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Individuals living in regions of intense malaria transmission exhibit natural immunity that allows them to be without fever and other symptoms for most of the time despite frequent parasitization. Although this tolerance of parasitemia appears to be more effective in children than in adults (as evidenced by lower parasitemia fever thresholds with age), adults do exhibit a degree of tolerance but the mechanism(s) underlying this are unclear. Asymptomatic malaria-exposed children have higher levels of nitric oxide (NO) than children with severe disease, and NO has been proposed as a mediator of malarial tolerance. However, the ability of highly malaria-exposed asymptomatic adults to generate high-level basal NO is unknown, as is the relationship between NO and malaria tolerance in adults. The relationship between NO and malaria parasitemia was therefore determined in asymptomatic adults from Papua, Indonesia. Adults with Plasmodium falciparum parasitemia had markedly increased basal systemic NO production relative to a parasitemic Papuan controls, who in turn produced more NO than healthy controls from a region without malaria. Immunoglobulin E levels were universally elevated in malaria-exposed Papuan subjects, suggesting that the prevalence of intestinal parasitosis may be high and that nonmalarial infection may also contribute to high basal NO production. Basal peripheral blood mononuclear cell (PBMC) NO synthase activity was elevated in Papuans but poorly correlated with systemic NO production, suggesting that NO production in this setting arises not only from PBMCs but also from other tissue and cellular sources. NO production was associated with and may contribute to malaria tolerance in Papuan adults.

The natural history of malaria in regions where it is endemic is characterized by long periods of asymptomatic parasitemia punctuated by episodic clinical attacks that decrease in frequency with age (40, 57). Although the immune processes preventing symptoms such as fever in chronically parasitized individuals are poorly understood, this aspect of immunity (malarial tolerance) is thought to be most efficient in childhood and then declines with age (18, 41, 70). This is because the threshold of parasitemia associated with fever appears to be age dependent and higher in children than adults from geographically diverse regions where malaria is endemic (56, 65). Nitric oxide (NO) has been proposed as the mediator of tolerance in populations in regions where malaria is endemic (15) on the basis that NO production in asymptomatic malaria-exposed children exceeds that of children with severe malaria (1, 5) and that parallels exist between the malaria tolerant state and endotoxin tolerance (72). Indeed, in 1965 it was shown that cross-tolerance to bacterial lipopolysaccharide could be induced by experimentally infecting prisoners with Plasmodium vivax (61); this finding is in accord with present molecular models of tolerance (21). Downregulation of the endogenous pyrogen tumor necrosis factor alpha (TNF-α) and upregulation of NO are thought to be typical manifestations of endotoxin tolerance in mononuclear cells (72). In addition, NO is thought to play a key role in tolerance induction (23, 58, 77). This latter role of NO is the basis on which the above hypothesis was formulated (15).

Much less is known of NO production in asymptomatic malaria-exposed adults than in children. The majority of studies examining NO production in malaria-exposed adults have reported NO metabolite levels in the setting of clinical disease (7, 22, 34, 46, 48, 54, 67, 69), reflecting altered NO production in clinical malaria. Most of these studies did not control for dietary nitrate ingestion (7, 22, 34, 46, 48, 54) or altered nitrate handling in renal impairment (4, 7, 34, 48, 54, 69). Very few studies have reported NO metabolite levels in asymptomatic malaria-exposed adults (7, 15, 48, 54), and none of these controlled for the confounding effect of dietary nitrite-plus-nitrate (NOx) ingestion. Some studies have hypothesized that malaria-exposed adults are less tolerant of parasitemia than children (15, 56) and that their systemic NO production would mirror that of healthy non-malaria-exposed adults due to purported hyporesponsiveness of peripheral blood mononuclear cells (PBMCs) to nitric oxide synthase 2 (NOS2) induction (15). However, adults from regions where malaria is highly endemic rarely experience clinical illness and have long been reported to tolerate parasitemias (sometimes exceeding 500 parasites/}

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nitric oxide in the absence of symptoms (41, 52). These basic epidemiological observations are consistent with the notion that tolerance in adults is less effective than in children (56) and also, importantly, with the notion that these adults are nevertheless more tolerant than would be expected of malaria-naïve subjects (26, 42).

