Immune Profiling of Pregnant Toxoplasma-Infected US and Colombia Patients Reveals Surprising Impacts of Infection on Peripheral Blood Cytokines

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In North America (NA) and Europe, the majority of toxoplasmosis cases are benign and generally asymptomatic, whereas in South America (SA) toxoplasmosis is associated with much more severe symptoms in adults and congenitally infected children. The reasons for these differences remain unknown; currently, there is little information from patients in either region on how the immune system responds to infection with Toxoplasma gondii. Here, we report the relative abundance of 51 serum cytokines from acute and chronic toxoplasmosis cohorts of pregnant women from the United States, where approximately one-half of clinical isolates are Type II, and Colombia, where clinical isolates are generally “atypical” or Type I-like strains. Surprisingly, the results showed notably lower levels of 23 cytokines in acutely infected patients from the United States, relative to uninfected US controls. In acutely infected Colombian patients, however, only 8 cytokine levels differed detectably with 4 being lower and 4 higher relative to uninfected controls. Strikingly, there were also differences in the cytokine profiles of the chronically infected patients relative to uninfected controls in the US cohort. Hence, Toxoplasma appears to specifically impact levels of circulating cytokines, and our results may partly explain region-specific differences in the clinical spectrum of toxoplasmosis.

Keywords. acute; chronic; Colombia; congenital, cytokine profile; cytokines; pregnant; toxoplasmosis; Toxoplasma gondii.

Toxoplasma gondii is an obligate intracellular parasite with an unparalleled global distribution and mammalian host range. This Apicomplexan organism is estimated to infect about one-third of the world’s human population [1], with certain geographical regions having seroprevalence rates as high as 84% [2]. Although generally an asymptomatic infection in immunocompetent adults, toxoplasmosis may cause blindness in such individuals, and it can result in life-threatening disease in immunocompromised individuals [3–5]. Congenital Toxoplasma infection can also produce serious disease with the severity depending on how recently acquired an infection is and the gestational age of the fetus at the onset of infection.

After ingestion of food or water contaminated with Toxoplasma tissue cysts or oocysts, the parasites develop into rapidly multiplying tachyzoites, which reproduce within gut tissue of the intermediate host. Tachyzoites are responsible for establishing the acute phase of infection during which they disseminate via the bloodstream to the various tissues in which they proliferate. Although eventually cleared from most organs due to developing host immunity, surviving tachyzoites differentiate...
into latent bradyzoites, or tissue cyst forms. It is thought that tissue cysts have a predilection for neural, muscular, and ocular tissue and persist indefinitely for the life of the host.

Encystation marks the chronic phase of infection, and if a chronically infected individual becomes immunocompromised, these tissue cysts serve as a reservoir from which local infections and further dissemination can develop. Similar to the lifelong tissue cysts, Toxoplasma-specific immunoglobulin G (IgG) levels can also persist for the life of the host [6], likely due to cycles of reactivation and, perhaps, reinfection. It is possible to differentiate between acute infections (initiated within the prior 12 months) and chronic infections (>1 year since initial infection) by a panel of serological tests [7, 8].

Remarkably, given the ubiquity of this parasite, the majority of human and animal isolates of Toxoplasma collected to date in Europe and North America (NA) have been found to be one of 3 genotypes: Types I, II, and III [9]. Interestingly, recent studies report variation in clinical symptoms by geographic area and hypothesize that these variations might be associated with parasite genotype [10–12]. Type II infections have been recognized as predominant in Northern American and European populations [13], and in these regions, the majority of Toxoplasma infections are generally asymptomatic [14] and less likely to result in ocular toxoplasmosis [10]. In contrast, Type I and “atypical” strains (ie, not Type I, II, or III) predominate in South America (SA) [15], where toxoplasmosis is associated with much more severe progression and symptoms [11, 16].

