Epidemiological studies on STIs in heterosexuals and MSM

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CHAPTER 5

EFFECT OF HIV AND CHLAMYDIA INFECTION ON RECTAL INFLAMMATION AND CYTOKINE CONCENTRATIONS IN MEN WHO HAVE SEX WITH MEN

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Submitted.
Chapter 5

ABSTRACT

OBJECTIVE AND DESIGN: Asymptomatic *Chlamydia trachomatis* (CT) infections are common in HIV-infected men having sex with men (MSM). Although CT with HIV likely enhances inflammation, the asymptomatic course suggests otherwise. We assessed local inflammation, mucosal damage and cytokine concentrations in rectal mucosal fluid from patients with HIV (with or without combination anti-retroviral therapy (cART)), with or without rectal CT.

METHODS: Rectal swabs from 79 MSM with and without CT, HIV, and cART, who reported receptive anal sex, were analyzed for neutrophil activation (myeloperoxidase [MPO]), mucosal leakage (albumin, alpha-2-macroglobulin) and pro-inflammatory and anti-inflammatory cytokines in rectal swabs.

RESULTS: CT infection, HIV infection and cART use in MSM had no differential effect on rectal neutrophilic inflammation and mucosal damage. IL-8 correlated with MPO, and MPO with markers of mucosal damage. In HIV-negative participants, men with CT had lower concentrations of MCP-1, IL-1α and IL-1RA compared to men without rectal CT (p=0.005, p=0.007 and p=0.07). We found no difference in anal cytokine concentrations in HIV-infected participants in relation to CT infection or cART use. In participants with rectal CT, those who were HIV-negative had lower median concentrations of IL-8 and IL-1α than those with HIV (p=0.05 and p= 0.06). The slope of the regression line between MPO and IL-8 was reduced in participants with rectal CT.

CONCLUSIONS: CT dampens cytokine concentrations, but not in HIV-infected patients. Mucosal damage was comparable in all patient groups. The apparent reduced neutrophil response to IL-8 in HIV-infected patients with CT is in accord with its asymptomatic course.
INTRODUCTION

Urogenital infection with *Chlamydia trachomatis* (CT) is the most commonly reported sexually transmitted infection (STI) and continues to be a major public health problem worldwide. Untreated CT infections can lead to serious complications, such as pelvic inflammatory disease in women and epididymitis in men (1). Asymptomatic CT infections are common, including in men who have sex with men (MSM) who are infected with human immunodeficiency virus (HIV). (2-5) This is thus a major clinical problem, since CT infection is regarded as an important co-factor in incident HIV infections among MSM. (6,7) CT infection is associated with increased HIV shedding, (8,9) and the treatment of CT urethritis reduces the seminal HIV-RNA concentrations in HIV-infected men. (10) The exact mechanisms by which CT infections increase viral shedding and why CT infections are often asymptomatic are not well understood. It has been suggested that sexually transmitted infections (STI), such as CT, enhance HIV transmission through increased release of pro-inflammatory cytokines, (8) attenuating the local mucosal immune defense.

Several studies have assessed concentrations of pro-inflammatory cytokines in CT infections and other STI. In vitro, CT has increased the concentration of cytokines in fibroblasts like synovial and epithelial cells. (11,12) Furthermore, in vivo increases in local pro-inflammatory and anti-inflammatory cytokines in CT infections were found in cervical-lavage specimens in women (13) and on urethral swabs in men. (14,15) Increased concentrations of cytokines were also detected in urine of men infected with *Neisseria gonorrhoeae* (NG) before the onset of symptoms, and they peaked at the onset of symptoms. (16)

Taken together, it is likely that HIV-infected patients co-infected with CT have more pronounced local inflammation than patients with either HIV or CT infection. In addition, HIV-infected patients, especially those who are not treated with combination antiretroviral therapy (cART), may have a decreased cellular immunity, (17) which could impede the adaptive immune response to CT and further aggravate inflammation. The enhanced inflammation, however, is not consistent with the
commonly asymptomatic course of CT in HIV-infected patients. To our knowledge, mucosal damage and the inflammatory and immune response of the rectal mucosa have not been previously studied. The aim of this exploratory study is to assess whether rectal CT infection, HIV infection and use of cART affect local markers of mucosal damage (leakage) and inflammation (neutrophil activation), as well as the concentrations of several pro-inflammatory and anti-inflammatory cytokines and chemokines (IL-8, MCP-1, MIP-1β, IL-1α, IL-1β, IL-1RA, IL-6 and IL-10) in the rectum of MSM who have had receptive anal intercourse.

