Dynamic Immune Profile in French Toxoplasmosis Patients

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Background. Toxoplasma gondii infection is usually benign in Europe due to the strong predominance of type II strains. Few studies have been conducted to examine the immunological course of infection in humans and have yielded conflicting results, maybe influenced by heterogeneous parasite strains.

Methods. We measured 23 immune mediators in 39, 40, and 29 sera of French noninfected, acutely infected, and chronically infected immunocompetent pregnant women, respectively.

Results. Four different cytokine patterns were identified regarding their dynamics through infection phases. For 11 of the cytokines (IFN-β, IFN-γ, IL-4, IL-5, IL-6, IL-10, IL-12, IL-15, CXCL9, CCL2, and CSF2) the serum levels were significantly elevated during acute infection. The inflammatory mediators IL-1β, IL-17A, IL-18, TNF-α, and CSF3 remained unchanged during acute infection, while they were significantly lower in chronically infected compared to noninfected patients. As for the anti-inflammatory cytokines TGF-β and CCL5, their levels remained significantly elevated during chronic infection. We also observed a significant negative correlation of several cytokine concentrations with IgG levels, indicating a rapid decline of serum concentrations during the acute phase.

Conclusions. These results indicate an anti-inflammatory pattern in chronically infected patients in a type II dominated setting and demonstrate the highly dynamic immune situation during acute infection.

Keywords. Toxoplasma gondii; toxoplasmosis; acute; chronic; cytokine profile; cytokines; pregnant; France.
levels of colony-stimulating factor 2 (CSF2), granulocyte-macrophage CSF (GM-CSF), CXCL11, transforming growth factor-β (TGF-β), CXCL9, and CXCL10 in the acute infection phase compared to the chronic phase [16,17]. CXCL9, CCL2, and CSF2 were even reported to distinguish between acute and chronic toxoplasmosis with an accuracy of 76% [17].

Clearly, the cytokine profile of response to toxoplasmosis seems to vary according to many parameters, such as the infection stage, the underlying clinical conditions, but also crucially the parasite strain [18]. These studies were often conducted in cohorts of pregnant women, which certainly introduced some bias but has the great advantage of providing a quite homogeneous and clinically well-defined group. One study compared a US cohort, where approximately 50% of infections were due to type II strains, with a similar Colombian cohort, where the setting was highly variable and more pathological [3]. Surprisingly, the North American patients showed decreased levels of half of the immune mediators, including IFN-γ, IL-17, CCL4, and CSF3 (G-CSF), during acute infection, while in Colombian patients, only few mediator levels were decreased during acute infection and some others were elevated.

Thus, although the cytokinome in infected patients has been well studied in South America and partly in the United States, these studies always include the uncertainty of strain variations. To characterize this cytokinome in a nearly homogenous type II setting, we therefore evaluated the cytokine profile of European pregnant women, infected or not with toxoplasmosis. In addition to distinguishing between acute and chronic infections, we also took into account the evolution of the acute phase by quantifying immunoglobulin G (IgG) antibody levels and avidity [19]. This allowed us to demonstrate the dynamics of cytokine responses during the course of the acute phase of infection.

**METHODS**

**Patients**

In this retrospective study, we collected sera from pregnant women who took part in routine toxoplasmosis serological screening at the Strasbourg University Hospitals, France, and who did not present any underlying pathology or signs of immunosuppression. Overall, 108 sera from 101 pregnant women were included in this study between 2011 and 2021. Toxoplasmosis serological screening consisted of an IgM assay (ARCHITECT Toxo IgM; Abbott) and an IgG assay (ARCHITECT Toxo IgG; Abbott), as well as an IgG avidity measurement in those patients with positive IgM and IgG levels (ARCHITECT Toxo IgG Avidity; Abbott) [19]. IgM were considered negative below 0.5 IU/mL, equivocal between 0.5 and 0.6 IU/mL, and positive above 0.6 IU/mL. IgG were considered negative below 1.5 IU/mL, equivocal between 1.6 and 3 IU/mL, and positive above 3 IU/mL. Avidity was considered weak below 40% and high above 60%. The patients were accordingly classified into 3 groups: 39 patients not infected with toxoplasmosis (IgM and IgG negative), 40 sera of 33 patients with acute-phase toxoplasmosis (IgM positive and/or IgG weakly positive and avidity below 40%), and 29 patients with chronic toxoplasmosis (IgM negative, IgG positive).