Infections of immunocompetent adults in French Guyana have even resulted in lethal disseminated disease [17] and in Colombia an increased frequency of active lesions and severity of symptoms has been reported in ocular cases [18, 19]. A recent study addressing the role of Toxoplasma strain type in ocular toxoplasmosis (OT) reported that of the tested OT cases, Colombians were infected with atypical strains and French patients were uniformly Type II-infected. An analysis of intraocular cytokine levels between these patients revealed increased levels of interleukin 6 (IL-6) and interleukin 13 (IL-13) and decreased levels of interferon γ (IFN-γ) and interleukin 17 (IL-17) in the Colombian cohort [20]. In North America, otherwise healthy adults with severe, atypical ocular toxoplasmosis were found to be often infected with Type I or atypical strains but not the more common Type II strains [21]. To date, however, no definitive study has been performed to address this question in large numbers of individuals.

There is also a paucity of data on the effects of acute and chronic toxoplasmosis on the immune response in infected humans and whether these responses might differ between individuals in North America vs South America. To remedy these gaps in our understanding, we analyzed the relative abundance of 51 cytokines in peripheral blood sera of acutely and chronically infected pregnant women from the United States and Colombia. For both cohorts, our results show a difference between the acutely infected patients relative to uninfected. The nature of those differences, however, was not the same between the 2 regions, consistent with an impact of Toxoplasma strain type on the host response to infection. We also saw very surprising evidence of differences within the US cohort between chronically infected and uninfected individuals. The implications of these results on the different patient groups are discussed.

METHODS

US Patients

A total of 144 serum samples (Supplementary Table 1) from human immunodeficiency virus (HIV)-negative, consecutive pregnant women were collected. At the time the samples were obtained, the Toxoplasma-infection status of these women was unknown, and thus no treatment was administered prior to sample collection. In sum, 48 (average age 29; range 17–43) were from uninfected women, 49 were from chronically infected women (average age 29; range 16–47), and 47 (average age 30; range 18–43) were from acutely infected women as established by serological test results performed at the Toxoplasma Serology Laboratory, Palo Alto Medical Foundation (PAMF-TSL, http://www.pamf.org/serology/) [7, 8]. Age and state of residence for each of the patients was collected, but the gestational age of the fetus was not (it was later determined for those who were found to be acutely infected but at the time this study was initiated, it was not considered relevant for the uninfected or chronically infected individuals). Serum was stored at –80°C until analysis. Comparison of mean age in all US cohorts by unpaired t test yielded no statistically significant differences. Informed consent was obtained from all patients.

Colombian Patients

A total of 113 samples (Supplementary Table 2) were obtained from a previously existing bank of sera isolated from HIV-negative pregnant Colombian women that was originally obtained for epidemiological studies aimed at determining the seroprevalence of T. gondii infection [22, 23]. As with the American cohort, the Toxoplasma-infection status of these women was unknown when the blood was drawn, and thus no treatment was administered prior to sample collection. From 22 July 2005 to 31 December 2011, 3428 pregnant women from 12 healthcare facilities consented to answer a questionnaire and allow investigators to take serum samples. Pregnant women were enrolled at the time they were attending their routine prenatal care visit. Women who had a suspected or confirmed diagnosis of T. gondii infection during pregnancy prior to being enrolled in the epidemiological study were excluded from the group studied here. Sera for our study were from 50 consecutive uninfected women (average age 29; range 17–46), 50 consecutive chronically infected women (average age 29; range 16–43), and 13 consecutive acutely infected women.
(average age 27; range 18–36). As for the US patients, gestational age was not systematically obtained. All sera were initially placed at 4°C and within 1 week tested for anti-Toxoplasma IgG and IgM at the Fundacion Valle del Lili (Cali, Colombia) per the manufacturer’s instructions using a microparticle enzyme immunoassay (MEIA) method (AxSYM System, Abbott Laboratories, Chicago, IL) and a bioMerieux VIDAS IgG and IgM (Marcy l’Etoile, France). The VIDAS IgG reference ranges are: negative <4 IU/mL, equivocal 4 to <8 IU/mL, positive ≥8 IU/mL; and for IgM: negative <0.55, equivocal 0.55 to <0.65, positive ≥0.65. The IgM test value is the ratio of the relative fluorescent value of the sample to that of the calibrator. Based on these tests, the pregnant women were classified as never infected (negative IgG/negative IgM), chronically infected, that is, infected more than 12 months (positive IgG/negative IgM), and possibly acutely infected (positive IgG/positive IgM). The remainder of each serum sample was stored at −20°C until shipped to the United States using a transportation company that uses a cold-chain system. Serum samples were stored at PAMF-TSL at −20°C until the day of cytokine testing. Positive IgG/positive IgM sera underwent confirmatory testing at PAMF-TSL to determine whether an acute infection had occurred within 1 year using the same criteria as above for the US samples. Comparison of mean age in all cohorts by unpaired t test yielded no statistically significant differences between any of the Colombian cohorts or between US and Colombian cohorts.

