Correspondence

Transforming Growth Factor-β, Interleukin (IL)-18, and IL-12: Effect on the Clinical Course and Complications of Plasmodium falciparum Malaria

To the Editor—We read with interest the recent article by Dodoo et al. [1], and we fully agree with them that secretion of proinflammatory cytokines is essentially to clearance of Plasmodium falciparum malaria. This statement is based mainly on several crucial points: (1) early production of interferon (IFN)-γ, stimulated by induction of interleukin (IL)-12 or IL-18 from macrophages [2, 3]; (2) the correlation of high levels of production of malaria-specific INF-γ and decreased risk of development of fever or of clinical malaria during follow-up [1]; (3) the ability of live parasites—rather than dead parasites—to mount an early INF-γ response to malaria antigens, in conjunction with the ability of either γ/δ T cells or NK cells to respond preferentially to live parasites [4]; and, finally, in severe malaria, (4) suppression of the protective effects of these cytokines by TGF-β [5].

Taken together, these findings show that innate immune response—and, in part, adaptive immune response, through increased production of INF-γ—limits the initial reproduction of the parasite and subsequently provokes its elimination [6, 7].

In our opinion, resolution of P. falciparum malaria is strictly dependent on a very early and effective activation of macrophages, stimulated by various malaria antigens, to produce and secrete IL-12 and IL-18. In the early phase of the immune response, innate immunity—that is, prompt production of IL-12 along with IL-18—may be able to directly activate NK cells and CD8 T cells to produce INF-γ. In addition, activation of NK cells and T cells by IL-18 and IL-12 is also able to produce tumor necrosis factor (TNF)-α, which cooperates with INF-γ to lead to resolution of malarial infection [8].

In a subsequent phase, adaptive immunity, CD8 T cells and γ/δ T cells are critical in the immune response, in that they produce and secrete INF-γ [9, 10]. This adaptive immune response, along with the early immune response, leads to a rapid and effective resolution of malarial infection.

In severe and complicated P. falciparum malaria, including cerebral malaria, there is a dramatic increase and persistent production of TNF-α, paralleled by decreased production of IL-10 and TGF-β [8, 11]. In contrast, the early activation of IL-18 and IL-12 may be weakened, since there is suppression of the protective effects of these cytokines by TGF-β [5].

TGF-β seems to be an important cytokine for maintaining the balance between protection against and progression toward P. falciparum malaria. However, the role that this cytokine plays in malaria has not been completely clarified, and several studies have shown contrasting results with respect to proinflammatory or anti-inflammatory response during the infection. Dodoo et al. [1] have shown that TGF-β production, induced by phytohemagglutinin, is associated with a significantly reduced risk of fever, and they have concluded that regulation of the proinflammatory cytokine cascade by cytokines such as TGF-β may contribute to protection from malarial pathology.

It is conceivable that, in severe P. falciparum malaria, including cerebral malaria, increased production of TGF-β may be associated with anti-inflammatory effects, through inhibition or decrease of proinflammatory cytokines such as IL-18 and IL-12 during the early phase of immune activation. In fact, Omer et al. [12] have reported that TGF-β has proinflammatory activity at low levels, whereas high levels of TGF-β are associated with anti-inflammatory activity.

Thus, we can postulate that, at low levels, TGF-β stimulates the early proinflammatory cytokine response—such as the production of IL-12, IL-18, and INF-γ—to lead to a prompt and complete resolution of malarial infection. In contrast, high levels of TGF-β up-regulate the anti-inflammatory cytokine response, which provokes reduced production of IL-12, IL-18, and INF-γ, with increased and persistent production of TNF-α, a series of events that is responsible for severe P. falciparum malaria infection as well as for cerebral malaria.

In conclusion, it seems that an early proinflammatory cytokine response, including production of IL-12, IL-18 and TGF-β (at low concentrations), mediates protective immunity, whereas a persistent and late anti-inflammatory cytokine response, including production of TNF-α and TGF-β (at high concentrations), contributes to severe and complicated P. falciparum malaria. However, further studies are needed to confirm the reciprocal and balancing effects that TGF-β, IL-12 and IL-18 have in determining the resolution or persistence and worsening of malaria infection.

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**Reply**

To the Editor—We agree with Torre and Speranza [1] that both innate and adaptive immune responses are likely to play an important role in the control of blood-stage malarial infections. Indeed, we have directly addressed this issue in a recent review article [2]. We had previously shown that γδ T cells are a significant source of interferon (IFN)–γ [3], and, more recently, we have demonstrated that human NK cells can be activated by *Plasmodium falciparum*-infected red blood cells to produce IFN-γ. NK activation is extremely rapid (<12 h) and, not surprisingly, is both interleukin (IL)–12 dependent and IL-18 dependent [4]. In our studies, at least, the activated NK cells do not produce large amounts of tumor necrosis factor (TNF)–α, but several sources of TNF-α, including monocyte macrophages and γδ T cells, have been identified by others [5, 6].

