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Functional CCR5 receptor protects arthritis patients from high synovial burden of infecting *Chlamydia trachomatis*

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Abstract

Objective—The CCR5 chemokine receptor occurs in a wild-type (wt) and a non-functional, deleted form ($\Delta 32$). Reports suggested that *Chlamydia*-induced reproductive tract pathology is attenuated in women bearing $\Delta 32$. We asked whether the mutation affects synovial prevalence/burden of *C. trachomatis*.

Methods—PCR defined CCR5 genotype in synovial tissue DNA from 218 individuals: 21 controls, 110 with reactive arthritis (ReA), 83 with undifferentiated oligoarthritis (UO), 4 with osteoarthritis (OA). Disease durations were 0.5–21 yr. Additional PCR assays defined the presence of *C. trachomatis* DNA. Bacterial load was assessed by real time PCR in selected samples.

Results—5 controls were wt/ $\Delta 32$, 16 were wt/wt; 2/21 controls (both wt/wt) were PCR-positive for *C. trachomatis*. 85 (44%) arthritis patients were PCR-positive for *C. trachomatis* (69 ReA, 16 UO). For ReA patients, 14 (13%) had wt/ $\Delta 32$, 10 (71%) of whom were PCR-positive. 19 UO patients (23%) were wt/ $\Delta 32$, with 1 (1%) PCR-positive. No differences existed for gender or other factors. 1 OA patient had wt/ $\Delta 32$. In ReA and UO samples, wt/ $\Delta 32$ heterozygotes had a 5–10-fold higher bacterial burden than did wt/wt patients ($p = 0.03$), regardless of diagnosis.

Conclusion—These results indicate that the wt/wt genotype is associated with attenuated synovial bacterial load compared to loads in wt/ $\Delta 32$ patients. While no alleles other than $\Delta 32$ were assessed, our data suggest that this allele provides little/no protection from ReA in chlamydiae-infected patients but it may provide some protection in UO patients. The basis of this possible differential effect of CCR5 genotype is under study.

Keywords

Chlamydia trachomatis; reactive arthritis; pathogenesis; inflammation; chemokine receptor

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Introduction

Current thinking holds that many rheumatic diseases result from interaction between infectious agents and various known or unknown aspects of host genetic background^{1,2}. For example, genital infection with the intracellular bacterial pathogen *Chlamydia trachomatis* is known to cause an acute inflammatory arthritis in approximately 5% of those infected; about half of those patients will progress to chronic arthritis³. Given that more than a million new genital chlamydial infections are reported to the Centers for Disease Control and Prevention each year in the US, *Chlamydia*-associated arthritis represents a significant number of both new and chronic patients. Further, reports indicate that 20–40% of individuals with inflammatory arthritis but no documented prior genital infection with *C. trachomatis*, and who are given a diagnosis of undifferentiated oligo- or mono-arthritis, are PCR-positive in synovial tissue and/or fluid for DNA from this organism^{e.g.}⁴. At this point, we have a poor understanding of why only some individuals develop arthritis following genital chlamydial infection, and we do not know why only a portion of those with acute disease progress to chronicity. However, it is clear that aspects of disease induction, severity, and chronicity are a function of the strain of the infecting *Chlamydia* and of as yet unknown properties of host genetic background⁵.

The CCR5 protein is a promiscuous chemokine receptor that occurs in two forms on human cells: a standard wild-type (wt) form and a truncated, non-functional form that results from a 32 bp deletion in the encoding gene, referred to as the $\Delta 32$ form⁶. It has been well established that CCR5 is a co-receptor on T cells for HIV-1, the virus causing AIDS. Individuals who are homozygous wt for the receptor are fully susceptible to infection with the virus, while $\Delta 32$ homozygotes cannot be infected; wt/ $\Delta 32$ heterozygotes can be infected but show long clinical courses^{6,7}. This receptor and its deleted form also have been associated with survivability and/or pathogenicity of several bacterial and viral infections, including plague caused by *Yersinia pestis*, although these associations remain controversial^{e.g.}^{8,9}. Interestingly, one recent report indicated that women with the CCR5- $\Delta 32$ mutation showed lower levels of tubal pathology during *C. trachomatis* infection than did women who lacked the mutation¹⁰. Because of these observations, we asked whether the CCR5 receptor allele type correlated with susceptibility to *Chlamydia*-induced arthritis, and/or with the bacterial burden in synovial tissue in patients with that inflammatory arthritis.

Patients and Methods

Patient samples

DNA prepared from synovial biopsies was analyzed as described below. These “freezer library” samples were procured over several years by HRS and JDC under approved protocols to study *Chlamydia*-associated arthritis. DNA preparations from 218 individuals were studied, including those from 21 normal control individuals. Patients had diagnoses of reactive arthritis (ReA, N=110), undifferentiated oligoarthritis (UO, N=83), and osteoarthritis (OA, N=4). Disease durations ranged from 0.5–21 yr (Table 1). All diagnoses were made according to current criteria of the American College of Rheumatology (ACR)¹¹. UO is not a definitive diagnosis. However, patients do occasionally present with an inflammatory arthritis that is difficult to classify. If these patients have 2–4 joints involved but do not meet the criteria for a specific type of inflammatory arthritis, then this is termed UO. Because patients with spondyloarthritis, such as ReA, often have an oligoarthritis these types of inflammatory arthritides are often grouped together.

