THE SOURCE OF THE PARASITEMIA IN THE RELAPSE OR THE LONG-TERM LATENT ATTACK

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At the Xth International Congress of Tropical Medicine and Malaria in Manila, we reported the discovery of a previously unknown tissue stage of the simian relapsing malaria parasite, Plasmodium cynomolgi, by a sensitive immunofluorescence (IFA) technique, and demonstrated its presence in liver as a uninucleate body, from 3 to 105 days following sporozoite inoculation. On the basis of this startling discovery, we proposed that this stage, to which the term "hypnozoite" or sleeping animalcule was applied, represented the true relapse body or latent form in relapsing malaria, and, in subsequent publications, suggested corollaries of this hypothesis. Simply stated, if the hypnozoite is indeed the true relapse body, it should be present in all relapsing malarias; it should be absent in all non-relapsing malarias; and its numbers should be directly related in some manner to observed relapse patterns, both in terms of decrease in number with time, and, perhaps more importantly, in terms of relationship to schizont numbers in defined P.vivax relapse types.

During the last 4 years, we have succeeded in demonstrating the presence of hypnozoites in two different strains of P.cynomolgi (P.c. bastianellii, P.c. cynomolgi) in rhesus monkeys, and in two distinct strains of P.vivax (Chesson, North Korean) in the chimpanzee, but have failed to detect any trace of these forms in infections with the non-relapsing parasite, P.knowlesi. Furthermore, as predicted, both schizonts and hypnozoites were found in the frequently relapsing Chesson strain of P.vivax, at 7 and 10 days after sporozoite inoculation, but only hypnozoites (without schizonts) were found at 7 days in the North Korean strain, which frequently exhibits a markedly prolonged pre-patent period. Finally, hypnozoites were found at 229 days after sporozoite inoculation, and the numbers did fall off with time over the entire period; one form was found with two nuclei at day 53, thus presumably being in the earliest stages of activation.

In short, we suggest that the hypnozoite theory of relapses is becoming ever more firmly established, and appears to be preferable to the hypothesis of cyclic schizogony originally proposed by Shortt and Garnham in 1948.

KILLING OF DENGUE-INFECTED CELLS BY HUMAN NATURAL KILLER CELLS AND ANTIBODY DEPENDENT CELL-MEDIATED CYTOTOXICITY

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Peripheral blood mononuclear cells (PBMC) from humans without antibodies to dengue 2 virus lysed dengue 2 virus - infected Raji cells to a significantly greater degree than uninfected Raji cells. Addition of mouse antidengue antibody increased the lysis of dengue-infected Raji cells by PBMC. Dengue 2 immune human sera also increased lysis of dengue-infected Raji cells by PBMC. These results indicate that both PBMC-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) can cause significant lysis of dengue-infected Raji cells.

In cold target competition experiments dengue 2 virus-infected Raji cells competed significantly better than uninfected Raji cells with ^{51}Cr-labelled dengue 2-infected Raji cells in the natural killing (NK) assay. The lysis of infected Raji cells in the ADCC assay correlated with the dilution of dengue-specific antibody which was added. These results show the specificity of the lysis of dengue virus-infected Raji cells.

Alpha interferon (IFN) was detected in the culture supernatant of PBMC and dengue-infected Raji cells. However, enhanced lysis of dengue-infected Raji cells by PBMC may not be due to the IFN produced, because neutralization of all IFN activity with anti-IFN antibody did not decrease the lysis of dengue-infected cells, and effector cells pretreated with exogenous IFN also lysed dengue-infected cells to a greater degree than uninfected cells.

The effector cells responsible for lysis of dengue virus-infected Raji cells in the NK and ADCC assays were analyzed. Non-adherent PBMC caused more lysis than did adherent cells. Characterization of non-adherent cells with monoclonal antibodies showed that the predominant responsible effector cells were $OKM1^+$ and $OKT3^-$ in the NK and ADCC assays. $Leu11^+$ and $Leu11^-$ cells were both active in killing dengue infected cells, but $Leu11^-$ cells do not kill K562 cells. Thus, there is more than one lymphocyte subset which preferentially kill dengue infected cells, unlike the natural killer cell which lysed K562 cells.

METHODS USED FOR SCREENING SIMULIUM LARVICIDES IN THE ONCHOCERCIASIS CONTROL PROGRAMME AND RECENT RESULTS

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The occurrence of resistance to two organophosphate compounds in some strains of the Simulium damnosum complex in West Africa has given an added impetus to the search for alternative larvicides for use in the Onchocerciasis Control Programme. Due to the high level of effectiveness required and the necessity to limit ecological damage, the requirements of the Programme are not easily met.

Screening of candidate larvicides must be carried out against Simulium damnosum under African conditions. Results with mosquito larvae in other screening programmes are used as indicators of candidate compounds. Lack of simple and reliable colonization techniques makes a purely laboratory technique with S. damnosum s.l. impossible. Initial screening is done in a closed-circuit trough system, complemented by an open-circuit "mini-gutter" system. When these results are promising, river trials are carried out. Large-scale operational trials are organized for the most promising compounds.

Effects on non-target fauna are measured carefully during the screening process.