Serological Testing at PAMF-TSL
Each of the pregnant women in the acute Colombian cohort and all of the US patients whose sera were used for this study were tested at the PAMF-TSL. All samples were tested with the T. gondii dye test (measuring primarily IgG antibodies [24]) and IgM enzyme-linked immunosorbent assay (ELISA), reported as arbitrary units and performed based on in-house standards [25]. Pregnant women were classified into 3 groups according to their serological test results: (1) uninfected, negative IgG/negative IgM; (2) chronically infected, positive IgG/negative IgM; (3) acutely infected, positive IgG/positive IgM. In order to confirm that the patient had been infected within 1 year prior to the date of serum sampling, the positive IgG/positive IgM samples were tested further with T. gondii-specific tests, including the differential agglutination or AC/HS test [26], IgG avidity test [3], IgA antibody test [27], and IgE antibody test [28].

Cytokine Measurement in Serum Samples
Serum samples were analyzed at the Human Immune Monitoring Core (Stanford, CA) by Luminex Human 51-plex kits purchased from Affymetrix (Santa Clara, CA) and used according to the manufacturer’s recommendations with modifications as described below. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower bound of 100 beads per sample per cytokine. Luminex measures fluorescent intensities (FI s) of the 51 cytokines and produces a distribution of typically 200 to 300 FIs. The median FI (MFI) for each distribution is computed. Because every subject’s sample was entered in 2 wells, 2 MFIs per cytokine were computed for each subject. Assay plate layout consisted of 9 standards in duplicate, 1 blank well and 60 μL duplicates of each serum sample, each run with a 1:3 dilution. Cytokines assayed were: CD40L, ENA78, eotaxin, FGF-β, GCSF, GMCSF, CXCL1, HGF, IFN-α, IFN-β, IFN-γ, IL-1α, IL-1β, IL-1Rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL12p40, IL12p70, IL-13, IL-15, IL-17, IL17F, CXCL10, Leptin, LIF, CCL2, CCL7, MCSF, CXCL9, CCL3, CCL4, NGF, PAI-1, PDGF-β, β, CCL5, RETN, SCF, FasL, ICAM-1, VCAM1, TGF-α, TGF-β, TNF-α, TNF-β, TRAIL, and VEGF.

Statistical Analysis
Comparisons within a country were made on the same plate, so data were normalized for each feature via centering and scaling as (Feature − mean (Feature))/SD(Feature). We fit a separate regression model to each feature as the outcome with dummy-coded (0 = absent, 1 = present) predictor variables for status (acute, chronic, uninfected) and plate. Regression coefficients were estimated via generalized maximum entropy [29] using a set of uniform support points over the interval [−5, 5]. Both raw and adjusted P-values are reported. All P-value adjustments were via an adaptive 2-stage linear step-up procedure [30] for control of false discovery rate (FDR), a method that has been shown in a comparative study to maintain close control of the FDR and achieve good statistical power [31].