Analyses of samples

PCR assays were used to determine the presence of chromosomal DNA from *C. trachomatis*, as described by us previously^{e.g.,12}. PCR assays also were used to define the presence/absence of the 32 bp deletion in the DNA sample from each patient and control studied. The primers employed for this latter purpose were: 5'-caaaaagaaggtcttcattacacc-3' and 5'-cctgtgcctcttcttcatttcg-3'. Products were displayed on standard 1% agarose gels stained with ethidium bromide. A first approximation of chlamydial load in PCR-positive DNA samples was determined by real time PCR targeting a sequence in the 16S rRNA gene of *C. trachomatis*, as described extensively by us; data were normalized to host 18S rDNA and calculated also as described previously¹³.

Statistical analyses

Statistical analyses were performed on the data in Tables 1 and 3. The Fishers Exact Test was utilized to determine the p-values on categorical data (Tables 1), and an unpaired t-test was used to determine the p-value of continuous data (Table 3).

Results

In screening assays, samples from 69 ReA patients (63%), 16 UO patients (19%), and 2 control individuals (10%) were PCR-positive for *C. trachomatis* DNA. No samples from OA patients were similarly PCR-positive.

In DNA samples from normal control individuals, 16/21 (76%) were homozygous wt for the CCR5-encoding gene (genotype wt/wt), and 5/21 (24%) were heterozygous for the $\Delta 32$ deletion allele (genotype wt/ $\Delta 32$). We identified no control samples homozygous for the deleted allele (genotype $\Delta 32/\Delta 32$). Both samples from control individuals that were PCR-positive for chlamydial DNA were wt/wt genotype for the CCR5 gene product (Table 2).

In DNA from ReA patients, 96/110 samples (87%) were wt/wt at the CCR5 locus, and 14/110 (13%) were wt/ $\Delta 32$. While DNA samples from 69/110 (63%) ReA patients were PCR-positive for chlamydial DNA, only 10 of these were wt/ $\Delta 32$ (14% of positives). For UO patient samples, 64/83 (77%) were wt/wt at CCR5, and 19/83 (23%) were heterozygous for the deleted form of the gene. 16/83 samples (19%) were PCR-positive for *C. trachomatis* DNA, but only 1 of these was from a patient with the wt/ $\Delta 32$ genotype (6% of positives). In the 4 OA patient samples, 3 were wt/wt and 1 was wt/ $\Delta 32$, and none were PCR-positive for *C. trachomatis* DNA. In none of the patient groups did we identify samples homozygous for the deleted allele ($\Delta 32/\Delta 32$) (Table 3).

Real time PCR was used to give an initial assessment of the bacterial burden in *C. trachomatis*-PCR-positive DNA samples from 15 wt/wt and 10 wt/ $\Delta 32$ ReA and UO patients. These were chosen for this quantitative analysis because each sample included sufficient high quality DNA to support this portion of the study; all samples so analyzed were from patients with disease duration of 12 mo or more. Interestingly, samples from patients heterozygous for the deleted gene showed bacterial burdens averaging 5–10-fold higher than did those of patients who were homozygous wild type at CCR5. That difference was statistically significant ($p = 0.03$) in these assays (Figure 1). The number of patients examined here was relatively small, of course, but the results do indicate that the average bacterial load among wt/wt ReA patients was higher than that of wt/wt UO patients (Table 4). In contrast, the average load in the wt/ $\Delta 32$ UO patients was higher than that of heterozygous ReA patients. More patient samples must be studied to confirm this trend.

Discussion

In this brief report we provide initial insight into the effect of genotype at the *CCR5*-encoding locus on disease association and bacterial load for *C. trachomatis* in synovial tissue from patients with *Chlamydia*-induced arthritis, and those with UO. The data presented suggest that presence of the 32 bp deletion in the gene in heterozygous individuals confers no protection from disease induction in patients with diagnosed ReA due to *C. trachomatis*, but it may provide some protection from induction of UO. In analyses not given here, we showed that presence of this deleted allele type does not influence relative bacterial load or induction of arthritis as a function of patient gender or patient age at first presentation. Yet another analysis suggested that presence/absence of the $\Delta 32$ allele does not affect relative synovial bacterial load in PCR-positive individuals having different disease durations.