For the moment, formulations of Bacillus thuringiensis H-14 are the only alternative larvicides in operational use. Azamethiphos is the only acceptable organophosphate which does not show cross-resistance. No effective carbamates have been found, although there is no cross-resistance. A pyrethroid, permethrin, is being considered for a large-scale operational trial. The IGR-type compounds are attractive, but present practical problems for application in moving water. A separate protocol is being developed to screen them.

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INHIBITION OF HUMAN MONOCYTE LOCOMOTION BY PRODUCTS OF AXENICALLY GROWN E. HISTOLYTICA

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The supernatant fluid of axenically grown E. histolytica inhibits chemotaxis (towards three different attractants i.e. ZAS, LDCF and FMLP), chemokinesis and random mobility of human monocytes (MP) as measured in Boyden chambers. Human polymorphonuclear locomotion is apparently unaffected. The inhibitory factor was found in comparable amounts in the supernatant fluid of axenic cultures of four strains of E. histolytica that differed widely in their human pathogenicity and virulence, as well as in two strains non-pathogenic for man. This dialyzable and thermolabile MP-locomotion inhibiting entamoeba released product (ERP) can be absorbed out by incubation with MP, but no with lymphocytes, while partial absorption was observed using PMN. The MP locomotion inhibitory effect of this ERP was cancelled by inhibiting protein synthesis in the MP by means of cycloheximide. In vivo this ERP caused a delay in MP migration in Rebuck skin windows in human volunteers. The mol wt of this ERP lies between 478 and 765 dalton by ultrafiltration and gel sieve chromatography. These results and ultrastructural data suggest a direct inhibitory effect upon the cytoskeletal and locomotive apparatus of the MP. This MP locomotion inhibiting ERP could contribute to the paucity of the inflammatory reaction observed in the advanced stages of invasive amebiasis and consequently also to the lack of scar tissue formation upon healing of amebic lesions. The MP locomotion inhibiting ERP joins the ranks of factors produced by parasites that may help them evade the immune and/or inflammatory responses of the host, and thus intervene in the modulation of the host-parasite relationship.

INSECTICIDAL TREATMENT OF WIDE-MESH NET CURTAIN FOR VECTOR MOSQUITO CONTROL

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The use of insecticide-treated curtain made of wide mesh net has been developed for the protection of human against mosquito-borne disease. It is easy and economical to utilize it as an alternative to the indoor spray of residual insecticides. The nets, which had openings large enough to allow the mosquito to pass through, were impregnated with the candidated insecticides at the different doses per square meter. Contact toxicity tests were conducted in a laboratory with unfed female *Anopheles stephensi*, 3-5 day old. The mortality was observed periodically and LT-50 values were determined for each of concentrations. As a result, three types of net treated with 0.2 g of phenothrin retained their insecticidal effectiveness for more than 23 weeks.

In order to assess the behaviour of mosquitoes under the influence of insecticides, a series of cage tests were carried out using an apparatus. This is composed of plastic bag served as a release chamber and the other is a wire cage (30x30x30 cm) served as a baited chamber. The netting, impregnated with the insecticides, was interposed between a chamber of avid mosquito and a baited cage. There was a reduced entry of mosquitoes through the phenothrin-treated net, but the degree of inhibition to fenitrothion was low. Both chemicals, however, applied at 0.2 g/m^2 showed high mortality level over two months.

RESISTANCE TO INSECTICIDES IN THE SIMULIUM DAMNOSUM COMPLEX. OPERATIONAL CONSEQUENCES. DEVELOPMENT OF NEW CHEMICALS AND FORMULATIONS

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Resistance of blackflies to DDT was suspected in earlier control operations in West Africa in the early 1970's. It was also detected in untreated populations in several West African countries at the beginning of organophosphate (temephos) treatments by the Onchocerciasis Control Programme (O.C.P.).

Resistance to temephos, the primary larvicide, however, did not occur until 1980, the 5th year of vector control operations, in spite of the widespread and intense selection pressure imposed by weekly aerial treatments.

At that point it was quickly followed by resistance to its replacement chlorphoxim.

This resistance has remained limited to forest cytospecies of the S. damnosum complex with the exception of one small, apparently short-lived savanna population isolated in the forest zone in the south of the Programme Area. Within the treated zone of the Programme Area, resistance affects populations of forest cytospecies in Ivory Coast and neighbouring parts of Ghana. Forest cytospecies larvae resistant to chlorphoxim and temephos have also been found in an untreated area in south-western Ghana.

There is cross-resistance to most organophosphates, except azamethifos and some other organophosphates characterised by high water solubility. Other groups (pyrethroids, carbamates, chlorinated hydrocarbons) are not affected. In fact, there is even some evidence that there is a "negative correlation" between resistance to organophosphates and susceptibility to pyrethroids.

Studies with synergists and related compounds have shown that MEF (an inhibitor of esterases) restores susceptibility to temephos as does the use of temephos sulfoxide. Neither effect is useful operationally. Piperonyl butoxide, on the other hand, reduces susceptibility to temephos, probably by inhibition of mixed function oxidase enzymes which normally activate temephos.

Electrophoretic studies of the esterases of susceptible and resistant strains reveal qualitative and quantitative differences but unfortunately resistance could not be diagnosed by a simple whole body staining technique.

ABSTRACT AND POSTER VOLUME

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