 days. Using serial t-test the differences of Geho exposed cultures compared to controls were significant (*p = 0.0007), whereas the differences of MCP-1 expression in B31 exposed cultures were not significant (p = 0.90) compared to control.

posed samples compared to controls (Fig. 5c). A synopsis of chemokine mRNA expression and protein concentration assessed at day 3 and 5 together is shown in Table II.

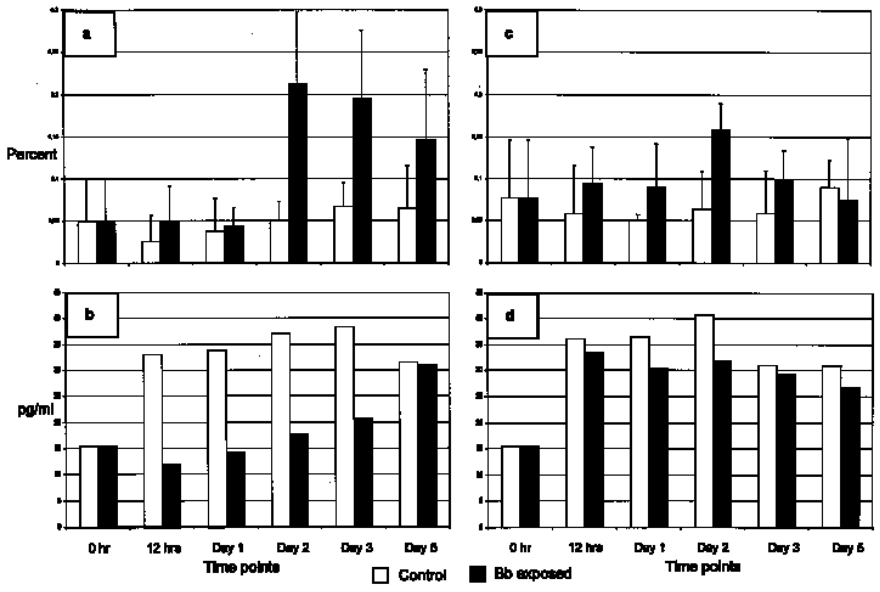
Discussion

One pathogenetic factor of Lyme arthritis is the invasion of the causative bacteria *B. burgdorferi* into articular tissues, demonstrated by the detection of Borrelia DNA in synovial tissue and fluid (35). There is evidence of spirochetal presence in synovial tissue of patients with chronic Lyme arthritis (36). Spirochetal structures have been identified in tissue biopsies by immunoelectron microscopy using monoclonal antibodies to *B. burgdorferi* OspA (36). Recruitment and activation of leukocytes is one of the important features of inflammatory arthritis. Chemokines play a central role in mediating the inflammatory events. Chemokine production appears to be ubiquitous. Almost every cell and tissue type in the body has been shown to produce chemokines upon stimulation, including endothelial cells, fibroblasts and synovial cells (37).

In Lyme borreliosis, heavy inflammatory infiltrates dominated by mononuclear cells are typically found at lesional sites (1). There have been several reports demonstrating that *B. burgdorferi* strongly activates monocytes which consecutively form cell aggregates and release proinflammatory cytokines including IL-1beta, IL-6, and tumour necrosis factor alpha (TNF-alpha) (27). The outer surface lipoprotein A (OspA) was identified as a main Borrelia-derived factor with cytokine inducing activity. However these cytokines can not contribute directly to the generation of mononuclear infiltrates in the synovium due to lack of chemotactic properties.

To investigate the role of chemoattractants in the pathogenesis of Lyme borreliosis, we analysed the expression of different chemokines in synovial cells after exposure to different strains of *B. burgdorferi.* The strains B31 and Geho showed different kinetics of chemokine expression. This could be due to different pathogenetic factors of these strains (9). Synovial cells are of particular interest, since their differential expression of chemokines may provide clues for the pathogenesis and clinical variability of Lyme arthritis. We found that the proinflammatory cytokine IL-8 was significantly upregulated from 12 hrs to day 5 using strain B31, and remains unchanged using strain Geho. Straubinger *et al.* also showed a local upregulation of IL-8 in canine synovial cell cultures infected with viable *B. burgdorferi* (38). These findings suggest that IL-8 is probably a prime initiator of polymorphonuclear cell (PMN) migration during acute Lyme arthritis. Receptors for IL-8, CXCR1 and CXCR2 are mainly expressed on neutrophils. In addition, IL-8 may act as a T cell chemoattractant (39, 40).

