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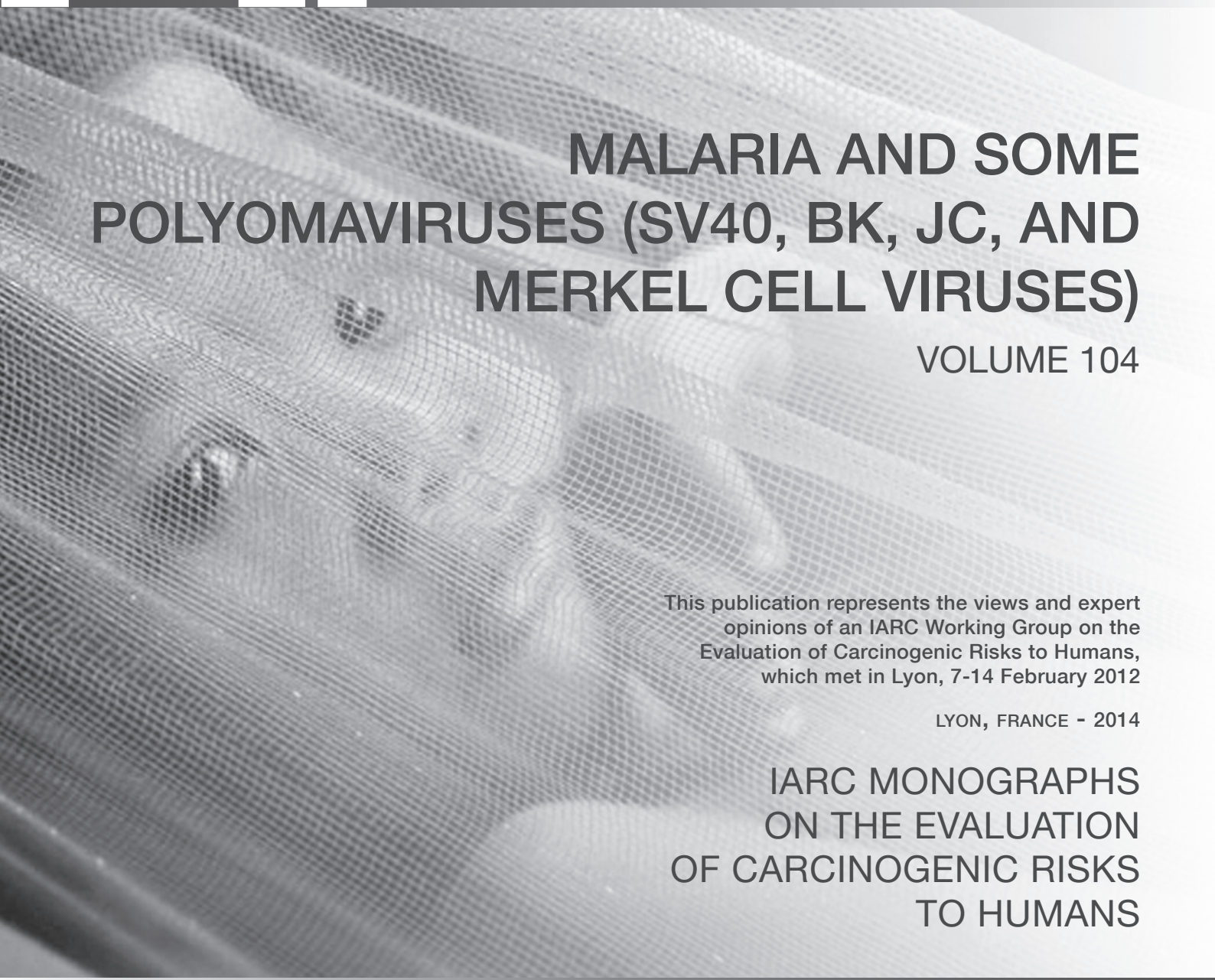

MALARIA AND SOME POLYOMAVIRUSES (SV40, BK, JC, AND MERKEL CELL VIRUSES)

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International Agency for Research on Cancer

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MALARIA

1. Exposure Data

Malaria is a human disease caused by infection with a parasite of the genus *Plasmodium*.

1.1 Taxonomy, structure, and biology of *Plasmodium*

1.1.1 Taxonomy

Plasmodium is a genus of parasites belonging to the family Plasmodiidae, order Haemosporidia and phylum Apicomplexa. The genus *Plasmodium* is subdivided into 10 subgenera. Malaria parasites in humans are all classified in the subgenera *Plasmodium* and *Laverania*. Four are well-characterized, strict human pathogens (e.g. *P. falciparum, P. vivax, P. ovale, P. malariae*), and one (*P. knowlesi*) is a recently identified human pathogen (Cox-Singh *et al.*, 2008; Kantele & Jokiranta, 2011).

Plasmodium has two obligate hosts in its life cycle: a mosquito host, which also serves as vector to a vertebrate host. The insect vector of the parasite is used as one biological criterion for classification of the different species of *Plasmodium*. Other biological criteria include the host range, the type of host cell infected, the length of the different stages of the life cycle, the presence or absence of relapse/recrudescences, and geographical distribution.

The morphology of the parasite is also used to characterize species. Morphological criteria include the shape of the trophozoite, the gametocyte and the oocyst, the number of nuclei in the erythrocytic and exo-erythrocytic schizonts, the aspect and distribution of the hemozoin pigment from the metabolism of the haemoglobin, and the nature of the damage induced by the parasite in the host cell. As more *Plasmodium* species are sequenced, newer taxonomic criteria based on molecular characteristics such as the 18S small subunit rRNA, the genes for the circumsporozoite protein and for cytochrome b are now being included to define *Plasmodium* species and generate phylogenetic trees (Outlaw & Ricklefs, 2011).

All *Plasmodium* species examined to date have 14 chromosomes, one mitochondrion and one plastid. Sequencing data have resolved the question of the origin of *P. falciparum* and its relationship with other *Plasmodium* parasites of primates and humans. Extensive sequence analysis of primate *Plasmodium* DNA indicates that *P. falciparum* is genetically related to a gorilla *Plasmodium* parasite in the *Laverania* subgenera (Liu *et al.*, 2010).

1.1.2 Structure

Specialized complexes of apical organelles known as micronemes, rhoptries and dense granules are distinguishing morphological features of parasites belonging to the phylum Apicomplexa. In addition, apicomplexan parasites have a vestigial plastid organelle, the apicoplast, which has its own genome and gene-expression

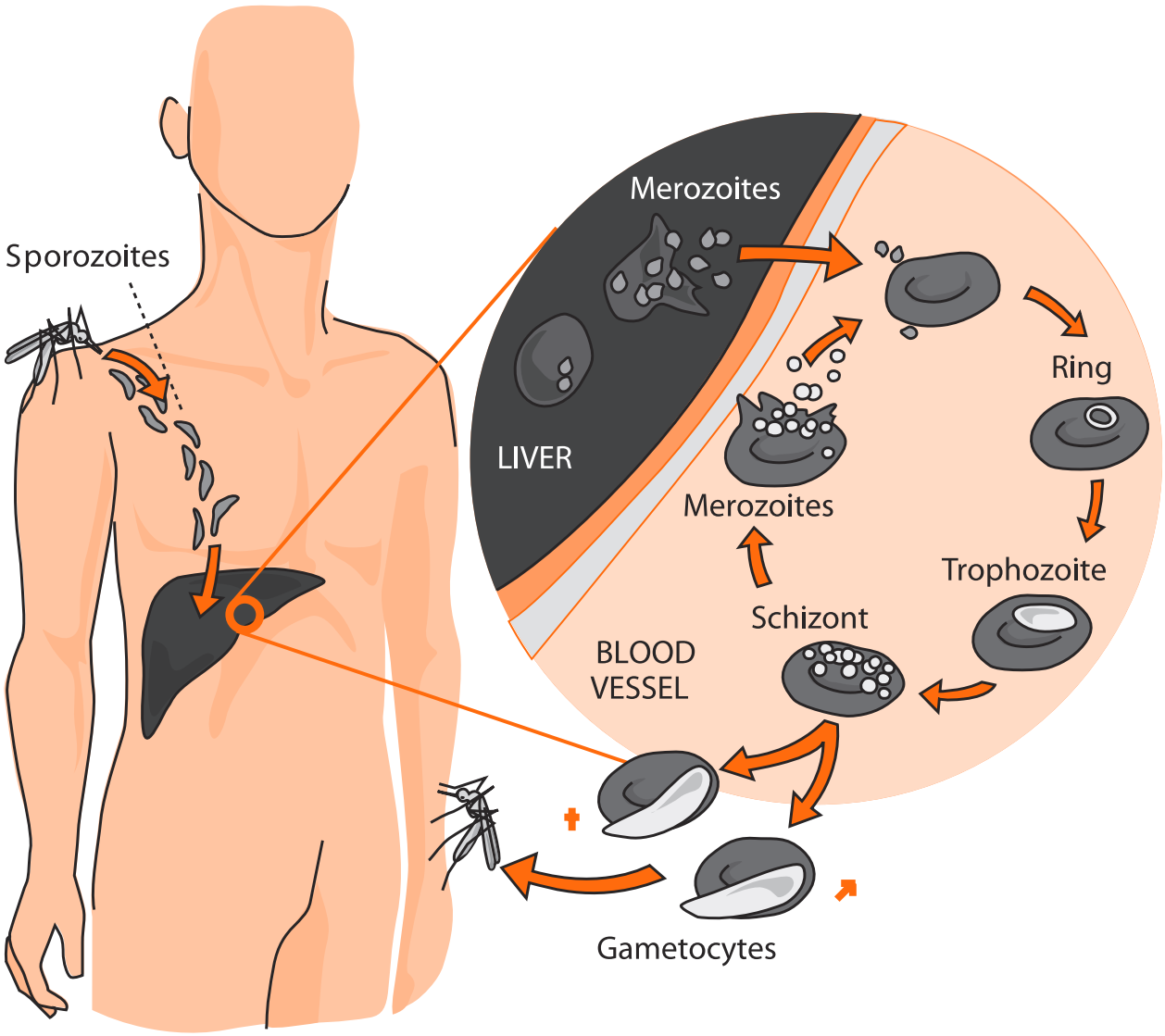
machinery (Waller & McFadden, 2005). Apical organelles have been implicated in the process of host-cell invasion. *Plasmodium* parasites have a complicated life cycle as they infect both mosquitoes and humans and, within each host, there are several stages of development. In the human host, the parasite first invades and replicates in hepatocytes. In this hepatic development phase, the parasite is known as a liver schizont. Liver schizonts appear as clusters of small basophilic bodies (merozoite nuclei) located in vacuoles within host hepatocytes, measuring 40–80 µm in diameter when mature. After replication in the hepatocyte, the parasite is released as a merozoite, which then infects erythrocytes. Four developmental stages are found in erythrocytes: trophozoites, schizonts, merozoites, and gametocytes. Each of these stages has well-defined morphological features and, combined with characteristic modifications of the host erythrocyte, these features are used to distinguish between the four primary species of human *Plasmodium* during infection of the human host *via* microscopic examination of Giemsa-stained peripheral-blood smears. The trophozoites are small and rounded, known as ring forms, and measure 1–2 µm in diameter. The schizont stage is amorphous and multinucleated, measuring up to 7–8 µm in length. The schizont can either divide into merozoites and repeat the erythrocyte-infection cycle, or differentiate into gametocytes. Gametocytes are the sexually reproductive stage and are approximately 1.5 times the diameter of the erythrocyte in length.

