Cytokine and Chemokine mRNA Produced in Synovial Tissue Chronically Infected with *Chlamydia trachomatis* and *C. pneumoniae*

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ABSTRACT. ***Objective.*** We used a highly quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) assay system to define the steady-state levels of mRNA encoding a large panel of soluble mediators of inflammation in synovial tissues from patients with chronic arthritis infected with *Chlamydia trachomatis* versus *C. pneumoniae*.

Methods. RNA/cDNA was prepared from synovial biopsies of 4 patients with chronic arthritis and joint infection with *C. trachomatis*, 6 with *C. pneumoniae* at that site, 3 uninfected healthy controls, and 3 patients with undifferentiated oligoarthritis (UO) who were PCR negative for all organisms assayed. Real-time RT-PCR was used to assess relative mRNA levels from 12 cytokine and 2 chemokine genes (IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-γ, TNF-α, MCP-1, RANTES). Input loading was normalized to 18S rRNA. Data were obtained for each mRNA from each sample in triplicate in comparison to the same mRNA level in the controls.

Results. In most *C. trachomatis* infected synovial tissue samples, high levels of IL-10 mRNA were present, with less mRNA for IL-8, IL-15, IFN-γ, and TNF-α. Synovial tissues from chronic arthritis patients with synovial *C. pneumoniae* showed significant levels of mRNA solely for IL-8 and IL-1ß. All other cytokine messengers assessed in each sample from each patient group were at or near control level. One patient with *C. pneumoniae* showed a high transcript level for RANTES, and one patient with *C. trachomatis* showed a high transcript level for MCP-1. No patient with UO showed elevated messenger level for any cytokines assayed, but RANTES mRNA was elevated in each.

Conclusion. Our data suggest that while both *C. trachomatis* and *C. pneumoniae* have been associated with inflammatory joint disease, each elicits a somewhat different steady-state profile of mRNA encoding relevant cytokines and chemokines during chronic infection of synovial tissue. Precisely how these differing profiles relate to clinical aspects of synovial inflammation will require further study, but the observations confirm and extend data indicating potentially important differences in the pathobiology of these 2 bacterial species. (J Rheumatol 2002;29:1827–35)

Key Indexing Terms:
CYTOKINES — SYNOVIAL INFECTION
CHLAMYDIAE — TRANSCRIPTION

Synovial presence of any of several bacterial species can generate, or be associated with, inflammatory arthritis. By American College of Rheumatology diagnostic criteria[1], these organisms include the genital pathogen *Chlamydia trachomatis*, and increasing evidence from several laboratories suggests that *Chlamydia pneumoniae* may be involved in synovial pathogenesis in some individuals; however, this latter organism's contribution to joint disease has yet to be definitively established[2-4]. Inflammation of the joint elicited by antigens from *Chlamydia* is a function of the proinflammatory molecules produced in response to those antigens, and many groups have described one or a few relevant

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cytokines, or the mRNA encoding them, in synovial material from patients with *Chlamydia* associated arthritis and/or in various tissue culture models[5–9]. Most such reports, though, have focused on patients relatively early in the disease process[6]. Importantly, no study has examined simultaneously and in quantitative terms the production of a large panel of cytokines, chemokines, or their messengers in joint materials from patients chronically infected with *C. trachomatis* or *C. pneumoniae*, and to our knowledge none has provided a large scale, comparative, steady-state analysis of synovial inflammatory mediators elicited by these 2 related organisms during chronic infection. This information would be of significant value, since it would provide insight into an important aspect of host-parasite interaction for these pathogens during persistent synovial infection.

C. trachomatis and *C. pneumoniae* are both obligate intracellular bacterial pathogens, and as with all *Chlamydiae* they share many biological characteristics[10]. For example, both species undergo a similar biphasic developmental cycle ending with release of new elementary bodies via host cell lysis or exocytosis. Both species seem to prefer epithelial or epithelia-like cells as host, although both are capable of infecting other cell types both *in vivo* and *in vitro*. Under some circumstances, these organisms undergo persistent infection, i.e., longterm infection of host cells, and in this state both *C. trachomatis* and *C. pneumoniae* display aberrant morphology, unusual transcriptional characteristics, and other attributes not observed during normal active infection[11-15]. When these bacteria enter the persistent state *in vivo*, chronic diseases can result. For *C. trachomatis* these can include often severe reproductive dysfunction in women, as well as reactive arthritis (ReA) in both sexes[12,16,17]. For *C. pneumoniae*, asthmatic bronchitis, and possibly atherosclerosis and arthritis can result[2-4,18,19].

As suggested by this latter observation, the biology of *C. trachomatis* and *C. pneumoniae* does differ in several aspects, despite the common developmental cycle and other characteristics exhibited by both species. *C. trachomatis* is primarily a pathogen of the urogenital system in developed countries, while its sister-species is a respiratory pathogen; thus, transmission of the 2 to mucosal surfaces involves profoundly different routes[20]. Moreover, biological characteristics associated with the major outer membrane protein of each species appear to differ, as do various aspects of elementary body morphology, inclusion structure, etc[21]. We reported that some as yet unknown aspect of pathogenesis for *C. pneumoniae*, but not *C. trachomatis*, is enhanced in human host cells bearing the ε4 allele type at the *APOE* locus on chromosome 19[22]. For these and other reasons, the controversial suggestion has been made to reclassify *C. pneumoniae* into the new Genus *Chlamydophila*, rather than retaining it within *Chlamydia*[23].