RESULTS
Cytokine Profiling of Infected US Cohorts
Sera from 47 pregnant US women who were asymptomatic but acutely infected with Toxoplasma were analyzed and compared to cytokine profiles from 48 uninfected pregnant females. Acute infection was defined as one estimated to have commenced within the prior 12 months as determined by serology tests at PAMF-TSL. Surprisingly, of the 51 cytokines profiled, 23 were significantly decreased in the acute cohort relative to the uninfected cohort with a stringent P value < .05 at an FDR of 5% (Table 1, Figure 1A and 2B). These included CXCL1, IL-17, IL1-α, IL-2, IFN-γ, IL-4, and several chemokines including CCL5 and CCL7. Macrophage colony stimulating factor (MCSF) alone was increased in the acutely infected cohort. Differences in levels of the remaining 27 cytokines (Supplementary Table 3) were not statistically significant, including TGF-β and PDGF-β which have previously been demonstrated to mediate inhibition of intracellular growth of T. gondii [32].

An analysis of the same panel of cytokines using sera from 49 asymptomatic, chronically infected women (ie, >1 year since
initial infection) yielded similar trends: 9 of the 24 cytokines depressed in the acute patients were also depressed in the chronic patients relative to the uninfected cohort (raw \( P \) value < .05), although none of these differences were significant using an FDR of 5% (Table 1, Figure 1A and 1B).

**Cytokine Profiling of Infected Colombian Cohorts**

To determine the cytokine profile in peripheral blood from Colombian patients, we compared serum cytokine levels of 13 acutely infected pregnant Colombian women to a control group of 50 uninfected pregnant women from this same country. The small number of acutely infected individuals in the Colombian cohort, however, meant that large differences between the 2 patient groups were needed to reach statistical significance (especially given the naturally large variation in cytokine levels typical between individuals). In contrast to the differences in 24 cytokines between the US uninfected and acute cohorts, only 8 profiled serum cytokines were significantly different in Colombian patients with acute toxoplasmosis relative to uninfected controls with a \( P \) value < .05 at an FDR of 5%. Four of these, ICAM1, IL-1\( \alpha \), CXCL10, and CXCL9, were higher on average in the acute patients, whereas CD40L, IL-8, CCL5, and RETN were observed at lower levels relative to uninfected controls (Table 2; Figure 2A and 2B).

