Upregulation of ICAM-I by *Plasmodium falciparum*: in vitro and in vivo studies

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Abstract

Aims—To monitor the expression of intercellular adhesion molecule I (ICAM-I) in vitro after stimulation of human macrophages with *Plasmodium falciparum* antigens, as well as the plasma concentrations of soluble ICAM-I (sICAM-I) in vivo in malarial patients.

Methods—Human mononuclear leucocytes were cultured and stimulated for four hours with 300 ng/ml exogenous *P falciparum* antigens. CD14 and CD54 (ICAM-I) expression was monitored using flow cytometry. Soluble ICAM-I (sICAM-I) was also measured in the blood of 122 outpatients with malaria before and after treatment (Rio Branco, Acre, Brazil).

Results—ICAM-I expression increased from 15% to 375% after four hours of stimulation. When sICAM-I was analysed in the plasma of 122 patients with *P falciparum* or *Plasmodium vivax* malaria by enzyme immunoassay, significant increases were found. These were more pronounced in patients with *P falciparum* malaria, compared with healthy controls, and with the same patients four weeks after treatment.

Conclusion—ICAM-I expression may also be upregulated in human macrophages by exogenous *Plasmodium* antigens as well as by cytokines during the acute phase of malaria. sICAM-I concentrations are downregulated after treatment, probably caused by the absence of circulating *Plasmodium* antigens.

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Intercellular adhesion molecule I (ICAM-I) is a sialylated glycoprotein with tissue specific differential glycosylation. It therefore has a wide molecular weight range between 70 and 120 kilodaltons.¹ ICAM-I can be readily induced in various cell types in response to cytokines such as γ -interferon (IFN- γ), tumour necrosis factor (TNF), and interleukin 1 (IL-1).²⁻⁴ The production of these last two can be upregulated by exogenous heat stable malarial antigens.^{5,6} ICAM-I also circulates in a soluble form (sICAM-I) in at least three different isoforms.¹ Increased blood concentrations of sICAM-I have been observed in a range of different conditions, such as malignant melanoma,⁷ uveitis,⁸ kidney and liver transplantation,^{9,10} acute myocardial

infarction,¹¹ rheumatoid arthritis,¹² idiopathic lung fibrosis¹³ and neonatal sepsis.¹⁴ ICAM-I has also been identified as an endothelial cell surface receptor for erythrocytes infected with *Plasmodium falciparum*.¹⁵ While erythrocytes parasitised by *Plasmodium vivax* and immature ring-stage *P falciparum* circulate without adhesion in the blood, erythrocytes with mature asexual stages of *P falciparum* adhere to the endothelium. Soluble molecules of ICAM-I might interfere with the adherence of infected red blood cells, thus mitigating the course of *P falciparum* malaria.¹⁶ By contrast, in *P vivax* malaria, there is no such potential interference.

In this study the induction of ICAM-I expression in human macrophages was investigated after stimulation with malarial antigens, as well as the variations in the concentrations of sICAM-I in the blood of different groups of malarial patients before and after treatment.

Methods

For in vitro experiments, human mononuclear leucocytes were isolated from buffy coats from healthy adults, using Ficoll-hypaque density gradients, as described before.¹⁷ The separated cells were cultured and stimulated for four hours with malarial antigens.¹⁷ CD14 and CD54 (ICAM-I) expression was monitored by flow cytometry (FACSCAN, Becton Dickinson, Heidelberg, Germany).

One hundred and twenty two outpatients attending a health care centre in Rio Branco, Acre, Brazil had either untreated, uncomplicated *P falciparum* malaria (n = 44; nine women, 35 men; median age 22 years, range 16-50 years) or *P vivax* malaria (n = 78; 18 females, 60 males; median age 22 years, range 3-81 years). In an arbitrary classification,¹⁸ patients with *P falciparum* malaria were considered "non-immune" if they had had two or fewer malaria attacks during their life (n = 28). Those who had had malaria three or more times in the preceding two years were classified as "semi-immune" (n = 16).

Fifteen healthy subjects from Rio Branco of similar gender and age distribution served as controls. None of them developed parasitaemia during the follow up period of 28 days.

All the patients infected with *P falciparum* received oral clindamycin 5 mg/kg twice daily for five consecutive days, as described elsewhere.^{19,20} Those infected with *P vivax* received a total of 25 mg/kg oral chloroquine

for two days and 0.25 mg/kg primaquine daily for 14 consecutive days. Plasma samples were collected from all patients on admission (day 0) and 28 days after treatment had started and stored at -20°C until analysis.

