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Distinct peripheral versus mucosal T cell phenotypes in chlamydia-infected women

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Abstract

Problem—Differences in circulating (peripheral) and mucosal T cell phenotypes in chlamydia-infected women remain largely unknown.

Method of study—Thirteen paired mononuclear cell specimens from blood and cervicovaginal lavages collected from chlamydia-infected women were stained and analyzed using ten-color cell surface flow cytometry for T cell distribution, activation status, homing, and T helper (Th)-associated chemokine receptors (CKRs).

Results—A higher proportion of genital mucosal T cells were activated (CD38⁺HLA-DR⁺) and expressed CCR5 and Th1-associated CKR CXCR3⁺CCR5⁺ compared to peripheral T cells, but a lower proportion of mucosal T cells expressed homing CKR CCR7, Th-2 associated CKR CCR4, and CXCR3⁺CCR4⁺ for both T cell subsets.

Conclusion—T cell phenotypes differed in the peripheral vs. genital mucosa compartments in chlamydia-infected women. Since chlamydia infects mucosal epithelial cells, the finding of a higher frequency of activated T cells and Th-1 phenotypes in the mucosa likely reflects an adaptive immune response to infection.

Keywords

mucosal; peripheral; *Chlamydia trachomatis*; T cell; phenotype; chemokine; receptor

1 | BACKGROUND

Chlamydia trachomatis (CT) infection is highly prevalent and associated with adverse reproductive outcomes. Our limited understanding of the T cell phenotypes involved in CT

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

infection comes primarily from murine models. In particular, the concomitant expression of CXCR3⁺ and CCR5⁺ on CD4⁺ T cells has been shown in murine models to play a critical role in localizing protective effector T cells to the genital mucosa, and loss of either receptor impairs protective immunity [1]. A single human study by Ficarra et al. evaluated CD3⁺ T cell phenotypes in the peripheral blood and genital mucosa of women with uncomplicated CT infection, but did not differentiate CD3⁺ T cells into CD4⁺ or CD8⁺ T cells when evaluating homing and activation markers and they only evaluated a limited number of T cell phenotypes [2]. No study in CT-infected humans has comprehensively investigated CD4⁺ and CD8⁺ T cell phenotypes in the peripheral blood (the compartment where circulating lymphocytes reside) and genital mucosa (the site of active CT infection), which was the aim of our study.

2 | METHODS

2.1 | Ethics statement

This study was approved by the UAB IRB and the Jefferson County Department of Health. All subjects provided written consent prior to enrollment in the study.

2.2 | Study participants

Women presenting to a STD clinic in Birmingham, AL, USA for treatment of a positive urogenital screening CT nucleic acid amplification test were enrolled in a CT immune response study, in which they had blood collected for peripheral blood mononuclear cells (PBMCs) and a cervicovaginal lavage (CVL) for mucosal mononuclear cells (MMCs) and were treated with azithromycin 1g. Women co-infected with gonorrhea, syphilis, or HIV infection were excluded. In this study, we evaluated T cell phenotypes in 13 CT-infected women who had sufficient counts of both PBMCs and MMCs. The majority of the enrolled women were non-Hispanic African American, with an average age of 22.6 years. Only 3 of 13 (23.1%) did not have a history of prior CT infection and 8 of 13 (61.5%) reported use of hormonal contraception. There were 4 of 13 (30.8%) diagnosed with cervicitis and 1 of 13 (7.7%) with pelvic inflammatory disease. Vaginal co-infection with *Trichomonas* occurred in 1 of 13 (7.7%), with bacterial vaginosis in 3 of 13 (23.1%), and with candidiasis in 2 of 13 (15.4%).

2.3 | Mononuclear cell isolation

PBMCs and MMCs were isolated in accordance with the UAB Center for Clinical and Translational Sciences (CCTS) Specimen Processing and Analytical Nexus (SPAN) protocol for centrifugation through lymphocyte separation medium (Mediatech, Inc., Manassas, VA). PBMCs were then frozen (in 90% FBS+10% DMSO) in liquid nitrogen until later thawed for immune studies. Immune studies were performed on fresh viable MMCs, not frozen, due to the low recovery rate upon thawing frozen MMCs.

