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# DNA PROBE FOR THE DIAGNOSIS OF VIVAX MALARIA

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I. Quarterly Progress Report

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The first stages of this project were devoted mainly to establish basic technologies needed for the successful performance of the various steps outlined in the grant proposal. Due to the lack of available P. vivax DNA at this time we used P. berghei from infected mice and P. falciparum from cultures as "surrogate" DNA. Although we are aware, that DNA from different species of Plasmodium differs both in composition and in structure, we considered it an appropriate model system.
Numerous extractions of DNA from Saponin treated P. berghei infected mouse red blood cells yielded about 1-2 ug DNA per ml infected blood at 30% parasitemia. It was obvious that, P. vivax DNA samples will never be of this magnitude, and therefore only the most frugal experiments could be performed in the future. P. berghei DNA served also as the model for the preparation of a genomic library using EMBL3 vector. We concentrated on this vector to complement and expand the library already prepared by the Thai-Australian team, so that two different libraries obtained by different means could be compared in the future. To achieve maximal cloning efficiency the DNA had to be purified to a high degree. Various techniques of DNA preparation have been compared to achieve purity with minimal loss during this process, so that even tiny amounts of DNA extracted from P. vivax samples will yield useful preparations.

Among the techniques examined, which included direct extraction from gel slices, electrolution and "Elutip D" columns and other accepted procedures, we chose Schleicher & Schull

Elutip D as the most efficient and DNA conserving. For maximal efficiency the appropriate physical conditions had to be established. The standardization of this step took most of our efforts prior to Prof. Pramuans visit and the delivery of P. vivax samples.

The first experiments using P. vivax from infected blood collected from patients in Thailand and concentrated using the method described in the grant proposal, were DNA extractions using the Maniatis method, as employed by the Thai-Australian team. This extraction procedure was later compared to the method used by Wallach et al. for P. falciparum and P. berghei.

At present we are attempting to find more probes that would hydridize to different isolates of P. vivax and not hybridize to DNA from other species of Plasmodia which could be used to analyze blood samples from patients suspected of being infected with the parasite. P. vivax shows a high level of keryotype polymorphism as has been shown by Langsley (1988) on isolated parasites obtained from different patients. Our approach is to find a repetitive sequence that can be used as a highly sensitive DNA probe. For this purpose, we extracted DNA from P. vivax parasites isolated from four pools of infected patients, cut with different restriction enzymes in order to see whether they contain repetitive sequences. The enzymes used were HaeIII, XhoI, PstI, AVUI, KpnI, BglII, HindIII and TaqI. The samples were run on 0.8% agarose gel, and then stained with ethydium bromide. No detectable discrete band proving the presence of repetititve

sequences have been detected. To improve on sensitivity, in addition to direct ethydium bromide Southern blotting was also used to detect discrete bands but this highly sensitive method also could not show repetitive sequences in our samples.

The results of our Southern blotting experiments thus far showed that no hybridization was obtained using 100-200 ng. P. vivax DNA, 100 ng human DNA or clone VPL101 (20 ng) as probes on a blot containing 60-100 ng of P. vivax DNA cut with the restriction enzymes BamHI and HindIII and hybridized overnight. We then performed blotting experiments to determine the level of sensitivity of these probes. Human DNA used as a probe could detect as little as 1 ng of human DNA on a blot. In addition, we were able to detect 100 pg of clone VPL101 in an overnight exposure. Thus it would appear that P. vivax DNA contains either very low amounts of repetitive DNA, or extreme heterogeneity of repetitive sequences which do not cross-hybridize within a given isolate. These results also show that there is very little (less than 1%) human DNA present in the P. vivax samples so far analyzed.

In order to distinguish between the possibility of either little or no repetitive DNA in the P. vivax genome, or a very high level of heterogeneity between repetitive elements, Southern blotting experiments will be carried out under conditions of low stringency. Should hybridization be detected, than various restriction enzymes would be tested to produce a pattern most suitable for cloning. However, if under low stringency conditions

little or no hybridization is detected, we would conclude that finding a DNA probe with greater sensitivity and specificity than VFL101 is very unlike.