As indicated, persistent synovial infection with *C. trachomatis*, and possibly *C. pneumoniae* as well, can elicit arthritis, although important aspects of the joint disease engendered by the 2 organisms differ[4,12]. For example, unpublished data from this group indicate that as many as 45% of patients with chronic ReA are polymerase chain reaction (PCR) positive for *C. trachomatis* in the joint. In contrast, a lower proportion of synovial samples was shown to be PCR positive for *C. pneumoniae*; indeed, our results indicated about 13% of synovial tissue samples, and even fewer synovial fluid samples, are so positive[4]. Importantly, while the extraarticular and other attributes of *C. trachomatis* associated arthritis are well established, we could identify no set of clinical characteristics uniquely associated with the synovial presence of *C. pneumoniae*. Because of these differences in clinical attributes and biology, because both *C. trachomatis* and *C. pneumoniae* are known to elicit a strong inflammatory response at sites of their residence, and because we have only limited information concerning host-parasite interaction for persistent *Chlamydiae*, we undertook a quantitative, comparative study of a large panel of cytokine and chemokine mRNA in synovial tissues from arthritis patients chronically infected with each of these organisms (but not both at once). For comparison, we included in the analyses synovial tissue samples from patients with undifferentiated oligoarthritis who had no identifiable bacteria in their synovia. The results reveal a major difference in the steady-state pattern of cytokine and chemokine messengers present in synovial tissue chronically infected by the 2 *Chlamydia* species, and both patterns differ significantly from those of PCR negative patients with undifferentiated oligoarthritis.

MATERIALS AND METHODS

Patient samples. DNA/RNA preparations from patient synovial tissues were selected for analysis from our extensive freezer library of such nucleic acid preparations on the basis of: (1) diagnosis of inflammatory arthritis plus PCR positivity for either *C. trachomatis* or *C. pneumoniae* but not both at once (see below); or (2) diagnosis of undifferentiated oligoarthritis plus PCR negativity in each of the *Chlamydia* directed PCR assay systems and in a pan-bacterial system[24]; all samples were also selected for analysis on the basis of their having adequate amounts of high quality RNA for assay and relatively long duration of disease. Biopsy samples were originally obtained from patients presenting at the Arthritis Clinics of the DVA Medical Center and the University of Pennsylvania Hospital, Philadelphia, PA, and the Hôpital Lariboisière, Paris, using the Parker-Pearson technique[25]. RNA preparations derived from synovial biopsies similarly obtained from healthy volunteers were used as controls; the original tissue samples were obtained with informed consent, according to an approved protocol at the NIAMS, National Institutes of Health, Bethesda, MD. At procurement, all samples were immediately snap-frozen at –70°C, transported to the laboratory, and subjected to nucleic acid preparation (see below). A summary of patient characteristics is presented in Table 1. Diagnoses were made according to American College of Rheumatology criteria where possible[1]; diagnoses of undifferentiated oligoarthritis followed published criteria[26].

Nucleic acid preparations. Total nucleic acids originally were prepared from synovial biopsies as described[4,27]. Each preparation was screened first

Table 1. Clinical information from patients.

Patient	Age/Sex	Diagnosis	Disease Duration, mo	Screening PCR Ct	Screening PCR Cpn	Primary rRNA*
Control						
1	55M	NV	NA	—	—	NA
2	55M	NV	NA	—	—	NA
3	26F	NV	NA	—	—	NA
C. trachomatis						
1	59M	ReA	60	+	–	+
2	45F	ReA	36	+	–	+
3	55F	ReA	30	+	–	+
4	46F	ReA	60	+	–	+
C. pneumoniae						
1	44M	UM	60	–	+	+
2	45F	ReA	60	_	+	+
3	33F	UO	4.5	_	+	+
4	71F	UO	30	_	+	+
5	28M	ReA	96	–	+	ND
6	52F	UM	Not available	_	+	ND
Undifferentiated†						
1	54M	UO	5	–	–	NA
2	44F	UO	4	–	–	NA
3	49F	UO	176	–	–	NA

* RT-PCR to assess primary transcripts from the bacterial rRNA operons; presence of such transcripts indicates viable, metabolically active organisms. See Materials and Methods.

† Patient samples screened extensively by PCR using a pan-bacterial primer system, as well as the *C. trachomatis* and *C. pneumoniae* directed primers. All samples were negative in all assays.

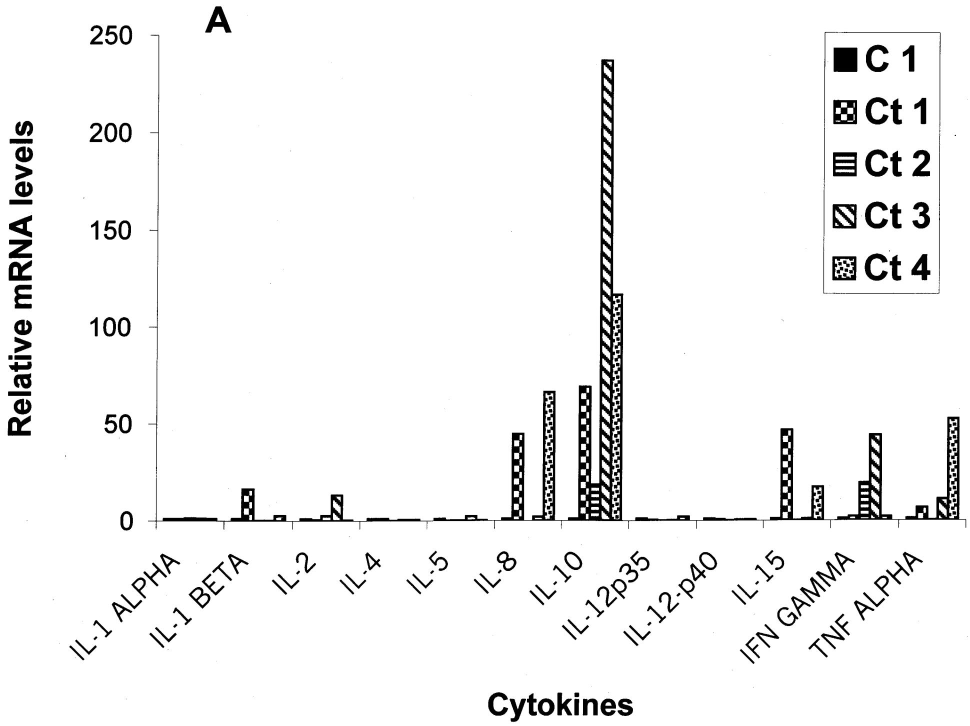
NV: normal volunteer; ReA: reactive arthritis; UM, UO: undifferentiated mono/oligoarthritis; NA: not applicable; ND: not done.