Several observations suggest that the chemokine RANTES is also an important mediator of inflammatory response and it displays significant chemotactic activity for eosinophils (41, 42), monocytes (43, 44) and the CD45RO+ memory T cell subpopulation (43, 44). Most



	B. burgdorferi strain Geho		*B. burgdorferi* strain B31	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
Control	5.0 *	1.6	6.7 *	1.4
Exposed	11.7 *	7.6	9.9 *	3.1

Fig. 3. MIP-1alpha mRNAand protein expression by synovial cells exposed to *B. burgdorferi* strain Geho (**a**, **b**, respectively) and strain B31 (**c**, **d**, respectively)

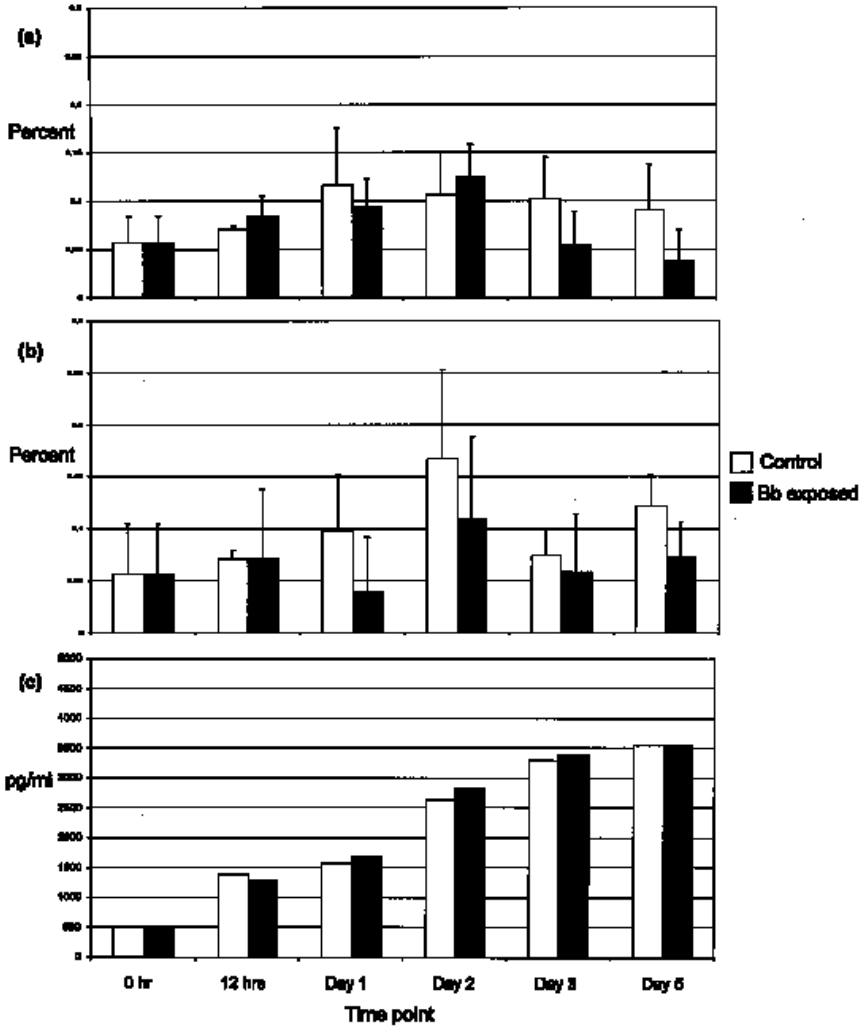
MIP-1alpha mRNA expression was assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**c**) after 12 hrs, 1 day, 2 days, 3 days and 5 days. In parallel, protein concentration of MIP-1alpha was measured by ELISAin the culture supernatant collected from *B. burgdorferi* strain Geho (b) and strain B31 (d) exposed samples. Results of the mRNA expression represent the means of three different consecutive experiments. ELISAdata is shown from one representative experimental series.