1.1.3 Life cycle, natural history of infection, persistence, latency

The life cycle of *Plasmodium* species in humans is shown in Fig. 1.1. The parasite transits between the *Anopheles* mosquito vector and the human host. The pre-erythrocytic stage begins when an infected female *Anopheles* mosquito inoculates *Plasmodium* sporozoites into the skin or into the bloodstream of humans during a blood meal. The sporozoites circulate transiently in the bloodstream before invading hepatocytes, where an asexual life cycle occurs. Recent studies have shown that sporozoites can remain for up to 6 hours at the site of injection (Yamauchi *et al.*, 2007), and that 25% of those leaving the injection site may enter the draining lymph nodes *via* the lymphatic vessels (Amino *et al.*, 2006). When sporozoites reach the liver parenchyma, they migrate through several hepatocytes before definitive infection. This migration seems to be advantageous for malaria infections in at least two different ways: to activate the sporozoites for infection and to increase the susceptibility of the host hepatocytes (Mota & Rodriguez, 2004). It is thought that invasion of the hepatocytes first requires invasion of Küpffer cells found in the liver (Pradel & Frevert, 2001). A hepatic phase of development begins that lasts approximately 1–2 weeks. Replication of *Plasmodium* in the liver is known as the exo-erythrocytic stage. The merozoites develop in unique vacuoles in the hepatocytes known as "parasitophorous vacuoles". For *P. falciparum* and *P. malariae*, this stage of the life cycle always proceeds to rupture and release of merozoites. However, the liver stages of *P. vivax* and *P. ovale* can either result in release of merozoites or the establishment of the hypnozoite stage. Hypnozoites are a latent phase of infection in the liver and can remain so for years (Markus, 2011). Very little is known about the biology of the hypnozoite phase. Malarial relapse due to emergence of hypnozoites is characteristic of *P. vivax* and *P. ovale* and distinguishes infections with these pathogens from those with *P. falciparum* and *P. malariae*.

Plasmodium parasites are highly species-specific and this has limited understanding of the biology of these parasites in their human cellular targets, e.g. hepatocytes and erythrocytes. Of the human parasites, most is known about *P. falciparum* in the erythrocytic phase of replication, because this parasite can be cultured

Fig. 1.1 Life-cycle of *Plasmodium* species in humans

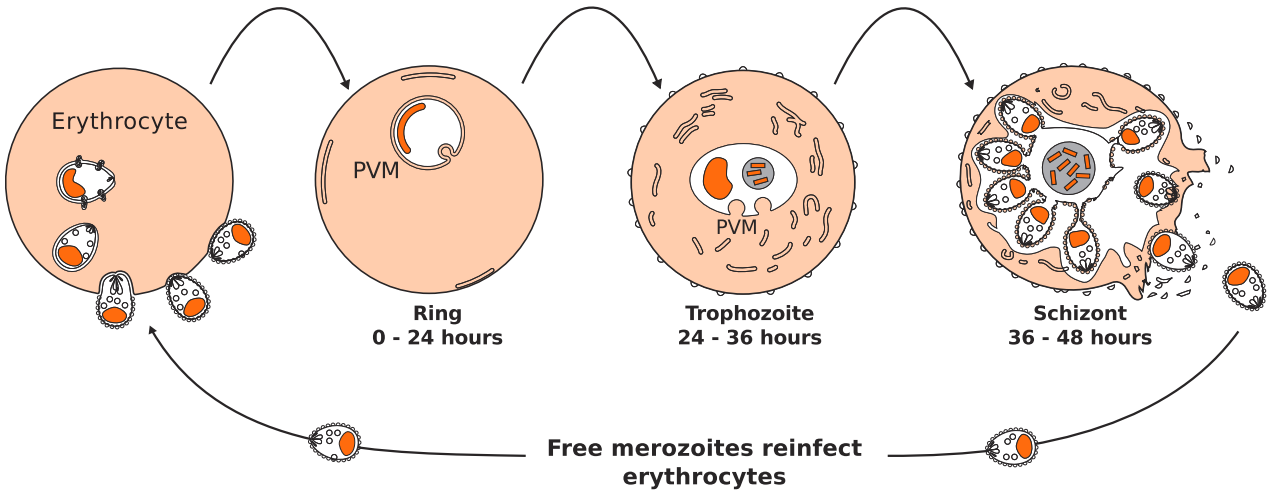


Prepared by the Working Group

in erythrocytes *ex vivo*. *P. vivax* preferentially infects reticulocytes, the immature form of erythrocytes; only recently has a system of culture *in vitro* been developed that allows for limited continuous culture of *P. vivax* (Panichakul *et al.*, 2007).

The *P. falciparum* merozoite can use many erythrocyte receptors for invasion; in contrast, invasion by *P. vivax* requires the Duffy antigen/chemokine receptor (as known as FY glycoprotein or cluster of differentiation 234, CD234) (Miller *et al.*, 1975). The detailed life cycle of *P. falciparum* within erythrocytes (the intra-erythrocytic stage) is shown in Fig. 1.2. In the newly infected erythrocyte, the parasite becomes enclosed in a vacuolar membrane and microscopic examination shows the clear presence of a ring in infected cells (i.e. the "ring" stage). The parasite remains in the ring stage for 24–32 hours and then matures to the trophozoite stage.

Fig. 1.2 Life-cycle of *Plasmodium falciparum* in erythrocytes



P. falciparum infects the erythrocyte and proceeds through several stages of maturation.
PVM, parasitophorous vacuole membrane
Prepared by the Working Group

What makes *P. falciparum* different from other malaria parasites in the trophozoite stage is that the infected erythrocytes become sequestered in the microvasculature by adhering to endothelial cells (Sherman *et al.*, 2003). Parasite-induced membrane changes are thought to be responsible for the adherence of infected erythrocytes.

The trophozoites mature into schizonts, which then divide into merozoites. Rupture of the erythrocyte releases merozoites into the bloodstream and repeated cycles of erythrocyte invasion and rupture result in exponential increases in parasite burden. These high levels of parasite burden are linked to the pathogenesis of *P. falciparum*, which is characterized by haemolytic anaemia. In contrast, because *P. vivax* preferentially targets immature erythrocytes, which are found at low levels in the peripheral blood, the parasite burden is significantly less than 1% of peripheral erythrocytes (Panichakul *et al.*, 2007). In some infected erythrocytes, instead of maturating into merozoites, the shizont differentiates into male and female gametocytes. These can be ingested by a *Anopheles* mosquito during a blood meal to complete the life cycle of *Plasmodium* in humans (the life cycle in mosquitoes is not described in this *Monograph*).

Infection of erythrocytes by *P. falciparum* results in extensive remodelling of the erythrocytic membrane, especially during the trophozoite phase (Cooke *et al.*, 2004). This results in functional changes in the erythrocyte, including increased membrane rigidity, reduced cell deformability, increased permeability and increased adhesiveness to the endothelium and other host cells. Although many of the parasite proteins essential for these alterations are unknown, several key proteins have been identified in recent years. For example, the parasite erythrocyte membrane protein 1 (PfEMP1) is a surface adhesin detected in the membrane of infected erythrocytes and is thought to be responsible in part for the adherent properties of the infected erythrocyte by forming knob-like structures on the surface of the infected cell. Proteomic analysis of *P. falciparum* has identified more than 400 proteins that could be exported to the erythrocyte (Marti *et al.*, 2004). A common export domain was identified on an additional 72 *P. falciparum* genes, suggesting that the proteins

encoded by these genes could be exported to the surface of the erythrocyte (Sargeant *et al.*, 2006).

1.2 Epidemiology of *Plasmodium* infection

1.2.1 *Global distribution of* Plasmodium *infection in humans*

Assessing the global epidemiology of malaria is a complex process involving evaluation of environmental ecology, vector-species identification, the local dominant parasites, dynamics of the exposed population, chemotherapeutic trends in clinical malaria, and the outcome of control strategies (Ototo *et al.*, 2011). Variation in the genetics of host susceptibility also contributes to the distribution of malaria globally. Overestimates of the burden of malaria can result when diagnosis is based on symptoms alone, since the symptoms typical of malaria (e.g. fever, chills, malaise) are also exhibited by other microbial infections characterized by acute febrile illness and the correct diagnosis of clinical malaria remains a challenge (Ari *et al.*, 2011). In contrast, reliance on passive national reporting of malaria has likely led to an underestimate of the true burden of malaria worldwide (Snow *et al.*, 2005).

Globally, populations in 107 countries and territories (about 50% of the world population) are at risk of malaria, with an estimated annual incidence of 216 million cases of malaria (WHO, 2011), and up to 1.2 million deaths due to malaria in 2010 (WHO, 2011; Lancet, 2012). An estimated 86% of all deaths occur in children aged less than 5 years (WHO, 2011). The *Plasmodium* species that infect humans are confined to the tropical and subtropical areas of the world where their insect vectors, mosquitoes of the genus *Anopheles*, are found. The large majority of infections can be linked to *P. falciparum*, with the remainder predominantly caused by *P. vivax*, and a very small number caused by *P. malariae*, *P. ovale* and *P. knowlesi* (WHO, 2011). The geographical distribution of *Plasmodium* species infecting humans is shown in Table 1.1.

In sub-Saharan African, the highest rates of morbidity and mortality are associated with infection with *P. falciparum*. *P. vivax* is less frequent than *P. falciparum* in Africa, but is the dominant *Plasmodium* species causing malaria in many localities outside Africa. Infection by *P. falciparum* and *P. malariae* occurs worldwide, while *P. ovale* is limited to Africa and parts of Asia.