**METHODS**

**Study population**

The outpatient STI clinic of the Public Health Service of Amsterdam (GGD Amsterdam) offers free and anonymous STI testing and treatment (18). From November 2010 to February 2011, consecutive MSM attendees of the clinic who reported receptive anal sex in the preceding 6 months and who were visiting the clinic for an STI screening were invited to participate in the study. Further inclusion criteria were an age of 16 years or more and sufficient understanding of the study in Dutch or English. Before routine consultation with the nurse, the study was explained, and the patient was invited to participate. After obtaining informed consent for participation, we asked HIV-infected patients for written approval to obtain data on CD4 cell count, HIV-RNA load, and cART use from the physician treating their HIV infection. Patients who declined to permit us to obtain data from their treating physicians were still allowed to participate. We aimed to select 90 participants from the total group who gave informed consent for cytokine testing. To study the effect of CT, HIV and cART on markers of inflammation and mucosal damage and on cytokine concentrations, we intended to recruit 15 participants in each of the following 6 groups:

- group A: CT-negative HIV-negative MSM
- group B: CT-negative HIV-infected MSM not receiving cART
- group C: CT-negative HIV-infected MSM receiving cART
- group D: CT-positive HIV-negative MSM
Chlamydia and rectal cytokine concentrations in MSM

- group E: CT-positive HIV-infected MSM not receiving cART
- group F: CT-positive HIV-infected MSM receiving cART.

Participants who were diagnosed with lymphogranuloma venereum, rectal gonorrhea, rectal herpes, anal warts, or non-CT proctitis were excluded. Also, participants who were diagnosed with syphilis or acute hepatitis B or C infection were excluded. For the analysis, we selected consecutive participants for each of the six groups. However, for groups consisting of HIV-infected MSM, we preferred those who permitted us to obtain data from the physician treating their HIV infection. Also, we preferred participants without any other STI except rectal CT. The medical ethics committee of the Academic Medical Center approved the study. All participants provided written informed consent.

Procedures and laboratory testing
All patients were screened for STI according to the clinic’s protocol (19). A nucleic acid amplification test (NAAT) (Gen-Probe Aptima Combo 2 Assay, Gen-Probe Incorporated, San Diego, USA) was used for the diagnosis of *Chlamydia trachomatis*. HIV screening was performed via a third-generation commercial microparticle enzyme immunoassay system (AxSym HIV Ag/Ab Combo; Abbott, Abbott Park, IL, USA). HIV-positive test results were confirmed by immunoblot (INNO-LIA HIV I/II Score; Innogenetics N.V., Gent, Belgium).

An additional anal swab for cytokine measurements was taken from all participants with a dry cotton tip swab before the standard rectal swabs for CT and NG tests. The swab was placed 5 cm into the rectum, twisted for 10 seconds, and placed in 1 mL phosphate-buffered saline (PBS; pH 7.4) at 4°C and cleared from debris by centrifugation at 260 g for 10 minutes within 24 hours of collection. Supernatant was stored in aliquots at -20°C until analysis.

The amount of mucosal-lining fluid on the swabs differed among participants. Albumin (Alb) is a relatively small molecule in the circulation, which passes the mucosal barrier relatively unrestricted. Thus, the concentration in the mucosal-lining fluid is almost in equilibrium with the concentration in the circulation (20), unless
there is leakage across the mucosal barrier, such as that caused by inflammatory damage. Consequently, the concentration of albumin (Alb) in the supernatant of the swab reflects the dilution of the mucosal-lining fluid. The $Q_{Alb}$ \( ([\text{Alb}]_{\text{supernatant}}/ [\text{Alb}]_{\text{serum}}) \) was calculated for each participant. We adjusted for the dilution of mucosal-lining fluid for each participant by dividing the observed values for the various parameters in the swab by $Q_{Alb}$. Alb was determined using nephelometry (modular pre-analytics evo [MPA]; Roche).

