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Autor: Bygbjerg, I.C. / Jepsen, S. / Theander, T.G.

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[1] Lymphocyte Laboratory, Department of Communicable and Tropical Diseases, Rigshospitalet, Copenhagen, Denmark
[2] Malaria Research Laboratory, Treponematoses Department, Statens Seruminstitut, Copenhagen, Denmark

Lymphocyte response to purified *Plasmodium falciparum* antigens during and after malaria

I. C. BYGBJERG[1], S. JEPSEN[2], T. G. THEANDER[1]

Summary

The peripheral blood lymphocyte response to affinity purified soluble *Plasmodium falciparum* antigens from in vitro cultures was studied in seven patients with acute falciparum malaria, on eight occasions, and in 15 persons having had malaria, at various times post infection, on 24 occasions. During infection, the response was low or absent in most patients (median stimulating index=[SI]=1.4). One week post infection, a specific antigen response rose (SI = 2.9), but not to the levels found two weeks to one year post infection (SI = 5.8). At two to four years post infection, it was still present. During a recrudescence of malaria in a single patient, it was lost temporarily. The response to optimal concentrations of lectin mitogens and to tuberculin antigen was not suppressed in acute malaria.

Key words: immunosuppression; lymphocyte proliferation; malaria; *Plasmodium falciparum;* parasite antigen.

Introduction

We have previously shown (Bygbjerg et al., 1985) that immunoadsorbent purified soluble antigens from *Plasmodium falciparum* long-term in vitro cultures induced proliferation of lymphocytes from persons, who had had malaria recently. Lymphocytes of unsensitized individuals were little affected. Preliminary experiments indicated that the lymphocytes from most persons with acute

Correspondence: Dr. I. C. Bygbjerg, Department of Communicable and Tropical Diseases, Righospitalet, Tagensvej 18, DK-2200 Copenhagen N, Denmark

malaria did not react to these antigens (Bygbjerg et al., 1983). Non-specific suppression (Lelchuk and Playfair, 1980) and specific suppression (McDonald and Sherman, 1980) of cellular immunity during infection have been described in murine malaria. In man, data on malaria-induced non-specific and specific immunosuppression are few (Weidanz, 1982). Specific suppression in patients with acute falciparum malaria to sonicates of schizonts has been reported (Troye-Blomberg et al., 1984).

In the present study, we examined the proliferative responses of peripheral blood lymphocytes to mitogens and antigens, including purified malaria antigens, in persons with acute falciparum malaria and at various times post infection.

Material and Methods

Subjects. Seven patients with acute, uncomplicated falciparum malaria and 15 persons having had falciparum malaria within the last two weeks to one year were studied on 8 and 24 occasions, respectively.

The patients, seven males, median age 26 years, were non-immune, having stayed in Africa (Benin, Kenya, Sudan, Tanzania, Zambia, Zimbabwe, Malawi) for 3 weeks to 13 months, median 3 months. Their levels of parasitemia were from 1 to 100 trophozoites per high power field of Giemsa-stained thick films, median 10 to 25. The duration of symptoms before treatment and testing was from 1 to 14 days, median 6 days. None were seriously ill, and all were cured by orally given antimalarials. The persons, 3 females and 12 males, median age 34 years, were non-immune, having stayed in Africa (Angola, Sudan, Kenya, Tanzania, Zambia, Zimbabwe, Malawi) for 2 weeks to 30 months, median 2 months. The levels of parasitemia were from less than 1 to more than 100 trophozoites per high power field, median 10 to 25. The duration of symptoms before oral antimalarials were given was from 1 to 14 days, median 5 days. Three of the persons comprized patients who were also tested during the acute malaria attack. Seven persons were also studied one week after the acute malaria attack and three persons at 1 to 4 years post infection. Some persons (including half of the patients studied in the acute phase) had had 1 to 3 malaria attacks previously, but all those tested must be considered as non-immune.

Lymphocyte cultures. Blood mononuclear cells isolated on a Ficoll-Isopaque gradient were washed in RPMI 1640 medium with 5% pooled human serum and penicillin plus streptomycin, as previously described (Bygbjerg et al., 1985). Antigens from supernatans of in vitro grown *P. falciparum* (F_{32}/Tanzania) were employed. The antigens were affinity purified on CNBr-Sepharose 4 B columns containing IgG from an immune Liberian adult (Jepsen and Andersen, 1981). After elution and concentration, 50 µl of various dilutions of antigens were added to each vial of 10^5 lymphocytes, as previously described (Bygbjerg et al., 1985). The optimal dilution was 1:10. Other lymphocyte cultures were stimulated with various mitogens (phytohaemagglutinin P = PHA, pokeweed mitogen = PWM, concanavalin A = Con A) or with purified protein derivative of tuberculin (PPD). Antigen stimulated cultures were incubated for 5 days, mitogen stimulated cultures for 3 days. Unstimulated cultures were always included, as controls. ^{14}C-thymidine was added to the cultures 24 h before termination. The cells were harvested on glass fibre filters, and incorporated radioactivity (expressed in counts per minute = cpm) was measured in a liquid scintillation counter.