Malaria is endemic in many countries within the tropical regions of the world. The greatest burden of falciparum malaria occurs in Africa (Table 1.2; WHO, 2011).

1.2.2 *Transmission of* Plasmodium

Plasmodium parasites are transmitted to humans by an infective bite from the *Anopheles* mosquito. The distribution of *Anopheles* species is highly variable from region to region, with considerable species variation between proximate geographical areas due to differences in environmental and climatic variables (e.g. altitude) that support or limit vector burden. Out of 390 species of *Anopheles* mosquitoes, only 50 species are known to transmit *Plasmodium*, with 20 species showing more localized global geographical distribution (Sinka *et al.*, 2010a, b, 2011). The most common vectors of human *Plasmodium* parasites in many parts of Africa are *Anopheles gambiae* and *Anopheles funestus* (Sinka *et al.*, 2010a).

The burden of infection with *P. falciparum* within a population is described ecologically on the basis of transmission intensity (Gething *et al.*, 2011). Transmission intensity can be measured in numerous ways, including the entomological inoculation rate (EIR). EIR is an estimate of the annual number of bites by infectious mosquitoes received by one person (WHO, 2010). In Africa, the average EIR due to *P. falciparum* is 112 and ranges from < 1 in Sudan, to 814 in Equatorial

Table 1.1 Geographical distribution of *Plasmodium* species that infect humans

Parasite	Host	Geographical distribution
P. falciparum	Human	Africa, Asia, South/Central America
P. vivax	Human	Africa, Asia, South/Central America
P. malariae	Human	Africa, Asia, South/Central America
P. ovale	Human	Africa
P. knowlesi	Human/monkeys	Asia

Data from WHO (2011)

Guinea (Kelly-Hope & McKenzie, 2009), and 1594 in Uganda (Yeka *et al.*, 2005). There can be great diversity in EIR rates even within limited geographical areas (Kelly-Hope & McKenzie, 2009). Alternative indicators of transmission intensity have been suggested, such as prevalence of antimalaria antibodies (Corran *et al.*, 2007) (see Section 1.2.3), or multiplicity of infection (e.g. as defined by the measurement of number of *P. falciparum* strains infecting a single host) (Arnot, 1998; Babiker *et al.*, 1999), but neither of these methods has been widely adopted in large-scale epidemiological studies.

Categories of transmission intensity for *P. falciparum* malaria have been described based on exposures in children aged 2–10 years and measured by EIR rates, parasite reproductive numbers, the prevalence of parasites in peripheral blood, and frequency of splenomegaly. Holoendemic malaria transmission can be defined as parasite prevalence of > 70% and splenomegaly in > 80% of children. Shown in Fig. 1.3 is a world map of *P. falciparum* endemicity (Gething *et al.*, 2011). In this map, malaria endemicity has been categorized based on the age-standardized annual mean *P. falciparum* parasite rate in children aged 2–10 years ($PfPR_{2-10}$). Four endemicity classes are shown, with the highest being holoendemic (e.g. $PfPR_{2-10}$ > 40%, intense, stable year-round transmission). The highest endemicity of *P. falciparum* transmission is seen across equatorial Africa and in Papua New Guinea. The categorization of transmission intensity is especially relevant for understanding the burden of disease due to *P. falciparum* malaria, for which there is an epidemiological correlation between holoendemic malaria and increased risk of endemic Burkitt lymphoma (see Section 2). Papua New Guinea is the only other region of the world where the intensity of *P. falciparum* transmission approaches that seen in Africa (Gething *et al.*, 2011).

The ability of the mosquito vector to survive in a given environment is a critical factor in determining the transmission intensity of malaria. For example, malaria transmission has long been known to decrease with increasing altitude. Transmission of *P. falciparum* is common at altitudes of < 1500 m. However, episodic transmission of malaria has been reported in areas at altitudes above 2000 m, depending on vector dynamics and environmental influences (Cooper *et al.*, 2009). Modifiable factors that influence the existence of the disease include climate change, effective and available antimalarial therapy, intense use of chemically treated bednets, and residual indoor spraying. Recent reports of reduction in the levels of *P. falciparum* transmission in Africa have been attributed to implementation of control strategies (WHO, 2011), but the risk of infection rebounds if vector-control strategies are relaxed.

Table 1.2 Estimated number of cases of malaria and percentage due to *Plasmodium falciparum* in 2010

WHO Regional Office	Total no. of cases of malaria	Cases of malaria due to *P. falciparum* (%)	Total no. of malaria deaths due to *P. falciparum*
Africa	174 000 000	98	596 000
Americas	1 000 000	34	1000
Eastern Mediterranean	10 000 000	82	15 000
Europe	200	5	0
South-east Asia	28 000 000	54	38 000
Western Pacific	2 000 000	77	5 000
All	216 000 000	91	655 000

Data from WHO (2011)

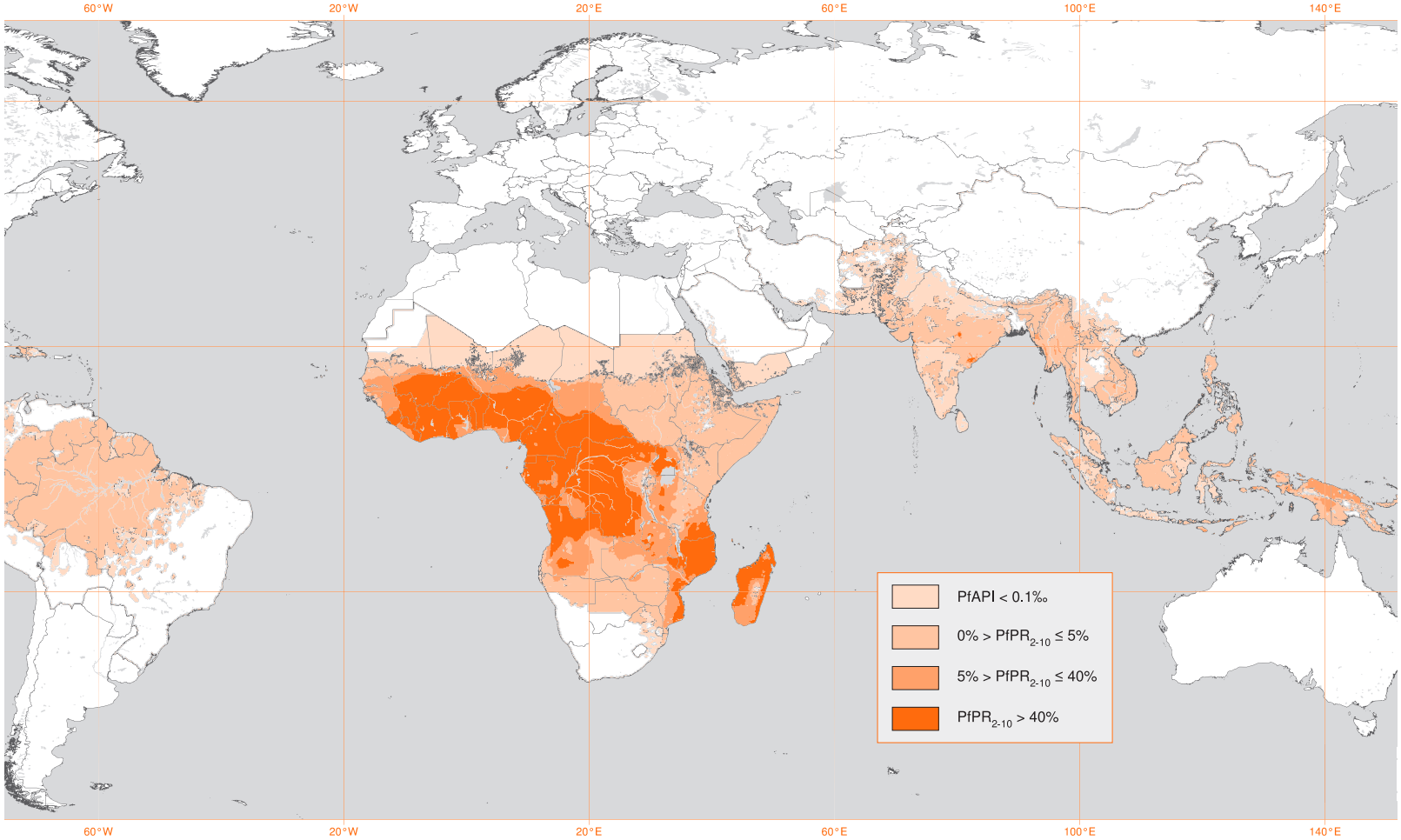
1.2.3 Biological markers of infection and susceptibility

Diagnosis of the erythrocytic stage of infection can be a challenge in many countries. Routine laboratory diagnosis is done by microscopic examination to detect parasitized erythrocytes in Giemsa-stained thick and thin films, from samples of blood obtained by finger prick (Krafts *et al.*, 2011); however, this method relies on the availability of good microscopes, power sources, and trained microscopists. Because of these limitations, WHO has recommended that biomarker-based rapid diagnostic tests become the standard if feasible (WHO, 2010). These tests are based on presence of the parasite lactose dehydrogenase antigen or histidine-rich protein 2 (HRP-2) antigen in the blood (Wongsrichanalai *et al.*, 2007). The sensitivity and specificity of these tests were found to be similar to those of microscopic detection (Abba *et al.*, 2011). In the research setting, quantitative polymerase chain reaction (PCR) is used alongside microscopy (Malhotra *et al.*, 2005). While serology is useful in understanding the epidemiology of malaria, it is not used diagnostically.

Defining the correlations of protective humoral immunity to *P. falciparum* has presented a challenge to scientists for several reasons (reviewed in Marsh & Kinyanjui, 2006). This is even more evident in malaria holoendemic regions, where antibodies to *P. falciparum* are often short-lived in children (Kinyanjui *et al.*, 2007; Crompton *et al.*, 2010) and only increase with age and repeated infections (Crompton *et al.*, 2010). Thus, because of the short half-life of immunoglobulin G (IgG)-specific antimalaria antibodies in children, use of antibodies as a marker of past exposure to *P. falciparum* is not always a reliable indicator. For example, in one study, children with a documented infection with *P. falciparum* and a *P. falciparum* IgG-antibody response to merozoite surface antigen 1 (MSP-1_{19}), (MSP-2) type A and B, apical merozoite antigen 1 (AMA-1) ectodomain, and region II of the 175 kDa erythrocyte-binding antigen (EBA-175II) was detected, but follow-up of the same children showed that the specific antibody response was lost (Kinyanjui *et al.*, 2007). In addition, the choice of malaria antigen to be used as a marker of infection can be problematic because of the well-documented genetic variation in the surface antigens of *P. falciparum* (Marsh & Kinyanjui, 2006).