**Serum Cytokine Assay**

The sera were stored at −20°C. The following 23 cytokines were measured on the Luminex MAGPIX system: IL-17, CCL5, CSF3, CSF2, IFN-α, IFN-β, IFN-γ, IL-1α, IL-1β, IL-10, IL-12p70, IL-15, IL-17A, IL-18, IL-4, IL-5, IL-6, CXCL8, CXCL10 (IP-10), CCL2 (MCP-1), CXCL9 (MIG), TNF-α, and TGF-β (PROCARTAPLEX Luminex kit). Assays were performed in duplicate on 25 μL of serum and analyzed using the Luminex Xponent software version 4.2.

**Statistical Analysis**

Statistical analysis was conducted with GraphPad Prism version 8.2. Nonparametric tests were used throughout, as a preliminary test showed a nonnormal distribution of cytokine levels. The comparisons between the noninfected, acutely infected, and chronically infected groups were performed using a Kruskal-Wallis test with Dunn correction for multiple comparisons. Correlations between *Toxoplasma*-specific IgG and cytokine levels were analyzed by Spearman correlation test. The Spearman test was also used to explore the correlation between avidity and IgM or IgG levels. *P* values <.05 were considered as statistically significant.

**Ethics Approval**

The serum samples were obtained in compliance with the quality assurance scheme and legal policies. They are part of a biobank of the Strasbourg University Hospital that has been authorized by the local Clinical Research Department and declared to the French Ministry of Health (No. DC-2019-3727).

**RESULTS**

In our cohort, 39, 33, and 29 patients (39, 40, and 29 sera) were classified as not infected with *T. gondii*, acutely infected, and chronically infected, respectively. The patients’ data are summarized in Supplementary Figure 1.

The levels of 23 cytokines were measured in the sera of these patients. We first compared the levels in each of the 3 infection groups (Figure 1 and Table 1). Four distinct patterns were identified. The first pattern (pattern A) comprised half of the examined mediators, including major Th1, Th2, and regulatory cytokines, namely IFN-γ, IL-12p70, CSF2, CXCL9, CCL2, IFN-β, IL-4, IL-5, IL-10, IL-6, and IL-15 (Figure 1A). These mediators were significantly upregulated in the sera from patients with acute toxoplasmosis. In chronically infected patients, cytokine levels returned to preinfection values, so that the noninfected and chronic groups were not statistically different. The
Figure 1. Serum cytokine concentration of the noninfected patients and patients with acute or chronic toxoplasmosis: (A) cytokines showing significant upregulation only in patients in the acute phase of infection; (B) cytokines with significant downregulation in patients in the chronic phase; and (C) cytokines with significantly elevated levels in patients from both the acute and chronic groups compared to the noninfected patients. Pattern plots show the relative dynamics between the 3 subgroups for each pattern. Abbreviations: CCL, CC chemokine ligand; CSF, colony-stimulating factor; CXCL, C-X-C motif chemokine ligand; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.
and CCL5 (RANTES), with their levels being significantly compared to both the noninfected and acute groups.

In studies characterizing the immune response in T. gondii infection, we regularly noticed a great variability of values, and of used to be high during chronic infection.

The third pattern (pattern C) comprised only TGF-β, IL-1α, IL-18, and IL-17A) and was characterized by significantly lower levels in the chronic group as compared to both the noninfected and acute groups (Figure 1B). None of these immune mediators showed a statistically significant difference between the noninfected and acute groups. The third pattern (pattern C) comprised only TGF-β and CCL5 (RANTES), with their levels being significantly more elevated in the acute and chronic groups than in the noninfected group (Figure 1C). Finally, the remaining cytokines (CXCL8, IL-17F, CXCL10, IFN-α, and IL-1α), corresponding to pattern D, showed no differences between the groups or were expressed at very low levels throughout (Supplementary Figure 2A). Thus, whereas most cytokines were upregulated only during the acute infection phase with no difference between noninfected and chronically infected patients, some mediators, notably inflammatory cytokines, continued to be low and others, namely the anti-inflammatory TGF-β, continued to be high during chronic infection.