The potential role that transforming growth factor (TGF-β) plays in the regulation of antimalarial cytokine responses is intriguing. We have previously postulated a dual role for TGF-β, in which it (1) promotes inflammation, when present at low concentrations early in the infection, and (2) subsequently, at high concentrations, feeds back to dampen the inflammatory response once the infection has been resolved [2, 7]. The experimental evidence so far seems to support this hypothesis, although more data are needed, especially from carefully characterized clinical studies. One problem with many of the clinical data is that they are based on measurements, by immunoassay, of total cytokine concentration in plasma or serum; such assays give little indication of the actual levels of bioactive cytokine and may reflect gradual accumulation of inactive or receptor-bound proteins over a period of several days. It is thus dangerous, on the basis of such data, to infer causal relationships between levels of different cytokines or to interpret cytokine ratios. We believe that one important feature of our recent paper [8] is that we looked at cytokine production in vitro, a situation in which such constraints are less of an issue.

We do take issue, however, with the suggestion that high levels of TGF-β production may potentiate TNF-α production, thereby leading to severe malaria. We are not aware of any data that would support this notion, and data from murine models would tend to indicate exactly the opposite—namely, that failure to up-regulate TGF-β and/or IL-10 may lead to prolonged TNF-α production and severe disease [7, 9]. More data from humans are required before the pathways of cytokine regulation during malaria infection can be inferred with any degree of confidence.

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References

9. Li C, Corraliza I, Langhorne J. A defect in interleukin-10 leads to enhanced malarial disease in *Plasmodium chabaudi* chabaudi infection.
Clinical Value of Adjuvant Interleukin-2: Clarifications and Corrections

To the Editor—I read with interest the recent article by Marchetti et al. [1]. Besides the fact that 22 individuals (3 of whom were lost to follow-up) represent a rather small sample size to assess the clinical value of adjuvant interleukin (IL)—2, the authors claim that IL-2 adjuvant therapy is associated with a reduced risk of human immunodeficiency virus (HIV)—related clinical events; however, given the small sample size, this statement should be reserved for currently ongoing larger phase 2 and 3 trials (e.g., SILCAAT [Study of IL-2 in People with Low CD4+ T Cell Counts on Active Anti-HIV Therapy] and ESPRIT [Evaluation of Subcutaneous Proleukin in a Randomized International Trial]).

Moreover, it is incorrect to call their results the “first evidence, in a randomized study, of a potential clinical benefit of IL-2 therapy” [1, p. 613] by referring to the incidence of thrush and herpes zoster. In 1998, we published a study suggesting a clinical benefit in individuals treated with adjunctive highly active antiretroviral therapy (HAART) [2]. In that study, 44 patients were randomly assigned to receive IL-2 (9 × 10^9 IU/day) for 5 consecutive days, either every 6 weeks or whenever the CD4 cell count dropped below the 1.25-fold of baseline, and were compared with 20 control patients who were receiving HAART alone. Besides significant increases in CD4 cell counts and reduced lymphocyte activation markers (major histocompatibility complex class II, CD25, and CD38), we looked for delayed-type hypersensitivity reactions against common recall antigens that increased in both IL-2 groups but did not reach statistical significance. Of note, delayed-type hypersensitivity reactions against the recombinant gp41 MN protein of HIV improved significantly (P < .05) in this trial, and no opportunistic infections in either of the IL-2 groups were observed, compared with 3 control patients with Kaposi sarcoma. Moreover, dermatological indicator diseases of declining immunocompetence, such as thrush, condyloma, and herpes simplex, occurred less frequently in the IL-2 groups.

In addition, the authors refer to CD25 as the “high-affinity IL-2 receptor” [1, p. 613], which is incorrect. IL-2 binds to 2 different receptors, which are composed of 3 different chains: the α (CD25), the β (CD122), and the γ (CD132). IL-2 binds to the β-γ receptor with intermediate affinity (dissociation constant factor [Kd] 10^-7) and to the α-β-γ receptor with high affinity (Kd10^-9), whereas low-affinity receptors (CD25) lack an intracytoplasmic tail with signaling function [3]. The γ, or common cytokine chain (CD132) is also shared by IL-4, IL-7, IL-9, and IL-15 and is constitutively transcribed in lymphocytes [4, 5]. Although expression of CD25 is classically induced by antigen stimulation, IL-2 alone is sufficient to induce expression of CD25 and progression of T cells through the cell cycle [6]. These discrepancies are frequently found in the literature and probably relate to the lack of reagents in the past; consequently, the expression levels of the β and γ chains of the IL-2 receptor have not been tested. Therefore, it remains unknown whether the expression of CD25 is an indication of cells expressing the high-affinity IL-2 receptor and, thus, are more likely to proliferate in response to IL-2.