Several different assays have been used to measure antibody responses to *P. falciparum* (Marsh *et al.*, 1989), but the most common is currently an enzyme-linked immunosorbent assay (ELISA). New technologies involving multiplex bead-based immunoassays have also been used and have the advantage of measuring more

Fig. 1.3 Spatial distribution of *Plasmodium falciparum* malaria in 2010 stratified by endemicity class



Estimates of the age-standardized annual mean *Plasmodium falciparum* parasite rate in children aged 2–10 years ($Pf\text{PR}_{2-10}$), within the spatial limits of stable transmission, stratified into four levels of risk. Areas of no risk and unstable risk (*Pf*API < 0.1‰) are also shown.
Adapted from © Malaria Atlas Project (2010) and © Gething *et al.* (2011)

than one antigen in a limited sample volume (Asito *et al.*, 2010). While no direct comparison was made in this particular study, other reports have found that sensitivity is similar to that of ELISA-based methods (Smits *et al.*, 2012). Most epidemiological studies of *P. falciparum* infection and susceptibility are based on candidate vaccine antigens, because most research has focused on development of a vaccine against this parasite. Several different antibodies against antigens derived from either the blood stage [e.g. merozoite surface protein 1 (MSP-1), serine repeat antigen 5 (SERA5, SE36) and apical membrane antigen 1 (AMA-1)] or the liver stage of infection [e.g. liver stage antigen 1, LSA-1; circumsporozoite protein 1, CSP-1] have been evaluated, but the value of using these antibodies as markers of exposure to *P. falciparum* in children living in areas where holoendemic transmission occurs is unclear. However, measurement of antibodies to antigens derived from whole schizont extracts is thought to be a good estimate of past exposure to *P. falciparum*. The serology for detection of malaria infection is complicated, and no clear consensus has emerged about which antibodies are protective (Asito *et al.*, 2010).

The evolutionary burden of malaria on the human population is seen in the large number of inheritable genetic mutations occurring in erythrocytes including, for example, a variant of the haemoglobin beta gene, haemoglobin S (*HbS*), that causes sickle-cell disease in homozygotes. The advantage for the host is suggested by epidemiological studies demonstrating that heterozygotes for the sickle-cell gene (i.e. genotype *HbAS*; who exhibit the sickle-cell trait) are protected from the high parasite densities and severe disease that characterize infection with *P. falciparum* (Lell *et al.*, 1999; Aidoo *et al.*, 2002). Other human haemoglobinopathies such as thalassaemias and glucose 6-phosphate dehydrogenase deficiency also provide protection against infection. Table 1.3 summarizes genetic polymorphisms that are known to be involved in attenuating the severity of disease (see also Section 4.1.3).

Unlike *P. falciparum*, the distribution of *P. vivax* in African populations may be restricted by the absence of the erythrocytic Duffy antigen in many Africans. It has been suggested that the Duffy antigen is necessary for the entry of the *P. vivax* merozoite into an erythrocyte (Howes *et al.*, 2011), although a very recent study has cast doubt on the strict requirement for this antigen for infection (Mendes *et al.*, 2011).

1.2.4 Pathology of infection: malaria

The pathology associated with exposure to *Plasmodium* differs significantly by species of *Plasmodium* parasite, age of the infected person, and intensity of transmission of the parasite. All infected hosts experience fever and chills associated with the periodicity of the rupture of the infected erythrocytes. Differences in the symptoms and severity of disease caused by the different *Plasmodium* species are described in Table 1.4.

Uncomplicated malaria occurs after infection with any *Plasmodium* species and is characterized by fever, chills and sweating in the majority of patients. If host immunity is adequate the infection can be cleared, but without treatment with effective antimalarial drugs, recrudescence of parasitaemia and relapse of symptoms can occur. Other symptoms include headache, nausea, myalgia, and vomiting (Trampuz *et al.*, 2003). In contrast, complicated or severe malaria is characterized by anaemia, cerebral malaria and metabolic acidosis, the main causes of death due to malaria infection. Other complications of severe malaria include pulmonary oedema, acute renal failure, and hypoglycaemia (Newton *et al.*, 1998).

The greatest burden of morbidity and mortality is associated with complicated falciparum malaria and the most vulnerable

populations are pregnant women (Section 4.1.2), children aged less than 5 years, and the elderly.

In sub-Saharan Africa where *P. falciparum* is holoendemic, more than 50% of children are parasitaemic at any given point in time (Høgh, 1996). Under these conditions, *Plasmodium* infection in children is recurrent, and the child's immune system is under constant stress from repeated *Plasmodium* infections (see Section 4.1.2). Complicated malaria is associated with severe anaemia, the major reason for malaria-related hospital admissions in Africa. Severe malarial anaemia is characterized by: haemoglobin, < 5 g/dL; haematocrit (erythrocyte volume fraction), < 15%; respiratory distress; and peripheral parasitemia (Marsh *et al.*, 1995). The mechanisms of pathogenesis are poorly understood, but are linked to enhanced clearance of erythrocytes in the spleen from the periphery (Weatherall *et al.*, 2002). Cerebral malaria is another complication of severe falciparum malaria and is linked to sequestration of the infected erythrocytes in the cerebral vasculature (Weatherall *et al.*, 2002).

In sub-Saharan Africa where malaria transmission is endemic, pregnant women can be infected with *P. falciparum* and have evidence of infection of the placenta at the time of birth (Desai *et al.*, 2007). Infection of pregnant women with *P. falciparum* has considerable adverse effects on maternal health and fetal morbidity and mortality (Desai *et al.*, 2007; Rogerson *et al.*, 2007). Infection of the placenta can cause intrauterine growth retardation and premature delivery, resulting in low-birth-weight infants or stillbirth (Desai *et al.*, 2007). Although the greatest burden of placental malaria is in primagravid women, women of all gravidities are at risk (Desai *et al.*, 2007).

While initial studies on placental malaria focused on birth outcomes, more recent studies have found that infants born to mothers with malaria have altered innate and adaptive immune responses. Several groups have reported that sensitization to parasitic antigens occurs *in utero*. This exposure affects the development of specific antimalaria antibodies to prevent infection and also infant T-cell responses at birth, with a shift towards T-helper 2 (Th2) mediated responses in cord blood mononuclear cells from parasitized placenta (Ismaili *et al.*, 2003; Dent *et al.*, 2006; Metenou *et al.*, 2007; Flanagan *et al.*, 2010). Skewing of the gamma delta T-cell repertoire and altering responses to toll-like receptor (TLR) signalling in cord blood of infants born to mothers with placental malaria also indicate an effect of placental malaria on innate immunity (Adegnika *et al.*, 2008; Cairo *et al.*, 2008). Another mechanism by which placental malaria could affect infant immunity was suggested by the recent finding that tolerance to antigens encountered *in utero* develops through establishment of regulatory T cells (Mold *et al.*, 2008). In support of this, cord blood of infants born to mothers with placental malaria showed an expansion of both malaria-specific and general regulatory $CD4^+$ T cells, as characterized by expression of FOXP3 (Malhotra *et al.*, 2009; Flanagan *et al.*, 2010). Long-term follow-up of these infants found that they had a greater risk of malaria over time, and evidence of T-cell anergy (Malhotra *et al.*, 2009). Thus a consequence of exposure to malaria *in utero* could be significantly reduced ability of an infant to respond to *Plasmodium* and to other infections encountered early in life, and an increased susceptibility to infectious diseases encountered subsequently during infancy.

Placental malaria could have an additional role in increasing infant susceptibility to heterologous infections. An important factor in protection against infections during infancy is the transfer of protective maternal antibodies across the placenta. However, several studies have demonstrated that placental malaria and maternal hyper-gammaglobulinaemia are associated with a significant reduction in the transfer of maternal IgG, including antibodies specific for measles, herpes simplex virus 1 (HSV-1), respiratory syncytial virus (RSV), tetanus, and

Table 1.3 Some genetic polymorphisms involved in resistance to malaria caused by *Plasmodium falciparum*

Condition	Gene [variant]	Proposed protective mechanisms	References
Sickle cell	Haemoglobin beta		
	[Haemoglobin C]	Reduced cyto-adherence of infected erythrocytes	Agarwal *et al.* (2000), Modiano *et al.* (2001)
	[Haemoglobin E]	Reduced erythrocyte invasion by merozoites, lower intra-erythrocytic parasite growth, and enhanced phagocytosis of infected erythrocytes.	Hutagalung *et al.* (1999), Chotivanich *et al.* (2002)
	[Haemoglobin S]	Selective sickling of infected sickle-trait erythrocytes leading to enhanced clearance by the spleen. Reduced erythrocyte invasion, early phagocytosis, and inhibited parasite growth under oxygen stress in venous microvessels. Enhancement of innate and acquired immunity.	Cholera *et al.* (2008)
α-Thalassaemia β-Thalassaemia	Haemoglobin alpha Haemoglobin beta	Reduced rosetting. Increased micro-erythrocyte count in homozygotes reduces the amount of haemoglobin lost for given parasite density, thus protecting against severe anaemia.	Allen *et al.* (1997), May *et al.* (2007), Fowkes *et al.* (2008)
-	Glucose-6-phosphate dehydrogenase	G6PD deficient erythrocyte sensitive to oxidant stress causes its protection against parasitization.	Allison & Clyde (1961), Bienzle *et al.* (1972), Ruwende *et al.* (1995), Tishkoff *et al.* (2001)
-	Pyruvate kinase	Invasion defect of erythrocytes and preferential macrophage clearance of ring-stage-infected erythrocytes.	Durand & Coetzer (2008)
Ovalocytosis	Solute carrier family 4 anion exchanger 1	Inhibition of merozoite entry into the erythrocyte, impairment of intracellular parasite growth and prevention of the erythrocyte lysis that occurs with parasite maturation, leading to release of merozoites into the blood stream.	Cortés *et al.* (2004, 2005)
Elliptocytosis	Alpha spectrin	Erythrocytes resistant to invasion	Facer (1995)
-	Glycophorins A, B, E	Erythrocytes resistant to invasion	Wang *et al.* (2003)
-	ABO blood groups	Reduced *P. falciparum* resetting in group O individuals	Rowe *et al.* (2007), Barragan *et al.* (2000), Paré *et al.* (2008)
-	*HLA-B*	Development of specific immunity	Hill *et al.* (1991), Gilbert *et al.* (1998); Young *et al.* (2005)
-	Haptoglobin	Oxidative damage to uninfected cells might be more marked in haptoglobin-polymorphic individuals since haptoglobin proteins bind less efficiently to heamoglobin, increasing premature destruction of erythrocytes and stimulating cytokine release by these circulating cells.	Elagib *et al.* (1998), Quaye *et al.* (2000), Cox *et al.* (2007)

