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Leading Article

Malaria in the liver

G.C. Cook

Hospital for Tropical Diseases, St Pancras Way, London NW1 0PE, UK

Introduction

The protozoan parasitaemia that characterizes malaria, results from infection with *Plasmodium* spp., which is inoculated by the female *Anopheles* mosquito – usually between dusk and dawn.[1] Transmission occurs over much of the globe; one estimate is that 46% of the world's human population lives in this area, and a recent WHO guesstimate indicates that 300 million individuals (90% of them in tropical Africa) are infected, of whom 2 million (1.2 million in Africa) die annually[1,2] (most belonging to the under 5 year old group).

From an hepatic viewpoint, it is important to appreciate that whereas *P. falciparum* infection (the severe form of the disease and the only one to cause acute mortality) and *P. malariae* directly utilize the hepatocyte during their life-cycle within *Homo sapiens*, *P. vivax* and *P. ovale* also undergo a dormant phase (the hypnozoite stage) in these cells, which allows recrudescence(s) to occur.[1,3-5]

The question as to whether malaria is a precursor of hepatitis, and later cirrhosis, is one which has perplexed physicians, including hepatologists, for many years. In 1898, Dr (later Sir) Patrick Manson wrote:[6] 'Under the influence of a succession of acute attacks, hepatic congestion may acquire a more or less permanent character'. He continued: 'If this ... be long maintained, it tends to bring about various kinds and degrees of chronic hepatitis with hypertrophy of the interlobular connective tissues, and in time leads to hypertrophic, or to different forms of atrophic, cirrhosis. Thus irremediable organic disease of the liver, portal obstruction, and ascites may ensue'. And in 1913, Lucius Nicholls wrote:[7] 'Cirrhosis of the liver is a common condition of many tropical countries, and numerous authorities have asserted that some cases are caused by repeated attacks of malaria ...'; in that paper he included a figure showing a shrunken liver, together with two histological preparations, claimed to demonstrate monolobular biliary cirrhosis – a result of repeated attacks of malaria, which had compressed and consequently injured the biliary system.

Elucidation of the hepatic component of the *Plasmodium* spp. life-cycle

Clear demonstration of the human *Plasmodium* spp. parasite was first accomplished by Alphonse Laveran in Algeria in 1880.[8] In 1897-1898, Ronald Ross, working in India, elucidated the host-mosquito-host cycle of avian malaria,[9,10] and in 1900 Manson and his colleagues, in a study carried out in Italy and London, demonstrated conclusively the man-mosquito-man cycle.[11] It was not, however, until 1948 that Shortt and Garnham – working at the London School of Hygiene and Tropical Medicine – elucidated the hepatocyte stage of the life-cycle.[12,13] Their work involved both experimental malaria using *P. cynomolgi*, and also human infection (induced as a therapeutic measure in neurosyphilis, at Horton Hospital, Epsom) using *P. vivax*. This group subsequently extended their work to *P. falciparum* infection. It was only in 1980 that Krotoski and his colleagues were able to demonstrate the 'missing link' (the hypnozoite stage) in the *P. vivax* group of infections.[14]

Following injection by the infected mosquito, sporozoites are rapidly taken up by the hepatocyte (possibly via Kupffer cell intervention), within 20-30 min.[1,3-5] Incubation within the hepatocyte takes several days to weeks – in the case of *P. falciparum* 9-14 days; at that point, schizogony results in the production and subsequent liberation of numerous merozoites, which after discharge from the hepatocyte, invade erythrocytes, giving rise to the characteristic cycle leading to periodic fever.[1] Prolonged *Plasmodium* spp. parasitaemia results from continued release of merozoites from the hepatocyte.[15] In *P. vivax* and *P. ovale* infections, the hepatocyte component of the life-cycle usually possesses a longer incubation period (12 days to several months) in the hepatocyte; however, arrested sporozoite development within these cells gives rise to the hypnozoite (5-6 μm) stage, which,

Correspondence: G.C. Cook, M.D., D.Sc., F.R.C.P., F.R.A.C.P., F.L.S.

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in the absence of satisfactory chemotherapy, persists for months or years.

