

## ADENOSINE DEAMINASE IN HUMAN MALARIA INFECTION

Principal Investigators : H. Kyle Webster, MAJ, MSC  
William P. Wiesmann, LTC, MC  
Peter E. Daddona, Ph.D.

Associate Investigators : Marvin D. Walker, SFC, USA  
Barnyen Permpanich, BS  
Somchit Tulyayon, BS  
Prasit Sookto, BS  
Niphon Chuanak  
Jose Solivan-Perez, SP5, USA

**OBJECTIVE :** To determine whether peripheral whole blood adenosine deaminase (ADA) is increased in human malaria infection and to biochemically characterize the malaria parasite ADA enzyme.

**BACKGROUND :** Hereditary deficiency of the purine enzyme, adenosine deaminase (ADA) is associated with severe combined immunodeficiency disease (SCID) - a condition in which both T-and B-lymphocyte function is impaired (1). Partial restoration of lymphocyte function can be achieved in SCID patients by enzyme replacement therapy involving whole blood or packed RBC transfusion. It thus appears that the ADA in normal RBC is sufficient to correct in part the purinogenic defect in ADA deficient lymphocytes. The precise mechanism for this effect is not understood although it may involve the role of the red cell mass in systemic adenosine metabolism in a way that influences purine metabolism in lymphocytes. Acute human malaria infection is characterized by immune suppression. In particular there is a decreased functional responsiveness of mononuclear cells - especially T-lymphocytes. It is also known that the intraerythrocytic malaria parasite produces major changes in the purine metabolism of the host red cells mass (2, 3). The purpose of these initial studies was to determine the level of adenosine deaminase activity of parasitized RBC (PRBC) in the RBC mass of peripheral whole blood. High levels of PRBC ADA activity would be predicted to perturb systemic blood adenosine metabolism. It is also important to characterize the parasite ADA enzyme in comparison to the host ADA enzyme.

**METHODS :** *Processing of whole blood.* Heparinized whole blood was obtained from malaria infected patients with *P. falciparum* malaria following informed consent. Normal healthy Thai adults served as controls. Lysates for enzyme determinations were prepared by adding 1 ml of whole blood to 1 ml of distilled water (1:2 lysate) and also to 2 ml of distilled water (1:3 lysate). Nucleotide extracts were prepared by adding 1 ml whole blood to 1 ml of 1 M perchloric acid followed by centrifugation and neutralization with 10 M KOH. Samples were stored at -70°C.

*Assay of adenosine deaminase.* The ADA assay was done by a radiochemical method using an automated PHLC system (5). ADA activity was measured by the

conversion of ( $^{14}\text{C}$ ) adenosine to ( $^{14}\text{C}$ ) inosine (any labelled hypoxanthine formed was included as part of the inosine product). The standard reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), ( $^{14}\text{C}$ ) adenosine 1.8 mM, 1.6 mCi/mmol) and erythrocyte lysate (200 ul) in a total volume of 700 ul. The range of protein concentration was 162 to 308 mg/ml. The reaction was linear with time and protein concentration. Specific activity was expressed as nanomoles/min/mg of protein.

*Nucleotide assay.* Purine nucleotides were determined by an anion-exchange gradient HPLC method (4). This method separates all major purine nucleotides as well as the ribonucleotide from the deoxyribonucleotide form.

**RESULTS :** *Adenosine deaminase activity in malaria infection with P. falciparum.* Lysates prepared on infected whole blood showed an increase in ADA activity compared to normal uninfected controls (Table 1). Basically, as parasitemia increased there was an increase in ADA activity (regression of malaria ADA activity versus parasitemia: correlation = 0.9875, R-squared = 0.8789, SE estimate = 0.3631 with 1 degree of regression; parasitemia =  $1.95 \pm 0.73$  (0.6 - 8.3% PRBC) *P. falciparum* infection). The deviation in correspondence between expected ADA activity and level of parasitemia was most notable at high parasitemias. This effect may be due to inhibitory factors in the host peripheral blood specific for the parasite ADA.