Table 1.3 (continued)

Condition	Gene [variant]	Proposed protective mechanisms	References
-	Nitric oxide synthase 2	Increased NO production induces T-helper 1 cytokines, which activate macrophages and could thus be an antimalarial resistance mechanism.	Kun *et al.* (2001), Hobbs *et al.* (2002)
-	Haemoxygenase I	Release of free haem in the bloodstream	Pamplona *et al.* (2007), Garcia-Santos & Chies (2010)
-	*TLR1, TLR4, TLR9*	*P. falciparum* glycosylphosphatidylinositol induces signalling *via* TLR4 and hemozoin-induced immune activation involves TLR9.	Coban *et al.* (2005), Mockenhaupt *et al.* (2006), Leoratti *et al.* (2008a)

Adapted from Driss *et al.* (2011)
HLA, human leukocyte antigen; TLR, tool-like receptor; NO, nitric oxide

Table 1.4 Clinical course of infection with *Plasmodium* in humans

Parasite	Liver schizogony period (days)	Relapse	Erythrocytes parasitized	Severe malaria	More severe complications
P. falciparum	5–6	No	All	Yes	Cerebral malaria, acute renal failure, severe anaemia, adult respiratory distress syndrome
P. vivax	8	Yes	Reticulocytes	Yes (occasionally)	Splenomegaly
P. ovale	8	Yes	Reticulocytes	Rare	Rare
P. malariae	13	No	Mature erythrocytes	Rare	Nephrotic syndrome

Prepared by the Working Group

varicella zoster virus (VZV) (Okoko *et al.*, 2001a, b; Cumberland *et al.*, 2007).

The parasite burden associated with *P. vivax* infection is lower than that associated with *P. falciparum* infection, and the morbidity associated with *P. vivax* infection is thus less severe.

1.2.5 Prophylaxis and treatment of malaria

The treatment of malaria targets primarily the blood stage of *Plasmodium* infection. While chloroquine has been the therapeutic drug of choice, increasing resistance among all *Plasmodium spp.*, particularly *P. falciparum*, has led to the adoption of artemisinin-based therapy (Burki, 2011). However, resistanceto artemisinins has now been reported in a growing number of countries in south-east Asia, and WHO recommends that oral artemisinin-based monotherapies be withdrawn from the market and replaced with artemisinin-based combination therapy (WHO, 2011).

Intermittent preventive treatment of malaria has been recommended by WHO for preventive treatment of pregnant women and infants living in areas of high transmission of *P. falciparum*. Currently, 35 of 45 countries in sub-Saharan Africa and Papua New Guinea have adopted this policy. While intermittent preventive treatment is also recommended for infants living in regions of moderate to high malaria transmission, no countries have adopted this policy (WHO, 2011). The past decades have seen the introduction of insecticide-treated bednets (WHO, 2011), and it is estimated that the number of bednets in sub-Saharan Africa increased from 5.6 million in 2004 to 145 million in 2010.

Studies on the efficacy of new drugs continue because the number of drugs that treat malaria effectively is limited and because of resistance issues (Burrows *et al.*, 2011). Primaquine is the only known drug that targets the liver stage of infection and eradicates hypnozoites; however, primaquine can cause haemolytic anaemia in individuals with glucose-6-phosphate dehydrogenase deficiency, so widespread use of this drug for malaria-elimination campaigns is not feasible (Beutler & Duparc, 2007).

There is no currently licensed vaccine for any of the human *Plasmodium* pathogens. A vaccine targeting the blood stage of *P. falciparum* was recently tested in phase III trials and found to be about 50% efficacious in preventing clinical and severe malaria in infants and children in Africa (Agnandji *et al.*, 2011).

2. Cancer in Humans

While malaria in humans is caused by several species of *Plasmodium*, including *P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi* (see Section 1), the majority of studies investigating

the link between malaria and cancer have focused on *P. falciparum* (the species linked to the most severe forms of malaria), or been conducted in areas where *P. falciparum* is highly prevalent. Unless otherwise stated, references to malaria in this chapter refer to malaria attributable to *P. falciparum*. The cancers investigated for a possible association with malaria include lymphoma (Ross *et al.*, 1982; Cook-Mozaffari *et al.*, 1998; Vineis *et al.*, 2000; Tavani *et al.*, 2000), especially Burkitt lymphoma (O'Conor & Davies, 1960; Burkitt, 1961, 1969; Burkitt & O'Conor, 1961; Burkitt & Wright, 1963, 1966; Wright, 1963), Kaposi sarcoma (Geddes *et al.*, 1995; Cottoni *et al.*, 1997, 2006; Ascoli *et al.*, 2001; Serraino *et al.*, 2003), cancer of the cervix (Odida *et al.*, 2002), cancer of the prostate (Thomas, 2005; Elson *et al.*, 2011), nasopharyngeal carcinoma (Yadav & Prasad, 1984; Chen *et al.*, 1990), and cancer of the liver (Welsh *et al.*, 1976; Lu *et al.*, 1988). Most research has focused on endemic Burkitt lymphoma and classical Kaposi sarcoma, both caused by gamma herpes viruses, Epstein–Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV), respectively. Research relating to other cancers is scant.

Exposure to malaria has been assessed by a variety of different methods: questionnaire, serology, or indirectly by measuring carriage of haemoglobin genotype abnormalities (e.g. sickle cell–haemoglobin C). The haemoglobin genotypes, because they are associated with partial protection against mild and severe malaria, are appropriate genetic markers for assessing lifetime exposure to malaria in populations living in areas where malaria is endemic. [Questionnaire measurement of exposure to malaria is subject to interviewer and recall bias, and asymptomatic malaria, if relevant, is impossible to measure by questionnaire. Measurement of antimalaria antibodies provides a more objective measure than the questionnaire approach, but is limited by several factors: the levels are subject to reverse-causality bias, misclassification, non-reproducibility, and incomplete knowledge of the relevant markers of cancer risk. The use of haemoglobin genotypes, or any other genetic marker of susceptibility to malaria, is reproducible and not subject to reverse causality. It should be noted that the controls should have comparable environmental exposure to malaria. General limitations of the malaria case–control studies included limited understanding of how to measure malaria exposure (patent malaria, malaria genotype, or malaria diversity) and how to compare results for different assays from different time periods].

2.1 Burkitt lymphoma

2.1.1 Background

(a) Historical aspects

The earliest mention of tumours consistent with Burkitt lymphoma in the medical literature can be traced back to the medical notes of Albert Cook, shortly after his arrival in Uganda in 1894. Anecdotal reports suggest that doctors who worked in the region were familiar with the different manifestations of the disease, and wood carvings depicting jaw tumours suggest that the disease preceded colonial intervention. However, it was a paper by Denis Parsons Burkitt describing "a sarcoma involving the jaws in African children" that established that "sarcomas" previously thought to be independent entities were in fact a single disease (Burkitt, 1958). Subsequently, the clinical (Burkitt & O'Conor, 1961), radiological (Davies & Davies, 1960), and pathological (O'Conor, 1961) features of the disease were described, and the eponym "Burkitt lymphoma" applied to tumours with similar presentation. Description of the morphological, histochemical and cytological features of Burkitt lymphoma (O'Conor & Davies, 1960; Burkitt & O'Conor, 1961; Wright, 1963) led to cases with histology consistent with Burkitt lymphoma in Africa being reported worldwide, including in Papua New Guinea (Reay-Young, 1974; Lavu

et al., 2005), Brazil (Luisi *et al.*, 1965), Colombia (Beltrán *et al.*, 1966), India (Bai & Agrawal, 1967; Date *et al.*, 1970), England, the USA (Dorfman, 1965; O'Conor *et al.*, 1965; Ziegler & Miller, 1966; Burkitt, 1967; Levine *et al.*, 1982), Malaysia (Krishnappa & Burke, 1967), China (Ji & Li, 1992), and the Republic of Korea (Myong *et al.*, 1990; Choi *et al.*, 2009).

(b) Disease characteristics

A monoclonal B-cell non-Hodgkin lymphoma, Burkitt lymphoma is considered to be a single disease that occurs as three epidemiological and clinical subtypes, namely: endemic, sporadic and immunodeficiency-associated (Leoncini *et al.*, 2008). Endemic Burkitt lymphoma is defined as affecting children in equatorial Africa and New Guinea, sporadic Burkitt lymphoma affects children and young adults throughout the world, and immunodeficiency-associated Burkitt lymphoma is primarily associated with HIV infection. These subtypes are indistinguishable by routine histopathological techniques. Endemic Burkitt lymphoma characteristically involves the jaw or other facial bones, distal ileum, caecum, ovaries, kidney or the breast. In the sporadic type, the ileum and caecum are the most common sites of involvement, and the jaw is less commonly involved.

(c) Molecular characterization

All three subtypes of Burkitt lymphoma are characterized by deregulation of the *MYC* gene, a master regulator of cellular differentiation, growth, and apoptosis (Klein, 2009). Deregulation of *MYC* is caused by chromosomal translocations that place the *MYC* coding sequence on chromosome 8 (8q24) next to the promoter sequences of genes encoding immunoglobulin heavy chains on chromosome 14 (14q32) in 80–90% of cases, or genes encoding kappa or lambda immunoglobulin light chains on chromosome 2 (2p12) and 22 (22q11), respectively, in 10–15% of cases (Leoncini *et al.*, 2008). Although translocation cannot be demonstrated in about 10% of cases (Leucci *et al.*, 2008), these cases still show evidence of *MYC* deregulation.

It has been reported that Epstein–Barr virus (EBV) is detected in nearly 100% of cases of endemic Burkitt lymphoma; this proportion is smaller in cases of sporadic and immunodeficiency-associated Burkitt lymphoma (Carbone *et al.*, 2008).

Endemic and sporadic Burkitt lymphomas differ at the molecular level, principally in the regions where chromosomal break-points occur (Gutiérrez *et al.*, 1992). No specific epidemiological differences, other than geographical origin of the tumours, have been linked to the molecular differences in chromosomal break-points. One idea is that chromosomal break-points might be related to the timing of the translocation relative to B-cell differentiation and/or infection of the B cell with EBV (Kuhn-Hallek *et al.*, 1995). No correlation has been shown between EBV positivity and specific chromosomal break-points (Gutiérrez *et al.*, 1992).