2.3 | Flow cytometry

PBMCs and MMCs were stained with fluorescent antibodies against cell surface markers and analyzed using 10-color flow cytometry by previously reported methodology [3]. Briefly, PBMCs and MMCs were labeled with LIVE/DEAD fluorescent reactive dye

(Invitrogen, Carlsbad, CA) and stained with surface antibodies against CD3-APCefluor780 (UCHT1; eBiosciences, San Diego, CA); CD4-Qdot 655 (S3.5) and CD8-Qdot 605 (3B5) (both from Life Technologies, Eugene, OR); CXCR3-APC-CD183 (1C6), CCR4 BV421-CD194 (1G1), CCR5-PE-Cy7-CD195 (2D7), CCR7-Alexa Fluor700-CD197 (150503), CD38-FITC (HB7) and HLA-DR-PE (L243) (all antibodies were purchased from BD Biosciences, San Jose, CA). Cells were then incubated for 30 minutes and centrifuged at 1200 rpm for 10 minutes. Both PBMCs and MMCs were fixed with 2% formalin before analysis. Using an LSR II flow cytometer (BD Immunocytometry Systems, San Jose, CA), CD3⁺ events were acquired from each sample. Data were analyzed using FlowJo software (v9.8.5, TreeStar, Ashland, OR). Lymphocytes analysis was based on forward and side scatter profiles after exclusion of dead cells (Fig 1). Gates were either set using negative sample or Fluorescence Minus One (FMO) control and were then applied to all samples from each participant.

2.4 | Statistical analysis

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.2 (SAS Institute, Cary, NC). Comparisons of T cell phenotypes between paired PBMCs and MMCs were done using the Wilcoxon signed-rank test.

3 | RESULTS AND DISCUSSION

We first investigated differences in the proportion of T cell subsets (CD4⁺ and CD8⁺) among the entire mononuclear cell population in the peripheral blood vs. genital mucosa. Our analysis revealed that the peripheral blood contained a greater percentage of CD4⁺ T cells when compared to CD8⁺ T cells, but in the genital mucosa, these T cell subsets were detected at similar frequencies (Fig 2A), although the proportion of both CD4⁺ and CD8⁺ T cells were lower in the mucosa compared to the periphery (CD4⁺: $P=0.0105$; CD8⁺: not significant [NS]) (Fig. 2A). It is unclear whether this is a normal difference in these T cell subsets between peripheral blood and the genital mucosa or if this is a change due to the presence of chlamydia. These results differ from those of Ficarra et al, who reported an equal percentage of CD4⁺ and CD8⁺ T cells at both the peripheral blood and endocervical sites. These differences could be due to the different sample collection site and methods. In our study, we collected mucosal samples from the cervicovaginal region by lavage, whereas in the Ficarra et al. study they sampled the endocervix using a cytobrush.

We next evaluated activation status of T cells with the hypothesis that mucosal T cells would be more activated than peripheral blood T cells, as CT is a mucosal pathogen. We found a higher proportion of T cells expressing the activation markers CD38 and HLA-DR as well their co-expression (CD38⁺HLA-DR⁺) at the genital mucosa for both CD4⁺ and CD8⁺ T cells (CD4⁺: $P=0.0266$, $P=0.0134$, $P=0.0007$; CD8⁺: $P=0.0005$, NS, $P=0.0012$) (Fig. 2B). Our HLA-DR finding is consistent with Ficarra et al. finding higher HLA-DR expression in mucosal CD3⁺ T cells; they did not evaluate CD38 [2].

We then evaluated expression of homing chemokine receptors (CKRs) at the peripheral blood vs. mucosal site anticipating a lower proportion of these CKRs at the mucosa due to down-regulation of these receptors upon arrival at the site of infection. We found lower

expression of CCR7, an important T lymphocyte homing and memory marker [4], in the genital mucosa for both CD4⁺ and CD8⁺ cells (both $P=0.0002$) (Fig. 2C). We found no significant difference in the expression of CXCR3, a marker of effector and memory cells that is also important in T cell trafficking, on CD4⁺ cells between compartments; however, CXCR3 expression on CD8⁺ cells trended towards being higher in the peripheral blood ($P=0.0771$) (Fig. 2C). We found expression of CCR5, an inflammatory homing marker that influences trafficking, to be higher in the genital mucosa for both CD4⁺ and CD8⁺ T cells ($P=0.0266$ and $P=0.0105$) [2]. Our finding of lower CCR7 expression and higher CCR5 expression in the genital mucosa for both CD4⁺ and CD8⁺ T cells is consistent with the Ficarra et al. findings showing the same differences in CD3⁺ T cells; they did not evaluate CXCR3 expression [2]. Overall, our evaluation of homing markers suggests CCR5 but not CXCR3 in humans may be a key marker for homing of effector T cells to the genital tract for an adaptive immune response against CT. While CCR7 is an important homing and memory marker [4], our finding of a low frequency of CCR7 expression at the genital mucosa is consistent with the idea that CCR7 is down-regulated once cells exit the lymphatic system and enter mucosal tissues, perhaps due to a shift towards these cells becoming tissue resident memory (Trm) T cells. Due to the limited number of fluorescence channels in our flow cytometer and limited number of viable mucosal mononuclear cells, we were unable to test additional markers that could further differentiate memory cell populations (CD45RA and CD62L) and also markers that allow us to evaluate for Trm T cells; this is a future research interest.