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References
our study is preliminary and small, it still demonstrates a significant difference between the IL-2-treated patients and the control patients, who received highly active antiretroviral therapy (HAART) alone. However, we agree that a definitive conclusion awaits larger trials. Unfortunately, we are unlikely to obtain a conclusive answer soon.

The article by Hengge et al. [2] results in a better understanding of IL-2 use in HIV-infected patients. However, the authors describe the IL-2 effect in a cohort of HIV-infected patients who are quite different from those we studied. All the patients enrolled in their study [2] had a baseline CD4 cell count >200 cells/µL and were divided into 2 groups; group A started 5-day IL-2 cycles every 6 weeks, and group B postponed IL-2 initiation whenever their CD4 cell counts fell to <1.25-fold of baseline. In contrast, the achievement of a CD4 cell count >200 cells/µL was the primary end point of our study [1]. We, in fact, selected a specific group of HIV-positive patients who had confirmed immunological failure after receiving HAART for at least 1 year. Our patients had very low CD4 cell counts at both nadir (mean, 48.8 cells/µL; range, 2–140 cells/µL) and baseline (mean, 147.3 cells/µL; range, 28–222 cells/µL), despite long-term HAART (mean duration, 3.9 years).

With regard to the role of CD25 (IL-2 receptor [R] α chain), we agree with Dr. Hengge [3] that there is controversy about the actual meaning of its expression on the cell surface (i.e., whether it identifies high-affinity IL-2R-expressing cells). The point we wanted to emphasize in our article was related to the differential IL-2R phenotype on early thymocytes (IL-2Rα, IL-2Rβ, and IL-2Rγ) [4], compared with naive cells in the periphery, which express the β and γ signaling chains of the receptor but lack the α chain. Thus, the evaluation of CD25 expression on T cell receptor excision circle–positive naive cells could provide further insight into the IL-2 effect in boosting de novo T cell synthesis or peripheral expansion.

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The Role That the Functional Asp299Gly Polymorphism in the Toll-Like Receptor–4 Gene Plays in Susceptibility to Chlamydia trachomatis–Associated Tubal Infertility

To the Editor—Recently, in the first genetic epidemiological study of the Toll-like receptor 4 (TLR4) in gram-negative infections, Read et al. [1] reported that a functional polymorphism, Asp299Gly, in the TLR4 gene is not associated with the likelihood or severity of meningococcal disease. Chlamydia trachomatis, which, like meningococcal disease, is a natural gram-negative infection, is the most prevalent sexually transmitted infection and is associated with severe complications in women with tubal infertility. Different C. trachomatis strains, called “serovars,” are not clearly associated with upper-genital-tract progression [2, 3]. It is likely that other potential bacterial and host genetic factors play an important role in the susceptibility of C. trachomatis infection and, subsequently, in the development of tubal infertility. To further assess the role that the Asp299Gly polymorphism in the TLR4 gene plays in human gram-negative infections, we investigated its relation with C. trachomatis infection and tubal infertility.

In 1992, a prospective study was initiated among 240 white Dutch subfertile women (ages 19–40 years). All underwent laparoscopy to assess the presence of tubal pathology [4]. C. trachomatis IgG antibody titers were determined by microimmunofluorescence, as described elsewhere [4]. All 48 women with positive IgG antibodies were included in the study. Of the 240 women studied, 35 had tubal pathology (and therefore were considered to be case patients) and 49 had no tubal pathology (and therefore were considered to be control subjects).

The functional A→G missense mutation at 896 bp, Asp299Gly, in the human TLR4 gene was identified, as described elsewhere [5]. The TLR4 gene is a potentially interesting candidate for study in C. trachomatis infections, because signaling through TLR4 is activated by both lipopolysaccharide and heat-shock protein 60, of both mammalian and microbial origin [6]. This, in turn, may contribute to the development of autoimmune reactions to C. trachomatis infections in the tubae. Surprisingly, not only the study presented by Read et al. [1], concerning the likelihood and severity of meningococcal disease, but also our study, of C. trachomatis and tubal infertility (table 1), did not result in an over- or underrepresentation, among case patients and control subjects, of the functional polymorphism of the TLR4 gene (P > .5). No statistical differences were observed between our allele frequencies and those reported by Read et al. [1]. Furthermore, the allelic frequency of the Asp299Gly polymorphism in the TLR4 gene did not differ from that in a control
population in The Netherlands (authors’ unpublished data). Our results support the hypothesis that the functional polymorphism in the gene encoding TLR4 function may not be a rate-limiting component for containment, by the human innate immune system, of natural gram-negative infection (due to extra- and/or intracellular pathogens). Additional information is available at the Web site of the Laboratory of Immunogenetics (http://www.med.vu.nl/immunogenetics/).