When there is substantial leakage across the rectal mucosa, $Q_{Alb}$ should not be used to adjust for dilution of the mucosal-lining fluid. To check whether there was substantial leakage, we also assessed alpha-2-macroglobulin (A2M). A2M is a large molecule in the circulation, and its passage across the mucosal membrane is more restricted than that of albumin, unless there is mucosal leakage (21). This mucosal leakage can be expressed as $Q_{A2M}$ \( ([\text{A2M}]_{\text{supernatant}}/ [\text{A2M}]_{\text{serum}}) \) or by comparison of the leakage of A2M with that of albumin in the relative coefficient of excretion ($RCE; Q_{A2M}/Q_{Alb}$; (22)). In case of dilution of the mucosal-lining fluid, the $Q_{Alb}$ and $Q_{A2M}$ are attenuated in a similar manner. In case of leakage across the mucosal barrier, the QA2M values will be close to those for Alb, raising the RCE. A2M was determined by enzyme-linked immunosorbent assay (ELISA) (21).

Myeloperoxidase (MPO) released by activated neutrophils in the supernatant was taken as a marker of neutrophilic inflammation and was measured by ELISA (lower detection limit, 1.5 ng/ml) (23). MPO values were adjusted for variable dilution by dividing by $Q_{Alb}$.

Concentrations of IL-8, MCP-1, MIP-1\(\beta\), IL-1\(\alpha\), IL-1\(\beta\), IL-1RA, IL-6 and IL-10 were analysed in supernatant with a multiplex fluorescent bead assay according to the standard protocol (BioRad Laboratories, Clinical Diagnostics Group, Hercules, CA, USA). Fluorescence was quantified with a Bioplex 200 (Bio-Rad Laboratories, Clinical Diagnostics Group, Hercules, CA, USA). Cytokine concentrations were adjusted for variable dilution by dividing by $Q_{Alb}$. 
Chlamydia and rectal cytokine concentrations in MSM

Statistical analyses
A value of half the lower limit of detection was assigned to samples with cytokine or MPO concentrations below the assay’s lower limit of detection. The effect of CT infection, HIV infection, and cART use on markers of mucosal damage and inflammation and on cytokine concentrations were analysed by the following comparisons:

- effect of CT infection: by comparing HIV-negative participants without and with CT infection (group A vs. D), and by comparing HIV-infected participants without and with CT infection (groups B+C vs. E+F)
- effect of HIV: by comparing HIV-negative and HIV-infected participants with CT infection (group D vs. E+F)
- effect of cART use: by comparing HIV-infected participants with CT infection without and with cART use (E vs. F)
- participants with self-reported anal symptoms vs. participants without self-reported anal symptoms

Differences between $Q_{A2M}$, $Q_{Alb}$, RCE, MPO and cytokine concentrations were calculated by the Wilcoxon test. No corrections for multiple testing were done. In scatter plots, fitted regression lines were calculated. We plotted $Q_{A2M}$ against $Q_{Alb}$ because a marked correlation would suggest no leakage across the rectal mucosa. We also plotted MPO against IL-8 for CT-negative participants and CT-positive participants to assess whether there were differences in MPO increases in reaction to IL-8 increases; IL-8 is a major chemokine for neutrophil recruitment and activation, and increases of IL-8 should correspond with increases in MPO.

Data analyses were performed with STATA 11.2 (STATA Intercooled, College Station, TX, USA). Differences were significant at P value ≤0.05 and near-significant at P value >0.05 and ≤0.1. In figures, a log10-scale was used. Adjusted values are presented, unless indicated otherwise.
RESULTS

Study population

Of 961 MSM who were screened for eligibility, 288 were excluded, because they reported not having had receptive anal sex in the 6 months preceding the study visit (flowchart, Figure 1). Of the 673 eligible MSM, 91 (13.5%) refused to participate. Of the remaining MSM, 79 consecutive participants were selected for groups A to F. The numbers in each group do not total exactly 15, as too few men fit the criteria for some groups (E & F) and some men ultimately had a different HIV and cART status than was initially reported (B & C). Participant characteristics are depicted in Table 1. MPO, Alb, A2M or cytokine concentrations could not be analysed in samples of three participants. Only five (6.3%) MSM had self-reported rectal symptoms, such as itching or discharge; four of the five were diagnosed with rectal CT infection.