Recent work has established that experimentally induced Plasmodium falciparum infection strongly induces NOS in PBMCs in malaria-naïve adult human volunteers at low levels of parasitemia that are detectable by PCR but not by microscopy (53). This finding agrees with in vitro studies (66) and a previous in vivo study linking P. falciparum to NO production in Tanzanian children, in which children with microscopically proven asymptomatic P. falciparum parasitemia produced significantly more NO than children negative for P. falciparum on both microscopy and PCR (6). It is also possible that other subclinical infections (such as intestinal parasitosis [46]) contribute to basal NO production in tropical regions via activation of monocyte CD23 receptors (33) or other mechanisms (24, 64).

In the present study, we sought to explore the relationship between NO production and tolerance in adults in malaria-endemic areas. We hypothesized that basal NO production in asymptomatic adults exposed to intense malaria transmission would be higher than in healthy controls from a region of low malaria endemicity, who in turn would produce more NO than non-malaria-exposed controls. In asymptomatic adults exposed to intense malaria transmission, we hypothesized that, as in children, NO production and/or PBMC NOS activity would be higher in adults with asymptomatic parasitemia than in those without microscopically detectable parasitemia. Further, we expected that PBMCs would be the predominant source of NO production and therefore that the NOS activity of PBMCs would correlate with levels of systemic NO production.

MATERIALS AND METHODS

Study sites. Subjects were recruited in March 1999 from 11 villages within close proximity of Genyem township (Papua province, Indonesia), which is located ca. 100 km by road from the provincial capital Jayapura and 20 km inland from the northern coast. The climate of this heavily forested region is typically tropical with a rainy season between November and May. Malaria transmission is reported to be perennial in this region but more intense during the rainy season, and all four malaria species parasitizing humans have been observed (68). In a comprehensive malariometric survey performed between November and December 1998, the prevalence of asexual parasitemia in children <10 years in age was 39% (messoendemic) and the spleen enlargement rate was 51% (hyperendemic [68]). These rates are inclusive of nonindigenous transmigrants (mostly Javanese) and are thought to under-estimate the true rates in indigenous Papuans due to less frequent use of bed nets and closer proximity to the forest in the latter group (68). The clinical epidemiology of malaria in Genyem is characterized by a very low incidence of severe disease and clinically indistinguishable uncomplicated attacks due to P. falciparum and P. vivax (68). Malarial endemicity in the urbanized city of Jayapura is difficult to estimate with certainty but is estimated to be significantly lower than in Genyem (20a, 20b).

Enrollment of subjects and controls. Ethical approval to conduct the study was obtained from the Joint Institutional Ethics Committee of the Royal Darwin Hospital and Menzies School of Health Research of Australia and from the Ethics Committee of the National Institute of Health Research and Development, Indonesian Ministry of Health, Jakarta, Indonesia. Approval to conduct the study was also sought and obtained from the relevant provincial, regional, and local administrative and health authorities within Papua province. Permission to work in each village was obtained from the respective village leaders after explanation of the study protocols and consent process. Written informed consent was obtained from individual participants after explanation of the study protocol in Bahasa Indonesia. All healthy adults aged 16 years and older living within the study area were eligible to enter the study. Volunteers presenting for screening underwent a clinical assessment questionnaire administered by the study team, measurement of axillary body temperature by using a digital clinical thermometer, and collection of thick and thin blood smear by fingerprint.

The clinical criteria for enrollment were as follows: no fever history or treatment for malaria within the past week, no clinical evidence of malaria or other infection, no diarrhea, and no current pregnancy. Screened subjects who fulfilled these criteria were provisionally allocated into one of three study groups on the basis of screening microscopy: (i) asymptomatic P. falciparum infection, (ii) asymptomatic P. vivax infection, and (iii) microscopically aaparastemic health controls. Potential enrollees were then selected from those eligible with the aim of including balanced numbers of parasitemic and aparastemic individuals and approximately equal sex and age distributions in each group. Enrollees were supervised overnight at the local health center, where clinical details were rechecked at interview in the evening and measurement of axillary temperature was repeated. A third axillary temperature was recorded the following morning. Urban Papuan adult controls were recruited in Jayapura between July 1999 and July 2000, and urban adult non-malaria-exposed Australian Research Institute employee controls were recruited in Darwin in February 2002 with similar criteria and methodology as for the subjects. Axillary temperatures were measured once in Jayapura controls and twice in Darwin controls, 16 h apart.