for chromosomal DNA of *C. trachomatis* and *C. pneumoniae* by PCR, as described[4,27]. Preparations positive in these assays were screened using assays targeting *Yersinia, Salmonella, Shigella*, and other relevant enteric organisms, and all were negative for these other organisms; samples from patients diagnosed with undifferentiated oligoarthritis were PCR negative in all assays (Table 1), including a pan-bacteria assay system[24]. Pure RNA was prepared from total nucleic acid preparations by treatment with DNaseI (Promega, Madison, WI, USA)[27]. Such preparations were confirmed DNA negative by PCR targeting the host ß-actin gene, in the absence of reverse transcription (RT). RT was done using 3 μg RNA from each patient sample incubated with MuLV reverse transcriptase (Life Technologies, Gaithersburg, MD, USA) and random hexamers as primers[28]. For most RNA preparations in which *Chlamydiae* were targeted, primary transcripts from the *C. trachomatis* and *C. pneumoniae* rRNA operons were assessed[19,27] to confirm viability and metabolic activity of the infecting organisms. Prior to analyses, confirmation of the quality of each cDNA preparation was assessed by standard PCR targeting the host actin gene.

Real-time transcript analyses. We used real-time RT-PCR[29-31] to simultaneously and quantitatively assess relative mRNA levels from 12 cytokine genes (IL-1α, IL-1ß, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-γ, TNF-α) in RNA/cDNA from synovial tissue. The control for comparison in the assays was RNA/cDNA from normal uninfected synovia (controls 1–3, Table 1). Real-time PCR on cDNA preparations was performed using the TaqMan Cytokine Gene Expression Plate™ (PE Biosystems, San Jose, CA, USA) as specified by the manufacturer. As supplied, this is a 96 well reaction plate arranged in 12 columns, one for each cytokine. Each column comprises 8 identical wells containing TaqMan primers and probes for assay of one human cytokine mRNA and an 18S rRNA endogenous control; probes used 6-carboxy-fluorescein as the reporter and 6-carboxy-tetramethyl-rhodamine as the quencher at the 5' and 3' ends, respectively. The primer/probe systems for the 12 cytokine cDNA are designed to amplify their target sequences with equal efficiency, allowing comparison among the various messenger levels in each sample. As assayed, each well contained 5 μl of cDNA, 1× Taqman buffer A, 5.5 mM $MgCl_2$, 200 μM dATP, dCTP, dGTP, 400 μM dUTP, 0.01 U/μl AmpErase, and 0.025 U/μl AmpliTaq Gold™ DNA polymerase in a total volume of 50 μl. Each well was closed with MicroAmp Optical caps after loading of reagents. For MCP-1 and RANTES mRNA, the SYBR green method for real-time RT-PCR analysis was used[31], and each assay was done in triplicate as for the cytokines given above; these assays were as described[11]. Primers for the assays were designed using GeneRunner™ software (Hastings Software, Hastings, NY, USA), and care was taken in the design to insure that each primer system amplified its target sequence with equivalent efficiency. The primers for MCP-1 were: 5'-gtcacctgctgctataacttc-3' and 5'-tgctgctggtgattcttcta-3' to generate an amplification product of 79 bp; primers for RANTES were: 5'-cctcgctgtcatcctcat-3' and 5'-acttgccactggtgtagaaa-3' to generate a 149 bp product. Amplification conditions for all assays were: 2 min at 50°C, 10 min at 95°C, and then 40 cycles of 95°C/15 s, 60°C/1 min. Amplification reactions were performed in a PE Biosystems model 7700 sequence detector. Assays for each cytokine and chemokine mRNA were run in triplicate for each sample, with triplicate controls; data were analyzed using the v. 1.7 software provided by PE Biosystems.

RESULTS

Steady-state cytokine and chemokine mRNA levels in C. trachomatis infected synovial tissue. We used real-time RT-PCR to quantitatively examine mRNA levels encoding 12 relevant cytokines in synovial tissue from each of 4 patients



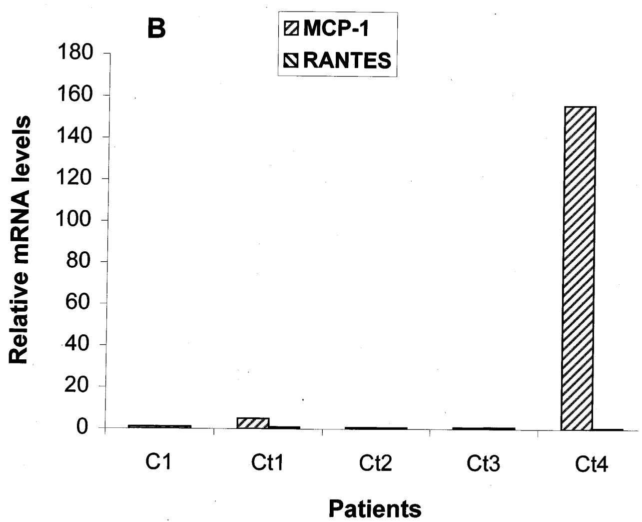


Figure 1. Relative levels of mRNA encoding 12 cytokines (Panel A) and the chemokines MCP-1 and RANTES (Panel B) in RNA preparations from synovial tissue from each of 4 patients with chronic arthritis infected with *C. trachomatis*. Total nucleic acids were prepared from patient samples, and RNA, then cDNA was made from those preparations. Patient designations as in Table 1; C1: control.