Data on plasma HIV-RNA and CD4 cell count were missing for participants who were newly diagnosed with HIV (n=7) and for participants who declined to give permission for obtaining information about plasma HIV-RNA and CD4 counts from the physician treating their HIV infection (n=7). One HIV-infected participant had never received cART, but nevertheless, he had an undetectable viral load.
Chlamydia and rectal cytokine concentrations in MSM

Figure 1. Flowchart of participants included in study on rectal cytokines & Chlamydia trachomatis, Amsterdam, 2010-2011.

STI, sexually transmitted infections; CT, Chlamydia trachomatis; HAART, highly active antiretroviral therapy.
<table>
<thead>
<tr>
<th>Demographics</th>
<th>CT-</th>
<th>n (%)</th>
<th>CT+</th>
</tr>
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<tbody>
<tr>
<td>Age in years, median (IQR)</td>
<td>(n= 15)</td>
<td>(n= 13)</td>
<td>(n= 19)</td>
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<tr>
<td>Ethnicity</td>
<td>(n= 15)</td>
<td>(n= 8)</td>
<td>(n= 9)</td>
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<tr>
<td>Dutch</td>
<td>11 (73%)</td>
<td>11 (85%)</td>
<td>17 (89%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (27%)</td>
<td>2 (15%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Anal symptoms</td>
<td>No</td>
<td>15 (100%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
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<td>HIV clinical data</td>
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<td></td>
<td></td>
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<tr>
<td>HIV plasma viral load, copies/ml, median (IQR)</td>
<td>-</td>
<td>1930</td>
<td>20 (20-20)</td>
</tr>
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<td>-</td>
<td>1 (8%)</td>
<td>19 (100%)</td>
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<tr>
<td>≥50</td>
<td>-</td>
<td>5 (39%)</td>
<td>0</td>
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<tr>
<td>missing</td>
<td>-</td>
<td>7 (54%)</td>
<td>0</td>
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<td>CD4⁺ T cells (cells/mm³), median (IQR)</td>
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<td>735 (400-880)</td>
<td>534 (430-690)</td>
</tr>
<tr>
<td>CD4⁺ T cells (cells/mm³), &lt; 200</td>
<td>-</td>
<td>0</td>
<td>1 (5%)</td>
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<tr>
<td>≥ 200</td>
<td>-</td>
<td>6 (46%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>missing</td>
<td>-</td>
<td>7 (54%)</td>
<td>0</td>
</tr>
</tbody>
</table>

CT, *Chlamydia trachomatis*; MSM, men having sex with men; cART = combination antiretroviral therapy; * = based on 6 participants with available test results; †, based on 3 participants with available test results; ‡, based on 7 participants with available test results.
Markers of inflammation and mucosal damage (A2M, albumin, RCE and MPO)

Values for $Q_{A2M}$, $Q_{Alb}$, RCE and MPO concentrations for each group are shown graphically in Figure 2 and in numbers in Table Supplemental Content 1.

Markers of mucosal damage

There were no significant differences in $Q_{A2M}$, $Q_{Alb}$ and RCE between groups (Figure 2). The relationship between $Q_{A2M}$ and $Q_{Alb}$ in sampled mucosal fluids is shown in Supplemental Content 2. The correlation between $Q_{A2M}$ and $Q_{Alb}$ indicated no mucosal leakage and thus adjustment by $Q_{Alb}$ for dilution of mucosal-lining fluid was justified. Only one sample deviated markedly from the fitted line, which suggest that the mucosal barrier was reduced for this participant.

![Figure 2. Adjusted MPO concentrations and $Q_{A2M}$, RCE and $Q_{Alb}$ concentrations for each group in study on rectal cytokines & Chlamydia trachomatis, Amsterdam, 2010-2011. $Q_{A2M}$ and $Q_{Alb}$: values were multiplied by 1000. MPO, myeloperoxidase; A2M, alpha-2-macroglobulin; $Q_{A2M}$ ratio of A2M in supernatant to A2M in serum; $Q_{Alb}$ ratio of albumin in supernatant to albumin in serum; RCE, $Q_{A2M}/Q_{Alb}$; MPO concentration was adjusted by dividing by $Q_{Alb}$.