Sample collection. To control for the confounding effect of dietary nitrate ingestion on NOx levels (27), subjects and controls were fasted for a minimum of 12 h overnight after a low nitrate meal of chicken and rice (2). Chicken from the three study sites was confirmed to be preservative- and additive-free through discussion directly with the suppliers and was cooked and then consumed without additives or condiments. Liberal quantities of locally sourced nitrate-free water were permitted during this period, but smoking and chewing of betel nut were prohibited. Adherence to the fasting protocol was confirmed by direct supervision of subjects and by using a questionnaire for controls. First-void urines were collected after the fast, after which urine and venous blood was collected. Urine from subjects was examined by dipstick (Bayer Diagnostics) to detect urinary tract infection and then stored on isopropanol (20% final volume) to prevent bacterial overgrowth. Whole blood was processed within hours, from which plasma was removed and PBMCs separated by density centrifugation and then cryopreserved at −70°C until assay.

Laboratory assays. Two thick and thin blood smears from consecutive days were taken to account for periodic fluctuation of parasite densities (10, 20). Smears were stained with 4% Giemsa solution and examined by a microscopist (S.S.) with more than 20 years of experience. Negative smears were those with no parasites seen on examination of 100 high-powered (S.S.) fields. The difference among ages (P = 0.001) was assessed with the Kruskal-Wallis test.

<table>
<thead>
<tr>
<th>Subject group</th>
<th>No.</th>
<th>Median age (yr) (IQR)</th>
<th>% Male</th>
<th>No. of subjects (median parasitemia/μl infected with: P. falciparum</th>
<th>P. vivax</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genyem</td>
<td>40</td>
<td>24.5 (19–33.5)</td>
<td>58</td>
<td>12 (311)</td>
<td>5 (67.5)</td>
<td>1 (48/37)</td>
</tr>
<tr>
<td>Jayapura</td>
<td>43</td>
<td>24 (18–30)</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Darwin</td>
<td>22</td>
<td>33 (26–42)</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* a The difference among ages (P = 0.001) was assessed with the Kruskal-Wallis test.  
  b The difference among ages (P = 0.001) was assessed with the Kruskal-Wallis test.  
  c One subject was infected with both P. falciparum and P. vivax, with parasitemia of each in parentheses, respectively.

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NOx)/(urine/plasma Cr). NOS activity was measured in PBMC pellet lysates by measuring the amount of [14C]arginine that was converted to citrulline per mg of total cellular protein per h (71). PBMC lysates were analyzed for NOS2 antigen content by immunoblot, as previously described (62). An immunoblot was considered positive if a clear band was visible at 130 to 131 kDa.

Statistical methods. All statistical analyses were performed by using Stata 7.0 software (StataCorp.). In general, summary statistics are presented as means with standard deviations for normally distributed data and as medians with interquartile ranges (IQR) for nonparametric data. The significance of binomial probabilities was assessed by using an exact method (Stata Bitest). Pairwise comparisons of nonparametric continuous data were performed by using the Mann-Whitney U test. Correlation between continuous measures was checked by using Spearman rank test. In all statistical tests, P values of <0.05 were considered to indicate statistical significance.

RESULTS

Baseline characteristics of subjects. There were 179 volunteers from Genyem screened for entry into the study, from which 56 subjects were enrolled. A total of 16 subjects met the exclusion criteria subsequent to their initial screening: 3 had a measured axillary temperature of ≥37.5°C, 9 gave a history of fever, 12 gave a history of other symptoms consistent with malaria, and 4 reported recent ingestion of antimalarial medication. One other subject had a urinary tract infection and was excluded from analyses involving urine results. There was no significant difference in the age of men or women in the 40 included subjects whose baseline characteristics are shown in Table 1. One subject was from Sulawesi and had been living in Genyem for 2 years, and the remainder were coastal Melanesians. The mean duration of residence in Genyem was 20 years (standard deviation of 10 years), with 25 of the subjects having never lived elsewhere. All subjects were supervised overnight at the Genyem Health Center, and no breaches to the fasting protocol were observed. Two thick and thin smears were available for examination by the experienced microscopist in 34 of the 40 subjects, and one smear only was available from 6 (all aparasitemic). The parasitemias of the included group were broadly representative of the screened group.