When looking more closely at the cytokine levels during acute infection, we regularly noticed a great variability of values, and often 2 or more distinct populations. Therefore, we dissected the evolution of infection during this phase using IgG titers as a marker. The results are shown in Figure 2, with cytokines displayed in the same groups as in Figure 1. Obviously, for most of the immune mediators with lower concentrations in chronically infected than in acutely infected patients, we observed a significant negative correlation between IgG levels and cytokine concentrations, regardless of whether concentrations were higher in acute compared to noninfected patient groups (IFN-γ, IL-12p70, CSF2, IFN-β, IL-4, IL-5, IL-6, and IL-15; Figure 2A) or not (TNF-α, IL-17A, CSF3, and IL-1β; Figure 2B). For those cytokines that remained elevated in the chronic phase, no correlation with IgG levels was noted (Figure 2C). This lack of correlation was also observed for mediators whose levels did not differ between the 3 infection groups (Supplementary Figure 2B). Thus, most cytokines that were temporarily upregulated during acute infections showed their highest concentrations early in the infectious process, followed by a rapid decline to the levels observed in the chronic phase.

**DISCUSSION**

In studies characterizing the immune response in T. gondii-infected patients, parasite- or host-related factors will always

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|---|---|---|
| **Table 1. Median Cytokine Concentrations in the Noninfected, Acutely Infected, and Chronically Infected Pregnant Women, and Adjusted P Values for Each Pairwise Comparison** |
| Cytokine Concentration, Median (Range), pg/mL | Noninfected | Acute | Chronic | **Noninfected vs Acute** | **Noninfected vs Chronic** | **Acute vs Chronic** |
|---|---|---|---|---|---|---|
| IFN-γ | 0.0 (0–0) | 0.0 (0–8.3) | 0.0 (0–0) | <.0001 **** | >.9999 NS | <.0001 **** |
| IL-12p70 | 0.0 (0–0) | 0.0 (0–48) | 0.0 (0–0) | <.0001 NS | >.9999 NS | <.0001 **** |
| CSF2 | 0.0 (0–0) | 8.6 (0–31) | 0.0 (0–14) | <.0001 **** | >.9999 NS | <.0001 **** |
| CCL5 | 0.935 (0–664) | 81.78 (0–980) | 0.0 (0–610) | <.0001 **** | >.9999 NS | <.0001 **** |
| CXCL8 | 22.605 (0–145) | 31.8 (0–183) | 6.49 (0–62) | .9435 NS | <.0001 **** | <.0001 **** |
| IFN-β | 0.0 (0–28.6) | 0.0 (0–13) | 0.0 (0–1.47) | .0004 *** | >.9999 NS | <.0003 *** |
| IL-4 | 0.0 (0–2.8) | 1.095 (0–9.5) | 0.0 (0–2.8) | <.0001 **** | >.9999 NS | <.0001 **** |
| IL-5 | 0.0 (0–83) | 1.34 (0–52) | 0.0 (0–86) | <.0001 **** | >.9999 NS | <.0001 **** |
| IL-10 | 0.0 (0–11.5) | 1.505 (0–3.12) | 0.0 (0–8.9) | <.0001 **** | .3228 NS | .0027 ** |
| IL-6 | 0.0 (0–472) | 0.89 (0–32) | 0.0 (0–16.2) | .0042 ** | >.9999 NS | .001 *** |
| IL-15 | 0.0 (0–34.1) | 0.0 (0–23.5) | 0.0 (0–9.5) | .0003 *** | >.9999 NS | .0012 ** |
| TNF-α | 0.0 (0–65) | 0.0 (0–30) | 0.0 (0–0) | .0824 NS | <.051 NS | <.0001 **** |
| IL-17A | 0.0 (0–12) | 0.0 (0–23) | 0.0 (0–6.4) | .6155 NS | <.0001 **** | .005 ** |
| CSF3 | 19.74 (0–46) | 14.56 (0–75) | 0.0 (0–40) | .1177 NS | <.0001 **** | .0009 *** |
| IL-1β | 3.97 (0–177) | 3.895 (0–30.1) | 0.0 (0–24) | >.9999 NS | <.0001 **** | <.0001 **** |
| IL-18 | 5.865 (0–93.9) | 6.88 (0–38) | 0.92 (0–48) | >.9999 NS | <.0001 **** | <.0001 **** |
| TGF-β, ng/mL | 675.1 (0–2607) | 5.1644 (1.8–29.3) | 2781.18 (465–5942) | <.0001 **** | <.0001 **** | <.0001 **** |
| CCL5, ng/mL | 3618.25 (132–10970) | 13.22182 (1.1–93) | 10 748.08 (1270–37729) | <.0001 **** | <.0001 **** | >.9999 NS |
| IL-1α | 0.0 (0–9) | 0.0 (0–4.2) | 0.0 (0–52) | .1746 NS | .6157 NS | >.9999 NS |
| CXCL8 | 29.995 (0–1332) | 21.405 (0–1332) | 18.94 (0–255) | >.9999 NS | >.9999 NS | >.9999 NS |
| IL-17F | 0.39 (0–2.2) | 0.22 (0–2.3) | 0.22 (0–1.6) | >.9999 NS | >.9999 NS | >.9999 NS |
| IFN-α | 0.0 (0–0.855) | 0.0 (0–0.55) | 0.0 (0–0.38) | >.9999 NS | >.9999 NS | >.9999 NS |
| CXCL10 | 6.11 (2.5–171) | 49.775 (2–205) | 6.91 (0–96) | .1969 NS | >.9999 NS | .1112 NS |