*Biochemical characterization of malaria (P. falciparum) adenosine deaminase.* A unique approach was used to obtain parasite ADA enzyme free of host enzyme for biochemical study. ADA deficient (ADA-) RBC were obtained from a SCID patient at UCSF. A laboratory strain of *P. falciparum* (FCR 3) maintained in continuous erythrocyte culture was seeded into a culture prepared from ADA(-) RBC. The infection established in the ADA(-) RBC served as a source of enzyme for biochemical characterization. The ADA activity in ADA(-) infected RBC was determined in comparison to ADA(-) uninfected RBC, ADA(+) infected RBC and uninfected RBC (Table 2). Biochemical properties for human and malaria ADA enzymes are given in Table 3. Table 4 compares substrate specificity of host and malaria parasite ADA enzymes. Immunotitration studies with human ADA antibody (done at the University of Michigan) show that the malaria parasite enzyme is nonimmuno-reactive and thus distinct from the host enzyme. The malaria enzyme was similar to human ADA with respect to the Michaelis constant ( $K_m$ ) for adenosine and deoxyadenosine, inhibition ( $K_i$ ) for deoxycoformycin, pH optimum,  $S_{20}$  and PI. The  $K_i$  for EHNA however was about 7000 times greater than for the malaria parasite ADA. Comparison of the apparent substrate specificity for the two enzymes was unremarkable. The parasite ADA enzyme can be readily distinguished from host enzyme using native PAGE gel electrophoresis.

#### REFERENCES :

1. Donofrio J, Coleman MS, Hutton JJ, Daoud A, Lampkin B, Dyminski J. (1987). Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. *Journal of Clinical Investigation* 62: 884-887.

2. Webster HK, Haut MJ, Martin LK, Hildebrandt PK (1982). Purine and pyrimidine nucleotide profiles during synchronous malaria infection (*Plasmodium knowlesi*) in the Rhesus Monkey. *International Journal for Parasitology* 12: 75-79.
3. Webster KH, Whaun JM (1981). Purine metabolism during continuous erythrocyte culture of human malaria parasites (*P. falciparum*). *Progress in Clinical and Biological Research* 55: 557-573.
4. Webster HK, Whaun JM (1981). Application of simultaneous UV-radioactivity high-performance liquid chromatography to the study of intermediary metabolism. I. Purine nucleotides, nucleosides and bases. *Journal of Chromatography* 209: 283-292.

Table 1. Adenosine Deaminase Activity in Malaria (*P. falciparum*) Infection

	<u>ADA Activity</u> (nmol/min/mg)
Control	0.83 ± 0.10 (0.38 - 1.29) <sup>1</sup>
Malaria infected <sup>2,3</sup>	2.41 ± 0.31 (1.36 - 4.89)

<sup>1</sup> Mean ± SEM (range), n = 10.

<sup>2</sup> Difference is significant at p < .005

<sup>3</sup> Parasitemia = 1.95 ± 0.73 (0.6 - 8.3)

Table 2. Adenosine Deaminase Activity in Normal (ADA +) and Deficient (ADA -) *Plasmodium falciparum* Infected Erythrocytes *In vitro*.

<u>Erythrocyte culture</u>	<u>Specific Activity</u> (nmol/min/mg)
ADA (-) uninfected	< 0.008
ADA (-) infected*	2.03
ADA (+) uninfected	1.02
ADA (+) infected	3.05

\* Parasitemia 8% PRBC

Table 3. Properties of Human Host Erythrocyte and Malaria Parasite Adenosine Deaminase

	Adenosine deaminase	
	Host	Parasite
Km adenosine	49 ± 6 M	51 ± 5 M
Km deoxyadenosine	53 ± 5 M	57 ± 6 M
Ki deoxycoformycin	3.73 x 10 <sup>-10</sup> M	3.15 x 10 <sup>-10</sup> M
Ki EHNA <sup>2</sup>	1.7 x 10 <sup>-8</sup> M	1.31 x 10 <sup>-4</sup> M
pH optimum	5-8	5-8
S20 W	3.8 ± .2	3.7 ± .1

<sup>1</sup> Mean ± SEM, n = 5

<sup>2</sup> Erythro-9-(2-Hydroxy-3-nonyl) adenine

Table 4. Comparison of Substrate Specificity of Human Erythrocyte and Malaria (*P. falciparum*) Parasite Adenosine Deaminase.

Substrate	Percent inhibition	
	Erythrocyte	Parasite
Adenine	27	13
Cordycepin	86	88
6-methylamino riboside	93	79
6-methyl mercapto purine riboside	15	16
2, 6-diamino purine	45	21