Baseline characteristics of controls. Of the 43 controls recruited from Jayapura (Table 1), 14 (33%) were coastal Melanesians, 6 (14%) were Melanesians from the highlands, and the remainder had emigrated from other parts of Indonesia. Male Jayapura controls (median age, 28 years; IQR, 22 to 32) were significantly older than female controls (median age, 18 years; IQR, 16 to 25; P = 0.002). Indonesian immigrants (median age, 30 years; IQR, 24 to 33) were significantly older than both coastal Melanesians (median age, 19 years; IQR, 16 to 22; P = 0.001) and Melanesian highlanders (median age, versus Darwin, P = 0.001; and Jayapura versus Darwin, P = 0.02. (B) Natural logarithms of NOx in plasma (in micromoles/liter). The significance levels of pairwise comparisons are as follows: Genyem versus Jayapura, P = 0.008; Genyem versus Darwin, P < 0.001; and Jayapura versus Darwin, P < 0.001. (C) Natural logarithms of PBMC NOS activity (in picomoles of [14C]arginine converted to citrulline per milligram of total cellular protein/hour). The significance levels of pairwise comparisons are as follows: Genyem versus Jayapura, P = 0.17; Genyem versus Darwin, P < 0.001; and Jayapura versus Darwin, P < 0.001. In each figure, the horizontal line represents the median and the box extends from the 25th to the 75th percentiles. Vertical lines extend no further than the most distant datum point or 1.5 times the IQR. Outlying values are represented as small diamonds.
was significantly higher in male Jayapura controls (median, 31 μmol/liter; IQR, 23 to 40 μmol/liter) than in females (median, 12 μmol/liter; IQR, 10 to 18 μmol/liter; P < 0.001). In that group, plasma NOx was also significantly higher in coastal Melanesians and controls from elsewhere in Indonesia than in six Melanesian highlanders (five of whom were women). After the NOx level in plasma was transformed to normality, the significance of sex was retained in a multiple linear regression model but that of ethnicity was not (nor was the effect of age significant in the model). Plasma NOx was also significantly higher in male Jayapura controls or Darwin controls and were also higher in Jayapura controls than in Darwin controls (Fig. 1A and B). Levels of Cr in plasma were within the normal range for all controls and for all but 1 subject from Genyem, whose Cr was 140 μmol/liter.

The results of all pairwise comparisons remained significant when this subject was excluded from the statistical analyses. Furthermore, the significance of each pairwise comparison was maintained if the ratio of NOx/Cr in plasma was used in place of plasma NOx in the statistical analysis (data not shown). As a marker of renal tubular NOx handling, the median FE_{NOx} levels in the Genyem subjects and Jayapura and Darwin controls were 30, 27, and 33%, respectively. Neither urine NOx/Cr nor plasma NOx were significantly correlated with age in any of the groups.

Urine NOx/Cr did not vary with sex in any of the groups (nor with ethnicity in Jayapura controls). However, plasma NOx was significantly higher in male Jayapura controls (median, 31 μmol/liter; IQR, 23 to 40 μmol/liter) than in females (median, 12 μmol/liter; IQR, 10 to 18 μmol/liter; P < 0.001). In that group, plasma NOx was also significantly higher in coastal Melanesians and controls from elsewhere in Indonesia than in six Melanesian highlanders (five of whom were women). After the NOx level in plasma was transformed to normality, the significance of sex was retained in a multiple linear regression model but that of ethnicity was not (nor was the effect of age significant). PBMC NOS activity was higher in Genyem subjects than in Jayapura controls, but this difference was not significant (Fig. 1C). NOS activity was significantly lower in Darwin controls than in both Genyem subjects and Jayapura controls (Fig. 1C). NOS activity was not significantly correlated with plasma NOx or urine NOx/Cr in any of the three study locations, nor was it significantly related to age or sex (or ethnicity in Jayapura controls). Sufficient PBMC lysate for measurement of NOS2 antigen content was available for 33 of the 40 Genyem subjects and for 33 of the 43 Jayapura controls. NOS2 antigen was detected in all but one of the Genyem subjects and in all 33 of the Jayapura controls. Figure 2 shows a representative immunoblot, with a clear band visible at 130 to 131 kDa.