n = 39, 40, and 29 sera for the noninfected, acute, and chronic groups, respectively. The comparisons between noninfected, acutely infected, and chronically infected groups were analyzed by Kruskal-Wallis test with Dunn correction for multiple comparisons. * P < .05, ** P < .01, *** P < .001, **** P < .0001. Abbreviation: CCL, CC chemokine ligand; CSF, colony-stimulating factor; CXCL, C-X-C motif chemokine ligand; IFN, interferon; IL, interleukin; NS, not significant; TGF, transforming growth factor.
Figure 2. Correlation profiles between Toxoplasma-specific IgG and cytokine levels in patients with an acute toxoplasmosis. Cytokines are displayed as in Figure 1: (A) cytokines showing significant upregulation only in the acute phase of infection; (B) cytokines with significant downregulation in the chronic phase; and (C) cytokines with significantly elevated levels in both the acute and chronic groups compared to the noninfected group. Correlations were analyzed by Spearman correlation test. * \( P < .05 \), ** \( P < .01 \), *** \( P < .001 \). Abbreviations: CCL, CC chemokine ligand; CSF, colony-stimulating factor; CXCL, C-X-C motif chemokine ligand; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; ns, not significant; TGF, transforming growth factor; TNF, tumor necrosis factor.
exert an influence. This was evident in the different studies looking at peripheral cytokine response in acutely or chronically infected populations. Most of them were performed in South America, where high infection rates and virulent parasite strains facilitate the enrollment of sufficient numbers of patients. However, the highly variable strains in this region make it more difficult to draw conclusions regarding the patients’ response. The particular character of the South American setting has been demonstrated repeatedly by comparison with European or North American patients [3,4]. The latter study [3], comparing US and Colombian pregnant women, showed a relatively small impact of infection on serum cytokine levels in Colombia. Similar observations were made when exploring cytokine aqueous humor levels, as evidenced by one of our previous studies and by others in South America [18,20–22]. Interestingly, US patients, however, showed diminished levels of a substantial number of immune mediators, which we did not observe for any mediator in our French patients. However, heterogeneity of parasite strains cannot be excluded in this US setting, where approximately 50% of infections were due to non-type II strains [8]. In French, and other European populations, as in our study, there was nearly homogenous type II infection, even if a few non-type II infections may have been included [23,24]. Furthermore, the routine French surveillance protocol for T. gondii infections in pregnant women enabled us to recruit patients in different infection phases, with minimal interference of concomitant infections. Avidity measurement allowed us to delimit more precisely the time frame of the acute phase to about 3 to 4 months following infection according to the manufacturer’s instructions for the different detection kits, in contrast to IgM expression only, where the time frame can reach 1 year and may thus misinterpret early chronic infection as acute infection. It is also noteworthy that some of the cytokines that were poorly or not expressed in our noninfected group showed substantial serum concentrations in the American noninfected cohort [3]. This disparity, perhaps due to patient recruitment bias, could explain some of the differences compared to our observations.

