Elevated Plasma Levels of P-Selectin (GMP-140/CD62P) in Patients with Plasmodium falciparum Malaria

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Abstract: The plasma concentration of soluble P-selectin (GMP-140/CD62P/PADGEM), a selectin produced by activated platelets and endothelial cells, was quantitated in a group of adults and East African negro children presenting with either non-severe or severe (cerebral) malaria caused by Plasmodium falciparum. Sixty percent of adults with non-severe malaria had immunoreactive levels of P-selectin above 200 ng/ml (the maximum recorded for any normal healthy adult in the assay) and 86% of all African children with malaria had concentrations above normal irrespective of their clinical categorization, and most exceeded the maximum limits of the assay (>640 ng/ml). There was no correlation between P-selectin levels and parasitemia. These results raise the possibility that elevated soluble P-selectin in malaria may have an important beneficial anti-inflammatory function.

Key words: Malaria, P-selectin, Inflammation, Cerebral malaria

P-selectin (GMP-140/CD62P/PADGEM) is a 140-kDa granule membrane protein found in the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells. It is expressed on the cell surface upon cell activation and is thought to play an important role in adhesive interactions particularly between granulocytes, platelets and vascular endothelial cells (notably those of post-capillary venules) during inflammation (1). Other members of the selectin family include the endothelial adhesion molecule (ELAM-1/E-selectin) and the leukocyte adhesion molecule-1 (LAM-1/L-selectin). All these molecules share a common structure: an NH2 terminal calcium-dependent lectin domain, an epidermal growth factor motif, a variable number of repeats of a sequence found in complement regulatory proteins, a transmembrane domain and a short cytoplasmic tail (7). The three selectins are >60% identical in their NH2 terminal 120 amino acid residues, the lectin binding domain (1). The cysteine-rich, heavily glycosylated region of the molecule is a feature compatible with a receptor function.

Following activation of endothelial cells with proinflammatory agents such as histamine, cytokines and oxygen radicals, P-selectin is rapidly mobilized to the cell surface where it acts in concert with other cell adhesion molecules such as the intracellular adhesion molecule 1 (ICAM-1) to mediate the rolling and subsequent adhesion of leukocytes to the endothelium (12). In a similar manner, activated platelets with their upregulated expression of P-selectin, rosette with a variety of leukocytes (11). Both activated platelets and endothelial cells secrete into plasma a soluble form of P-selectin, lacking the transmembrane domain, which is thought to have an anti-inflammatory function (3).

The role of P-selectin in malaria is unknown. An essential pathological feature of severe malaria caused by Plasmodium falciparum is sequestration of erythrocytes containing mature forms of the parasite in the deep vascular beds, a phenomenon which is greatest in the brain (13). Parasitized erythrocytes cytoadhere to endothelial cells by means of a variety of adhesion molecules which act as receptors including ICAM-1 and ELAM-1 and which are upregulated by proinflammatory cyto-

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of the malaria-activated cytokine network (for review see Ref. 6).

To our knowledge, there are no reports of plasma or serum P-selectin concentrations in infections. In this pilot study, we have investigated plasma levels in normal healthy adults and compared the results with those obtained from adults and children with non-severe and severe (cerebral) *falciparum* infections.

### Patients and Methods

Venous blood was collected into ethylene-diamine tetraacetic acid (EDTA) (1.5 mg/ml) from the following donors and malaria patients: [1] 7 normal healthy Caucasian adults (age range 21-47 years), [2] 10 adults (5 Caucasian, 5 Negroid, age range 20-52 years) with uncomplicated *P. falciparum* infections (parasitemia range <1-10%) attending the Royal London Hospital, [3] 7 East African negro children (age range 1-5 years) with mild (non-severe) *falciparum* malaria (parasitemia range 180,000-600,000/μl), [4] 7 East African negro children with *falciparum* infection (parasitemia range 155,000-1,036,000), matched with the previous group for age, ethnic group and area of residence in East Africa, defined as having severe (cerebral) malaria with an admission score from 0-4 on a modified Glasgow scale (15). African children were residents of areas hyperendemic for malaria. A single blood sample was taken on admission to hospital and before any antimalarial chemotherapy. All blood samples were centrifuged at 3,000 × g for 10 min at 4 C to remove platelet microparticles (9) and the plasma was stored at −70 C until assay. The plasmas from African patients were transported in liquid nitrogen to London for testing.

Plasma P-selectin concentrations were quantitated using a sandwich ELISA (British Bio-technology Ltd.) that utilizes two mouse anti-P-selectin monoclonal antibodies (mAb) WGA-1 and PL7-6. Briefly, 96-well plates were coated with mAb WGA-1 and incubated overnight at 4 C. Plates were then washed and blocked with phosphate-buffered saline (PBS) containing 2% bovine serum albumin for 2 hr at 37 C. After washing three times, 100 μl sample (neat plasma or a 1/10 dilution in PBS) or standard concentrations of P-selectin (from 0–640 ng/ml), was added to duplicate wells and incubated for 1 hr at room temperature. After careful washing, aliquots of peroxidase-conjugated mAb PL7-6 were pipetted into each well, the plates left for 1 hr, washed and the substrate (O-phenylenediamine HCl) in hydrogen peroxide added and kept at room temperature for approximately 15 min. The reaction was stopped by the addition of 2 M sulphuric acid and absorbance was read at 492 nm in a microplate reader. Concentrations of P-selectin in test samples were calculated from the standard curve. In some cases, absorbance readings were off the scale (>640 ng/ml), so the ELISA was repeated on samples diluted 1/10. For reasons of non-parallelism in the assay, concentrations of P-selectin could not be accurately calculated from the diluted samples, so results for these cases are expressed as the number of standard deviations (S.D.) above the mean of the 7 normal controls. Results >2 S.D. (P <0.05) above the normal mean were considered significantly raised. The Mann-Whitney U-test was used to assess the significance of differences between groups and the Spearman rank test for correlation between parasitemia and P-selectin levels.

