

EFFECT OF ANTI-COAGULANTS ON COLD-REACTIVE
ANTI-LYMPHOCYTE ACTIVITY IN MALARIOUS
PATIENTS BLOOD

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OBJECTIVE : To compare lymphocytotoxicity in malarious patients plasma and serum.

SUMMARY : The effect of three different anti-coagulants on the level of cold-reactive anti-lymphocyte activity (ALA) in the peripheral blood (PB) of malarious individuals was assessed to determine if plasma could be substituted for serum in assays designed to characterize ALA. Plasma was obtained from PB previously treated with heparin, acid-citrate dextrose (ACD), or ethylenediamine tetraacetic acid (EDTA). An equivalent level of ALA was found in the serum and plasma obtained from the ACD or EDTA-treated blood, however, ALA in the heparin treated blood was substantially lower. Thus, it appears that plasma obtained by treating PB with ACD or EDTA, but not heparin can be used instead of serum to investigate the role of anti-lymphocyte factors in malarial infections. The major practical advantage of this procedure is the higher yield of MNC and plasma to investigate the interactions of lymphocytotoxic factors and autologous MNC.

MATERIALS AND METHODS :

Patients : Peripheral blood was obtained from Thai adult male patients with naturally acquired *P. falciparum* or *P. vivax* malaria who were diagnosed as having malaria by the medical staff at the Malaria Eradication Center (MEC), Phrabuddabat, Thailand. All the patients had low levels of parasitemia (<0.09%) and denied being on any medication at the time of testing. All patients were subsequently treated as "out patients" by the MEC medical staff. Healthy Thai male volunteers who had not previously had malaria served as controls.

Collection of serum and plasma : Blood from a single donor was collected into 4 sample tubes containing a standard concentration of either heparin (Heparin Sodium Injection, USP, Porcine Mucosa, 1,000 U.S.P. units/ml, Lypho-Med, Inc. Chicago, Illinois), acid citrate dextrose (ACD), ethylenediamine tetraacetic acid (EDTA) or one drop of saline. Mononuclear cells were isolated using Ficoll-Hypaque density gradient centrifugation methodology of Boyum (1968) as previously described by Wells et al. (1980). Briefly, MNC were washed three times and resuspended in final medium, RPMI 1640 (GIBCO) containing 2 mM glutamine; 24 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer (HEPES), 50 μ of penicillin, 50 μ g of streptomycin per ml, and 20% heat-inactivated fetal bovine serum (FBS).

Lymphocytotoxic antibody assays : The methodology for the lymphocytotoxic antibody assays has been described previously (Wells et al., 1980). Briefly, at either 15°C or 37°C, 0.1 ml of serum or plasma was added to duplicate microtiter wells containing 0.1 ml target indicator cells (2×10^6 cells/ml) and incubated for 30 min. followed by the addition of 0.1 ml of fresh rabbit serum (as a source of complement). Then after an additional 3 hours incubation at the respective temperature the percentage of dead cells was determined by using the eosin dye inclusion technique.

RESULTS AND DISCUSSION : Malarious patients' plasma was as effective as serum in killing mononuclear target cells in assays used to measure cold-reactive lymphocytotoxicity provided the plasma was obtained from acid citrate dextrose (ACD) or ethylenediamine acid (EDTA) treated peripheral blood. However, when heparin was used as an anti-coagulant the lymphocytotoxicity in plasma was substantially lower (Table 1). Furthermore, high serum lymphocytotoxicity was reduced or eliminated when heparin was mixed with the serum prior to testing it in the lymphocytotoxicity assay, yet the addition of saline, ADC or EDTA directly to aliquots of the serum has little or no effect on lymphocytotoxicity (data not shown). This observation suggests that ADC or EDTA-treated plasma can be used instead of serum to study cold-reactive lymphocytotoxic factors that are present in the peripheral blood of patients with *P. falciparum* or *P. vivax* malaria. Since it is often difficult to obtain a sufficient volume of blood from some malarious patients due to the patients' clinical condition, and personal, cultural or religious convictions the use of plasma has a major practical advantage in that it provides more materials and thus allows for : autologous MNC and cytotoxic factors simultaneously obtained, to be used in *in vitro* assays to investigate interactions of cytotoxic factors and the various cellular components of the immune response (MacDermott et al. 1980). Furthermore, the use of autologous MNC in the *in vitro* assays helps to rule out any nonspecific or HLA-associated interactions that often are seen when allogeneic cells are used in cellular assays. Although the use of anti-coagulants may adversely effect lymphocytotoxic factors present in patients with different tropical infections, some investigators may find that the plasma is as effective as serum and can serve as a valuable alternative to serum in situations where only a limited amount of the patients' blood can be obtained for use in cellular and humoral assays.

Table 1. Cold-reactive lymphocytotoxicity in malarious patients serum and plasma.

Patient	% cytotoxicity ^a				
	Serum		Plasma		
	Neat	Freeze thawed	ACD	EDTA	Heparin
P.f. ^b	20.0	18.0	-	-	-
P.f.	30.0	27.5	20.0	21.5	3.0
P.f.	11.5	10.0	-	-	-
P.f. + P.v.	32.5	N.D.	26.0	17.0	2.0
P.f.	27.0	27.0	26.0	22.0	10.5
P.f.	41.0	36.0	33.5	28.5	7.5
P.f.	27.5	23.5	20.5	15.5	6.5
P.v.	25.5	23.5	18.5	18.0	2.5
P.f.	25.5	27.0	25.0	17.0	4.5

^a Average cytotoxicity determined in duplicate cultures in assays done at 15°C as described in Materials and Methods.

^b P.f. indicates patients with *Plasmodium falciparum* infections;
P.v. indicates patients with *Plasmodium vivax* infections;
P.f. + P.v. indicates mixed infection.

^c (-) dash indicates no cytotoxicity was observed.

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