While focusing specifically on the different cytokines during the distinct phases of infection, we could identify different patterns of reactivity. Pattern A, with elevated serum concentrations only during the acute phase, comprises classical Th1 cytokines, such as IFN-γ, IL-12, CXCL9 (MIG), and CCL2 (MCP1). CXCL9 was also reported to be elevated in infected Brazilian patients, both adults [17,25] and newborns [16]. Interestingly, there also seemed to be a counterbalancing Th2 response during acute infection, as evidenced by elevated IL-4 and IL-5 levels, with similar observations also made in some mouse studies [26,27]. In contrast, our observation of increased IFN-β levels during this phase has not been reported in the aforementioned studies. This type I IFN has very well-described antiviral properties; however, its role in Toxoplasma infection is still controversial [28,29]. Our results indicate its involvement in the acute infection phase. Pattern B is characterized by significantly lower serum levels during the chronic phase, as compared to both the noninfected and acute groups. In contrast, the levels did not significantly differ between the acute phase and noninfected groups. Interestingly, we identified several inflammatory mediators, such as TNF-α, IL-17A, and CSF3, in this group. The pattern C mediators, whose serum levels remained elevated during the chronic phase, contained the anti-inflammatory cytokine TGF-β. These findings demonstrate that this long-lasting chronic infection creates a new immunological equilibrium towards an anti-inflammatory response. This changing equilibrium seems to be strain dependent. A Brazilian study of pregnant women actually also found lower TNF-α concentrations in chronically infected patients, but, in contrast to our results, a strong depression of IFN-γ production [3]. Additionally, TGF-β levels were only enhanced during acute infection, but not during the chronic phase [17]. The observed suppression of potentially harmful inflammatory responses is evidently protective, but could render an infected person more vulnerable to certain infections and other challenges, and it would therefore be interesting to compare T. gondii with other infections. Previous results have also established a link between certain clinical contexts and failure to reach such equilibrium. For example, an increased expression of the IL-6 and IL-1β genes was observed in pregnant women infected with T. gondii who have had a miscarriage [30]. During ocular toxoplasmosis, ocular cytokine profiles were characterized by increased levels of IL-17, IL-33, IL-10, CXCL9, and CXCL10 [16,22,31,32], and decreased levels of CCL11, CCL26, MIF, and CXCL12 [16]. Moreover, lower levels of CCL2 were associated with active ocular toxoplasmosis lesions, but more data are needed to define the subtle links between peripheral cytokine levels and localized disease, especially as these markers could differ between the local, ocular compartment, and peripheral circulation. A study on acute ocular toxoplasmosis reported enhanced TGF-β expression in peripheral blood mononuclear cells, while the corresponding aqueous humor levels were diminished [22]. The dynamics between local and circulating immune cells could explain the distinct local manifestations of Toxoplasma infection.

Our results demonstrated that the acute phase was the most dynamic part of the immune reaction during T. gondii infection. While this has been shown previously, no study specifically looked at the dynamics within this phase. Indeed, we observed a great variability of values, or sometimes 2 or more distinct populations, during this phase, whereas this was very rarely the case in noninfected and chronically infected patients. This led us to conclude that the heterogeneous cytokine levels were due to clear-cut dynamics within the acute phase. These were especially evident in pattern A, where the markedly
increased cytokine levels were clearly confined to the early phase of acute infection. Even if we cannot determine the exact timing corresponding to these IgG titers, the initially increased cytokine levels in this pattern seem to drop at an early time point. This result could be important to interpret the acute phase in future studies, as well as to determine the infection status of individual patients in difficult diagnostic situations, like persistent IgM production or acute infection without IgM antibodies.

In conclusion, we characterized the serum cytokine levels in a European setting with predominantly type II strains and observed substantial secretion of Th1 and Th2 cytokines during acute infection, as well as an anti-inflammatory bias during chronic infection. Additionally, our results found that most cytokine activation occurred in the initial phase of the acute infection. These results could pave the way to better understand the immune dynamics of T. gondii infection, especially when including subsequent localized pathologies such as ocular toxoplasmosis in further studies, and thus help to guide diagnosis and treatment.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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