Tokai J Exp Clin Med., Vol. 23, No. 2, pp.99-100, 1998

Recrudescence of *Plasmodium falciparum* in Culture: Unaffectedness and Frequency of Dormant Parasites

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Recrudescence in falciparum malaria is considered as a sign of treatment failure in those who are treated with anti-malarial drugs and a failure of immune suppression in those who once developed protective immunity against parasites. The cause of this phenomenon after chemotherapy has been explained that resistant parasite strains are selected by the drug treatment or that blood levels of the drug are insufficient to further suppress the parasites. Recrudescence occurring in immuned hosts may be the results of antigenic variation. In practice, recrudescence is a frustrative problem because all recrudescent parasites are not examined to be drug-resistant in *in vitro* drug sensitivity tests. It was hypothesized that a small percentage of the parasites may be in a dormant state, so that they are unaffected by drug treatment; when such treatment is discontinued, these parasites may be transformed into active ones, resulting in a detectable parasitemia. To confirm this idea recrudescence was reproduced in *in vitro* culture.

Five types of *Plasmodium falciparum* were maintained in continuous culture. Three out of five types (Geneve/SGE-1, FCR-3, and ITG2) are pyrimethamine sensitive. The remaining two (7G8 and HB3) are pyrimethamine resistant. Pyrimethamine sensitivity tests of these parasites were carried out to determine the concentration at which the parasites were treated to examine whether they recrudesced after cessasion of the treatment. Geneve/SGE-1, FCR-3, and ITG2 were treated with 10^{-6}M pyrimethamine in standard medium for 4 days. Pyrimethamine resistant clones, 7G8 and HB3, were treated with 10^{-4}M pyrimethamine for the same period as were sensitive ones. On Day 4, parasitized erythrocytes were washed once with complete medium and twice with incomplete medium and were resuspended in complete medium for cultivation. To confirm recrudescence in different treatment, the parasitized erythrocytes were exposed to 5% D-sorbitol every 12 hours for 4 days.

During pyrimethamine treatment parasitemia was reduced and eventually erythrocytic stages of parasites were not detected. Several days later affer the treatment parasites reappeared and multiply in all culture dishes. In experiments using D-sorbitol, parasites also reappeared several days after the exposure in all culture dishes, although reduction of parasitemia was faster than that in experiments of pyrimethamine treatment. Recrudescent parasites were examined to be as sensitive to pyrimethamine and D-sorbitol treatment as were original ones.

Parasites were recrudesced in *in vitro* culture both after pyrimethamine and after D-sorbitol treatment. Because the effects of pyrimethamin and D-sorbitol on parasites are completely different, it is unlikely that both agents push parasites into inactive state or arrest parasites at some stage. Rather, it is more likely that inactive parasites are omnipresent component of many malaria parasites infections, and are resistant to drugs which act by introducing metabolic perturbations into active ones.

These observations provide evidence that dormant parasites may be present in erythrocytes and their stages may be ring-form. Parasites may not be induced into dormant form by unfavorable situations like pyrimethamine and D-sorbitol treatment,

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but may become dormant and active form by some mechanism which is inherent in them. Dormant parasites may escape antimalarial treatment with high concentration. Frequency of recrudescence may be decreased when treatment period is prolonged.

To explore the probability of these ideas, parasites were treated with chroloquine, mefloquine, quinine, and PBS containing sugar, washed and kept in standard medium to observe recrudescence. In other experiments parasites were treated with high concentrations of pyrimethamine and mefloquine. Various numbers of parasitized erythrocytes were treated with pyrimethamine or mefloquine for several days to measure the proportion of dormant parasites in culture.

Recrudescence was observed in culture treated with above drugs and PBS sugar. Frequency of recrudescence was decreased with prolonged periods of treatment. This observation suggested that some parasite population may contain dormant parasites with different dormant periods.