Given the small size of the acute Colombian cohort, we applied a less stringent threshold (FDR at 20%) to see if other cytokines trended in similar directions. We observed that, using this threshold, CCL7, Trail, and TNF-\( \beta \) were also present at increased levels in acutely infected vs uninfected patients, whereas CCL4 and HGF were present at decreased levels (Table 2; Figure 2A and 2B). No differences were observed in the other 38 cytokines assayed (Supplementary Table 3). A similar analysis of the chronic and uninfected Colombian cohorts, both consisting of 50 individuals, showed no statistically significant differences between the two groups. These data suggest that, at least with these cohorts of pregnant Colombian women, acutely infected patients exhibit dramatically different immune profiles in comparison to uninfected patients, whereas chronic patients are not detectably different from the uninfected group.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Uninfected</th>
<th>Acute</th>
<th>Chronic</th>
<th>Uninfected vs Acute</th>
<th>Uninfected vs Chronic</th>
</tr>
</thead>
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<td>CD40L</td>
<td>439.5</td>
<td>268.3</td>
<td>421.8</td>
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<td>.1955 .0345</td>
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<td>Eotaxin</td>
<td>93.5</td>
<td>57.0</td>
<td>84.6</td>
<td>&lt; .0001 &lt; .0001</td>
<td>.5594 .3973</td>
</tr>
<tr>
<td>GCSF</td>
<td>80.1</td>
<td>65.2</td>
<td>73.9</td>
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<td>.3045 .0847</td>
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<td>109.1</td>
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<td>.1494 .0167</td>
</tr>
<tr>
<td>HGF</td>
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<td>354.7</td>
<td>523.6</td>
<td>&lt; .0001 &lt; .0001</td>
<td>.3045 .1062</td>
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<tr>
<td>IFN-( \gamma )</td>
<td>31.9</td>
<td>21.6</td>
<td>28.6</td>
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<td>.3045 .0887</td>
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<td>IL-15</td>
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<td>41.0</td>
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<td>.6578 .4952</td>
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<td>IL1-( \alpha )</td>
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<td>.2045 .0844</td>
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<td>70.6</td>
<td>90.6</td>
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<td>.3856 .1663</td>
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<tr>
<td>IL-4</td>
<td>97.4</td>
<td>71.9</td>
<td>90.0</td>
<td>&lt; .0001 &lt; .0001</td>
<td>.3045 .1049</td>
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<tr>
<td>IL-8</td>
<td>2039.8</td>
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<td>657.3</td>
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<td>.4225 .2191</td>
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<tr>
<td>LIF</td>
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<td>38.7</td>
<td>47.2</td>
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<td>.1441 .0113</td>
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<td>.4225 .2191</td>
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<td>210.0</td>
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<td>.7025 .5648</td>
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<td>CCL4</td>
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<td>967.0</td>
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<td>.1494 .0176</td>
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<td>CCL3</td>
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<td>887.1</td>
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<td>NGF</td>
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<tr>
<td>CCL5</td>
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<td>10 351.5</td>
<td>11 794.6</td>
<td>.0008 .0004</td>
<td>.5594 .3820</td>
</tr>
<tr>
<td>RETN</td>
<td>12 293.1</td>
<td>10 616.0</td>
<td>10 650.2</td>
<td>.0191 .0121</td>
<td>.1786 .0280</td>
</tr>
<tr>
<td>SCF</td>
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<td>87.7</td>
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</tr>
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<tr>
<td>TRAIL</td>
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<td>607.7</td>
<td>.0105 .0056</td>
<td>.3856 .1447</td>
</tr>
</tbody>
</table>

\( N = 48, 47 \) and 49 for uninfected, acute, and chronic cohorts, respectively. Data shown are only for those cytokines (\( P < .05, \) FDR at 5\%) that yielded a significant difference between either pairwise comparison. FDR \( P \) value is adjusted to control the FDR as described in methods.

Abbreviations: FDR, false discovery rate; IL, interleukin; TNF, tumor necrosis factor.
**DISCUSSION**

Prior studies in vitro or in animal models have shown that protective and inflammatory immune responses to the parasite are mediated by elevation of several cytokines, including IL-12, IFN-γ, IL-6, IL-10, and TNF-α in mice, and CD40L during human infection [33, 34]. In contrast, we observed that 23 of 51 cytokines assayed, including IFN-γ, IL1-α, IL-2, TNF-α,
IL-17, CD40L, and IL-4 were lower in the serum of acutely infected pregnant US patients relative to uninfected pregnant patients. This was also true of several chemokines, including CCL5 and CCL7. In support of these findings, an independent study from Spain revealed that levels of circulating CCL2 were depressed in patients who had active ocular toxoplasmosis.

Figure 2. Cytokine profiling of infected Colombian cohorts. A, MFI values (y-axis) represent the ratio of the average MFI per cytokine between acute and uninfected, and chronic and uninfected pregnant Colombian women. All cytokines shown had statistical significance for the acute to uninfected comparison (raw P < .05). Eight cytokines (denoted by *) were also significant (P < .05) using the more stringent test where the FDR is set to 5%. No significant differences were detected between the chronic and uninfected cohorts. Dotted line at 1 represents no difference in MFI values between uninfected and infected cohorts. B, MFI values (y-axis) reported for all cohorts (x-axis): Uninfected (U), Acute (A), and Chronic (C) for those cytokines found to be significantly different between uninfected and acutely infected cohorts. Abbreviations: FDR, false discovery rate; MFI, median fluorescent intensity.
when compared to patients with inactive disease or uninfected controls [35]. Like CCL5 and CCL7, CCL2 is a member of the β-chemokine family and mediates the recruitment of inflammatory cells to target tissues [36, 37].