Chlamydia and rectal cytokine concentrations in MSM
Chapter 5

Markers of inflammation

Figure 2 shows the MPO concentrations for each group. There were no significant differences in MPO concentrations between groups. $Q_{a2m}$ and RCE correlated with MPO (not shown): spearman $\rho = 0.6$ and $\rho = 0.5$, respectively), suggestive of neutrophil activation causing mucosal damage.

Cytokine concentrations

Concentrations of pro-inflammatory and anti-inflammatory cytokines are shown by group in Figure 3, and differences between the groups below $p<0.1$ are shown in box plots. Numbers are shown in the table in Supplemental Content 1. Supplemental Content 3 shows a table with an overview of the differences between adjusted and unadjusted cytokine concentrations. Although adjustment of cytokine concentrations for dilution resulted in lower $P$ values, three out of the five unadjusted values already showed (near-)significant differences.

Influence of CT on cytokine concentrations

Among HIV-negative participants, median concentrations of MCP-1, IL-1$\alpha$ and IL-1RA were unexpectedly higher (at $P$ value $<0.1$) in those without rectal CT (group A) as compared to those with CT (group D). In HIV-infected participants, there were no differences in median concentrations of cytokines between those without and those with rectal CT infection.

Influence of HIV, and of cART, on cytokine concentrations

In participants with rectal CT infection, HIV-negative MSM (group D) appeared to have lower median concentrations of IL-8 and IL-1$\alpha$ than participants with HIV (groups E+F) (unadjusted $P=0.09$ and unadjusted $P=0.04$). Adjustment of concentrations for dilution resulted in similar differences ($P=0.06$ and $P=0.05$). There were no differences in median cytokine concentrations between HIV-infected participants with and without cART, irrespective of the presence or absence of CT infection.
Asymptomatic versus symptomatic patients

The median concentrations of IL-6 and IL-10 were higher in asymptomatic patients (n=74) than the median concentrations in patients with symptoms (n=5) (P=0.04 and P=0.01; adjusted IL-6 (0.8 vs. 0.2 pg/ml) and adjusted IL-10 (0.3 vs. 0.01 pg/ml)).

MPO and IL-8 concentrations

Since IL-8 is a major chemokine for neutrophil recruitment and activation, we assessed the relationship between MPO and IL-8 concentrations in men without and with CT (Supplemental Content 4), with different results. We found that increases of IL-8 corresponded with higher increases in MPO in MSM without CT (groups A, B and C) than in those with CT (groups D, E and F). In MSM with CT, however, more neutrophil activation was seen at low IL-8 concentrations.

Figure 3. Adjusted cytokine concentrations by group in study on rectal cytokines and Chlamydia trachomatis, Amsterdam, 2010-2011.

Cytokine concentrations were adjusted by dividing by Q_{Alb}. IL, interleukin; MIP, macrophage inflammatory protein, Q_{Alb}, ratio of albumin in supernatant to albumin in serum.
DISCUSSION

The most important finding in this study is that mucosal damage and neutrophil inflammation were not different between patients with or without CT, HIV and cART. Interestingly, CT infection was associated with a suppressed cytokine (IL-8, MCP-1, IL-1α and IL-1RA) expression in HIV-negative patients, but not in those infected with HIV (IL-8 and IL-1α). CT infection also was associated with an attenuated neutrophil response to IL-8, which may explain why there was no difference in inflammation and mucosal damage between the patient groups. To our knowledge, this is the first report of a study on rectal cytokine concentrations and rectal parameters of inflammation. We found that rectal CT infection in MSM is not paralleled by enhanced inflammation, which may explain why rectal CT infections are often asymptomatic.