with chronic arthritis infected with *C. trachomatis* at that site. RNA from each patient sample was positive in RT-PCR assays targeting primary chlamydial rRNA transcripts, indicating viability of the organism in the synovial tissues from which the RNA was prepared (Table 1); *in situ* hybridization also was done on synovial tissues from each patient, confirming the PCR positivity of each sample for the organism (data not shown). Representative results of the comparative and highly quantitative cytokine transcript assays are shown in Figure 1A, as indexed to mRNA levels from the same cytokine genes in a synovial tissue sample from an uninfected control (designated C1). These data indicate that transcripts encoding IL-10 predominate in most of these infected patient samples. Indeed, in 3/4 samples showing the strongest level of transcript induction, IL-10 mRNA is increased 75 to nearly 250-fold (Patients Ct1, Ct3, Ct4). Two samples showed significant mRNA levels for IL-8 and IL-15 (Patients Ct1, Ct4), and other samples showed varying levels of messenger encoding IFN-γ and TNF-α. One patient sample showed an approximately 20-fold higher level of IL-1ß compared to control (Patient Ct2). Interestingly, none of these patients had significant mRNA specifying IL-12 subunits.

Enough cDNA remained from these *C. trachomatis* infected samples to perform real-time analyses for mRNA from the genes specifying MCP-1 and RANTES, both of which are important in modulation of an inflammatory reaction. Results of these assays are shown in Figure 1B. Only one sample (Patient Ct4) showed high transcript levels from the gene encoding MCP-1, but none of these samples had significant levels of RANTES mRNA.

Steady-state cytokine and chemokine transcript levels in C. pneumoniae infected synovial tissue. RNA/cDNA from 6 synovial tissue samples from chronic arthritis patients infected with *C. pneumoniae* also were analyzed using the comparative, highly quantitative real-time assay system targeting the 12 cytokine messengers; again, RNA preparations from 4 of these patient samples (Cpn1–Cpn4) were analyzed for primary rRNA transcripts from the organism, and each showed the presence of such transcripts, indicating viability and metabolic activity of the organism in synovial tissue at the time of biopsy (Table 1). Figure 2A shows representative results from the simultaneous cytokine mRNA assays, as indexed to congruent transcript levels in synovial tissue from an uninfected control (C2). In contrast to results for the chronically *C. trachomatis* infected synovial samples, the predominant cytokine transcripts in the *C. pneumoniae* infected joint tissues were IL-8 and IL-1ß. Because the assays in Figure 2A were not run simultaneously with those in Figure 1A, the 2 sets of results cannot be compared directly; however, the highest level of transcript induction for IL-8 and IL-1ß in the *C. pneumoniae* infected patients does appear to be lower than those for IL-10 in the *C. trachomatis* infected samples. That is, the highest level of induction for IL-8 mRNA was about 50-fold over that of control (Patient Cpn4); for IL-1ß, the highest level of induction was slightly less than 40-fold compared to control (Patient Cpn2). mRNA encoding IL-10 was essen-

