Serum laminin in malaria

C Wenisch, W Graninger, C Viravan, S Looareesuwan, B Parschalk, W Wernsdorfer

Abstract

**Aim**—To determine serum laminin concentrations in patients with uncomplicated *Plasmodium falciparum* malaria.

**Methods**—An enzyme linked immunosorbent assay (ELISA) was used to determine serum laminin concentrations in 54 patients with acute uncomplicated *P. falciparum* malaria during and after treatment, and in 17 control subjects in Bangkok, Thailand.

**Results**—Raised concentrations of soluble laminin were observed in patients (mean (SD) concentration 628 (225) ng/ml), compared with normal controls (490 (116) ng/ml), during the acute phase of the disease. During treatment, serum laminin concentrations decreased and returned to normal within three days. Serum laminin concentrations were correlated with parasite counts before treatment, and with the serum concentration of soluble intercellular adhesion molecule-1 (ICAM-1), soluble E-selectin, and soluble tumour necrosis factor receptor at 55 kilodaltons.

**Conclusions**—These findings are compatible with an increased production or release of laminin in *P. falciparum* malaria, which could indicate a role for the subendothelial basement membrane in the pathogenesis of the disease.

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Laminin, a 900 000 kilodalton multidomain glycoprotein, is produced by a variety of cell types, including epithelial cells, endothelial cells, muscle and Schwann cells. It is associated with several components of basement membranes, including collagen IV, heparan sulfate proteoglycan, and nidogen/entactin. The cruciform structure of laminin, with a number of functional domains, linked by pentameric elements, is well suited for bridging interaction sites and for mediating interactions between cells and extracellular proteins.

Serum laminin is increased in patients with hepatic fibrosis, several different types of tumours, essential cryoglobulinaemia, systemic sclerosis, and in hepatic schistosomiasis. Basement membrane degradation has been shown to be related to serum laminin P1 in patients with transitional cell carcinoma of the bladder. These patients had higher serum laminin concentrations than control subjects. The extracellular matrix can induce tumour necrosis factor alpha (TNFα) in vitro in the absence of an antigen in an MHC-II independent manner. TNFα down-regulates the expression of the α6 subunit of the α6, β1 integrin complex (laminin receptor), thus reducing the adhesion of TNFα activated endothelial cells to laminin in vitro.

As TNFα concentrations are raised in *P. falciparum* malaria, and the roles of extracellular matrix and circulating basement membrane proteins in this disease are unknown, we undertook an immunoserological study of serum laminin in patients with uncomplicated *P. falciparum* malaria from Bangkok, Thailand.

**Methods**

Patients acquire malaria from areas outside Bangkok (Thai-Myanmar border, Thailand). Transmission is therefore of the focal, "forest-fringe" type which affects mainly young adult migrant workers.

This study was approved by the local ethical committee and informed consent was obtained from each of the 54 patients.

All patients in the study met the following criteria: (1) age between 16 and 60 years; (2) infection with *P. falciparum* only; (3) no treatment during the preceding 14 days; (4) no evidence of clinical complications, such as cerebral or renal disease; and (5) male gender. Patients stayed at the Hospital for Tropical Diseases during the study period where no malaria transmission occurred. None of the 54 patients had a severe underlying disease (such as cancer, advanced coronary heart disease, etc.). Seventeen healthy men of similar age distribution served as controls. None of them developed parasitaemia during the follow-up period.

Patients were treated with hydroxynaphthoquinone (Atovaquone) 750 mg every eight hours four times a day plus 1000 mg tetracycline-hydrochloride four times a day for seven days, or with Atovaquone 750 mg every eight hours four times a day plus 200 mg Proguanil daily for seven days (Abstract presented at the XIIIth Congress of Tropical Medicine, Pattana, Thailand, 1992).

Daily clinical examinations were performed during the acute phase of the disease. Body temperature was measured until it became normal. All patients were followed up for 28 days.

The routine laboratory analyses included red blood cell count, haematocrit, haemoglo-
bin, white cell and differential counts, platelet count, serum electrolytes, total bilirubin, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, globulin, aspartate and alanine transaminases, uric acid, blood coagulation tests, and urinalyses.

Blood smears (thick and thin films) were obtained from fingerprick samples, stained with Giemsa, and parasite counts were done. Parasite density was determined by counting the number of parasites per 1000 red cells in a thin film, or the number of parasites per 200 white cells in a thick film, and was expressed as the number of parasites per microlitre of blood. Blood films were declared negative if no parasites were seen in 200 oil immersion fields on a thick film. Blood smears were performed every six hours from the start of treatment until blood films were negative for two consecutive examinations; thereafter smears were done daily.

Serum samples for serological investigation were taken on day 0, during treatment on day 3, and after treatment on days 7, 14, and 28. Serum concentrations of soluble laminin were determined using a commercially available enzyme immunoassay, following the outline of the manufacturer (Takara Shuzo Co. Ltd., Kyoto, Japan). Serum concentrations of soluble ICAM-1, endothelial leucocyte adhesion molecule 1 (ELAM-1), and TNF receptors were also determined by ELISAs (Bender and Co., Vienna, Austria).

Student's t test and Pearson's correlation matrix was used for comparisons between patients and controls. All the analyses were two-tailed, and differences with a p value of less than 0.05 were regarded as significant.