Although the results obtained may be affected by the Th2-polarization reported in pregnant women [38], it is intriguing that we see a decrease in levels of circulating cytokines in the acutely infected pregnant American cohort, presumably already immunosuppressed, relative to uninfected pregnant controls. These depressed cytokines included GCSF and TNF-α, previously reported to be often elevated during pregnancy [39] and leukemia inhibitory factor (LIF), a secreted cytokine with a critical role in blastocyst development [40], which remained depressed in chronic US patients relative to uninfected controls (FDR at 20%). The mechanistic basis for the depressed cytokine levels observed here is not known but could be the result of down-regulatory pathways activated in an attempt to neutralize potential immunopathology in various organs including the placenta.

We observed clear differences in cytokine profiles of the geographically distinct cohorts (Figure 3). Unlike the systemic suppression observed in the acutely infected Americans, however, only 4 cytokines were at significantly (FDR of 5%) lower levels in the acutely infected pregnant Colombian cohort relative to uninfected controls. Furthermore, Colombian acute patients exhibited increased levels of proinflammatory IL1-α and Trail, a known inducer of apoptosis, both of which were depressed in serum of US acute patients relative to uninfected US controls. These differences in cohorts may be attributable to the infecting Toxoplasma strain type; although we do not have definitive information on the infecting Toxoplasma strain in the tested cohorts, Type II Toxoplasma infections have been shown to dominate in Europe and North America, whereas “atypical” strains are more commonly found in South America. A recent study by de-la-Torre et al [20] comparing Toxoplasma strains and intraocular cytokine levels in OT patients in South America and Europe found that Colombian OT patients were infected with various Toxoplasma Types and had lower IFN-γ and IL-17 and higher IL-6 and IL-13 intraocular levels relative to French OT patients, who were determined to be uniformly infected with Type II Toxoplasma. The differing cytokine levels reported in our work and the de-la-Torre study may reflect differences observed in the local microenvironment (ie, ocular fluids) vs the systemic immune response (ie, serum), as previously reported during inflammation and infection [41, 42]. We cannot, of course, exclude the possibility that confounding factors could be wholly or partially involved in these regional differences, including the state of the infecting parasite, coinfection status, diet, medications, and host genetics. Regardless of the mechanism underlying the geographic differences reported here, our results may provide a partial mechanistic explanation for why the parasite appears to cause a “clinically benign” acute infection in the vast majority of individuals in the United States including pregnant women, whereas it is prone to cause symptomatic and more aggressive disease in South America: the immune response to infection in the 2 regions is very different and this could well impact the resulting pathology.

Interestingly, and very surprisingly, several cytokines including GCSF, IL-17, LIF, CCL3, and NGF were depressed (FDR at 20%) in chronically infected US patients relative to uninfected

### Table 2. Average MFI Listed per Cytokine for the Uninfected, Acute, and Chronically Infected Pregnant Colombian Cohorts