Parasitaemia was determined by drawing 10 μl blood (fingerprick), distributed equally on an 18×10 mm area of a glass slide. After Giemsa staining, the parasite species was identified and the number of parasites counted per 100 oil-immersion fields ($\times 1000$). The count was multiplied by four to yield the number of parasites per 1 μl of blood.²¹

Plasma concentrations of α_1 -proteinase inhibitor (API), α_1 acid glycoprotein (AAG), and C-reactive protein (CRP) were measured by radioimmunoassay using commercially available test kits (Partigen, Behring Austria). Plasma concentrations of sICAM-I were assayed using a commercially available enzyme immunoassay (Bender, Austria). All samples were tested in duplicate and sICAM-I concentrations were determined by comparing the mean absorption of duplicate samples with that of a standard curve. The sensitivity of the test was about 0.5 ng/ml.

Statistical analysis was performed using the Mann-Whitney U test and the Spearman rank correlation coefficient. The level of significance was set at 0.05.

Results

The effect of *P. falciparum* exogenous antigens on the in vitro expression of ICAM-I by human macrophages was analysed using fluorescein isothiocyanate (FITC) labelled anti-CD54 monoclonal antibodies in flow cytometry. The results of the experiment with maximal induction of ICAM-I are shown in fig 1. The separated human mononuclear leucocytes were incubated for four hours at 37°C with CRPMI medium without (fig 1A) and with (fig 1B) serum free preparations of *P. fal-*

ciparum exogenous antigens at a standard concentration of 300 ng/ml. Malarial antigens induced variable increases in ICAM-I expression (15% to 375%). Most of the cells showing upregulated ICAM-I expression were monocytes or macrophages, determined using simultaneously phycoerythrin (PE) labelled anti-CD14 antibodies.

In the study of malarial patients in Acre, Brazil, all patients had been radically cured by day 28 of follow up. The concentrations of sICAM-I in plasma from the different groups of analysed patients before and after treatment are shown in fig 2. Median values from "non-immune" (G2) and "semi-immune" (G3) patients infected with *P. falciparum* were compared with the corresponding median values from those infected with *P. vivax* (G4) and with healthy controls (G1). Significantly lower values were detected in the control group than in both the malarial groups. Both the "non-immunes" and the "semi-immunes" of the patients infected with *P. falciparum* had significantly higher concentrations of sICAM-I than patients infected with *P. vivax* before and after treatment. There was no correlation between parasitaemia and sICAM-I concentrations in any of the groups of patients studied. sICAM concentrations were still increased 28 days after treatment in all the patients and were most pronounced in infections with *P. falciparum*.

The concentrations of acute phase reactants in plasma were analysed in all patients before and after treatment (table). Blood API concentrations were significantly lower in the control group than in "non-immune" *P. falciparum* and *P. vivax* malarial patients. There was no significant difference between the subgroups of patients infected with *P. falciparum*. A correlation between parasitaemia and API concentrations was detected only in those with *P. falciparum* malaria. Plasma AAG concentrations were significantly increased in all malarial patients. "Non-immune" patients

Figure 1 CD54 (ICAM-I) fluorescence patterns in human mononuclear leucocytes after four hours of incubation without (A) and with (B) exogenous antigens from *P. falciparum* at a concentration of 300 ng/ml. The cell fractions indicated by * were mainly identified as monocytes or macrophages by using simultaneously phycoerythrin (PE) labelled anti-CD14 monoclonal antibodies as fluorescence 2. The fluorescence patterns are the results of the experiment with maximal induction of ICAM-I, using FITC labelled anti-CD54 (ICAM-I) monoclonal antibodies as fluorescence 1.

Figure 2 Soluble ICAM-I concentrations in plasma of patients infected with *P. falciparum* and *P. vivax* before (day 0) and after treatment (day 28). Values are medians for each group: G1 = healthy controls, G2 = "non-immune" *P. falciparum* infected patients, G3 = "semi-immune" *P. falciparum* infected patients, G4 = *P. vivax* malaria patients.