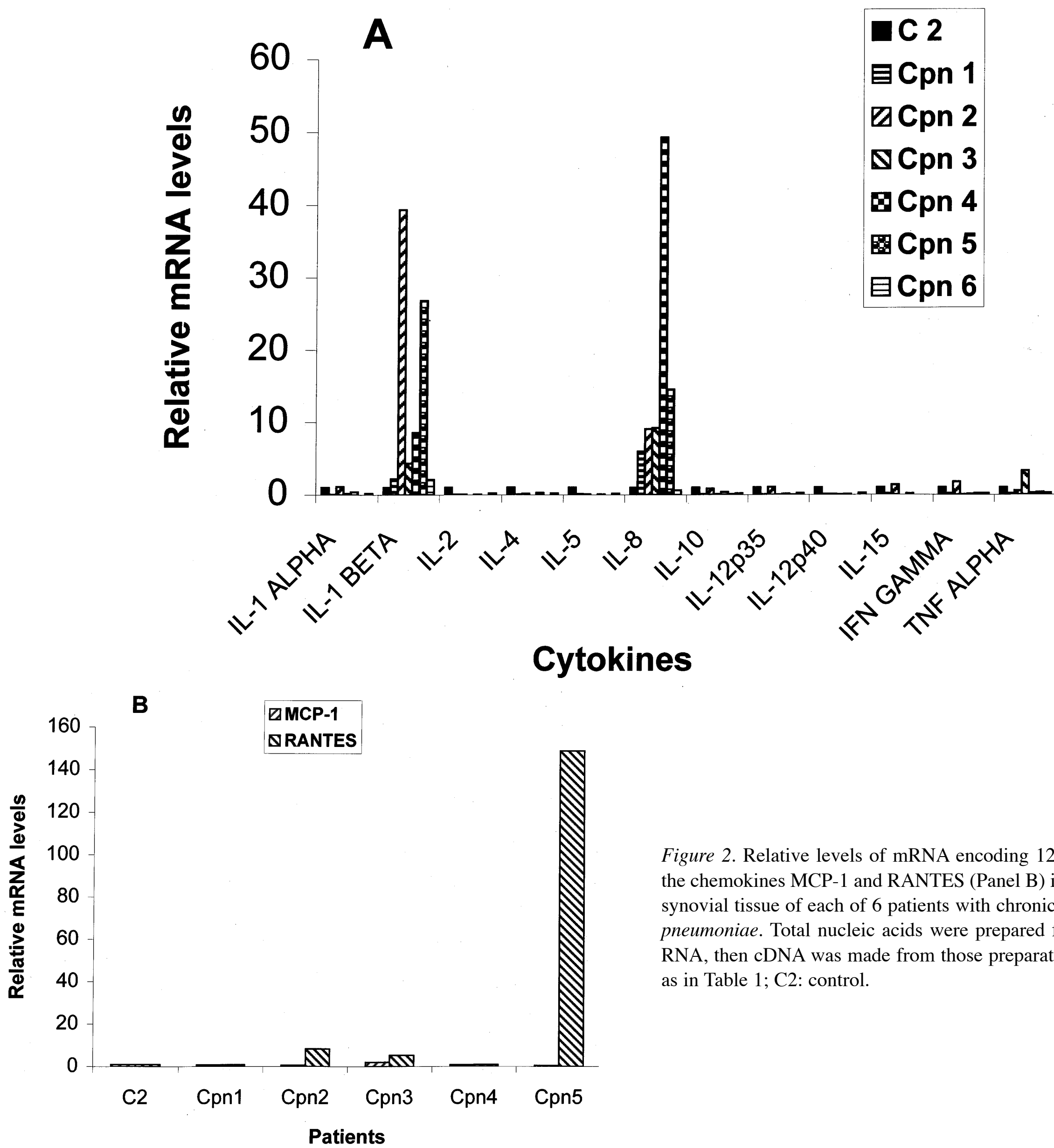


Figure 2. Relative levels of mRNA encoding 12 cytokines (Panel A) and the chemokines MCP-1 and RANTES (Panel B) in RNA preparations from synovial tissue of each of 6 patients with chronic arthritis infected with *C. pneumoniae*. Total nucleic acids were prepared from patient samples and RNA, then cDNA was made from those preparations. Patient designations as in Table 1; C2: control.

tially absent in all *C. pneumoniae* positive patient samples, as were transcripts encoding all other cytokines; one patient sample (Cpn3) showed a 3-fold increase in TNF-α mRNA.

cDNA remaining from 5 *C. pneumoniae* infected samples were subjected to real-time analyses for mRNA levels from the 2 chemokine genes. Results of these assays are shown in Figure 2B. Only one patient (Cpn5) showed a high transcript level from either gene, and this was for RANTES rather than MCP-1. Patients Cpn2 and Cpn3 showed low levels of RANTES mRNA, while Patient Cpn1 and Cpn4 showed essentially no transcripts at all from either chemokine gene.

Steady-state cytokine and chemokine transcript levels in PCR negative synovial tissue from patients with undifferentiated oligoarthritis. As an external control, we performed the same extensive real-time RT-PCR analysis on a set of 3 patient samples from individuals diagnosed with undifferentiated oligoarthritis; we chose these particular samples for study because they were PCR negative not only in the *Chlamydia* directed assays, but also in all PCR assays using a pan-bacterial primer system. As shown in Figure 3A, each of these patient samples showed mRNA levels for all the targeted cytokines that were essentially identical to the control. Similarly, none of these samples showed significant transcript level for MCP-1 (Figure 3B), although RANTES mRNA was elevated nearly 10-fold in one sample (Patient UO3) and 2–3-fold elevated in the other 2 samples.

DISCUSSION

Many methods have been used to define relative mRNA levels from various genes in patient or other samples, including Northern hybridization, RNase protection, and RT-PCR. The first 2 of these methods require relatively large amounts of RNA and thus are not usually suitable for analysis of small specimens. Standard RT-PCR systems have been used to analyze specific cytokine mRNA in synovium[6-8], but this method is only semiquantitative. Recently, real-time RT-PCR techniques have been developed that can overcome many of these limitations. Unlike standard endpoint analytical methods, real-time RT-PCR monitors amplification of target by fluorescence during the

