Absence of Circulating Interferon in Patients with Malaria and with American Trypanosomiasis (37539)

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A number of protozoa has been shown to be capable of interferon induction in experimental animals. It has been established for example that the organism of murine malaria —*Plasmodium berghei* (1), and *Trypanosoma cruzi* (2) (among others), can induce interferon in mice. Little is known, however, regarding interferon response in patients experiencing naturally acquired infection with these organisms. It was felt to be of interest, therefore, to determine whether interferon could be detected in sera of patients with malaria and those with Chagas disease (American trypanosomiasis). Humoral interferon has been reported previously in man during the course of clinical viral infections (3), and following administration of attenuated viral vaccines (4).

Materials and Methods. Source of sera. 1. *Malaria.* The patient population from whom serum specimens were obtained comprised 42 Viet Nam returnees treated for malaria at two V.A. hospitals. In 65% of the patients there was no previous history of clinical malaria. All patients were febrile and acutely ill on admission. None were on antimalarial prophylaxis immediately prior to the onset of illness. Thirty-six patients were found to have *P. vivax* malaria, four had *P. falciparum,* and two had a double infection. Acute sera obtained at the time when blood smears were positive for malaria were available on all of these patients. In addition, nine convalescent specimens were obtained 1 to 2 wk following initiation of therapy (generally with chloroquine and primaquine), at which time the patients were afebrile and asymptomatic.

2. *Chagas disease.* A recent field study of the epidemiology of Chagas disease, carried out by our departments in Bolivia, afforded an opportunity to study interferon levels in patients with documented *T. cruzi* infection. Forty sera were selected for interferon titration. All were strongly positive for *T. cruzi* antibodies by latex agglutination ("Latex-Chagas Reagent," Behringwerke Marburg-Lahn, Germany), indicating a recent infection. None of the patients had clinical evidence of Chagas disease however.

Human interferon titration. Twofold dilutions of serum were made in E-MEM with 2% fetal calf serum. Two milliliters of each dilution were added in duplicate to human foreskin fibroblast monolayers in 60 × 15 mm plastic petri plates (Falcon). Plates were incubated 6 hr at 37° in 5% CO_2 atmosphere. Interferon was aspirated and monolayers were washed with phosphate buffered saline (PBS). Cells were challenged with 50 plaque forming units (PFU) of vesicular stomatitis virus (VSV—Indiana strain). After 45 min of incubation at 37°, monolayers were overlayed with 5 ml nutrient enriched agar (Ionagar 50%, 2 × E-MEM 50%). Twenty-four hours following incubation a second agar overlay was added containing 0.005% neutral red. Plaques were counted 24 hr later and the interferon titer was expressed ("units") as the reciprocal of the highest dilution suppressing plaque formation by 50% compared to virus controls.

Results and Discussion. Concurrent titration of human interferon standards, consistently gave titers in the range of 128 to 512 units (authors' laboratory standard) during

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titration of malarial sera, and of 2560 to 5120 units (British Standard A, 69/19) during assay of Chagasic sera, indicating a high degree of sensitivity of our assay system. However, no interferon activity was detected in 50 of the 51 specimens from malaria patients at the initial serum dilution of 1:4. One acute specimen from a patient with *P. vivax* malaria had an interferon level of 4 units. Similarly, no interferon could be detected in the Chagasic sera.

There are several possible explanations for this failure to detect circulating interferon in clinical malaria: (a) Human malaria strains, unlike *P. berghei* are not good interferon inducers. (b) The inoculum in clinical infections is inadequate to induce interferon. (c) During the incubation period (prolonged in most cases), the patients developed tolerance or hyporesponsiveness to interferon induction by plasmodia. It is not likely that inhibition of interferon by chloroquine was responsible for the lack of detectable levels in at least the acute specimens, because none of the patients were taking the drug at that time. (This observation is pertinent because Kohno, Kohase and Suganuma (5) have reported that chloroquine produced a potent and specific inhibition of interferon synthesis by primary chick embryonic cells inoculated with uv-irradiated Newcastle disease virus.)

It is likely that similar mechanisms are responsible for the absence of humoral interferon in patients with *T. cruzi* infection. An additional consideration may be that unlike the patients with malaria who were clinically ill, these individuals did not have overt symptoms and signs of Chagas disease.

The possible role of interferon in resistance to malarial infection in man has been the subject of comment in medical literature (6). The present study, indicating lack of humoral interferon (for whatever the reasons) would weigh against such a role in clinical malaria.

Summary. Interferon could not be detected in 50 sera obtained during the acute illness from 42 patients with clinical malaria. Similarly negative results were obtained when 40 sera from patients with subclinical *T. cruzi* infection were studied.

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1. Huang, K. Y., Schultz, W. W., and Gordon, F. B., Science **162**, 123 (1968).

2. Rytel, M. W., and Marsden, P. D., J. Trop. Med. Hyg. **19**, 929 (1970).

3. Wheelock, E. F., and Sibleg, W. A., Lancet **2**, 382 (1964).

4. Petralli, J. K., Merigan, T. C., and Wilbur, J. R., N. Engl. J. Med. **273**, 198 (1965).

5. Kohno, S., Kohase, M., and Suganuma, M., Jap. J. Med. Sci. Biol. **21**, 239 (1968).

6. Weintraub, M., Ann. Intern. Med. **73**, 1045 (1970).

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