Acute phase proteins from serum of patients infected with *P falciparum* and *P vivax* before (day 0) and after treatment (day 28)

	Controls (n = 15)	<i>P falciparum</i>		<i>P vivax</i> (n = 78)
		"Semi-immunes" (n = 16)	"Non-immunes" (n = 28)	
Parasitaemia-(0)	0	1710 (12-10764)	3112 (16-32680)	3000 (10-18000)
API-(0)	209 (161-271)	252 (135-236)	297*†† (133-411)	285*†† (167-502)
API-(28)	184 (129-255)	211 (94-346)	226* (126-300)	235* (135-338)
AAG-(0)	72 (38-148)	120*†† (41-175)	146*†† (45-269)	122*†† (46-208)
AAG-(28)	71 (44-121)	61†† (23-94)	78 (30-640)	85 (27-272)
CRP-(0)	0 (0-3.0)	2.3*††††** (0.6-5.1)	5.4*††** (0-9.0)	5.2*††** (0-12.2)
CRP-(28)	0 (0-0.7)	0 (0-0.4)	0 (0-7.8)	0 (0-3.2)

Values are expressed as median and range. Parasitaemia is expressed in parasites per µl blood; API, AAG, and CRP in mg/100 ml plasma.
*Difference from controls was significant (p < 0.05).
†Difference from "non-immunes" was significant.
‡Difference from *P vivax* group was significant.
††Difference from day 28 value was significant.
**Correlation with parasitaemia was significant.

with *P falciparum* had higher initial concentrations than "semi-immune" patients, or than patients infected with *P vivax*. There was a positive correlation between parasitaemia and AAG plasma concentrations in patients with *P falciparum*. CRP concentrations discriminated best between the control group and all subgroups of malarial patients. There were also significant differences between "semi-immune" and "non-immune" patients infected with *P falciparum*, and between the "semi-immune" subgroup and the *P vivax* malarial patients. Correlations between the CRP concentrations and parasitaemia were found in both subgroups of *P falciparum* patients, as well as in the *P vivax* patients.

Discussion

As far as we are aware, this is the first report about the induction of ICAM-I by malarial antigens. This effect is to be expected, however, as exogenous malarial antigens have been found to induce both TNF and IL-1 secretion,^{5,6} which would consequently induce the expression of ICAM-I. Remarkably, the maximal increases in ICAM-I expression found after four hours of stimulation with malarial antigens correspond with maximal TNF production as a response to in vitro stimulation of human mononuclear leucocytes with *P falciparum* exogenous antigens under similar conditions.¹⁷ Therefore, it is not possible to conclude whether ICAM-I upregulation is caused by the antigens directly or indirectly via an autocrine stimulatory pathway involving TNF and IL-1.

The discovery of ICAM-I as one of the adhesion molecules responsible for sequestration in *P falciparum* malaria,²² as well as our own in vitro results, led to a further investigation of sICAM-I plasma concentrations in malarial patients. Indeed, high sICAM-I concentrations were found in patients with *P falciparum* malaria, and these decreased gradually after treatment with clindamycin. Although to a lesser degree, sICAM-I concentrations were also increased in patients with *P vivax*

malaria. In the latter, no parasite sequestration occurs, raising the question of how sICAM-I concentrations and cellular expression of ICAM-I are associated with each other. As to the numerous conditions in which increased sICAM-I concentrations have been described, it is obvious that sICAM-I plasma concentrations are related to the amounts of cytokines released. The plasma concentrations of the acute phase proteins API, AAG, and CRP, induced by monokines, behave in a similar manner to sICAM-I, with the highest concentrations seen in "non-immune" patients with *P falciparum* malaria and the lowest concentrations in the "semi-immune" *P falciparum* and *P vivax* groups. However, unlike sICAM-I, differences between the increased concentrations of acute phase proteins were not significant in patients with *P falciparum* or *P vivax* malaria. This indicates that there might be specific differences associated with the role of sICAM-I in both groups of malarial patients.

The high concentrations of API, AAG, and CRP returned to lower levels comparable with those of healthy controls within four weeks of starting treatment, and this was especially noticeable for CRP, where a correlation with parasitaemia was also observed. This agrees with other studies, which have also shown a link between parasitaemia and CRP concentrations in patients infected with *P falciparum*.²³⁻²⁵ Plasma sICAM-I concentrations behaved in a similar manner to those of acute phase proteins, but no correlation was found between sICAM-I concentrations and parasitaemia or the concentrations of any of the acute phase proteins analysed. This might have been due to the different kinetics of production and metabolism. Moreover, the upregulation of sICAM-I expression during the acute phase of malaria can be attributed to an increased release of cytokines,²⁶ but whether sICAM-I has any pathogenic role in malaria or merely represents an unspecific response to enhanced cytokine secretion and the presence of malaria antigens remains to be determined.

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