Effect of parasitemia in Genyem subjects on NO production and PBMC NOS activity. Plasma NOx was significantly higher in those with malaria parasitemia (median, 40 μmol/liter; IQR, 16 to 36) than in those without it (median level of NOx in plasma, 20 μmol/liter; IQR, 16 to 36; P = 0.020). Excluding the one subject with mixed infection, plasma NOx was highest in individuals with P. falciparum, intermediate in those with P. vivax, and lowest in aparasitemic subjects (Fig. 3A). The difference in plasma NOx between P. falciparum parasitemic subjects and aparasitemic subjects was significant, but those with P. vivax did not differ significantly from either of the other two groups. Urine NOx/Cr was also higher in parasitemic subjects (median, 0.14; IQR, 0.10 to 0.23) than in aparasitemic subjects (median, 0.07; IQR, 0.04 to 0.20; P = 0.049). Urine NOx/Cr was similar in subjects with P. falciparum and P. vivax parasitemia but lower in aparasitemic subjects (Fig. 3B). The difference in urine NOx/Cr between P. falciparum parasitemic subjects and aparasitemic subjects was significant, but those with P. vivax did not differ significantly from either of the other two groups. There was no significant difference in NOS activity between aparasitemic subjects and either subjects with any malaria parasite or those with only P. falciparum or P. vivax. There was no significant correlation between the densities of either P. falciparum or P. vivax parasitemia and either plasma NOx, urine NOx/Cr, or NOS activity.

Relation between NO production and IgE. IgE levels were elevated (normal, <100 kU/liter) in 35 of 36 Genyem subjects for whom a result was available (median, 3,350 kU/liter; IQR, 1,425 to 5,800). There was no significant difference in IgE levels between parasitemic and aparasitemic subjects, including those whose parasitemia was due only to P. falciparum, nor was IgE related to the level of parasitemia. The level of IgE in plasma was not significantly related to either plasma NOx, urine NOx/Cr, or NOS activity.

**DISCUSSION**

In contrast to what has previously been suggested (15), the results presented here show that adults from Papua province produce more NO than Australian adults and that P. falciparum parasitemia is associated with, and may be responsible for, an increment in basal NO production. As found in semi-immune African children (5, 49), we have shown that adults from both urban and rural areas of Papua have marked elevation of basal PBMC NOS activity and have near-universal basal expression of PBMC NOS2, a finding that is rare in residents of temperate regions (71). Surprisingly, basal NOS activity in PBMCs varied less according to the level of malaria exposure than did the total systemic NO production. This suggests that, in addition to inducing NOS2 in PBMCs, P. falciparum may induce NO production from other tissue and cellular sources.

**FIG. 2.** Representative immunoblot showing NOS2 expression (presence of a clear band at 131 kDa) in PBMC lysates from asymptomatic residents of Genyem. Lanes: 1 and 2 (P.f. neg), absence of P. falciparum on microscopy; 3, 4, and 5 (P.f. pos), presence of P. falciparum on microscopy. Neg, negative control (unstimulated J774 mouse macrophages); Pos, positive control (J774 cells stimulated with lipopolysaccharide/IFN-γ). Unrelated samples run in intervening lanes have been omitted.
exclusion of subjects meeting these stringent criteria. Furthermore, the strict dietary controls would have minimized extraneous influences on NOx interpretation and the normal renal function and similar fractional excretions of NOx ensured that results were comparable across the three study sites. Although urinary NOx excretion (estimated as the spot ratio of NOx to Cr) may be a more stable measure of total systemic NO production over time than the plasma NOx (9), both measures were well correlated in the present study, and similar conclusions can be drawn from interpretation of either data set.