**Influence of CT, HIV and cART on mucosal damage and inflammation**

In vitro and in vivo studies have suggested that CT infection in the urethra and vagina leads to increased release of inflammatory mediators (24-26), and thus, we expected an increased local inflammatory response and mucosal damage in participants with rectal CT infection. We used Q_{A2M/Alb} and RCE (Q_{A2M/Alb}) as markers of rectal mucosal damage and MPO concentration as a marker of the neutrophil inflammatory response. There were, however, no significant differences for these parameters, indicating no difference in mucosal damage between the patient groups. The correlation between Alb and A2M in rectal samples and the correlation between MPO and markers of mucosal damage underscore that these findings are genuine. Since mechanical stress can lead to inflammation (27), local mechanical stress in the rectum caused by receptive anal sex may have overshadowed differences in markers of mucosal damage and inflammation between the groups.

**Influence of CT, HIV and cART on cytokine concentration**

CT infection in HIV-infected MSM neither reduced nor increased cytokine concentrations. In HIV-negative MSM, however, we found reduced cytokine concentrations in those with CT infection as opposed to those without CT infection. These results are not due to the adjustment for dilution, since three out of the five
unadjusted values already showed (near-) significant differences. In previous studies, CT-infected epithelial cells were found to release more IL-1α (28), and CT infections of the urethra and cervix displayed increased IL-6, IL-8 and IL-10 concentrations (24-26,28,29). It is probable that the microbial load in the gastrointestinal tract is increased over that in the cervix and urethra (30), which may underlie the observed differences in inflammatory response to CT infection between the rectum and the cervix and urethra. An alternative explanation for the reduced cytokine concentrations in CT-infected HIV-negative patients is that CT infection induces indoleamine 2,3-dioxygenase (IDO) activity that inhibits immune responses (31). Since IDO expression is dependent on T-cell activation, a reduced T-cell response, as may occur in HIV-infected individuals, can prevent induction of the inhibitory effect of IDO. The lack of difference in cytokine concentrations between HIV-infected MSM with or without CT infection in our study supports that explanation.

In HIV-infected participants with rectal CT infection in our study, median cytokine concentrations did not differ between MSM who used cART and those who were not using it. This lack of difference is in contrast to a study in HIV-infected women without CT (32) that showed that concentrations of TNF-α, IL-6 and IL-1β in cervical lavages decreased after initiation of cART. Our results may also be explained by the absence of a difference in immune status. In our participants with and without cART, all had relatively high concentrations of CD4+ T cells; the median circulating CD4+ T cells were 670 and 460 cells/mm³, respectively.

**Asymptomatic versus symptomatic participants**

Most CT-infected participants (88%) were asymptomatic in our study. In another study, increased concentrations of cytokines were detected in the urine of NG-infected men before the onset of symptoms, and these concentrations peaked simultaneously with the onset of symptoms (16). The lack of symptoms in those with CT infection in our study might be explained by the fact that cytokine concentrations did not differ in HIV-infected MSM with and without CT infection and were reduced in HIV-negative MSM infected with CT. Another explanation is that the median concentrations of IL-6 and IL-10 were higher in asymptomatic participants. Since IL-6 and IL-10 can have...
anti-inflammatory effects, increased concentrations of these cytokines may lead to a decreased inflammatory response and, therefore, fewer symptoms.

A limitation of this study is the low number of participants in each group. As a result, the power of the study was limited, which means that we might have missed important differences. Since there were no data available on cytokine concentrations in rectal fluid, we performed no calculations for sample size. This was a pilot study, and we aimed to include 15 participants per group. In view of the dynamic range of most cytokines, strong effects on cytokine concentrations due to CT, HIV, or both would have been detected. No corrections for multiple testing were done. Since patients not receiving cART had a relatively high CD4+ T-cell count, the contrast between HIV-infected participants receiving cART and those not receiving cART was small. This may explain why we did not find a difference in cytokine concentrations between these groups.

Adjustment for dilution of the samples by Q_{ALB} might have resulted in over-adjustment or under-adjustment of results. However, as there were no significant differences in the measured parameters of mucosal damage and results were comparable without adjustment for dilution, overestimation or underestimation is not expected. We did not inquire about the most recent occurrence of receptive anal intercourse. Recent receptive anal sex might have caused local trauma, which might have affected local cytokine release, markers of inflammation, and mucosal leakage. It is possible that we did not find differences in inflammatory markers between the groups because all men may have had local trauma caused by receptive anal sex. Furthermore, we have no information about the estimated duration of the CT infection, which might have influenced our results.