(**e**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative MIP-1alpha gene expression measured in synovial cells exposed to *B. burgdorferi* strains Geho and B31 over 5 days. Using serial t-test the differences of Geho and B31exposed cultures compared to their controls were significant (*p = 0.0044, *p < 0.00005, respectively).

human adult and fetal tissues normally do not express RANTES or contain only a few scattered RANTES positive cells (45). We found that RANTES mRNA and protein expression in synovial cells were upregulated by both *Borrelia burgdorferi* strains. This *in vitro* data suggests that RANTES expression might increase at both mRNA and protein level after Borrelia infection of the joint. This molecule is strongly proinflammatory (45). It has been associated with arthritis (46) and sarcoidosis (47). Conti *et al.* reported that RANTES is present in great quantities in synovial fluid of patients affected with inflammatory synovitis (48).

MCP-1 is chemotactic for monocytes, T cells, NK cells and basophils (49). The main function of MCP-1 in the joint may be the recruitment of macrophages, as injection of MCP-1 into rabbit joints resulted in a marked macrophage infiltration of the synovial tissue (49). Expression of MCP-1 at mRNA level was not changed and concentration of MCP-1 protein was elevated using strain B31. At both mRNAand protein levels MCP-1 was significantly downregulated using strain Geho. High levels of MCP-1 have been detected in synovial fluid from rheumatoid arthritis patients (49,50). In the present study, we could document that B31 and Geho are differentially inducing MCP-1 expression in synovial cells. This might reflect differences in the interaction of different strains of *B. burgdorferi* with resident host tissue cells especially in the joint. We found that MCP-2 gene expression was upregulated by both strains B31 and Geho.

MIP-1alpha is a member of CC chemokine superfamily and is chemotactic for T, B, NK cells, basophils and eosinophils (51). The present study showed that MIP-1alpha chemokine mRNA expression has been upregulated by both the strains B31 and Geho. This finding is similar to a recent *in vivo* study, which showed that MIP-1alpha plays a significant role in the process of neutrophil recruitment to sites of inflammation following stimuli including lipopolysaccharide (52) and TNF-alpha (53). An abundant amount of MIP-1-alpha was also found in rheumatoid arth-