Although the hepatocyte is therefore a crucial component in the *Plasmodium* spp. life-cycle, significant morphological change does not occur during this process; although schizogony temporarily displaces the hepatocyte nucleus, other sequelae are absent.[4]

Plasmodium spp. infection in the long-term resident of an area of high transmission

In a malarious area, repeated infection occurs from the day of birth onwards. During the first 3–6 months of life, partial immunity – rendered by maternal antibodies – results in moderate protection; hepatic changes are minimal.[16] At >6 months, a high peripheral blood parasitaemia is a frequent occurrence, and significant morbidity (and occasionally mortality) occurs. Splenomegaly is frequently present. During this phase, hepatocyte damage is, however, minimal and the hepatic histological appearance is dominated by ingested *Plasmodium* spp. in sinusoidal erythrocytes.[16] However, marked reticuloendothelial (RE) activity (involving the Kupffer cells and macrophages) is present; this is accompanied by pigment deposition – well demonstrated using a bi-refringent staining technique. Later, with the onset of increased inherent immunity, the level of parasitaemia is reduced, malarial antibody titre rises and the splenomegaly rate reduced. Clearance of pigment – beginning at the centre of the hepatic lobule – is apparent histologically.[16]

In an indigenous population in an area of high transmission, RE changes therefore predominate; hepatocyte changes (which are minimal) consist of lipofuscin and occasionally haemosiderin (malaria pigment) deposition, fat-droplet formation, mitochondrial swelling, and microvillus loss.[5]

In a small percentage of individuals living in an area of high transmission, hyper-reactive malarious splenomegaly (HMS) – an aberrant immunological response to the four human *Plasmodium* spp. results.[17,18] In this syndrome, massive splenomegaly, often accompanied by significant hepatomegaly, is usual; sinusoidal lymphocytosis (mostly T-lymphocytes) (which may be intense), raised serum IgM (polyclonal) and an elevated malarial antibody titre in peripheral blood are co-existent features. IgM staining of a liver biopsy section reveals intense deposition within the RE elements. It is sometimes possible to detect immune complexes, cryoglobulins, rheumatoid-like factor and other auto-antibodies also.[5] In studies carried out in Africa, an increase in circulating B-lymphocytes has been demonstrated; T-cell concentration remains normal.[5] However, in Indonesia, the T-8 concentration is diminished, B-lymphocyte concentration remaining normal.[4] Immunological mechanisms underlying HMS therefore remain enigmatic.

Acute *Plasmodium* spp. infection in the non-immune individual

Malaria infection in the newly exposed individual is sometimes followed by profoundly devastating consequences.[16,19] Pathological changes are multisystemic, the brain and kidneys being most severely affected. However, the liver may be intensely congested and swollen; numerous parasitized red cells can be demonstrated within hepatic sinusoids, and there is a great deal of malaria pigment deposition. Owing to the fact that ethical constraints preclude intense clinical investigation in the presence of *severe* complicated *P. falciparum* infection, pathophysiological events in this disease have not to date been fully elucidated (see below).

Hepatotoxicity resulting from anti-*Plasmodium* spp. agents

Amodiaquine – formerly widely used as a chemoprophylactic against *Plasmodium* spp. – produces significant hepatocellular dysfunction;[20,21] it is now rarely used due to a causative association with bone-marrow depression. 'Fansidar' (pyrimethamine + sulphadoxine) has been extensively used in chemoprophylaxis, and remains an effective chemotherapeutic agent; it also produces significant hepatocellular dysfunction.[22] Mefloquine, a compound now widely used both in chemoprophylaxis and chemotherapy, can also produce significant changes in liver-function tests;[22] it has not, however, been associated with significant histological abnormality. Quinine, again the first-line agent against *P. falciparum* infection, is also hepatotoxic, albeit rarely.[1]

Clinical manifestations and differential diagnosis

In the presence of *Plasmodium* spp. (especially *P. falciparum*) infection, tender hepatomegaly, often associated with splenomegaly, is frequently present.[1,4,5,23] Jaundice usually has a haemolytic basis.[23] Anaemia (which may be severe) is also usually haemolytic in origin. Differential diagnosis is from other causes of tender hepatomegaly: e.g., the viral hepatitides, Epstein–Barr virus/cytomegalovirus hepatitis, *Coxiella burnetii* hepatitis, *Salmonella* spp. hepatitis, invasive hepatic amoebiasis, and the 'jaundice of acute systemic bacterial infection'.[4] Liver-function tests frequently exhibit an uncon-

jugated hyperbilirubinaemia; however, in *P. falciparum* infection, the conjugated component may be significantly elevated. In blackwater fever, cholestasis may predominate. In severe *P. falciparum* infection, serum aminotransferase and 5′-nucleotidase concentrations are elevated, prothrombin time is prolonged, and serum albumin reduced.[5] Other findings are lactic acidosis and hypoglycaemia (see below), together with changes in triglycerides, phospholipids, free fatty acids, and cholesterol (esterified and non-esterified).[5]