**Results**

All patients had acute uncomplicated *Plasmodium falciparum* malaria with a mean (SD) temperature on admission of 38·3 (0·8°C. The most common symptoms were fever, headache, nausea, and backache (table 1). The mean (SD) parasite clearance time was 65 (16) hours. The mean parasite counts before treatment were 12270/µl. There was no difference in parasitaemia or duration of fever between the two patient groups. Fever persisted, on average, for 45 hours after the start of treatment. No differences between the two treatment regimens were detected regarding parasite clearance time or fever clearance time (data not shown). Fever clearance time also correlated with serum laminin concentrations. No recrudescence in the patients studied occurred during follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n = 54)</th>
<th>Controls (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td><strong>Mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>26-4 (8-9)</td>
<td>24-3 (6-8)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164 (5-9)</td>
<td>166 (7-3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54-6 (7-1)</td>
<td>59-2 (8-2)</td>
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<tr>
<td>Days with fever before treatment</td>
<td>4 (2-4)</td>
<td>0</td>
</tr>
<tr>
<td>Previous malaria attacks</td>
<td>4-3 (4-4)</td>
<td>4-1 (3-9)</td>
</tr>
<tr>
<td>Highest temperature before treatment (°C)</td>
<td>38-3 (0-8)</td>
<td>36-2 (0-2)</td>
</tr>
<tr>
<td>Fever clearance time (hours)</td>
<td>45-2 (39-4)</td>
<td></td>
</tr>
<tr>
<td>Parasite clearance time (hours)</td>
<td>69-1 (15-9)</td>
<td></td>
</tr>
</tbody>
</table>

The serum laminin concentrations were significantly increased before antimalarial treatment (figure). The mean (SD) concentration was 628 (225) ng/ml before treatment. The mean (SD) serum laminin concentration in the control group was 490 (116) ng/ml. Serum laminin dropped to normal within three days of treatment being started. Parasite counts correlated with serum laminin concentration before treatment (r = 0·331). The serum concentrations of soluble TNF receptor, soluble ICAM-1, and soluble E-selectin in patients with *P falciparum* infection were also raised compared with normal controls. The serum concentration of these variables showed different kinetics during the course of the disease. The serum concentration of soluble ICAM-1 remained increased as much as 28 days after the acute malarial attack, whereas soluble TNF and soluble ELAM-1 returned to normal within 28 days (data not shown). Initial serum laminin concentrations also correlated with initial serum concentrations of soluble TNF receptor (55 kilodaltons) (r = 0·475), soluble ICAM-1 (r = 0·494), and soluble ELAM-1 (r = 0·586).

No significant differences could be detected between the two patient groups regarding serum laminin concentrations, soluble TNF receptor, soluble ICAM-1, and soluble E-selectin.

**Discussion**

Malaria caused by *P falciparum* still constitutes a worldwide problem. A growing amount of data have accrued in recent years regarding the pathogenesis of this disease. Sequestration of *P falciparum* infected red blood cells (termed cytoadherence) seems to have a crucial role in the pathogenesis of malaria, as, on the one hand, adhesion of parasitised erythrocytes to microvascular endothelium is a requirement for survival of *P falciparum* in vivo, and, on the other hand, it could trigger a cascade of deleterious events, including induction of toxic inflammatory mediators, metabolic disturbances, and tissue anoxia. Several adhesion molecules have been shown to act as in vitro adhesion receptors of erythrocytes infected with the
asexual mature stage of *P falciparum*. In vitro models of *P falciparum* infection have shown that CD36,21 thrombospordin,22 ICAM-1,23 E-selectin, and vascular cell adhesion molecule-124 are receptors for infected erythrocytes.25

It is not known if extracellular matrix proteins have a role in the pathogenesis of malaria. However, serum laminin concentrations were raised in patients compared with normal controls before treatment. The observed correlation with parasitaemia (P = 0.017) indicates a parasite related production of laminin. In other febrile illnesses with endothelial damage, such as Gram negative sepsis, an increase in serum laminin concentrations was shown to predict acute respiratory distress syndrome.26 However, raised serum laminin concentrations were also found in various non-infectious diseases27-29 and in patients with impaired liver function.30 It is therefore unlikely that laminin acts as an acute phase protein.

Increased interleukin-6 (IL-6) concentrations have also been found in patients infected with *P falciparum* and the bioactivity could be neutralised in patients’ sera using oligoclonal anti-IL-6 antibodies.27 Basement membrane laminin was observed to induce IL-6 secretion in vitro.31 Such a cytokine induction of laminin may modulate the immune response to *P falciparum*.

Multicellular interaction with extracellular matrix proteins, exposed as a consequence of vascular wall injury mediated by sequestrated *P falciparum* infected erythrocytes, can serve to stimulate the secretion of TNFα which induces the recruitment of additional cells to the developing lesion. The adhesion of endothelial cells to laminin in subendothelial basement membrane is reduced by TNFα mediated down-regulation of the endothelial laminin receptor (the α 6, β 1 integrin).32 This also leads to the exposure of subendothelial matrix to the blood stream.

Whether laminin is an adjuvant for cytoadherence for *P falciparum* infected red blood cells, mediating adhesion to subendothelial basement membrane, or a marker for endothelial destruction by adherent infected erythrocytes and subsequent release of basement membrane components in the blood, remains to be elucidated.

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