Statistics. The Wilcoxon rank sum test was employed.

Results

Table 1 shows that with regard to lymphocyte response to mitogens and PPD there was no significant difference between patients with acute malaria

Table 1. Response of lymphocytes to mitogens and antigens, expressed as ^{14}C-thymidine (^{14}C-TdR) incorporation, in cpm×10^{3}, and ratio between stimulated and unstimulated lymphocytes (stimulating index = SI), in persons having had falciparum malaria within two weeks to one year ("post infection"), compared with patients with acute falciparum malaria ("acute infection")

Day 3 cultures	PHA		PWM		Con A		Unstimulated
	^{14}C-TdR	SI	^{14}C-TdR	SI	^{14}C-TdR	SI	^{14}C-TdR
Post infection (N = 18–20) ...	15.1	101.1	3.0	21.6	14.6	94.6	0.1
range	6.5–24.8	36.7–224.6	1.5–4.1	9.3–46.8	6.5–24.7	55.1–209.3	0.1–0.4
Acute infection (N = 8)	21.9	155.7	3.5	22.3	15.0	113.2	0.1
range	7.8–30.8	34.1–422.4	0.8–5.4	3.9–49.0	5.7–22.3	21.5–305.9	0.1–0.4
Day 5 cultures	PPD		Malaria AG		Unstimulated		
	^{14}C-TdR	SI	^{14}C-TdR	SI	^{14}C-TdR		
Post infection (N = 19–24) ...	4.6	14.9	1.4	5.8	0.2		
range	0.8–11.2	2.9–78.9	0.4–5.6	1.5–16.9	0.1–0.7		
Acute infection (N = 6–8)	4.5	10.8	0.3*	1.4*	0.2		
range	1.0–5.4	2.6–24.8	0.2–4.3	0.8–36.2	0.1–0.5		

Malaria AG = Malaria antigen; N = Number of experiments; medians are given

* significantly lower than persons post infection, $p \leq 0.01$

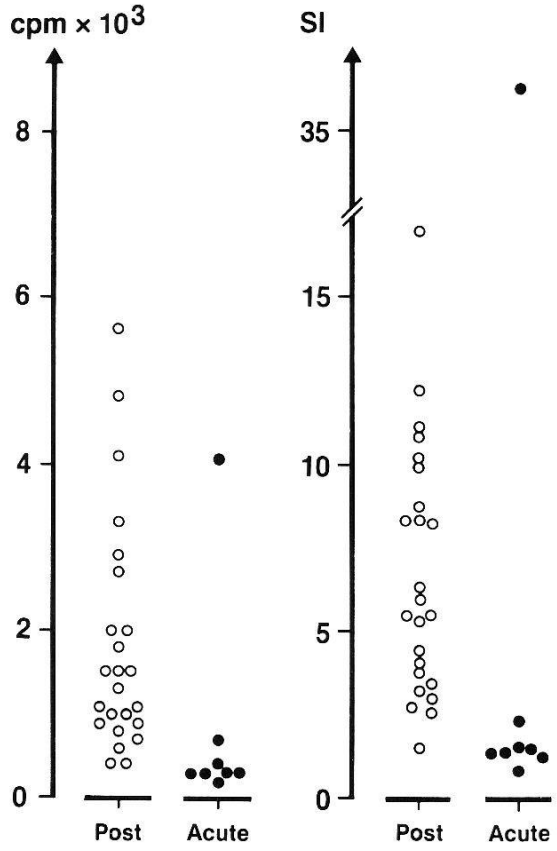

Fig. 1. Response of lymphocytes to malaria antigens, expressed as ^{14}C-thymidine incorporation (in cpm×10^3) and as ratio between stimulated and unstimulated lymphocytes (SI), in persons having had falciparum malaria ("Post") compared with patients with acute falciparum malaria ("Acute").

and persons having had malaria within two weeks to one year. However, the response to malaria antigens was significantly less in the group with acute malaria. Fig. 1 shows that only one patient in this group responded strongly to the malaria antigens; generally, the response to malaria antigens was weak or absent in these patients – as in unsensitized persons.

In seven additional experiments, the response to malaria antigens at one week post infection was studied. The median response was two to three times higher than in the group with acute malaria (0.9×10^3 cpm vs 0.3×10^3 cpm, SI 2.9 vs 1.4), but the difference was not significant (data not shown).

One patient, responding strongly (2.7×10^3 cpm, SI 8.2) to the malaria antigens at one and a half months after the primary attack, had a recrudescence of falciparum malaria. The response to malaria antigens dropped to 0.3×10^3 cpm (SI 1.3); at one week post infection it was 1.2×10^3 cpm (SI 3.7); at one and a half and three months after the recrudescence, the antigen response rose to 2.0×10^3 cpm (SI 10.8) and 4.1×10^3 cpm (SI 7.7), respectively. During a delayed primary attack of ovale malaria one year later in the same patient, the response fell to the same low level (0.3×10^3 cpm, SI 1.2) as during the recrudescence falciparum attack (data not shown).