Experimental and clinical investigations

Between 1945 and 1965, B.G. Maegraith and his colleagues, working at the Liverpool School of Tropical Medicine, carried out extensive studies on experimental malaria in *Macaca mulatta*.[24] This group demonstrated reduced hepatic blood flow, which was considered to result from sympathetic overactivity.[24–27] As a consequence, sinusoidal perfusion was shown to be reduced, with resultant anoxia and centrilobular hepatic necrosis. Satisfactory human studies have been few and far between and, due to ethical constraints, very few have addressed the situation in *severe* complicated infection.[28–42] Available data relate to 'therapeutic' *P. vivax* infection (see above), studies in British soldiers during World War II (1939–1945), and in American soldiers serving in the Korea and Vietnam wars. These older investigations utilized liver-function tests which are now outdated; in few instances was hepatic histology examined. There are, therefore, very few satisfactory studies relating to the state of the liver in complicated *Plasmodium* spp. infection.

Recently, Molyneux and his colleagues, working in Thailand, have studied hepatic blood flow both in complicated and uncomplicated *P. falciparum* infection – using an indocyanine green clearance technique.[43] In complicated infection, hepatic blood flow was shown to be significantly reduced when measurements were compared with those following recovery ($P < 0.002$); in uncomplicated infection, there was no significant change. The investigation was interpreted as reflecting reduced splanchnic flow in complicated *P. falciparum* malaria, a conclusion strengthened by further demonstration that intestinal absorptive function (based on studies involving three orally administered test sugars) was also reduced; this was also attributed to reduced splanchnic flow.[43] This study, therefore, corroborates experimental evidence indicating that in *severe* complicated infection, centrilobular necrosis – ischaemic in origin – results from a major reduction in splanchnic flow induced by heavy *P. falciparum* parasitaemia. Whether tumour necrosis factor (TNF) – greatly elevated in complicated *P. falciparum* infection – contributes to these changes is unknown.

Significance of hepatic blood-flow reduction in complicated *P. falciparum* infection

Hypoglycaemia, which results from defective hepatic gluconeogenesis, can be explained, in part at least, by relative ischaemia.[4,43] Recent evidence indicates that the segment of the glycolytic pathway between galactose and glucose is unimpaired in *severe* complicated infection.[44] Reduced clearance is also likely to explain the associated lactic acidaemia and endotoxaemia. Therapeutically, metabolism of anti-*Plasmodium* spp. compounds, for example, quinine, is likely to be significantly impaired.[4,43]

Management of hepatic abnormalities

Specific therapy to counteract hepatic involvement consequent on *severe* (complicated) *Plasmodium* spp. infection is *not* indicated. Curative measures should be targeted towards reducing parasitaemia, and in caring for the (frequently) unconscious patient. Specific aspects of management should be directed towards hypoglycaemia, pulmonary oedema, shock (hypotension), secondary infection(s), maintenance of renal function and an associated haemorrhagic diathesis/DIC.[4]

A *P. falciparum* vaccine in the foreseeable future?

Research has largely concentrated on the possibility of a sporozoite vaccine;[45,46] other investigators have sought a safe and effective merozoite or gametocyte preparation. Induction of protective immunity is made difficult by phenotypic/sequential antigenic variation, and antigenic diversity. Only in the hepatocyte cycle (see above) is there a possibility of inducing a cytotoxic lymphocyte response.[5] Host factors, which must be crucially important, leading to resistance to *severe* complicated infection are slowly being unravelled.[47]

Conclusions

Few, if any, would now give credence to early work that purported to invoke hepatitis and/or cirrhosis as long-term sequelae of *Plasmodium* spp. infection (see above). In the light of available data, however, the following conclusions seem justified:

1. Hepatocyte involvement, which is crucial to the life-cycle of *Plasmodium* spp., does *not* produce significant clinical disease;

2. The major hepatic insult in *Plasmodium* spp. infection involves the RE components;
3. Significant clinical symptoms/signs are unusual in a straightforward infection;
4. Severe, complicated *P. falciparum* infection can result in significant centrilobular necrosis; metabolic consequences include hypoglycaemia and lactic acidosis; and
5. No evidence of long-term sequelae (for example, hepatitis/cirrhosis) exists, apart from residual RE changes and the occasional development of HMS.

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