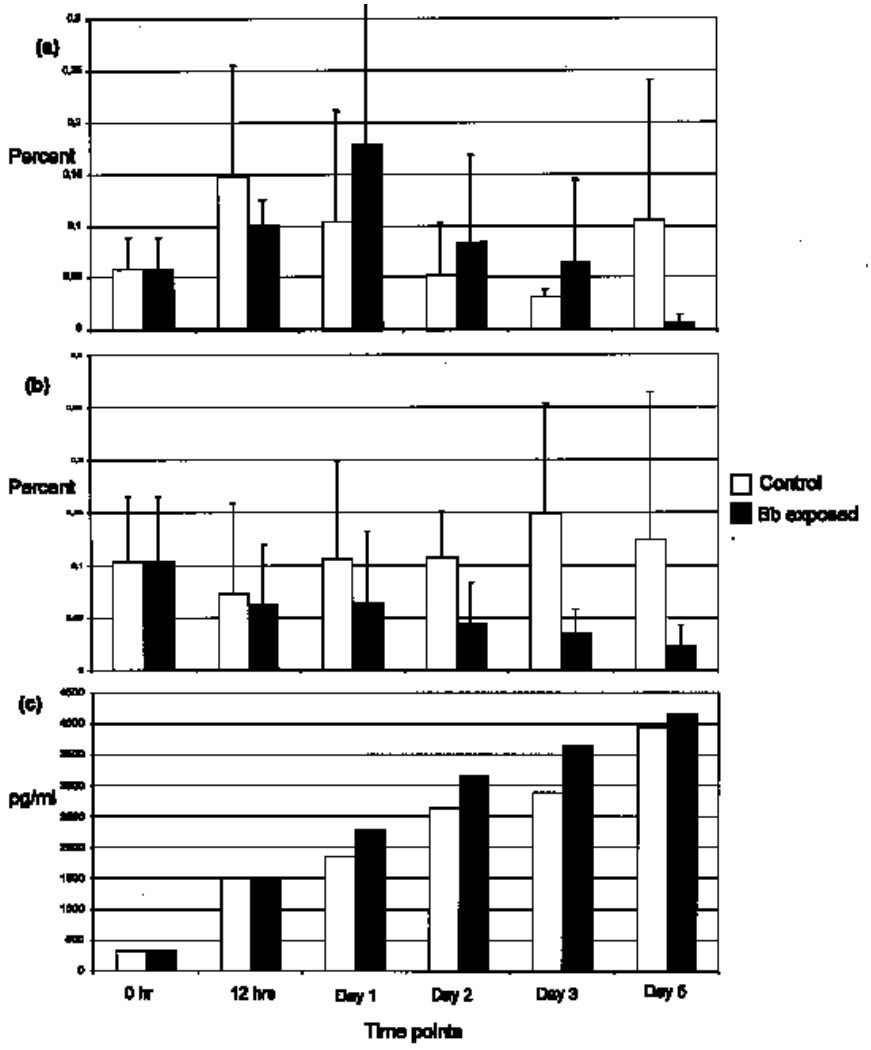


B. burgdorferi strain Geho

	Mean (%) (SDF-1α)	Standard deviation (SDF-1α)	Mean (%) (SDF-1β)	Standard deviation (SDF-1β)
Control	9.1	2.3	9.8 *	4.1
Exposed	7.6	3.2	6.8 *	2.4

Fig. 4. SDF-1alpha mRNA (**a**), SDF-1beta mRNA (**b**) and protein (**c**) expression by synovial cells exposed to *B. burgdorferi* strain Geho.

SDF-1alpha mRNAexpression (**a**) and SDF-1beta mRNAexpression (**b**) were assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho after 12 hrs, 1 day, 2 days, 3 days and 5 days. In parallel, protein concentration of SDF-1 (**c**) was measured by ELISAin the culture supernatant. Results of the mRNAexpression represent the means of three different consecutive experiments. ELISA data is shown from one representative experimental series. (**d**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative SDF-1alpha and SDF-1beta gene expression measured in synovial cells exposed to *B. burgdorferi* strain Geho over 5 days. Using serial t-test the differences of SDF-1alpha mRNAexpression in Geho exposed cultures compared to controls were not significant (p=0.21), whereas the differences of SDF-1beta mRNAexpression in Geho exposed cultures were significant (*p = 0.036) compared to control.



B. burgdorferi strain B31

	Mean (%) (SDF-1α)	Standard deviation (SDF-1α)	Mean (%) (SDF-1β)	Standard deviation (SDF-1β)
Control	8.4	4.3	11.5 *	2.5
Exposed	8.3	5.7	5.5 *	2.8