The possible role of the wt vs. the deleted gene products from *CCR5* in various rheumatic diseases has been examined in contexts other than that assessed here. In the case of rheumatoid arthritis (RA), for example, it is generally accepted that presence of the deleted form of the receptor is protective, even in individuals heterozygous at that locus^{see 14–16}. However, the role of the deleted gene product in other rheumatic diseases is somewhat unclear at this point^{e.g., 16, 17}. In contrast to the situation for RA, the data presented here indicate that homozygosity for the wild-type allele is protective; that is, the wt/wt genotype is associated with lower bacterial loads in the patient samples examined. In this context, it is not clear why heterozygotes for the deleted allele should be at risk for higher pathogen burdens, nor is it clear that those with higher burden have more severe disease; however, the latter prediction does seem reasonable. As to the former question, we speculate that individuals having two copies of the wild-type allele may be able to mount a more effective inflammatory immune response to the pathogen than do individuals having and expressing only one allele encoding a functional receptor, with the other allele producing only a defective receptor. We currently are testing this possibility. Consistent with this contention, in experiments not shown here we assessed the relative level of attachment of *C. trachomatis* elementary bodies to homozygous wild-type cells for *CCR5* (HEp-2 cells) and those cells with expression of *CCR5* knocked-down by more than 70% via a standard siRNA method. In those experiments, we found no difference in attachment level in wild-type vs. the knock-down strain, indicating that *CCR5* does not function as a co-receptor for attachment of *C. trachomatis* to host cells.

Because of the nature of our previous studies, the patient samples included in our “library” were mostly from individuals with chronic, rather than acute, inflammatory arthritis^{e.g., 12}. It would be of interest to assess *CCR5* genotype longitudinally in a large group of individuals presenting for the first time with genital *C. trachomatis* infections but who have not (yet) developed arthritis, and to determine who among them develop acute, and then chronic inflammatory arthritis. Thus, we have initiated analysis of *CCR5* genotype in patients presenting at an STD clinic and who display signs and symptoms of genital chlamydial infection. To date, we have screened DNA from more than 150 individuals, and analysis of this large and increasing patient group will be ongoing for the foreseeable future.

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Figure 1.

Bacterial burden in wt/wt vs wt/Δ32 DNA preparations from patient samples. Real time PCR was used to assess the number of *C. trachomatis* chromosome copies/5 mg tissue in synovial biopsies from 15 wt/wt and 10 wt/Δ32 ReA and UO patients. Real time analyses were done as described in Patients and Methods, using primers and calculations referenced there. Bars are the average bacterial load for each genotype. The difference in bacterial burden between the two groups was statistically significant ($p = 0.03$)

Table 1

General characteristics of the patients studied

Diagnosis	Number	Gender	Disease Duration	Ct^a PCR+
Normal	21	8M/13F	(na)	2 (10%)
ReA ^b	110	53M/57F	0.5–21 yr	66 (60%) ^c
UO	83	53M/30F	0.8–72 mo	16 (19%) ^d
OA	<u>4</u>	<u>3M/1F</u>	<u>0.5–16 mo</u>	<u>0 (0%)</u>
	218	117M/101F		84 (39%)

^a Ct, *C. trachomatis*^b Abbreviations are: ReA, reactive arthritis, UO, undifferentiated oligoarthritis, OA, osteoarthritis^c p<0.0001 for ReA vs Normal^d p=0.5179 UO vs Normal; p<0.0001 ReA vs UO

Table 2*CCR5* genotype/PCR+ in control samples

Gender	wt/wt	wt/Δ32	PCR+	PCR+ wt/Δ32
13F	11 (84%)	2 (15%)	2 (15%)	0 (0%)
<u>8M</u>	<u>5 (63%)</u>	<u>3 (37%)</u>	<u>0 (0%)</u>	<u>0 (0%)</u>
21	16 (76%)	5 (24%)	2 (10%)	0% of total

Table 3*CCR5* genotype/PCR+ in ReA^a and UO^a samples

ReA^a				
Gender	wt/wt	wt/Δ32	PCR+	PCR+ wt/Δ32
F 53	45 (85%)	8 (15%)	32 (60%)	8 (100%) ^b
<u>M 57</u>	<u>51 (90%)</u>	<u>6 (11%)</u>	<u>37 (65%)</u>	<u>2 (33%)</u>
110	96 (87%)	14 (13%)	69 (63%)	10 (14% of positives/9% of total)
UO^a				
F 53	41 (77%)	12 (23%)	9 (17%)	1 (8%) ^c
<u>M 30</u>	<u>23 (77%)</u>	<u>7 (23%)</u>	<u>7 (23%)</u>	<u>0 (0%)</u>
83	64 (77%)	19 (23%)	16 (19%)	1 (6% of positives/1% of total)
OA^a				
F 3	3 (75%)	0 (0%)	0 (0%)	0 (0%)
<u>M 1</u>	<u>0 (0%)</u>	<u>1 (25%)</u>	<u>0 (0%)</u>	<u>0 (0%)</u>
4	3 (75%)	1 (25%)	0 (0%)	0 (0% of total)

^a Abbreviations as given in the legend to Table 1^b p=0.5642 PCR+wt/Δ32 vs PCR+wt/wt^c p=0.1026 PCR+wt/Δ32 vs PCR+wt/wt

Table 4Average load of *C. trachomatis* in ReA^a vs UO^a patients as a function of *CCR5* genotype^b

	<u>wt/wt</u>	<u>wt/Δ32</u>
ReA	1.8±0.5×10 ⁶	6.2±0.8×10 ⁶
UO	0.8±0.2×10 ⁶	12.4±2.8×10 ⁶

^a Abbreviations as given in the legend to Table 1^b Data presented as number of chlamydial chromosome copies/5 mg tissue.