In conclusion, levels of mucosal damage and neutrophil inflammation did not differ between patients with or without CT, HIV, and cART. CT infection was associated with a suppressed cytokine (IL-8, MCP-1, IL-1α and IL-1RA) expression in HIV-negative patients, but not in those infected with HIV (IL-8 and IL-1α). CT infection was also associated with an attenuated neutrophil response to IL-8. Thus, CT infection in MSM
Chlamydia and rectal cytokine concentrations in MSM

is not paralleled by enhanced inflammation, which may explain why CT infections are often asymptomatic. Further study is required to clarify the exact process by which CT escapes the mechanisms in the rectum that initiate inflammation and eradication of a pathogen.

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We would like to thank all participants and colleagues of the STI clinic of the Health Service of Amsterdam, especially Anne Rutte for inclusion of participants and sample handling and Martijn van Rooijen for data management. We would like to thank our colleagues at the Public Health Laboratory (Health Service Amsterdam) for STI testing, Barbara Dierdorp, Tamara Dekker and Marianne van de Pol (AMC, Depts. Experimental Immunology and Respiratory Medicine) for the determination of inflammatory parameters and cytokines, Ronald Geskus, Jan Prins and Ferdinand Wit for critical review of the manuscript, and Sally Ebeling for editorial assistance.
REFERENCES


Chlamydia and rectal cytokine concentrations in MSM


Chapter 5


Supplemental content 1. Unadjusted and adjusted rectal inflammatory markers and cytokine levels in 76 MSM included in study on rectal cytokines and *Chlamydia trachomatis*, Amsterdam, 2010-2011.

<table>
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<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>HIV-</td>
<td>HIV+ CART-</td>
<td>HIV+ CART+</td>
<td>HIV-</td>
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<td>86 (43-1503)</td>
<td>473 (32-1060)</td>
<td>365 (82-1133)</td>
</tr>
<tr>
<td>Adj MPO</td>
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<td>807 (154-2143)</td>
<td>555 (134-4700)</td>
<td>648 (192-1436)</td>
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<td>Q(_{A2M})</td>
<td>0.8 (0-0.2)</td>
<td>0.2 (0.1-0.3)</td>
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<td>0.5 (0.1-1.9)</td>
<td>0.4 (0.2-1.9)</td>
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<td>0.4 (0.2-0.9)</td>
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<td>239 (46-1371)</td>
<td>150 (90-1140)</td>
<td>579 (240-1188)</td>
<td>276 (57-995)</td>
</tr>
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<td>470 (286-4207)</td>
<td>1527 (325-4521)</td>
<td>516 (202-1555)</td>
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<td>MIP-1β</td>
<td>32 (670)</td>
<td>18 (7-41)</td>
<td>36 (21-77)</td>
<td>43 (7-85)</td>
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<td>38 (26-121)</td>
<td>92 (30-348)</td>
<td>71 (21-122)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>2 (0-5)</td>
<td>1 (0-3)</td>
<td>4 (2-8)</td>
<td>0 (0-4)</td>
</tr>
<tr>
<td>adj MCP-1(^b)</td>
<td>10 (4-21)</td>
<td>3 (1-4)</td>
<td>5 (3-21)</td>
<td>3 (07)</td>
</tr>
<tr>
<td>IL-1α</td>
<td>77 (46-668)</td>
<td>320 (81-419)</td>
<td>117 (21-535)</td>
<td>37 (9-119)</td>
</tr>
<tr>
<td>adj IL-1α(^b)</td>
<td>1394 (124-2419)</td>
<td>432 (246-2233)</td>
<td>416 (33-2507)</td>
<td>89 (39-304)</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>16683 (1999-65169)</td>
<td>8850 (4758-33684)</td>
<td>17749 (4033-27767)</td>
<td>5525 (3308-52558)</td>
</tr>
<tr>
<td>adj IL-1RA(^b)</td>
<td>32606 (29104-148719)</td>
<td>20527 (6188-62724)</td>
<td>26130 (5414-135718)</td>
<td>21311 (4973-50483)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>19 (8-114)</td>
<td>83 (21-195)</td>
<td>47 (18-340)</td>
<td>62 (11-384)</td>
</tr>
<tr>
<td>adj IL-1β(^b)</td>
<td>187 (13-456)</td>
<td>276 (252-1008)</td>
<td>171 (18-1918)</td>
<td>194 (54-272)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.1 (0.1-0.1)</td>
<td>0.1 (0.1-0.1)</td>
<td>0.1 (0.1-0.4)</td>
<td>0.1 (0.1-2.2)</td>
</tr>
<tr>
<td>adj IL-6(^b)</td>
<td>1.4 (0.3-6.7)</td>
<td>13 (0.5-2.3)</td>
<td>1.4 (0.3-6.7)</td>
<td>0.6 (0.3-4.0)</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.1 (0.3-0.3)</td>
<td>0.1 (0.3-0.3)</td>
<td>0.3 (0.0-4)</td>
<td>0.1 (0.0-4)</td>
</tr>
<tr>
<td>adj IL-10(^b)</td>
<td>0.6 (0.1-2.5)</td>
<td>0.3 (0.1-6)</td>
<td>0.2 (0.1-2.1)</td>
<td>0.2 (0.0-9)</td>
</tr>
</tbody>
</table>