2.1.2 Epidemiology of endemic Burkitt lymphoma

(a) Geographical clusters

Denis Burkitt was the first to comment on the uneven geographical distribution of Burkitt lymphoma (Burkitt, 1961; Burkitt *et al.*, 1963; Burkitt & Wright, 1966). His maps showed that the highest incidence of Burkitt lymphoma was found in a broad belt around equatorial Africa, spanning from 10 degrees north to 10 degrees south, with an extension of the belt southward along a thin coastal rim in Mozambique (Burkitt, 1963). Within this belt, cases showed climatic restriction. Few cases were recorded at altitudes higher than 4000 m above sea level, where temperatures can fall below 16 °C, in places such as Kigezi in Western Uganda, Kilimanjaro in the United Republic of Tanzania, Rwanda, or Burundi. Many cases were recorded

in lowland regions with yearly rainfall of 50 cm or less (Burkitt, 1962a, b, c; Haddow, 1963). Outside Africa, cases of Burkitt lymphoma were seen in Papua New Guinea (Booth *et al.*, 1967; Lavu *et al.*, 2005), which has a tropical climate, and in tropical regions of Brazil (Burkitt, 1967).

The discovery of EBV in cultured cells from Burkitt lymphoma examined by electron microscopy in 1964 (Epstein *et al.*, 1964) suggested that EBV could be involved in Burkitt lymphoma etiology. EBV was also discovered to be ubiquitous and to be spread *via* contact with saliva (IARC, 2012). Dalldorf first proposed an etiological link between Burkitt lymphoma and malaria (Dalldorf, 1962; Dalldorf *et al.*, 1964), based on data from Kenya, Papua New Guinea, Malaysia, and Brazil, which suggested that Burkitt lymphoma was not common in any area where holoendemic or hyperendemic malaria did not exist. This hypothesis was supported by biological evidence showing that chronic infection with malaria stimulated the reticuloendothelial system and caused polyclonal B-cell stimulation and immunosuppression (Greenwood *et al.*, 1970; Barker & Powers, 1971), which could influence the risk of developing Burkitt lymphoma.

The geographical correlation between Burkitt lymphoma and holoendemic malaria persisted at a country level. For example, in Uganda, the highest incidence of Burkitt lymphoma was observed in low-lying districts of Lango, West Nile, Madi, and Acholi, and the lowest incidence was observed in high-lying districts of Kigezi (Wright & Roberts, 1966; Wright, 1973). Similar patterns were observed even at a micro-geographical level, such as in studies restricted to the districts of West Nile (Williams *et al.*, 1974). This geographical pattern in Uganda was also apparent in a recent study from the country (Ogwang *et al.*, 2008). In Kenya, the incidence is low in highland tribes, such as the Kalenjin, and high in lowland tribes, like the Luo in Kisumu (Dalldorf *et al.*, 1964; Rainey *et al.*, 2007a, b). In the United Republic of Tanzania, cases were less frequent in the Kilimanjaro highlands (Kitinya & Lauren, 1982), but more common in the lower regions of Mara (Brubaker, 1984). Similar patterns were also observed in Cameroon in West Africa (Wright *et al.*, 2009).

The geographical patterns of distribution of Burkitt lymphoma in Africa support the role of a mosquito-borne infection, now widely believed to be malaria (Fig. 2.1; Burkitt, 1962a, b ,c). The evidence supporting a link between Burkitt lymphoma and malaria is summarized in Table 2.1.

Cook & Burkitt (1971) analysed the proportional distribution of Burkitt lymphoma across large geographical areas using data on several selected tumour types at hospitals in Kenya, Uganda, and United Republic of Tanzania. The relative frequency of Burkitt lymphoma, as a fraction of the seven tumours investigated, was highest around the lake shores of Lake Victoria and in northern Uganda along the River Nile. Burkitt lymphoma was rare or not reported in areas where malaria transmission season was shorter than 6 months, suggesting that the incidence of Burkitt lymphoma is influenced both by duration and intensity of exposure lasting for 6 months or more. Because this study covered many malaria-free areas that were densely populated, but remained tumour-free, the proportional distribution of cases was not explained by the underlying population distribution (Kafuko & Burkitt, 1970).

Kafuko & Burkitt (1970) conducted a detailed analysis of malaria and incidence of Burkitt lymphoma at the country level in Uganda between 1963 and 1966. Patterns of malaria distribution were obtained by examining thin and thick blood smears for malaria parasitaemia, supplemented by spleen surveys, in children in 100 schools and in 86 mass surveys in the general population [limited detail was provided on how schools and survey villages were selected]. Malaria endemicity was classified as hypoendemic (absolute parasite prevalence

in the age group 2–10 years, < 10%), mesoendemic (parasite prevalence, 11–50%), hyperendemic (if constantly > 50%), and holoendemic (if constantly > 75%), and the proportion of areas in the area with each level of malaria endemicity was calculated. Although no statistical comparisons were made, there was an obvious gradient in the incidence of Burkitt lymphoma from 0 per 100 000 children in the Kigezi Highlands (where 80% of the areas surveyed were malaria-free and 20% were hypoendemic), to 5.49 per 100 000 in the West Nile district (where 59% of the areas were holoendemic and 35% of the areas were hyperendemic).

Schmauz *et al.* (1990) found similar geographical patterns of incidence of Burkitt lymphoma and malaria endemicity in 18 districts of Uganda between 1966 and 1973.

Kafuko *et al.* (1969) investigated the pattern of Burkitt lymphoma and malaria at a micro-geographical level within the West Nile district of Uganda. Data on malaria were obtained from children in 17 schools and from 11 mass surveys. Data on Burkitt lymphoma were obtained from the Kuluva cancer registry. A strong geographical correlation was observed between areas with a high incidence of Burkitt lymphoma and areas with a high prevalence of malaria. In addition, a correlation was observed between the age at which malaria parasite prevalence peaked (age 0–4 years) and the age at which Burkitt lymphoma cases peaked. However, the overall prevalence of malaria parasitaemia was generally > 70% at most times in all areas and increased to 91% during some of the months in children in some age groups. These results highlighted the fact that only a few children among those exposed repeatedly to very high levels of malaria parasitaemia develop Burkitt lymphoma.

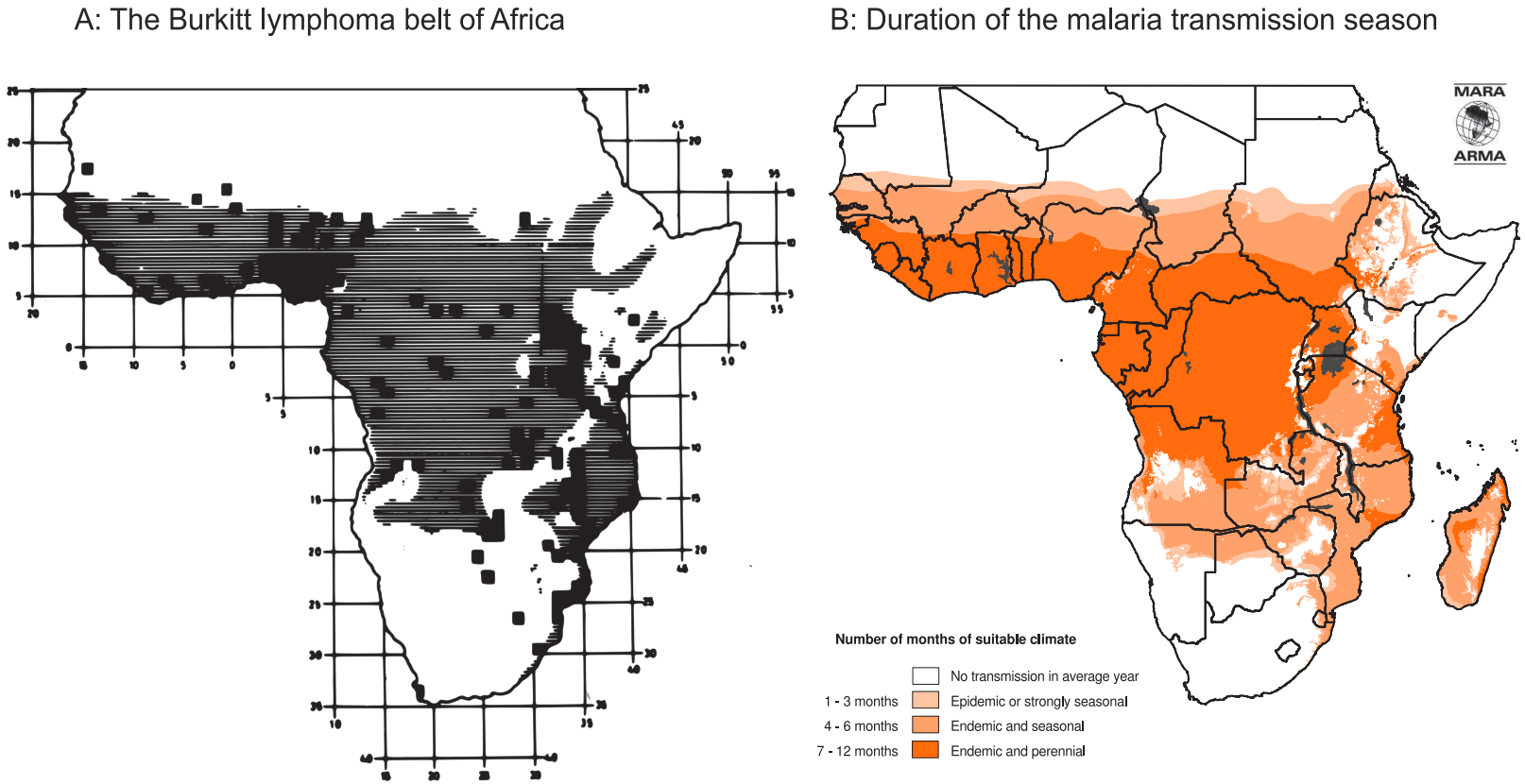
Morrow *et al.* (1976) investigated micro-geographical patterns of Burkitt lymphoma using data on 123 patients residing in the Mengo district of Uganda, diagnosed during 1959–68. The incidence rate of Burkitt lymphoma was lower in the counties at higher altitude, presumably because the prevalence of malaria was lower. In addition, there was seasonal variation in the incidence of Burkitt lymphoma, which was attributed to seasonal changes in the prevalence of malaria (Morrow *et al.*, 1977). The incidence of Burkitt lymphoma declined during the period of observation and was attributed to a fall in the burden of malaria in the Mengo district caused by socioeconomic and health-care improvements and widespread distribution of chloroquine. The fall in the incidence of Burkitt lymphoma appeared to be specific, since no changes were noted for other cancer diagnoses during the same period.

