

pyrimethamine and followed for 5 weeks by microscopy and collection of filter paper blood samples. At day 0, 52 subjects had asymptomatic low parasitemia while 57 had no parasitemia. Parasite DNA was extracted from filter papers and analyzed by PCR for the presence of DHFR mutations at amino acid positions 108, 51, 59 and 164. After beginning pyrimethamine prophylaxis 8 subjects (15%) showed a persistent parasitemia and 52 breakthrough infections (positive malaria smear following initial clearance of parasitemia) occurred in 46 subjects. The overall prophylaxis failure rate was 47%. The prevalence rate of DHFR mutations rose from 10% at day 0 to 90% in infections occurring after initiation of pyrimethamine. The relative risk of DHFR mutations for persistent or breakthrough infections was 1, i.e. DHFR mutations were not predictive of pyrimethamine prophylaxis failure. All 8 persistent infections (100%) had a sensitive DHFR genotype at day 0, but all showed DHFR mutations after 1 week of pyrimethamine prophylaxis. These data show that pyrimethamine is strongly selective for DHFR mutations *in vivo* and that mutants arise very quickly under drug pressure, even when undetectable in the initial infection.

- 380 MUTATION IN *PLASMODIUM FALCIPARUM* DHFR AND DHPS AND RESISTANCE TO PYRIMETHAMINE-SULFADOXINE; A MOLECULAR EPIDEMIOLOGIC STUDY IN VENEZUELA. Urdaneta L*, Tibayrenc M, Plowe C, Goldman I, and Lal A. Escuela de Malariologia y Saneamiento Ambiental Dr. Arnaldo Gabaldon, Maracay, Venezuela; UMR CNRS/ORSTOM: Genetique Moleculaire des Parasites et des Vecteurs, 34032 Montpellier, Cedex 01, France; Division of Geographic Medicine, University of Maryland School of Medicine, Baltimore, MD; and Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA

In vitro resistance of *Plasmodium falciparum* dihydrofolate reductase (DHFR) inhibitors is conferred by point mutations in parasite DHFR. A Ser to Asn mutation at amino acid position 108 results in pyrimethamine resistance, and Ser to Thr at 108 with an Ala to Val change at position 16 is associated with resistance to cycloguanil, the active metabolite to proguanil. Similarly, mutations in *P. falciparum* dihydroterotoate synthase (DHPS), the target enzyme of sulfa drugs, may mediate resistance to sulfa antifolates. The present study was designed to examine mutations in the DHFR and DHPS genes of *P. falciparum* in the Bolivar region of Venezuela, where clinical resistance to Pyrimethamine-sulphadoxine (PS; Fansidar-TM) has been documented. We used nested mutation-specific PCR and restriction digest protocols to measure: 1) the prevalence of DHFR mutations at the 108 and 16 codon positions, and 2) the prevalence of mutations in the 436, 437, 581, and 613 codon sites in the DHPS gene. The DHFR gene was amplified by PCR from 54 parasite DNA samples, followed by digestion with restriction enzymes. We found that 52 (96%) carried the Asn-108 mutation that conferred resistance to pyrimethamine. Only 2 samples (4%) showed the wild type Ser-108, and none showed Thr-108 and Val-16, specific for resistance to cycloguanil. To measure the prevalence of mutations at the 436, 437, 581, and 613 codon sites in the DHPS gene, we used nested mutation-specific PCR for 67 parasite DNA samples. We found that 64 (96%) carried Gly-581 mutation, and only 1(1%) showed Ser-613 mutation. Analysis of 436, 437, and 540 mutations by enzyme restriction is in progress now. The molecular data on DHFR and DHPS from the Bolivar region in Venezuela is in agreement with prior clinical observations of high rates of clinical PS resistance. Similar studies are underway in Venezuela and western Kenya to establish the relationship between DHFR and DHPS mutations and responses to PS.

- 381 USE OF A YEAST HETEROLOGOUS EXPRESSION SYSTEM TO STUDY DIHYDROFOLATE REDUCTASE BASED DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*. Hamilton KL*, Sirawaraporn W, and Sibley C. University of Washington, Department of Genetics, Seattle WA; Mahidol University, Department of Biochemistry, Faculty of Science, Bangkok, Thailand; and University of Washington, Department of Genetics, Seattle WA.

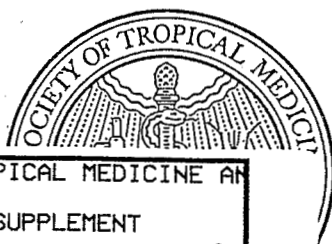
A system to rapidly and cheaply screen experimental antifolates that are both efficacious and fail to select for drug resistant parasites would be a valuable tool in fighting malaria. We are developing such a system in our lab. We began with a strain of the budding yeast, *Saccharomyces cerevisiae* that has a disrupted DHFR locus and is DHFR minus. A drug sensitive DHFR domain from *Plasmodium falciparum* was integrated into yeast chromosome XV near the *His3* locus. This strain of yeast, called pKHD6-His-IF3 (IF3), is sensitive to the antimalarial antifols pyrimethamine (pyr), cycloguanil (cyc), and the experimental antifol WR 99210 (WR), whereas wild-type yeast are not antifol sensitive. We have used this strain to isolate pyr resistant mutants utilizing both single-step and multi-step drug challenges. The single-step pyr challenge gave a mutation rate from drug sensitivity to drug resistance, for all causes, of 4.3×10^{-7} . We are currently analyzing the pyr resistant mutants from both sets of experiments to determine the molecular basis of the drug resistance phenotype. It has not been possible thus far, using the multi-step drug challenge, to isolate clones that are resistant to WR 99210, the active metabolite of PS-15, or cycloguanil, the active metabolite of proguanil. We are modifying the multi-step drug selection experiments in an effort to select for resistance to these drugs. Drug resistance in *P. falciparum* to antimalarial antifols has been correlated with a defined set of mutations in the DHFR domain coding region. We are also conducting experiments on DHFR alleles with mutations at amino acids 16, 51, 59, 108, 163, and combinations of mutations at two, three, or four of these positions to genetically dissect the contribution of each mutation and mutation combination to overall antifol



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