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

W Graninger, J Prada, S Neifer, G Zotter, F Thalhammer, P G Kremsner

**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 (s ICAM-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.
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 (finger prick), 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 (×1000). The count was multiplied by four to yield the number of parasites per 1 μl of blood.

Plasma concentrations of α1-proteinase inhibitor (API), α1 acid glycoprotein (AAG), and C-reactive protein (CRP) were measured by radioimmunodiffusion 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 leukocytes were incubated for four hours at 37°C with CRPIMI medium without (fig 1A) and with (fig 1B) serum free preparations of *P. fal-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** CD54 (ICAM-I) fluorescence patterns in human mononuclear leukocytes after four hours of incubation without (A) or with (B) exogenous antigen 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](https://example.com/figure2.png)

**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.

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
Uregulation of ICAM-I by P falciparum

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

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 15)</th>
<th>&quot;Semi-immune&quot; (n = 16)</th>
<th>&quot;Non-immune&quot; (n = 28)</th>
<th>P falciparum (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitaemia—(0)</td>
<td>0</td>
<td>1710 (12-10764)</td>
<td>3112 (16-32680)</td>
<td>3000</td>
</tr>
<tr>
<td>API—(0)</td>
<td>209</td>
<td>252 (3-161)</td>
<td>29784 (135-236)</td>
<td>28574 (167-502)</td>
</tr>
<tr>
<td>API—(28)</td>
<td>184</td>
<td>211 (129-255)</td>
<td>226* (126-300)</td>
<td>235* (135-338)</td>
</tr>
<tr>
<td>AAG—(0)</td>
<td>72</td>
<td>120** (41-175)</td>
<td>140*tt (45-269)</td>
<td>122**tt (46-208)</td>
</tr>
<tr>
<td>AAG—(28)</td>
<td>71</td>
<td>613* (23-94)</td>
<td>78 (3-9)</td>
<td>85 (0-6-5-1)</td>
</tr>
<tr>
<td>CRP—(0)</td>
<td>0</td>
<td>0 (0-3-0)</td>
<td>0 (0-9-0)</td>
<td>0 (0-12-2)</td>
</tr>
<tr>
<td>CRP—(28)</td>
<td>0</td>
<td>0 (0-0-7)</td>
<td>0 (0-7-8)</td>
<td>0 (3-2)</td>
</tr>
</tbody>
</table>

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 falciparum group was significant.

Discussion

As far as we are aware, this is the first report about the induction of ICAM-I by malarial antigens. The effect is to be expected, however, as exogenous malarial antigens have been found to induce both TNF and IL-1 secretion, 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. 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, 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 observed. This agrees with other studies, which have also shown a link between parasitaemia and CRP concentrations in patients infected with P falciparum. 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, but whether ICAM-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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