A role for malaria in the etiology of Burkitt lymphoma was also suggested by studies in migrants in Uganda. Burkitt & Wright (1966) reported that migrants from Rwanda, Burundi, and Kigezi district in Uganda (areas where both malaria and Burkitt lymphoma are rarely if ever seen) to Buganda (a subnational kindom within Uganda), where both malaria and Burkitt lymphoma are endemic, showed susceptibility to Burkitt lymphoma that was approximately equal to that of the indigenous population. Notably, the incidence of Burkitt lymphoma was 0.49 per 100 000 among adult immigrants aged 16–45 years *versus* 0.10 per 100 000 in this age group among the locally born, further suggesting that malaria infection serves as the trigger mechanism of onset of Burkitt lymphoma (Morrow *et al.*, 1976).

The age at diagnosis of Burkitt lymphoma varied inversely according to malaria endemicity. In Mengo district, in southern Uganda, the median age was 8.2 years for areas of Mengo where malaria transmission was mesoendemic, and 7.8 years in Acholi and 6.6 years in Lango, two areas where malaria transmission is holoendemic (Morrow *et al.*, 1977).

In Ghana, Biggar & Nkrumah (1979) observed that cases of Burkitt lymphoma originated from predominantly rural areas, based on a study of 236 cases treated at Korle Bu Hospital, Accra, in

Fig. 2.1 Estimated distribution of Burkitt lymphoma and of malaria in Africa



A. The shaded area represents that in which, on climatic grounds, Burkitt lymphoma might be expected to occur. The black squares show the distribution of the series of cases compiled by Denis Burkitt (Haddow, 1963)

B. The map is based on theoretical stably local climatic, and therefore the potential duration, onset and end of the malaria transmission season, in the average year.
The dark orange areas represent regions where hyperendemic or holoendemic transmission of P. *falciparum* malaria is expected to occur 7–12 months per year.
Note: Based on a theoretical model from available long-term climate data, the map is reasonably accurate but since it is not based on actual malaria data it may not reflect the real malaria status.
From MARA/ARMA (2001)

Table 2.1 Ecological evidence supporting a link between malaria and endemic Burkitt lymphoma

1	Geographical coincidence of Burkitt lymphoma and holoendemic or hyperendemic *P. falciparum* malaria (see Fig. 2.1, Panel A and Panel B)
2	Lower risk of Burkitt lymphoma in malaria-protected areas, e.g. urban areas or highlands, or areas with cool temperatures
3	Coincidence of peak age of maximum titres of antimalarial immunoglobulin and peak age at which Burkitt lymphoma develops
4	Coincidence of malaria splenomegaly, a marker of chronic exposure to malaria, and Burkitt lymphoma
5	Elevated risk of Burkitt lymphoma in migrants from areas with a low intensity of malaria to areas of high intensity, with the age at developing Burkitt lymphoma reflecting age at migration (Burkitt & Wright, 1966)
6	Protective effect of sickle-cell trait against development of Burkitt lymphoma
7	Protective effect of malaria suppression, eradication, or use of malaria prevention strategies
8	Time–space clustering and reports of seasonal variation in Burkitt lymphoma correlated with seasonal malaria

Prepared by the Working Group

1970–75. This pattern was consistent with a role for repeated exposure to malaria in the etiology of Burkitt lymphoma. In a separate cross-sectional survey of malaria, Biggar *et al.* (1981) reported a malaria prevalence of 1.4% in urban areas and 22% in rural areas. Unlike Uganda, where seasonal variation in the incidence of Burkitt lymphoma was reported (Morrow *et al.*, 1977), no seasonal variation was observed in Ghana (Biggar & Nkrumah, 1979).

Rainey *et al.* (2007a, b), have reported ecological evidence linking the incidence of Burkitt lymphoma to risk of malaria at a population level in two studies conducted in Kenya. In the first study (Rainey *et al.*, 2007a), the incidence of paediatric Burkitt lymphoma in Kenya was estimated using data on 960 histologically verified cases diagnosed between 1988 and 1997. Risk of malaria was classified based on recent estimates of transmission intensity at the district level as low risk, arid/seasonal, highland, endemic coast, and lakeside endemic. The 10-year average annual incidence rate varied by malaria endemicity from 0.39 per 100 000 in low-risk regions, 0.25 in arid/seasonal regions, 0.66 in highland, 0.68 in endemic coast and 1.23 in endemic lake areas ($P = 0.002$). The odds ratio (OR) for Burkitt lymphoma in regions with chronic and intense malaria transmission compared with regions with no or sporadic transmission was 3.47 (95% CI, 1.30–9.30).

In the second study, focusing on a smaller geographical region, Rainey *et al.* (2007b) conducted an analysis of micro-geographical variation of Burkitt lymphoma in Nyanza province, including areas with holoendemic as well as seasonal malaria. Data was obtained for cases diagnosed between 1999 and 2004 at New Nyanza Province General Hospital, the largest hospital with facilities to diagnose and treat Burkitt lymphoma in the province. Analysis was performed using two appropriate cluster-detection methods (Anselin's Local Moran test for spatial autocorrelation and a spatial scan test statistic) to identify significant clustering or "hot spots" with a very high incidence of Burkitt lymphoma and "cold spots" with a low incidence of Burkitt lymphoma. Significant clustering was identified in five locations in the western region of Kisumu district: Central Kisumu, East Seme, South Central Kisumu, South West Kisumu and West Kisumu, with incidence rates of 2.1–8.0 cases per 100 000 children; and in two locations in Nyando District: Kakola/East Kano and North Nyakach, with incidence rates of 3.1 and 4.4 cases per 100 000 children, respectively. A cold spot was identified in Nyamira, Kisii and Gucha districts, including some areas where

malaria transmission was holoendemic and some areas where malaria transmission was seasonal. [Although the hot spots may be influenced by access to infrastructure, including roads to the hospital, this factor was not considered an important cause of bias because a large administrative area (directly east of Kisumu city) with a large population with good access to roads had few cases. These results support a role of environmental cofactors in "hot spot" activity, possibly micro-geographical malaria endemicity.] This variation in the incidence of Burkitt lymphoma at a micro-geographical level is a consistent feature that was reported by Ogwang *et al.* in Uganda (Ogwang *et al.*, 2008). [Case ascertainment was incomplete.]

(b) Time–space clusters

Time–space clusters of Burkitt lymphoma (defined as cases occurring closer in time and space than expected by chance) were first reported by Pike *et al.* (1967) and Williams *et al.* (1969) in the West Nile district in Uganda. The first report included 36 cases of histologically confirmed Burkitt lymphoma diagnosed in 1961–65, and the second report included an additional 29 cases diagnosed in 1966–67. Clustering was assessed and the results were consistent with significant time clusters, at intervals ranging from 30 to 360 days, and distance clusters, ranging from 2 to 40 km. At least five cases were diagnosed in one village (Aliba) in West Nile within 2 years (Pike *et al.*, 1967). No epidemiological evidence of personal contact between the cases was demonstrated, [suggesting clustering was likely to be due to area-wide changes in cofactors rather than person-to-person contact]. Clustering activity was apparent in a larger analysis including 200 cases from West Nile in 1965–75 (Williams *et al.*, 1978).

Morrow *et al.* reported a cluster of seven cases occurring over 27 months (October 1966 to December 1968) in Bwamba county in Uganda (Morrow *et al.*, 1971). However, no evidence of clustering was observed in other parts of southern Uganda, including Mengo district (Morrow *et al.*, 1976), or elsewhere in Africa, including in the Mara region in northern United Republic of Tanzania (Brubaker *et al.*, 1973; Siemiatycki *et al.*, 1980), or in Ghana (Biggar & Nkrumah, 1979). Observation of clusters ignited interest in discovering cofactors that might influence prevalence and intensity of etiological exposures over lager areas or longer time intervals that might be responsible for area-wide drift of Burkitt lymphoma in Africa. However, negative data from other parts of Africa, coupled with lack of epidemiological data supporting interpersonal contact between affected individuals in the clusters, and the large time and space intervals in the reported clusters reduced enthusiasm for characterizing clusters of Burkitt lymphoma as a means of elucidating shared environmental etiology.

Van den Bosch *et al.* (1993b) investigated clustering in 146 cases of Burkitt lymphoma diagnosed during July 1987 and October 1989 in Malawi. Cases in children aged > 8 years were closer together in time and space than would be expected by chance.

2.1.3 Correlation between age at diagnosis of Burkitt lymphoma and malaria biomarkers

Emmanuel *et al.* (2011) correlated proportional age distribution of Burkitt lymphoma with age distribution of malaria biomarkers. The authors hypothesized that, given the rapid growth rate of Burkitt lymphoma (Iversen *et al.*, 1972), and estimates that the latent interval from onset to diagnosis might be as short as 6–8 months (Williams *et al.*, 1974), the malaria exposures that influence onset of disease should have an age-specific pattern similar to that of Burkitt lymphoma. Data on Burkitt lymphoma were obtained from four well-characterized data sets from Ghana (1965–89) (Nkrumah & Olweny, 1985), Uganda (1991–2006) (Parkin

et al., 2010), and (1997–2006) (Ogwang et al., 2008), and the United Republic of Tanzania (1960–2009) (Geser et al., 1989). Data on malaria were compiled age-specifically from published studies conducted in the same countries (Smith et al., 1999; Peyerl-Hoffmann et al., 2001; Owusu-Agyei et al., 2002). Data on malaria included prevalence of malaria parasites in peripheral blood, geometric mean parasite density, and mean multiplicity of malaria genotypes, defined as the number of malaria genotypes per positive blood sample based on the merozoite surface protein-2 (MSP-2) (Smith et al., 1999; Peyerl-Hoffmann et al., 2001; Owusu-Agyei et al., 2002). Strong and significant correlations were observed between the age-specific pattern of Burkitt lymphoma and the age-specific mean number of multiplicity of *P. falciparum* genotypes in all study regions (Pearson correlation coefficients ranging from 0.77 to 0.91 (Fig. 2.2 for two study regions in Ghana and United Republic of Tanzania). Both the incidence of Burkitt lymphoma and parasite multiplicity peaked between age 5 and 9 years, and then declined gradually. This was in contrast to the prevalence of parasites in peripheral blood and geometric mean parasite density, which both peaked at ages 2 and 3 years and decreased thereafter, gradually for the former and rapidly for the latter (Fig. 2.2). [The Working Group noted that these results were ecological, were based on data spanning many years when epidemiological techniques for the study of malaria were changing, the populations correlated may not have been overlapping, and the completeness and accuracy of the cases were uncertain. This study was valuable for highlighting the limits in our understanding of the exact nature of malaria exposure relevant to the pathogenesis of Burkitt lymphoma and hence the most appropriate biological measure of malaria exposure most proximally linked to risk of Burkitt lymphoma.]