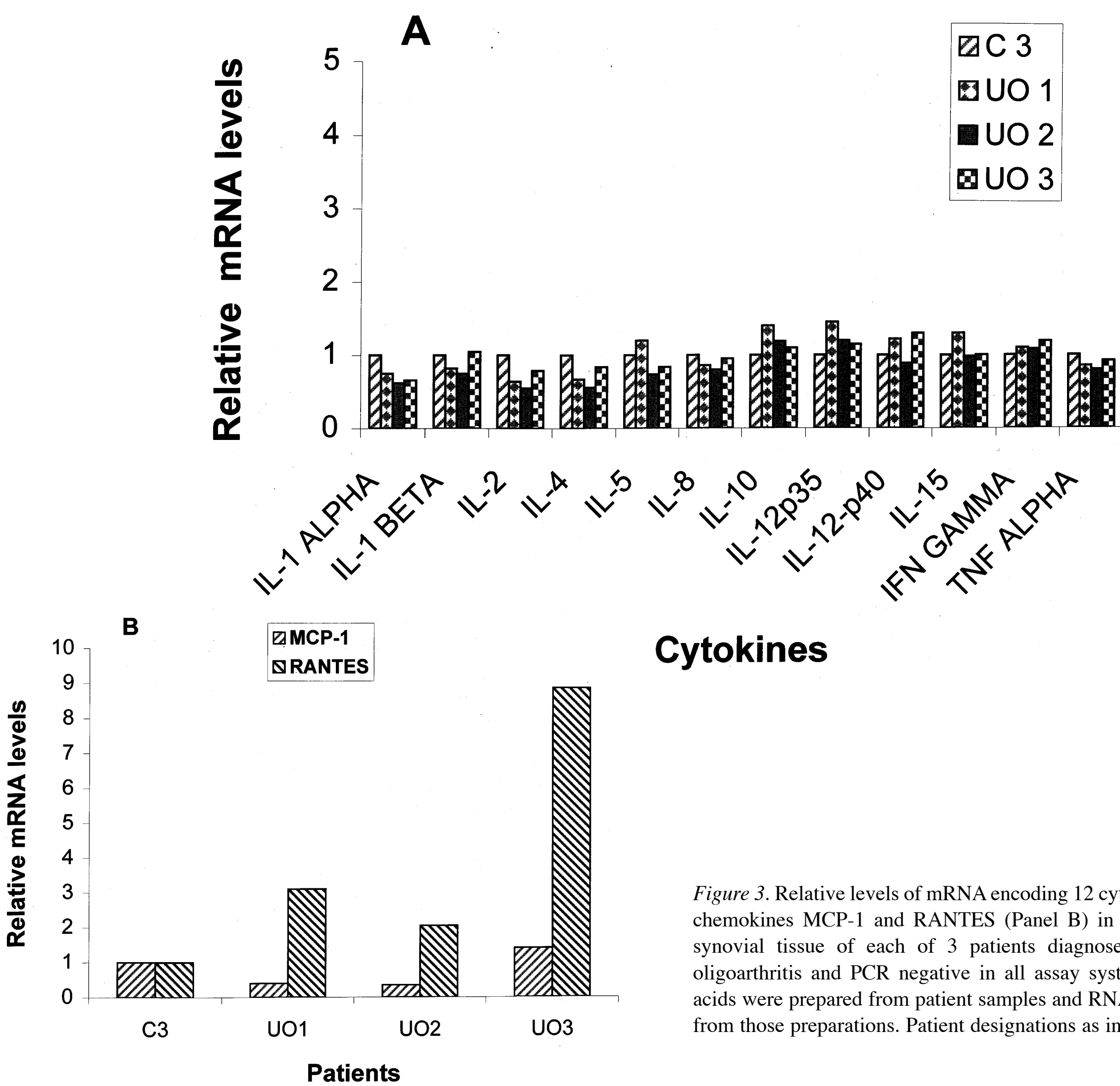


Figure 3. Relative levels of mRNA encoding 12 cytokines (Panel A) and the chemokines MCP-1 and RANTES (Panel B) in RNA preparations from synovial tissue of each of 3 patients diagnosed with undifferentiated oligoarthritis and PCR negative in all assay systems used. Total nucleic acids were prepared from patient samples and RNA, then cDNA was made from those preparations. Patient designations as in Table 1; C3: control.

earliest portion of the product accumulation curve, the portion in which that accumulation most closely approximates second-order kinetics[29-31]. Moreover, this technology allows rapid assessment of large panels of transcripts simultaneously in combination with cDNA from control preparations, permitting quantitative comparison of mRNA levels from many genes in several samples at once. We used real-time RT-PCR to simultaneously and quantitatively evaluate relative steady-state mRNA levels from 12 cytokine and 2 chemokine genes in synovial tissues from patients with chronic arthritis, 4 of which were infected with *C. trachomatis*, 6 with *C. pneumoniae*, and 3 whose synovial tissue had no detectable bacteria.

Analyses of the *C. trachomatis* infected patient samples showed that IL-10 mRNA levels were strongly elevated in the majority of these individuals; messengers encoding IL-8, IFN-γ, IL-15, and TNF-α mRNA were also elevated in some patients, although to a lesser extent than IL-10. In these samples, we found virtually no transcripts from the gene encoding RANTES, but one individual showed a high mRNA level from the gene specifying MCP-1. In patients infected with *C. pneumoniae* IL-8 transcripts were at high level, but no *C. pneumoniae* infected patient had significant mRNA encoding IL-10. Rather, transcripts for IL-1ß were elevated in these samples. In contrast to *C. trachomatis* infected patients, no individuals with synovial *C. pneumoniae* had significant mRNA specifying MCP-1; one patient had a high level of mRNA encoding RANTES. None of the PCR negative patients showed elevated transcript levels for any cytokine assayed or for MCP-1, but each showed an elevated mRNA level for RANTES.

At least in the limited number of patient samples we analyzed, both *C. trachomatis* and *C. pneumoniae* elicited to varying extent the production of IL-8 mRNA, encoding a chemoattractant molecule for neutrophils, but the steady-state level of messengers specifying this molecule appeared to be different between groups infected with the 2 organisms. It is of interest that, with the exception of IL-10 in the *C. trachomatis* group and IL-1ß mRNA in the *C. pneumoniae* group, transcripts specifying most cytokines other than IL-8 were at relatively low levels in synovia infected with these 2 related bacteria, particularly in those infected with *C. pneumoniae*. It is especially interesting that *C. trachomatis* but not *C. pneumoniae* strongly elicited the messenger specifying IL-10, an attenuator of inflammation and IFN-γ production, and that most macrophage derived cytokine mRNA were present in both infected patient groups only at quite low levels.

The data for chemokine production also reflect a differential profile between groups infected with these 2 pathogens. RANTES has been shown recently to be at higher level than MCP-1 in synovial fluid from patients with inflammatory arthritis[32], and our data for the 3 PCR negative patients and those PCR positive for synovial *C. pneumoniae* are consistent with that finding. However, the total lack of messenger specifying RANTES in all patients with synovial *C. trachomatis* indicates a difference in the details of host-parasite interaction between this organism and its sister-species, at least in the context analyzed. Indeed, we suspect that the differences observed here in steady-state mRNA production for both cytokines and chemokines reflect a difference, and probably an important one, in the details of pathogenesis engendered in the patients harboring persistent synovial *C. trachomatis* versus *C. pneumoniae*.