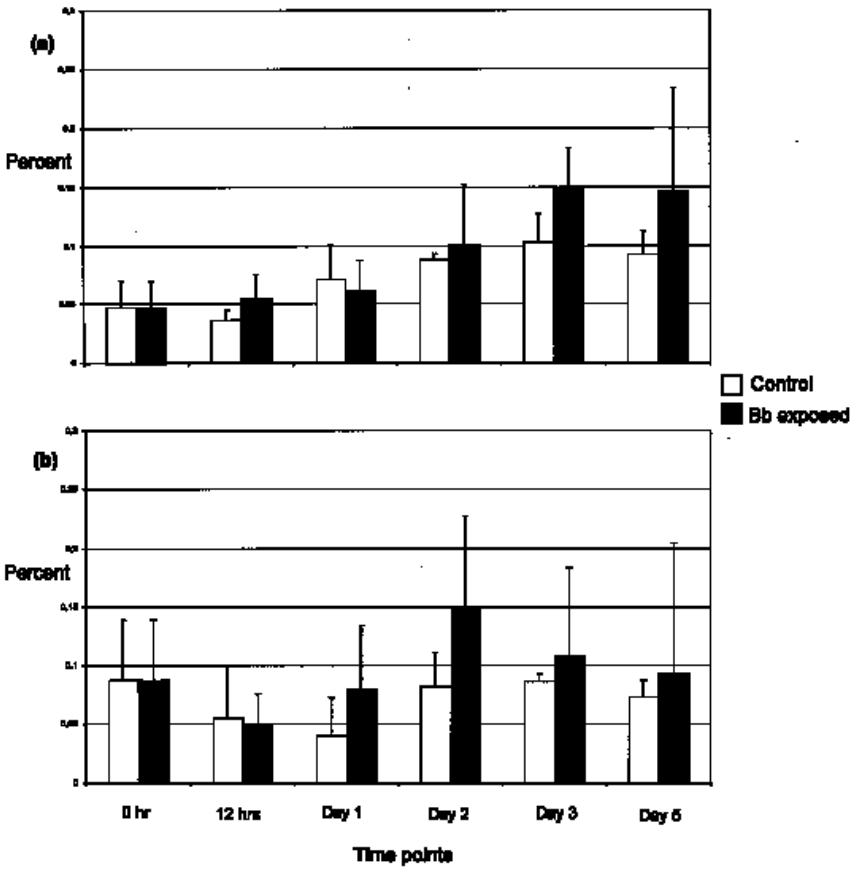
Fig. 5. SDF-1alpha mRNA (**a**), SDF-1beta mRNA (**b**) and protein (**c**) expression by synovial cells exposed to *B. burgdorferi* strain B31.
SDF-1alpha mRNAexpression (**a**) and SDF-1beta mRNAexpression (**b**) were assessed by semiquantitative RT-PCR, in addition to protein concentration of SDF-1 (c) was measured by ELISAin the culture supernatant before and after synoviocytes have been exposed to *B. burgdorferi* strain B31 after 12 hrs, 1 day, 2 days, 3 days and 5 days, as described in figure 4. The standard deviation of the exposed samples at day1 is 0.2298 (**a**).
(**d**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative SDF-1apha and SDF-1beta gene expression measured in synovial cells exposed to *B. burgdorferi* strains B31 over 5 days. Using serial t-test the differences of SDF-1alpha mRNAexpression in B31 exposed cultures compared to controls were not significant (p = 0.93), whereas the differences of SDF-1beta mRNAexpression in B31 exposed cultures were significant (*p = 0.018) compared to control.

ritis synovial fluid (54). Outer surface lipoprotein (Osp) or bacterial DNA of *B. burgdorferi* could act as a stimulatory factor for synovial cells in the present experiment. Even though mRNA levels were shown to be upregulated by both *B. burgdorferi* strains, MIP-1-alpha protein concentrations however were lower. This discordant mRNAand protein expression of MIP-1alpha might be due to reasons like a short half life of protein, poor mRNA stability and varied post translational mechanisms responsible for turning mRNA into protein (55, 56).
Stromal cell derived factor-1 (SDF-1), which belongs to the category of CXC chemokines (57), originally was identified as a growth factor for murine pre B cells (58). The chemotactic effect of SDF-1 is mediated specifically by the chemokine receptor CXCR4 receptor. It has been implicated in CD4+ T cell recruitment into rheumatoid arthritis synovium (33). It occurs in two alternative splice variants, SDF-1alpha and SDF-1beta. SDF-1 exhibits a chemoattractive activity for monocytes, neutrophils and early stage of B cell precursors (57,59). Even though we could demonstrate an intermittent upregulation of SDF-1 alpha especially by the arthritic strain B31, the overall SDF-1alpha and SDF-1beta gene expression seems to be suppressed by both Borrelia strains. In addition, protein concentrations were unchanged compared to controls. Thus, the SDF-1 chemokine expression appeared to be constitutive and it did not seem to be involved as a proinflammatory chemokine in our coculture model.
It is well known that microbial pathogens are able to subvert the host immune system in order to increase microbial replication and propagation (60). By this action microorganisms might escape the antimicrobial host responses. Altering the expression levels of chemokines can be achieved through the manipulation of transcription factors. For example, exposure with soluble factors produced by *Helicobacter pylorii* (61), *Bordetella pertusis* (62) or *Clostridium difficile* (63) resulted in the activation of transcription factors NF-κB and AP-1 in host cells. This can

result in the upregulation of IL-8. It is reported that OspA of *B. burgdorferi* is a potent stimulator of NF-κB nuclear translocation in endothelial cells (64). The lipid moiety of OspAwas essential for this activity (64).