Table 2. Response of lymphocytes to malaria antigens, expressed as ^{14}C-thymidine (^{14}C-TdR) incorporation, in cpm×10^3, and as ratio between stimulated and unstimulated lymphocytes (SI), in three persons at various times post infection

Person No.	Time post infection							
	≤1 year		2 years		3 years		4 years	
	^{14}C-TdR	SI	^{14}C-TdR	SI	^{14}C-TdR	SI	^{14}C-TdR	SI
1	2.1	4.9	1.8	6.8	–	–	–	–
2	0.7	4.0	–	–	0.8	4.0	–	–
3	1.1	3.2	–	–	–	–	5.1	14.0

To elucidate whether antigen recognition waned after one year, the response was studied at various times in three persons, who had responded well to the antigens one month to one year post infection (Table 2). The lymphocytes of all three persons responded to the antigens, even four years post infection: in one person the response was unaltered, in another the response was slightly higher, and in a third person it was considerably higher than when first examined. The potency of the antigen batches employed initially was weaker than that of the batches used later. Therefore, it can only be concluded that the ability to respond to the antigen did not wane after one year.

Discussion

The results confirm our preliminary findings (Bygbjerg et al., 1983) that the peripheral blood lymphocytes of most patients with acute falciparum malaria did not respond to affinity purified soluble malaria antigens. Half of the patients in the present study had had one to three previous attacks of malaria, i.e., they were probably sensitized to circulating antigens. In spite of this, they were unable to respond during a new attack. This malaria-specific suppression was most clearly demonstrated in the patient whose responsiveness was recorded both before, during and after a recrudescence of falciparum malaria. Interestingly, the response was also lost during a delayed attack of ovale malaria. We have previously shown (Bygbjerg et al., 1985) that both lymphocytes from persons having had falciparum and ovale malaria respond well to the falciparum antigens.

During acute infection, only one person responded strongly to the malaria antigens. He had never had malaria before. Duration and severity of symptoms did not differ from the remaining patients, and we have no explanation for why he responded so well to the antigens.

Suppression of parasite-specific lymphocyte responses in man with acute falciparum malaria has been reported by Troye-Blomberg et al. (1984). How-

ever, these authors found that the response to non-specific mitogen (PHA) was also suppressed (Troye-Blomberg et al., 1983). In the present study, the response to lectin mitogens (PHA, PWM, Con A) was not significantly different when comparing the patients with acute malaria with persons having had malaria two weeks to one year previously; in particular, the median response of the patients to PHA (at a concentration of 5 μg/ml) was normal. The response to PPD was also normal in the patients with acute infection.

Previous reports concerning lymphocyte responsiveness to non-specific antigens and mitogens in acute human malaria are conflicting. Greenwood et al. (1972), MacDermott et al. (1980) and Ballet et al. (1982) found that the response to PHA was normal. Moore et al. (1974), studying the PHA response of whole leukocyte cultures, showed that responses were depressed at a low dose (0.1 μg/ml) of PHA, but normal at a higher dose (1.0 μg/ml). Troye-Blomberg et al. (1983) found that the PHA response of lymphocytes was reduced even at a higher dose (5 μg/ml) of PHA, while the response to PWM was normal. However, these authors have later shown that purified T cells from patients with acute malaria did ot respond significantly differently to PHA, compared to T cells from immune, non-parasitaemic individuals (Troye-Blomberg et al., 1984). Brasseur et al. (1983) found that even in patients with high parasitaemia and cerebral malaria, proliferative responses to lectins were within normal ranges, while responses to candidin were suppressed in parallel with delayed cutaneous responses. Our results are in agreement with those of Druihle et al. (1983), who found that the response to PHA, PWM, Con A and candidin was normal in most patients with mild malaria infection.

McDonald and Sherman (1980) pointed to four different mechanisms of depression of cellular immunity in murine malaria: immune complexes, high titres of antibodies, a heavy antigen burden and migration of mononuclear cells to the spleen and the liver. The lack of responsiveness to malaria antigens in human malaria may simply reflect recruitment of reactive cells out of the blood stream (homing), into the spleen or the liver. Troye-Blomberg et al. (1984) pointed to certain irregularities in composition of T cell subsets – suppressed helper/suppressor ratios – as a possible mechanism of malaria specific impaired responsiveness. Weidanz (1982) concluded that no single mechanism could explain the depressive effects of malaria on host immunological activity.

Malaria-induced immunodepression of parasite-specific responses may be a complicating factor in immunization against malaria (Weidanz, 1982). On the other hand, the suppression found in the present study was only transitory, and the specific responsiveness was retained for up to four years post infection, and possibly longer. Accordingly, Wyler and Oppenheim (1974) showed that lymphocytes from persons, who had been infected with *P. falciparum* up to 15 years previously, underwent significant in vitro proliferation in response to malaria antigens.

Which subsets of lymphocytes that respond to the malaria antigens and the

mechanism(s) of suppression of specific antigen responses as well as their role in the slow development of immunity against malaria is currently being investigated. Also the possibility of homing of reactive cells, e.g. into the spleen, deserves clarification.

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