A good deal of variation exists among the patients in the relative level of mRNA from each of the cytokine and chemokine genes assessed; such variation probably results from differences in host genetic background, disease duration, etc. Nonetheless, it may be instructive to examine the cytokine profile observed in these experiments from individual patients in relation to the working diagnoses for those patients. Synovial tissues from patients Ct1, Ct3, and Ct4, individuals with ReA of long duration, each displayed powerful induction of IL-10 mRNA, with some induction of IFN-γ and TNF-α mRNA. Patients Ct1 and Ct4 also showed strong induction of IL-8 mRNA, and the latter patient had high levels of MCP-1 mRNA. The mixed induction of pro and antiinflammatory modulators in the chronically *C. trachomatis* infected patients is consistent with results of somewhat more limited cytokine studies published recently for patient materials[6,7,33], but they are not fully consistent with data from some *in vitro* studies[5,8]. We and others[9,12] suspect that in chronic *C. trachomatis* associated arthritis, especially in patients with disease of long duration in which the organism is metabolically active, the immunopathogenic response is induced to be mixed Th1 and Th2-type by the organism. Specifically, we suspect that persistently infecting *C. trachomatis* overtly induce production of the antiinflammatory IL-10, as do *Mycobacterium tuberculosis* and some viruses[34], thus helping to insure its own longterm survival in affected tissues. Along this line, it will be of interest to assess IL-10 levels in relation to those of proinflammatory molecules in patients with chronic *C. trachomatis* associated ReA during both active and quiescent disease.

In the patients infected with *C. pneumoniae*, in contrast, the situation for cytokine mRNA appears to be more internally consistent than for the *C. trachomatis* infected group. That is, each of the 6 patients whose synovial tissues were studied here had inflammatory arthritis, and most showed induction of IL-8 and IL-1ß mRNA, both of which encode proinflammatory molecules, although the relative level of induction of those messengers varied among patients. Patients Cpn3 and Cpn4, for example, each showed elevated transcripts from the IL-8 and IL-1ß genes, and in both patients the former predominated. In patients Cpn4 and Cpn5, however, both of whom showed significant mRNA induction from the same 2 genes, the pattern is different; i.e., in Patient Cpn4 IL-8 transcripts are the most strongly induced, while in Patient Cpn5 IL-1ß mRNA predominates. Of note, Patient Cpn5, with inflammatory arthritis of 96 months' duration, had a significant level of RANTES messenger, in addition to IL-8 and IL-1ß mRNA. Like MCP-1, RANTES is a CC group chemokine with chemoattractant activity for monocytes, basophils, eosinophils, and mast cells[35]. This molecule is strongly proinflammatory and has been associated with asthma[36], sarcoidosis[37], arthritis[38], and granuloma formation by *Mycobacterium bovis*[39]. *C. pneumoniae* has been associated with several of these clinical entities[18]. Moreover, important differences have been noted at the molecular level between the characteristics of chronic infection with *C. trachomatis* compared to C. *pneumoniae*[22] consistent with elicitation of RANTES mRNA by patients with the latter but not the former species.

At this point neither the clinical nor biological significance of these detailed differences in steady-state host-response characteristics of persistent synovial *C. trachomatis* and *C. pneumoniae* infection is clear. More study will be required to confirm whether the observations reported here, especially those relating to elicitation of IL-10 by persistent *C. trachomatis* and the lack of this mediator in patients persistently infected with *C. pneumoniae*, represent the situation in all or most cases of chronic *Chlamydia* infection of the joint. The approach employed in this study will undoubtedly prove to be useful to conduct such studies. Further, it will be important to elucidate the molecular mechanisms that govern host-parasite interaction during persistent synovial *Chlamydia* infection, since such information is likely to suggest new therapeutic approaches to the treatment of *Chlamydia* associated arthritis.

