

HUMAN LIVER CELL LINE

This application claims priority from U.S. Provisional Application No. 60/235,051 filed on Sep. 25, 2000.

INTRODUCTION

Malaria is one of the most serious health risks due to problems of multi-drug resistance and lack of an effective vaccine. Two human malaria parasites that can cause severe disease are known, *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*). Development of vaccines and drugs against the disease has been difficult since the parasites can develop resistance to most of the drugs quite rapidly and the development of the parasites in the liver where is the first place for transmitting the disease to human has not been well understood. Presently, there is no efficient in vitro human liver model available to support vaccine and drug development.

Malaria sporozoites are injected into human during the blood meal of a female anopheline mosquito and rapidly invade hepatocytes. One sporozoite can develop into 20,000 merozoites, which rupture from hepatocyte, enter the bloodstream and invade erythrocytes. This initiates a cycle of intra-erythrocytic stage development, rupture and re-invasion, leading to 10–20 fold increase in the number of the parasites in the blood stream every 48 hours. These asexual erythrocytic-stage parasites are responsible for the clinical manifestations and pathology of the disease. Some erythrocytic stages differentiate into gametocytes, which are infective for mosquitoes. Fertilization occurs in the mosquito midgut, and form oocysts. Sporozoites rupture from these oocysts and invade the salivary glands of the mosquito from where they are injected into a human host. The hepatic stage of the life cycle is an ideal target for vaccine-induced protective immune responses because this stage lasts for at least 5.5 days and is not associated with pathology. Development of causal prophylaxis drug is important for protection from the disease. The animal models such as mice, monkey and in vitro culture of rat liver cells have been used for efficacy test of new drugs.

Complete development of *P. vivax* and *P. falciparum* exoerythrocytic stage was shown in primary culture of human hepatocytes (Mazier et al., 1984, Science 227, 440–442; Smith et al., 1984, Lancet 29, 757–58; Mazier et al., 1984, Nature 307, 367–69). Primary hepatocyte cultures are not feasible to use as a tool to evaluate malarial drugs and vaccines since they are expensive and their delivery schedule is uncertain. Development of *P. falciparum* exoerythrocytic stage has also been demonstrated in a human hepatoma cell line, HHS-102 (Karnasuta et al., 1995, Am. J. Trop. Med. Hyg. 53, 607–11) and *P. vivax* in HepG2-A16 (Luo et al., 1994, Chung Kuo chi Sheng Chung Hsueh Yu Chi Sheng Chung Ping Tsa Chin. 12, 8–4) but with low density of growth. In addition, these cell lines are derived from a tumorigenic cell line and would not reflect growth conditions in a normal, non-tumorigenic cell line.

Hence there is a need for a cell line established from normal liver tissue and which can support high density, complete erythrocytic development of both *P. falciparum* and *P. vivax*.

SUMMARY OF THE INVENTION

This application describes such a cell line. The cell line of the present invention, HC-04, has two important characteristics. First, the cell line was developed from normal liver

tissue (hepatocytes) and second, this cell line supports in vitro development of human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*.

Therefore, it is an object of the present invention to provide a hepatocyte cell line HC-04, the cell line deposited under the Budapest Treaty at American Type Culture Collection, Manassas, Va. on 8 Jun. 2001, assigned accession no. PTA-3441. The cell has been propagated in vitro for more than 100 passages.

It is another object of the invention to provide a method for making an immortalized human hepatocyte cell line containing cells capable of supporting *Plasmodium* exoerythrocytic growth.

It is another object of the present invention to provide a method for screening to identify compounds that affect parasite growth. In one embodiment, the method includes incubating components comprising the compound and at least one hepatocyte of the present invention which has been infected with the parasite under conditions sufficient to allow the compound and cell to interact; and determining the effect of the compound on the cell and on the parasite. A function of the parasite or cell that may be modulated (e.g., inhibited or stimulated) by the compound includes, but is not limited to, differentiation, gene expression, production of factors, response to factors. In another aspect, the present invention provides for compounds identified as inhibitory to the development or differentiation of the parasite for use as a malaria drug or vaccine for protection against malaria.

In another aspect, the invention provides a method of using the cells of the present invention to produce a certain developmental stage of the parasite. In one embodiment, a hepatocyte is cultured under conditions effective to produce a developmental stage of the parasite. By studying gene expression in the presence of a potential vaccine compound, or by producing a compound which reacts directly with an antigen on the surface of the parasite such that its growth is inhibited compounds can be identified for prophylactic or therapeutic use against malaria.

These objects and others which will become obvious to the skilled artisan are deemed to fall within the spirit and scope of the present invention and are intended to be included herein.

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims, and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Phase contrast micrograph of human hepatocytes (HC-04).

FIG. 2. Chromosomes of HC-04 prepared from cell culture passage #6.

FIG. 3. Early exoerythrocytic parasites (P) were found in cytoplasm of liver cells (N=nucleus of liver cell) 3A. Giemsa staining. 3B. IFA staining with human antibody and mouse monoclonal antibody. The antibodies bind to surface of parasite. Liver cells were counterstained with Evan blue. 3C. The same slides as 3B after being washed and stained with Giemsa.

FIG. 4. Liver schizonts (S) were observed from day 7 to day 28. (4A) 7 days schizont. (4B) 21 days schizont. (4C) 28 days schizont.

FIG. 5. Schizont (S) were observed on day 14 with (5A) IFA staining with human antibody and MAB and compared with (5B) Giemsa staining of the same specimen.

FIG. 6. Liver merozoites (M) were found on day 28.