Table 1. Genotype and allele frequencies of the Asp299Gly polymorphism in the TLR4 gene, in Chlamydia trachomatis IgG-positive white Dutch women with (case patients) or without (control subjects) tubal pathology.

<table>
<thead>
<tr>
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<th>Genotype 1</th>
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<th>Genotype 3</th>
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<th>Group</th>
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<tr>
<td>Case patients</td>
<td>30</td>
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<td>0</td>
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<tr>
<td>Control subjects</td>
<td>41</td>
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<tr>
<td>Total</td>
<td>71</td>
<td>11</td>
<td>2</td>
<td>84</td>
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| Allele           | 88         | 10         | 98        |

NOTE. Data are no. (%).

References


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Reply

To the Editor—The Asp299Gly single-nucleotide polymorphism (SNP) of the human TLR4 gene is the most common hitherto described and is apparently functional in that airway epithelial cells from patients who carry it are hyporesponsive to lipopolysaccharide (LPS) and exhibit reduced expression of Toll-like receptor 4 (TLR4), a loss of function that is restored by ectopic transfection of the wild-type gene [1]. Furthermore, compared with individuals with wild-type TLR4, people with the Asp299Gly allele have been shown to have lower resting plasma concentrations of some (but not other) markers of inflammation, including interleukin-6, procalcitonin, fibrinogen, and soluble vascular-cell adhesion molecule 1 [2]. Therefore, it is reasonable to postulate that people with the Asp299Gly polymorphism should be more likely to manifest disease after exposure to a gram-negative infection, in which LPS is a biologically significant mediator of pathology.

The data presented here by Morré et al. [3], together with ours [4], suggest that this is not the case. We found, in our large study of patients with a history of meningococcal disease and blood-donor control subjects, that the polymorphism is not overrepresented in people with this prototypic gram-negative infection; the allele frequency of Asp299Glu was 5.9% in 879 blood-donor control subjects, 6.5% in 1047 patients with microbiologically proven meningococcal disease, and 4.1% among 86 patients who died of proven meningococcal disease [4]. These frequencies exhibited no significant partitioning among patients, including fatal cases. It is noteworthy that the overall allele frequencies reported by ourselves are strikingly similar to those observed by Morré et al., lending technical credence to each study.

Two studies originating from the laboratory that discovered the Asp299Gly polymorphism have reported overrepresentation of the SNP in patients with clinical disease. Kiechl et al. [2], in a study involving 810 middle-aged and older individuals, 53 of whom were heterozygous for the Asp299Gly allele, found that the frequency of putative, undefined bacterial infections was higher in carriers of the SNP and that the severity of atherogenesis was also diminished in carriers. Lorenz et al. [5] studied 91 patients with septic shock and 73 healthy blood-donor control subjects and found the Asp299Gly exclusively in the septic-shock cohort. The latter study was somewhat small, but the absence of the allele in any of the blood-donor control subjects was a little surprising, in light of the allele frequency that we—and Morré et al.—have reported.

Asp299Gly is not the only polymorphism in human TLR4; there is another, Thr399ile, that also impairs lipopolysaccharide signaling and inflammation [1]. We have sequenced full-length TLR4 of people who have experienced meningococcal disease, and we have found other, functionally insignificant polymorphisms that have no likely direct causal relationship with the disease (R.C.R., unpublished data). In C3H/HeJ mice, a point mutation within exon 3 modifies TLR4 within the cytoplasmic portion of the receptor, which

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is critical for signal transduction [6]. In a search for gene variation that is likely to be biologically significant, we examined a 500-bp segment of the same region of the gene in 51 patients with meningococcal disease, but we found no evidence of sequence variation [4].

Despite both the reported functional significance of Asp299Gly and the reported associations described elsewhere, we remain unconvinced that it has any effect on the manifestation of gram-negative infections, and we consider that inefficient TLR4 signaling may not be rate limiting during human responses to natural LPS-driven diseases. There are a considerable number of collateral pathways available for innate responses to infections, and 10 human TLRs have now been described, which differ in their cofactor requirements and in their pattern-recognition specificities [7]. In the case of Neisseria meningitidis, there is at least 1 non-TLR4 pathway for innate signal transduction; TLR2 can also signal the presence of the organism [8, 9], possibly in response to the presence of lipoprotein H8. It is likely that such pathways contribute to effective innate immunity even if TLR4 function or expression is impaired.

It is clear that there is some genetic influence on the expression of disease due to Chlamydia trachomatis [10, 11], but Morré et al. show that C. trachomatis-seropositive patients with tubal disease do not have an Asp299Gly allele frequency that differs from what would be expected. Although they used the best serological test available, we would offer the cautionary note that, compared with nucleic acid detection, serological tests have poor specificity in the diagnosis of genital infection due to this organism [12].

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