### Results

Plasma P-selectin concentrations in 7 healthy adult subjects ranged from 93–199 ng/ml with a mean of 130+/−35 ng/ml and a median of 137 ng/ml (Fig. 1). Six of the ten adult patients (60%) with uncomplicated malaria had raised P-selectin with
three patients exceeding the upper limit of the assay (>640 ng/ml). As shown in Fig. 1, P-selectin levels ranged from 74–640 ng/ml with a mean of 327 ± 225 ng/ml and a median of 230 ng/ml. There was thus a trend for higher P-selectin in the malaria patients (P=0.09) but because of low patient numbers, the statistical analysis between patients and controls was of limited usefulness. Three of the five negro patients (numbers 1, 4, 5, 6, and 10 in Fig. 1) in the uncomplicated malaria group, and three of the remaining five Caucasians (numbers 2, 3, 7, 8, and 9 in Fig. 1) in the same group had levels above 200 ng/ml. There was no statistically significant difference between P-selectin levels in negroes and Caucasians although there was a trend for the latter to present with higher concentrations.

Due to the high level of P-selectin in the African children with malaria (>640 ng/ml), plasma was diluted 1/10 and re-tested by ELISA. Control plasma was similarly diluted and included on the same plate for comparison. The results, shown in Fig. 2, are expressed as explained above. In contrast to uncomplicated malaria in adults, P-selectin levels were raised above normal (>2 S.D. above the normal mean) in all but one of the children, irrespective of their clinical categorization with 3/7 and 5/7 children in the non-severe and severe (cerebral) groups, respectively, having very high levels (>6 S.D. above the normal mean). There was no statistically significant difference between the mean for each group and no correlation between P-selectin levels and parasitemia.

Discussion

The pathophysiological and prognostic significance of raised plasma P-selectin concentration in malaria cannot be deduced from this small pilot study. However, the cause of the elevated selectin levels and the possible consequences of this should be considered.

Our results confirm the few earlier reports that normal human plasma contains soluble immunoreactive P-selectin at concentrations ranging from 90-200 ng/ml (3, 8). The markedly raised concentrations found in malaria, in particular in African children with non-severe or cerebral disease, where levels in 86% of the cases exceeded the maximum limits of the ELISA, requires explanation.

Since mRNA encoding the soluble form of P-selectin exists in both endothelium and platelets (7), plasma P-selectin must derive from either one or both of these sources on activation of the cells. The relative contribution of plasma P-selectin from these two sources in our malaria patients is unknown. One would predict that the plasma level could provide a useful marker of either platelet activation or endothelial perturbation. Certainly there is evidence of in vivo activation of platelets in malaria (4) and it is inferred that endothelial activation also occurs (16). P-selectin, like other adhesion molecules, is upregulated on the surface of endothelial cells by cytokines such as IL-1 and TNF-α (1) and the synthesis of P-selectin in TNF-injected mice is clearly elevated (17). The P-selectin measured in our assay did not derive from platelet microparticle contamination (9) nor could it arise from platelet activation on sample collection (8).

Current evidence suggests that P-selectin plays a fundamental role in both limiting and mediating the inflammatory response of neutrophils. Thus, surface-expressed P-selectin mediates the CD18-dependent adhesion of neutrophils to thrombin or TNF-stimulated endothelial cells (5), and soluble P-selectin serves an anti-inflammatory function by binding to the ligand on neutrophils, thereby
downregulating adhesion and the respiratory burst. Additionally, soluble P-selectin binds to chronically activated CD4+ T cells and modulates their production of proinflammatory cytokines (2). Neutrophils are activated in malaria and have been implicated in parasite killing (10) and overstimulation of CD4+ T cells is thought to be responsible for the high levels of proinflammatory cytokines (14). In this context, raised plasma P-selectin in malaria may have a beneficial effect by regulating inflammation. If this is the case, then one might expect to find lower P-selectin in parasitemic asymptomatic patients and we plan to test the plasma of such individuals to investigate an anti-inflammatory role for this selectin.

Deep vascular sequestration of parasitized erythrocytes (late trophozoites and schizonts) of P. falciparum is considered a central pathological event in causing cerebral malaria which carries a high fatality rate. Sequestration is initiated by cytoadherence of the parasitized erythrocytes to vascular endothelial cells involving interaction between parasite antigens expressed on the surface of the red cell and receptors on endothelial cells upregulated by cytokines such as TNF (6). At least four endothelial receptors have been identified and it appears that the different receptors are used by different parasite isolates (16). It remains to be seen whether P-selectin represents another receptor for adhesion of parasitized red cells. Certainly the appearance of P-selectin on endothelial cells stimulated by high plasma concentrations of TNF-α present in malaria supports its function as an alternative parasite receptor, and the presence of P-selectin on the surface of activated platelets might explain the in vitro rosetting of platelets around parasitized red cells (Facer, unpublished observations). Experiments are currently in progress to examine the potential of P-selectin as an additional receptor and to quantify P-selectin in a greater number of patients and African control subjects (both adults and children) to assess its usefulness as a prognostic indicator and regulator of the pathophysiology of severe malaria.

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References

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