\(^a\) Q\(_{A2M}\), Q\(_{Aβ}\), and RCE have no dimension; Q\(_{A2M}\) and Q\(_{Aβ}\): values were multiplied by 1000.

\(^b\) MPO and cytokine concentrations were adjusted by dividing by Q\(_{Aβ}\).

CT, *Chlamydia trachomatis*; MSM, men having sex with men; CART= combination antiretroviral therapy; IQR, interquartile range; MPO, myeloperoxidase; A2M, alpha-2-macroglobulin; Q\(_{A2M}\), ratio of A2M in supernatant to A2M in serum; Q\(_{Aβ}\), ratio of albumin in supernatant to albumin in serum; RCE, Q\(_{A2M}\)/Q\(_{Aβ}\); IL, interleukin; MIP, macrophage inflammatory protein; adj, adjusted.
Supplemental content 2. Scatter plot of $Q_{\text{A2M}}$ against $Q_{\text{Alb}}$ with regression line, in study on rectal cytokines & Chlamydia trachomatis, Amsterdam, 2010-2011.

For meaning of groups A through F see Methods. $Q_{\text{A2M}}$ and $Q_{\text{Alb}}$ values were multiplied by 1000.

A2M, alpha-2-macroglobulin; $Q_{\text{A2M}}$, ratio of A2M in supernatant to A2M in serum; $Q_{\text{Alb}}$, ratio of albumin in supernatant to albumin in serum.

Supplemental content 3. P-values of statistical differences in cytokine concentrations between groups, in study on rectal cytokines & Chlamydia trachomatis, Amsterdam, 2010-2011.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Compared groups</th>
<th>p-value</th>
<th>p-value adj. concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>D&lt;E+F</td>
<td>0.09</td>
<td>0.06</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCP-1</td>
<td>A&gt;D</td>
<td>0.2</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-1α</td>
<td>A&gt;D</td>
<td>0.04</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>D&lt;E+F</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>A&gt;D</td>
<td>0.7</td>
<td>0.06</td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Wilcoxon rank sum test was used for comparison of cytokine concentrations between groups. Differences between groups for unadjusted and adjusted cytokine concentrations are only shown when differences between groups for adjusted cytokine concentrations are below p < 0.1. Adjusted cytokine concentrations were adjusted by dividing by $Q_{\text{Alb}}$. < and > symbolize higher or lower concentrations respectively in the group following the symbol < or >. IL, interleukin; adj., adjusted; $Q_{\text{Alb}}$, ratio of albumin in supernatant to albumin in serum.
Supplemental content 4. Scatter plot of MPO against IL-8 and fitted lines, in study on rectal cytokines & Chlamydia trachomatis, Amsterdam, 2010-2011.

Figure shows scatter plot of MPO against IL-8 for study participants without CT (groups A, B and C) and with CT (groups D, E and F). IL-8 concentrations were adjusted by dividing by $Q_{Alb}$. MPO, myeloperoxidase; IL, interleukin; $Q_{Alb}$, ratio of albumin in supernatant to albumin in serum.