REFERENCES

1. Schumacher HR, Klippel JN, Koopman WH, editors. Primer on the rheumatic diseases. 10th ed. Atlanta: Arthritis Foundation Press; 1993.
2. Braun J, Laitko S, Treharne J, et al. Chlamydia pneumoniae — a new causative agent of reactive arthritis and undifferentiated oligoarthritis. Ann Rheum Dis 1994;53:100-5.
3. Hannu T, Puolakkainen M, Leirisalo-Repo M. Chlamydia pneumoniae as a triggering agent in reactive arthritis. Rheumatology 1999;38:411-4.
4. Schumacher HR, Gérard HC, Arayssi TK, et al. Chlamydia pneumoniae is present in synovial tissue of arthritis patients with lower prevalence than that of C. trachomatis. Arthritis Rheum 1999;42:1889-93.
5. Rothermel CD, Schachter J, Lavrich P, Lipsitz EC, Francus T. Chlamydia trachomatis-induced production of interleukin-1 by human monocytes. Infect Immun 1989;57:2705-11.
6. Kotake S, Schumacher HR, Arayssi TK, et al. IFN-γ, IL-10, and IL-12 p40 gene expression in synovial tissues from patients with recent-onset Chlamydia-associated arthritis. Infect Immun 1999;67:2682-6.
7. Yin Z, Braun J, Neure L, et al. Crucial role of interleukin-10/interleukin-12 balance in the regulation of the type 2 T helper cytokine response in reactive arthritis. Arthritis Rheum 1997;40:1788-97.
8. Rodel J, Straube E, Lungershausen W, Hartmann M, Groh A. Secretion of cytokines by human synoviocytes during in vitro infection with Chlamydia trachomatis. J Rheumatol 1998; 25:2161-8.
9. Braun J, Sieper J. Cytokines and the immunopathology of the spondyloarthropathies. Curr Rheumatol Rep 1999;1:67-77.
10. Hackstadt T. Cell biology. In: Stephens RS, editor. Chlamydia: intracellular biology, pathogenesis, and immunity. Washington: American Society for Microbiology Press; 1999:101-38.
11. Gerard HC, Krausse-Opatz B, Wang Z, et al. Expression of Chlamydia trachomatis genes encoding products required for DNA synthesis and cell division during active vs. persistent infection. Mol Microbiol 2001;41:731-41.
12. Inman RD, Whittum-Hudson JA, Schumacher HR, Hudson AP. Chlamydia-associated arthritis. Curr Opin Rheumatol 2000; 12:254-62.
13. Beatty WL, Morrison RP, Byrne GI. Persistent Chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. Microbiol Rev 1994;58:686-99.
14. Moulder JW. Interaction of Chlamydiae and host cells in vitro. Microbiol Rev 1991;55:143-90.
15. Byrne GI, Ouellette SP, Wang Z, et al. Chlamydia pneumoniae expresses genes required for DNA replication but not cytokinesis during persistent infection of Hep-2 cells. Infect Immun 2001;69:5423-9.
16. Kohler L, Zeidler H, Hudson AP. Etiologic agents in reactive arthritis: their molecular biology and phagocyte-host interactions. Baillieres Clin Rheumatol 1998;12:589-609.
17. Cates W, Wasserheit JH. Genital chlamydial infections: epidemiology and reproductive sequelae. Am J Obstet Gynecol 1993;164:1771-81.
18. Saikku P. Chlamydia pneumoniae — clinical spectrum. In: Stephens RS, Byrne GI, Christiansen G, et al, editors. Chlamydial infection. International Chlamydia Symposium, San Francisco 1998;145-54.
19. Gerard HC, Schumacher HR, El-Gabalawy H, Goldbach-Mansky R, Hudson AP. Chlamydia pneumoniae infecting the human synovium are viable and metabolically active. Microb Pathogen 2000; 29:17-24.
20. Schachter J. Infection and disease epidemiology. In: Stephens RS, editor. Chlamydia: intracellular biology, pathogenesis, and immunity. Washington: American Society for Microbiology Press; 1999:139-69.
21. Stephens RS. Genomic autobiographies of Chlamydiae. In: Stephens RS, editor. Chlamydia: intracellular biology, pathogenesis, and immunity. Washington: American Society for Microbiology Press; 1999:9-27.
22. Gerard HC, Wang GF, Balin BJ, Schumacher HR, Hudson AP. Frequency of apolipoprotein E (APOE) allele types in patients with Chlamydia-associated arthritis and other arthritides. Microbiol Pathogen 1999;26:35-43.
23. Everett KD, Bush RM, Andersen AA. Emended description of the order Chlamydiales, proposal of Parachlamyudiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic Genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol 1999;49:415-40.
24. Gerard HC, Wang Z, Wang GF, et al. Chromosomal DNA from a variety of bacterial species is present in synovial tissue from patients with various forms of arthritis. Arthritis Rheum 2001;44:1689-97.
25. Schumacher HR, Kulka JB. Needle biopsy of the synovial membrane: experience with the Parker-Pearson technique. N Engl J Med 1972;286:416-9.
26. Wollenhaupt J, Zeidler H. Undifferentiated arthritis and reactive arthritis. Curr Opin Rheumatol 1998;10:306-13.
27. Gerard HC, Branigan PJ, Schumacher HR, Hudson AP. Synovial Chlamydia trachomatis in patients with reactive arthritis/Reiter's

syndrome are viable but show aberrant gene expression. J Rheumatol 1998;25:734-42.
28. Harwood J, editor. Basic DNA and RNA protocols. Totowa, NJ: Humana Press; 1996.
29. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996;6:986-94.
30. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res 1996;6:995-1001.
31. Hiratsuka M, Agatsuma Y, Mizugaki M. Rapid detection of CYP2C9*3 alleles by real time fluorescence PCR based on SYBR green. Mol Genet Metabol 1999;68:357-62.
32. Conti P, Reale M, Barbacane RC, Castellani ML, Orso C. Differential production of RANTES and MCP-1 in synovial fluid from the inflamed human knee. Immunol Lett 2002;80:105-11.
33. Kotake S, Schumacher HR, Yarboro CH, et al. *In vivo* gene expression of Type 1 and Type 2 cytokines in synovial tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis. Proc Assoc Am Phys 1997;109:286-302.
34. Redpath S, Ghazal P, Gascoigne RJ. Hijacking and exploitation of IL-10 by intracellular pathogens. Trends Microbiol 2001;9:86-92.
35. Rossi D, Zlotnik A. The biology of chemokines and their receptors. Ann Rev Immunol 2000;18:217-42.
36. Knol EF, Roos D. Mechanisms regulating eosinophil extravasation in asthma. Eur Respir J 1996;22:136-40.
37. Iida K, Kadota J, Kawakami K, et al. Analysis of T cell subsets and ß chemokines in patients with pulmonary sarcoidosis. Thorax 1997;52:431-5.
38. Rathanaswami I, Hachicha M, Sadick T, et al. Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts: differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. J Biol Chem 1993;68:5834-9.
39. Chensue SW, Warmington KS, Allenspach EJ, et al. Differential expression and cross-regulatory function of RANTES during mycobacterial (type 1) and schistosomal (type 2) antigen-elicited granulomatous inflammation. J Immunol 1999;163:165-73.