The median plasma NOx of malaria-exposed adults in our study was closer to that reported in unfasted malaria-exposed Papua New Guinean children (median plasma NOx, 40 μmol/liter; mean age, 5 years) than Papua New Guinean adults whose malaria exposure was unspecified (median, 12.6 μmol/liter [range, 9.5 to 34]; age range, 18 to 40 years) (15). Our finding that systemic NOx was highest in individuals tolerant of malaria parasitemia is consistent with the notion that NO plays a role in the tolerance of malaria-exposed adults, as hypothesized in children (6, 15), but does not prove it. Although NO was previously thought to play a key role in mediating the related phenomenon of endotoxin tolerance (23, 58, 77), a growing body of evidence suggests that other mechanisms could be more important (21). In fact, recent evidence suggests that at least in NOS2 knockout mice, NO is dispensable for the development of endotoxin tolerance (76). The proposed glycosylphosphatidylinositol toxin of P. falciparum may instead initiate tolerance through repeated binding to the mononuclear cell Toll-like receptor 2 (TLR-2) (12, 60) and induce functionally similar events to that caused by bacterial lipopolysaccharide (which binds to TLR-4 [36]) (59). Induction of cross-tolerance by ligands interacting differentially with TLR-2 and TLR-4 has been demonstrated and shown to be independent of the activity of paracrine mediators (37), which further suggests that NO may not be critical to this process. An alternative possibility (that tolerance does not result from the characteristic downregulation of TNF-α production seen with endotoxin tolerance [72] but from resistance to the effects of TNF-α) is not supported by clinical data (1).

Whether NO is induced by malaria infection (66) and other stimuli proportionate to the sum total of stimulation and/or upregulation of NOS2 induction, as occurs with endotoxin tolerance (77), may be a moot point if the amount of NO produced correlates with the level of tolerance. Our finding that urban Papuan controls and aparasitemic subjects had similar levels of PBMC activity and systemic NO production (but that both were higher than Australian adults) may be consistent with genetic differences in NO regulation or alternatively result from stimuli present in those from Papua that were absent from those in Darwin. It is unlikely that submicroscopic malaria parasitemia alone accounted for these findings, since malarial endemicity in Jayapura is low, a fact which is supported by the absence of microscopically detectable parasitemia from all 43 Jayapura controls. The reason for the sex-related difference in plasma NOx (but not other measures of NO production) in Jayapura controls is unclear. A number of intestinal parasitic infections are endemic among individuals living in Papua province that are rarely seen in Darwin adults (Wayne Pederick, unpublished data). These include the helminths Trichuris trichiura, Ascaris lumbricoides, Necator americana...
canus, and Strongyloides sp., and protozoal infections such as Entamoeba histolytica, Entamoeba coli, and Giardia lamblia (8, 19, 25, 43). It is likely that helminth infections were a major cause of the very high levels of IgE measured in the Genem subjects (73) since, although P. falciparum has been linked with elevated IgE (51), the levels are highest in cases of severe malaria (50) and IgE was not significantly related to asymptomatic P. falciparum infection in the present study.

Helminth infection has been linked to NO production in a malaria-exposed population of Thai adults (46). This may reflect activation of monocyte CD23 receptors by IgE (33) or induction of NO-inducing cytokines by helminths. IgE was not associated with any measure of NO production in the present study but the possibility that IgE did induce NOS2 cannot be entirely discounted. Induction of NO production by IgE could become saturated at a threshold IgE level that was exceeded in most subjects or the contribution of IgE to overall NO production. This may not have been detected statistically due to a type 2 error. Alternatively, IgE alone may not be sufficient to activate CD23 and the presence of IgE complexes may be more important (45). Although helminth infections have typically been associated with Th2 cytokine responses (16, 74), ex vivo cytokine responses to T. trichura and A. suum antigens in the whole blood of children aged 4 to 15 years from Cameroon have been shown not to “easily fit the highly polarized Th1/Th2 paradigm,” given that production of TNF-α and the potent NO inducer gamma interferon (IFN-γ) was common among older subjects (24). This finding is in agreement with data from a murine model showing elevated IFN-γ and NOS2 mRNA levels in the spleens of A/J mice coinfected with Schistosoma mansoni and P. chabaudi (75) but is at odds with data from coinfection C57BL/6 mice, in which TNF-α production was reduced (28). Additionally, as part of the host defense against amoebiasis, E. histolytica has been shown to stimulate TNF-α release leading to NO production by IFN-γ-primed murine bone marrow macrophages in vitro (64).