The findings of the present study suggest that induction, in addition to suppression of different chemokines in synovial cells might alter the local environment in favour of the spirochetes. One mode of stimulation/suppression might be via borrelial lipoproteins and their interaction with CD14 and/or Toll like receptor 2 (65). The upregulation and downregulation of chemokines could be a form of exploitation of the chemokine system by Borrelia strains for different purposes like immune evasion. Immune evasion might lead to prolonged persistence of spirochetes in host tissues. The most obvious way in which microbes can take advantage of the chemokine system is to prevent chemotaxis of host leukocytes to allow immune evasion (60). This can be achieved either through preventing the inflammatory influx or biasing the influx to a less effective composition by downregulating the chemokine expression of the infected cells and tissues.

The exact mechanism by which *B. burgdorferi* induces or even regulates inflammation at the site of inflammation in the early stages of disease is not understood. The recruitment of proinflammatory cells, predominantly PMNs has been implicated in the development of arthritis. Our findings indicate that expression of stimulatory surface components of invading spirochetes might elicit inflammatory responses in synovial cells, resulting in an inflammation in the joint. When the intensity of induction and suppression of chemokines between the strains B31 and Geho were compared, a different pattern of induction and suppression of proinflammatory genes was noted. Two out of 4 chemokines were elevated in the supernatant with B31, whereas one of 4 chemokines was elevated using strain Geho (Table II). The latter, however, did show a more than 7 fold increase. Thus, the kinetic of differential expression of chemokines by synovial cells stimulated by different Borrelia strains could be important for the pathogenesis of early Lyme borreliosis. How these differing gene induction profiles relate to clinical aspects of synovial inflammation especially in later stages of disease will require further studies. In case of a favourable outcome of a spirochetal infection, indicated by clinical remission, the host's immune system ob-



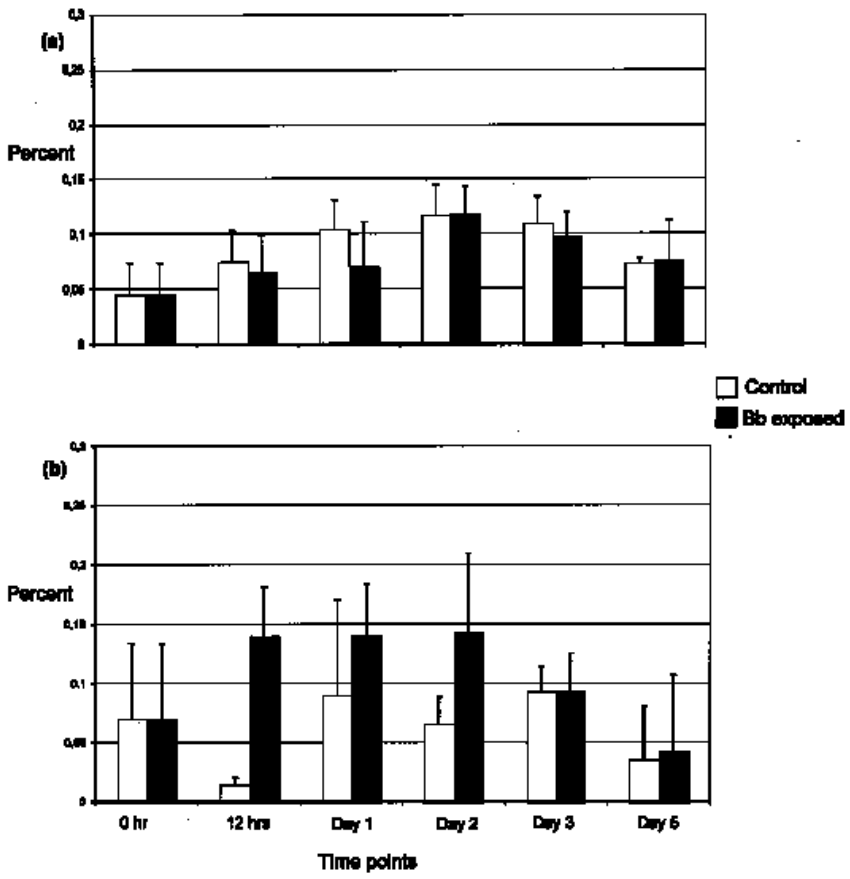
	B. burgdorferi strain Geho		*B. burgdorferi* strain B31	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
Control	7.3 *	2.7	7.1 *	1.9
Exposed	9.3 *	4.5	9.5 *	3.3