Finally, we investigated T helper (Th)-associated phenotypes, not studied by Ficarra et al. [2], to assess whether T cells preferentially polarized towards Th1 or Th2 phenotypes in peripheral blood and the genital mucosa, anticipating a higher expression of Th1-associated CKRs in both compartments, given that murine studies have shown CD4⁺ Th1 responses are essential for immunity to chlamydia [1]. Our data, on the contrary, demonstrated a higher expression of Th2-associated CKR CCR4⁺ in the peripheral blood (CD4⁺: $P=0.0002$) (Fig. 2D), which is in agreement with findings from a study by Miguel et al. [5] that showed that CT-infected women mounted a robust Th2 response. However, we found higher expression of Th1-associated CXCR3⁺CCR5⁺ in the genital mucosa (CD4⁺: $P=0.0046$), suggesting CD4⁺ T cells may polarize from Th2 to Th1 phenotypes once they arrive at the mucosa, the site of active infection. We observed a similar trend in expression of these markers in CD8⁺ T cells (CCR4⁺: $P=0.0002$; CXCR3⁺CCR5⁺: $P=0.0171$) (Fig. 2D). The comparable expression of CCR4 and CXCR3 CCR5 for both CD4⁺ and CD8⁺ T cells suggests that both of these T cell subsets may be involved in response to CT infection. We also found a higher expression of CXCR3⁺CCR4⁺ T cells in the peripheral blood (CD4⁺ and CD8⁺ both $P=0.0002$) (Fig. 2D); this T cell phenotype has been previously reported to be expressed on regulatory T cells in inflamed human liver tissue [6] and in synovial fluid of patients with juvenile idiopathic arthritis [7], suggesting it may regulate Th cell polarization and/or could contribute to immunopathology in CT infection.

4 | CONCLUSIONS

In summary, our investigation of differences in T cell phenotypes between peripheral blood and the genital mucosa of CT-infected women revealed a greater proportion of activated T

cells at the genital mucosa, a lower expression of the homing and memory marker CCR7 and higher expression of homing marker CCR5 in the mucosa, and more frequent polarization towards a Th2-associated phenotype in the blood vs. Th1-associated phenotype in the genital mucosa. Thus, T cell phenotypes are distinct at the peripheral blood vs. genital mucosa sites in women with CT infection. This highlights the importance of studying both peripheral and mucosal compartments in CT infection and our findings provide knowledge that will be important in future studies aimed at conducting a time-course analysis of T cell phenotypes from the time of CT infection to clearance and evaluating CT-specific function of these specific T cell phenotypes.

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Fig 1. Gating strategy used to analyze CD4+ and CD8+ T cells

Lymphocytes were first gated based on forward scatter (FSC) and side scatter (SSC). Single cells were then gated first by SSC-A vs. SSC-H and then by FSC-A vs. FSC-H, before gating for CD4+ or CD8+ T cells by selecting for CD3+CD4+ and CD3+CD8+ populations. Both CD4+ and CD8+ T cells were then subsequently gated for the expression of activation and chemokine markers.

Fig. 2. Differences in T cell phenotypes from the peripheral blood vs. genital tract mucosa in chlamydia-infected women

a–d Scatter-bar plots comparing mucosal (M, n=13 [closed circles]) vs. peripheral (P, n=13 [open circles]) T cell phenotypes. **a** T cell distribution of both T cell subsets; **b** Activation markers CD38+HLA-DR+; **c** Homing CKRs CCR5+, CCR7+, CXCR3+; **d** T helper associated CKRs CCR4+ (Th2-associated), CXCR3+CCR5+ (Th1-associated), CXCR3+CCR4+ expressed on both CD3+CD4+ (left) and CD3+CD8+ (right) T cell subsets. Findings show that at the genital tract mucosa in chlamydia-infected women, there is enhanced T cell activation along with a higher expression of CCR5, a lower expression of

CCR7, and association with a Th1 phenotype; in the peripheral blood, Th cells expressed predominantly a Th2-associated phenotype. * $P \leq 0.05$, ** $P < 0.001$, *** $P < 0.0001$, or NS (not significant) by the Wilcoxon signed-rank test. Data are represented as median and interquartile range (IQR).

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