

Lymphocytotoxic Factors in Malaria Patients

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OBJECTIVE : To identify lymphocytotoxic activity in the sera of patients infected with *falciparum* or *vivax* malaria.

BACKGROUND : There have been several reports in the literature of serum associated antibodies which are cytotoxic for autologous lymphocytes. Terasaki *et al.* reported such activity in sera from patients afflicted with either systemic lupus erythematosus or rheumatoid arthritis (1). These findings were confirmed by Michlmayr *et al.* (2) who indicated that both T lymphocytes and B lymphocytes were target cells although primarily the former were killed. Detto-ratius *et al.* (3) extended these findings with their report of lymphocytotoxicity in patients infected with hepatitis. Earlier work in this laboratory (4) indicated a marked suppression in the proportion of circulating T lymphocytes during infection with malaria. It was reasoned that lymphocytotoxicity might be one mechanism underlying this phenomenon. Following earlier attempts (Annual Report 1977) which had proved fruitless, we again assayed for cytotoxic activity with modified technique as reported herein.

METHODS : Cytotoxic assays were performed with the sera of patients infected with either *falciparum* or *vivax* malaria. The methodology of Terasaki (1) was utilized with modification. Control sera and target lymphocytes were obtained from normal volunteers. Mononuclear leukocytes isolated by ficoll hypaque centrifugation were adjusted to a concentration of 3×10^6 cells/ml in HBSS (pH 7.3) with 10% fetal calf serum. Experiments were conducted in flat bottom tissue culture trays. Each experimental set-up included 0.1 ml each of cell suspension, human serum and undiluted rabbit serum (as a source of complement). Assays run at 15°C were done in a refrigerated centrifuge while those at 37°C were done in an incubator. Total incubation times were 4 hours. The percent of dead cells per 200 mononuclear cells were determined in duplicate by eosin dye exclusion. All counts were made "blind" with the identity of the sera unknown to the investigator.

RESULTS : Serum associated cell death was demonstrated both at 15°C and 37°C - particularly at the lower temperature. Table 1 summarizes the results of these experiments. In fifty-seven sera from patients infected with *P. falciparum* there was an average percent cell death of 19.6 at 15°C and 6.9 at 37°C compared with 3.3 and 3.4 percent respectively for the normal control sera. The range of killing in the patient sera was considerable. There was no apparent relationship between the level of infection and cytotoxicity. Values for the *P. vivax* sera were higher with a mean of 29.5 percent cell death at 15°C and 8.5 percent

at 37°C. It is possible that this phenomena may, in part, serve to modulate cellular response to human malaria. This is a final report pending the re-design of protocols for more definitive investigations.

Table 1. Lymphocytotoxins in malaria

| | <i>P. falciparum</i> (57) | | <i>P. vivax</i> (50) | | Controls (29) | |
|---------|---------------------------|--------|----------------------|------|---------------|---------|
| | 15°C | 37°C | 15°C | 37°C | 15°C | 37°C |
| Range : | 3.5-72* | 2.5-24 | 4.5-78 | 3-32 | 2.5-4.5 | 2.5-4.5 |
| Mean : | 19.6 | 6.9 | 29.5 | 8.5 | 3.3 | 3.4 |
| SD : | 14.8 | 4.6 | 10.7 | 6.4 | 0.6 | 0.5 |

* Percent Cytotoxicity by Dye Exclusion

REFERENCES :

1. Terasaki, P.I., Mottironi, V.D. and Barnett, E.V. Cytotoxins in Disease : Autocytotoxins in Lupus. N. Engl. J. Med. 283:724-728, 1970.
2. Michlmayr, G. *et al.* Antibodies for T. Lymphocytes in Systemic Lupus Erythematosus. Clin. Expt'l. Immunol. 24:18-25, 1976.
3. Dettoratus, R.J., Henderson, C. and Strickland, R.G. Lymphocytotoxins in Acute and Chronic Hepatitis : Characterization and Relationship to Changes in Circulating T Lymphocytes. Clin. Expt'l. Immunol. 26:21-27, 1976.
4. Wells, R.A. *et al.* Loss of Circulating T Lymphocytes with Normal Levels of B and "Null" Lymphocytes in Thai Adults with Malaria. Manuscript in preparation.