The increased systemic NO in subjects with all-cause malaria parasitemia may have been unduly influenced by the larger proportion of subjects with P. falciparum, even though the NOx levels in subjects with non-P. falciparum parasitemia were closer to those of subjects with P. falciparum than subjects with other parasites. The evidence from the present study directly linking P. falciparum parasitemia with NO production is in agreement with our previous finding in Tanzanian children (6) and evidence from in vitro studies (66). The lack of correlation between PBMC NOS activity and systemic NO production in the present study suggests that malaria parasitemia may induce NO predominantly from other tissue and/or cellular sources. This differs from the recent finding that experimental P. falciparum infection strongly induced PBMC NOS in malaria-naive volunteers (53), although there may be differences in immune responses between natural infection and those induced by experimental very-low-dose intravascular infection. Differences in the background stimulation of PBMC NOS may also be present for the different relationships between parasitemia and NOS activity observed in the present study and the one in malaria-naive volunteers. Although the present study design precluded assessment of the cellular origins of NO beyond PBMCs, NOS2 expression has been demonstrated in the livers, spleens, muscles, and colons in rodent models secondary to induction by malaria and bacterial lipopolysaccharide (29, 32, 47, 55). Induction of NOS has also been demonstrated in human vascular endothelial cells in response to the glycosylphosphatidylinositol toxin of P. falciparum in vitro (66), and NOS2 expression has been found in the endothelium of a variety of tissues from patients with severe malaria (13, 39). The magnitude of any endothelial expression in asymptomatic infection is not known.

Genetic differences in NOS2 or related regulatory cytokine genes could also contribute to differences in basal NO production between Papuans and Australians given that protective polymorphisms may undergo selection in populations with a long history of exposure to malaria and other infections (30). Different genetic polymorphisms have been described in the NOS2 gene that were associated with increased (11) and reduced (31, 35) susceptibility to malarial disease severity, and basal NO production was higher in Gabonese children recovered from mild compared to severe malaria (49). Most recently, a polymorphism in the NOS2 promoter was associated with functional differences in NO production in asymptomatic fasted Tanzanian children (31). The coevolution of humans with malaria parasites and helminths and the possibility of interspecies interactions with respect to innate immune effector mechanisms (14, 17) further increases the potential complexity of this genetic selection (44). Although the present study was not designed to answer questions relating to the role of NO in malarial pathogenesis or resistance to the severity of disease once it has developed, our findings provide a framework within which to investigate the relationship between genetic polymorphisms and basal NO production, while controlling for the potential confounding effects of malaria infection and intestinal parasitosis.

In conclusion, the present study has shown that basal NO production is higher in asymptomatic P. falciparum-parasitized Papuan adults than in aparasitemic and urban controls, who in turn produce more NO than urban Australian researchers not expected to be infected with malaria or intestinal parasites common to Papua. Although genetic polymorphisms in the NOS2 gene or related regulatory genes may explain some of these differences, our data are consistent with a contribution from intestinal parasitosis, possible subclinical malaria infection, and an increment due to microscopically evident malaria parasitemia. The contribution of PBMCs to overall NO production was less than expected and suggests that other cellular sources are also involved, particularly in relation to NO induced by P. falciparum. These data renew the possibility that NO could contribute to tolerance of malaria parasitemia in adults, as may also be the case in children, which may in part reflect an increased genetic capacity to produce NO consequent upon selection by malaria and other infections such as helminths. The complex relationships between asymptomatic and clinical malaria, intestinal parasitosis, genetic polymorphisms, and NO production in populations such as these require further longitudinal study given the potential for targeted interventions aimed at reducing the disease burden of malaria (38, 63).

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