Fig. 6. MCP-2 mRNA expression by synovial cells exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**b**).

MCP-2 mRNA expression was assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**b**) after 12 hrs, 1 day, 2 days, 3 days and 5 days. Results of the mRNAexpression represent the means of three different consecutive experiments.

(c) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative MCP-2 gene expression measured in synovial cells exposed to *B. burgdorferi* strains Geho and B31 over 5 days.

Using serial t-test the differences of MCP-2 mRNAexpression in Geho and B31 exposed cultures compared to their controls were significant ($p = 0.0032$, *, $p < 0.00005$, *, respectively).



	B. burgdorferi strain Geho		*B. burgdorferi* strain B31	
	Mean (%)	Standard deviation	Mean (%)	Standard deviation
Control	8.7	2.8	6.2 *	3.1
Exposed	7.9	2.6	10.6 *	4.3

Fig. 7. IL-8 mRNAexpression by synovial cells exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**b.**)
IL-8 mRNAexpression was assessed by semiquantitative RT-PCR before and after synoviocytes have been exposed to *B. burgdorferi* strain Geho (**a**) and strain B31 (**b**) after 12 hrs, 1 day, 2 days, 3 days and 5 days. Results of the mRNA expression represent the means of three different consecutive experiments.
(**c**) Descriptive statistics (mean of expression in percentage and standard deviation of the mean) of the relative IL-8 gene expression measured in synovial cells exposed to *B. burgdorferi* strains Geho and B31 over 5 days. Using serial t-test the differences of IL-8 mRNAexpression in Geho exposed cultures compared to controls were not significant (p = 0.34), whereas the differences of IL-8 expression in B31 exposed cultures were significant (p = 0.0073, *) compared to control.

viously had been acting effectively to clear the pathogen. However, pathogenesis of manifested Lyme borreliosis might be triggered by strain specific properties either leading to neurological, dermatological or joint inflammation, which in part might be modulated by the pathogen itself.

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Table II. Comparative expression of chemokine mRNAexpression and protein secretion by synovial cells after exposure to *B. burgdorferi* strain Geho and B31 after 3-5 days.

	B. burgdorferi strain B31		*B. burgdorferi* strain Geho	
Chemokines	mRNA	Protein	mRNA	Protein
MCP-1	↑ (1.7)	↑ (1.4)	↓ (0.19)	↓ (0.39)
RANTES	↑ (1.9)*	↑ (1.3)	↑ (2.9)*	↑ (7.4)
SDF- 1 alpha+beta	↓ (0.5)*	– (1.1)	↓ (0.5)*	– (1.0)
MIP-1A	↑ (1.2)*	↓ (0.8)	↑ (2.6)*	↓ (0.7)

↑ Higher expression or concentration.
↓ Lower expression or concentration.

– No change in expression or concentration.
Numbers in brackets show the relation of up- or downregulation of mRNAexpression or change in protein concentration in the Borrelia exposed series at day 3 together with day 5 of exposure compared to the controls. This factor has been calculated as the mean percentage of mRNAexpression of exposed samples divided by the mean percentages of the controls of day 3 and day 5 together, in addition, as the mean of protein concentrations of the exposed samples compared to the mean concentration of the controls at day 3 together with day 5 of Borrelia exposed. The differences in mRNAexpression of the time periods 12 hrs to 5 days were expressed by using a serial t-test. The * indicates a difference of $p < 0.05$ when Borrelia exposed samples were compared to controls.

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