<table>
<thead>
<tr>
<th>Feature</th>
<th>Uninfected</th>
<th>Acute</th>
<th>Chronic</th>
<th>FDR P Value</th>
<th>Raw P Value</th>
<th>FDR P Value</th>
<th>Raw P Value</th>
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<td>.0037</td>
<td>.8663</td>
<td>.6282</td>
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<tr>
<td>HGF</td>
<td>671.7</td>
<td>539.0</td>
<td>640.1</td>
<td>.0712</td>
<td>.0199</td>
<td>.8663</td>
<td>.5660</td>
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<td>ICAM1</td>
<td>9026.4</td>
<td>12438.8</td>
<td>9887.8</td>
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<td>.0006</td>
<td>.6627</td>
<td>.2988</td>
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<td>IL1-α</td>
<td>129.1</td>
<td>243.5</td>
<td>131.8</td>
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<td>&lt;.0001</td>
<td>.9675</td>
<td>.9245</td>
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<tr>
<td>IL-8</td>
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<td>.0033</td>
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<td>&lt;.0001</td>
<td>.9405</td>
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<td>.9405</td>
<td>.7437</td>
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<td>.8663</td>
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<td>10 279.2</td>
<td>.0005</td>
<td>&lt;.0001</td>
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<td>.3255</td>
</tr>
<tr>
<td>TNF-β</td>
<td>108.9</td>
<td>152.7</td>
<td>127.1</td>
<td>.0642</td>
<td>.0164</td>
<td>.6627</td>
<td>.2945</td>
</tr>
<tr>
<td>TRAIL</td>
<td>495.0</td>
<td>568.5</td>
<td>527.5</td>
<td>.0895</td>
<td>.0271</td>
<td>.7034</td>
<td>.3586</td>
</tr>
</tbody>
</table>

N = 50, 13, and 50 for uninfected, acute, and chronic cohorts, respectively. Data shown are only for those cytokines that yielded a significant difference (raw P < .05) between either pairwise comparison.

Abbreviations: FDR, false discovery rate; IL, interleukin; MFI, median fluorescence intensity; TNF, tumor necrosis factor.
controls. This suggests that there may be significant, long-term effects of a chronic Toxoplasma infection in American pregnant women who are otherwise asymptomatic. For example, a chronic infection could have profound implications for how the pregnant woman’s immune system responds to other infections, allergens, or vaccines. Chronic immune stimulation has been shown to alter viral dynamics [43], and while little is known about the functional significance of steady-state blood cytokines, it is perhaps necessary to revisit the concept of a chronic infection being “asymptomatic.” Specifically, these results suggest that attention should be paid to minor sequelae that might occur in children born to even chronically infected mothers. We know of no reports that such children experience any symptoms but such might have been too minor to have been noted in studies performed to date. In aggregate, our data suggest that Toxoplasma infection is causing altered levels of several cytokines in the chronically infected US cohorts, although we cannot exclude the possibility that these chronically infected individuals are distinct in some other unknown way compared to the uninfected group. For example, it could be that extrinsic factors, like host genetics, coinfection status, lifestyle, etc, determine whether an individual maintains a measurable titer of anti-Toxoplasma antibodies for a prolonged period and that this other factor explains the differences between these 2 groups.

We recognize this study has some limitations. We did not include nonpregnant women in our cohort, and there could be an effect of gestational age on the immune response; hence, findings in this study may not be generalizable to nonpregnant females or males. Second, although the onset of infection was estimated for the acute and chronic classifications, we cannot know the exact date. In addition, the time of day of the blood draw was not recorded and may impact results because levels of circulating cytokines are known to be influenced by circadian rhythms, meals, exercise, and other factors. Within the United States and Colombia, the patients were sampled identically, so this is unlikely to explain within-country differences; however, it could contribute to the between-country differences seen here. Similarly, we cannot exclude that differences in storage protocols for samples in the 2 countries underlie some of the region-specific difference, especially the use of −20°C for the Colombia samples vs −80°C for the US samples. Cytokines may decay during storage, as a function of both time and temperature, and this could contribute to some of the differences seen between regions.

Despite these limitations, this work raises several important questions. Can knowledge of the cytokine network during infection enable us to design targeted therapeutic strategies? There is, in fact, considerable debate about whether to use immunosuppressing steroids to control excessive inflammation during ocular toxoplasmosis in adults or whether such drugs or treatments are antagonistic to the immune system’s efforts to control the infection [44]. From what cell types and tissues are these cytokines being made during infection? By addressing these questions, we can learn more about what shapes the immunological milieu during the initial infection and expansion of Toxoplasma, and how influencing this milieu can dictate the progression and pathogenesis of infection in pregnant women.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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