[Taken together, correlation studies have provided the most consistent evidence for a link between Burkitt lymphoma and falciparum malaria, but at a population level. Interpretation of the results must be cautious because the results do not measure relationship between exposure and risk at the individual level.]

2.1.4 Cohort studies

Only one cohort study has examined the link between Burkitt lymphoma and malaria. De-Thé et al. (1978) conducted a prospective study to examine the impact of infection with EBV on the risk of Burkitt lymphoma. In northern Uganda, 42 000 children aged 4–8 years were recruited and followed over time for development of Burkitt lymphoma. At recruitment, blood was collected from every child and presence of the malaria parasite was evaluated by thick and thin blood films. Subsequently, 14 cases of Burkitt lymphoma were diagnosed. Within this cohort, EBV was analysed in 14 cases and 69 representative controls from the same population. There were three types of control (four or five per case) selected as follows: (1) serum from a neighbour of the same age and sex selected at random from the main survey; (2) four controls from the serum bank from children of the same age, sex, and locality as the child with Burkitt lymphoma and bled at the same time; (3) serum from a random sample of the surveyed population. There were no marked differences between the number of malarial parasites in children with Burkitt lymphoma before diagnosis and in controls, but cases at diagnosis had significantly fewer parasites than controls [possibly because cases had been given antimalarial drugs. No numbers or levels of parasitaemia were available in the publication].

2.1.5 Case–control studies

Ten case–control studies have investigated the link between Burkitt lymphoma and malaria (Williams, 1966; Pike et al., 1970; Ziegler et al., 1972; Feorino & Mathews, 1974; Nkrumah &

Perkins, 1976; Nkrumah *et al.*, 1979; Carpenter *et al.*, 2008; Mutalima *et al.*, 2008; Asito *et al.*, 2010; Guech-Ongey *et al.*, 2011) (Table 2.2, Table 2.3. Most studies were previously reviewed in *IARC Monograph* Volume 70 (IARC, 1997) and in *IARC Monograph* Volume 100B (IARC, 2012). Two studies, conducted since the last IARC evaluation (Asito *et al.*, 2010; Guech-Ongey *et al.*, 2011) are reviewed here for the first time.

(a) Exposure assessed using antibody assays

Ziegler *et al.* (1972) investigated the frequency, type of malaria, and immunological response to malaria in 100 patients with Burkitt lymphoma treated at the Uganda Cancer Institute between 1967 and December 1970. No significant differences were noted in titres of antimalaria antibodies measured by immunofluorescence assay at admission among 17 cases and 18 controls [selection of controls was not fully described] (Table 2.2). [The Working Group noted that there were few controls in the study and the source population was not described, suggesting that neither may have been ideal. Although the controls were matched by tribe, age, and sex to the cases, no further information was provided on age and sex, both important risk factors for Burkitt lymphoma. The Working Group was uncertain how to evaluate the malaria markers, especially given that antibodies were not correlated with parasitaemia and splenomegaly, both measures of high malaria burden.]

Feorino & Mathews (1974) compared titres of antimalaria antibodies, measured by indirect immunofluorescence assay and indirect haemagglutination assay in 60 patients with Burkitt lymphoma aged 4–15 years *versus* 60 controls matched on age (aged 3–16 years), sex, tribe and residence. Titres of > 1:16 were considered as positive. No statistically significant difference was found in prevalence between cases and controls by indirect immunofluorescence (95% in cases *versus* 93% in controls) or by indirect haemagglutination (65% *versus* 67%) or in the geometric mean titres. [Antimalaria antibodies were measured in blood samples taken after use of chloroquine in the cases, which could explain the null association. In addition, the cellular immune response was not measured, which may have consequences for understanding the role of immunity to malaria infection. The limitations of this study included small sample size. The Working Group noted that the patients with Burkitt lymphoma, perhaps even controls, from Feorino & Mathews were a subset of those described in the study by Ziegler *et al.* (1972), but it was not possible to determine whether the 17 patients with Burkitt lymphoma evaluated for antimalaria antibodies were included in Ziegler *et al.* (1972). The subject population was not adequately described, so potential for selection bias, lack of information on potential confounders and the appropriateness of the exposure measures could not be evaluated.]

Nkrumah *et al.* (1979) investigated patterns of total immunoglobulin (Ig) as well as malaria-specific IgG, IgA, and IgM levels in 56 patients with newly diagnosed Burkitt lymphoma (age 4–14 years) and 56 apparently healthy, nearest-neighbour controls, individually matched on age, sex, tribe, and residence in Ghana. The malaria-specific IgG and IgM antibodies for the schizont antigen of *P. falciparum* and *P. malariae* were measured using the indirect immunofluorescence assay in cases and controls. Children with Burkitt lymphoma had lower values of total immunoglobulin (IgG, IgA, and IgM) than did the nearest-neighbour controls (Mann–Whitney U test statistic, $P < 0.001$ for IgG, IgA, and IgM). Antibody titres for *P. falciparum* were higher than those for *P. malariae*, reflecting higher intensity of transmission of *P. falciparum* and tendency of the human host to respond to higher antibodies to *P. falciparum* than *P. malariae*. IgG antimalaria antibody titres to *P. falciparum* (and *P. malariae*) did not differ between cases and controls. However, IgM antibody titres to *P. falciparum*, but not *P. malariae*, were statistically

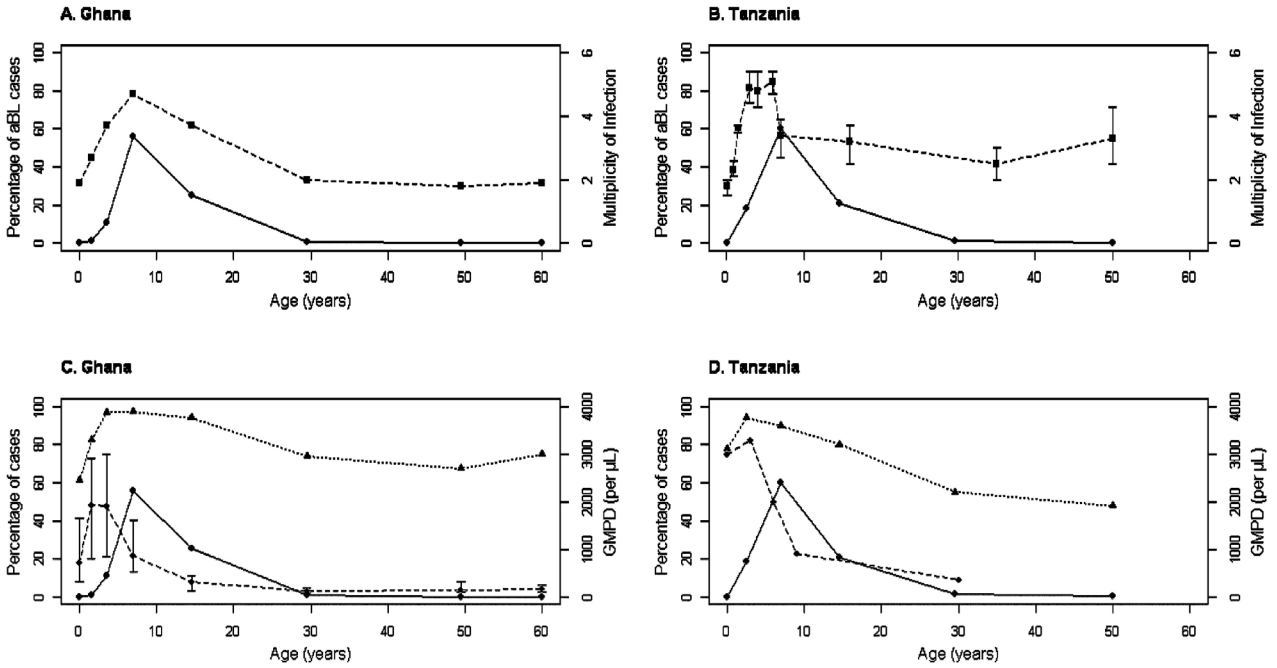
significantly lower in cases of Burkitt lymphoma than controls ($P < 0.001$). The inverse association between Burkitt lymphoma and IgM levels was unclear. [The Working Group noted that measurement of serum total, as well as malaria-specific, IgA, IgG, and IgM levels at 1–2 months and again at 12 months in 18 patients revealed no change in Ig levels in samples from pre-treatment and post-remission periods, suggesting that the low levels of Ig found might be related to abnormalities in Ig synthesis or catabolism in the patients, but not due to a disease effect. The Working Group noted small sample size and uncertainty about the reliability of assays for antimalaria antibodies as limitations of these early studies.]

Carpenter *et al.* (2008) investigated the association between Burkitt lymphoma and malaria in 325 cases and 579 controls aged 0–14 years in Uganda who were seronegative for HIV. The cases were clinically diagnosed and histologically confirmed as Burkitt lymphoma at the Uganda Cancer Institute and the controls were other children diagnosed with non-malignant conditions or non-lymphatic cancers at the same hospitals. Compared with controls, cases were more likely to report a history of frequent treatment for malaria in the past year (OR, 2.0; 95% CI, 1.3–3.1) and less likely to report use of insecticides at home (OR, 0.2; 95% CI, 0.1–0.3). Among 126 cases and 70 controls with data available on antibodies, compared with those with negative values for antimalaria antibodies (9.5% of cases *versus* 22.8% of controls), the odds ratio for Burkitt lymphoma was 2.5 (95% CI, 1.6–3.6) for children with low titres of antimalaria antibodies and it was 3.4 (95% CI, 1.7–6.7) for children with high titres of antimalaria antibodies (P for trend = 0.05). When stratified by urban *versus* rural districts, these associations were stronger and statistically significant in the rural districts. [A test for interaction was not provided; the stratum-specific analyses were sparse. The strengths of the study included using a combination of questionnaire methods and antibody measurement to assess exposure to malaria; restriction of analyses to HIV-negative subjects, and an effort to histologically verify diagnosis in many cases (recent studies of lymphoma pathology in low-resource settings have indicated variable accuracy of clinical and local pathology diagnosis, thus, misclassification is a concern. However, the results are typical for Africa). The limitations included the relatively small sample with results for antimalaria antibodies, which reduced the effective power of the study to observe interactions, and the possibility of reverse causality and biases in the study could not be excluded. The Working Group noted that all children in this study were definitely exposed to malaria and negative results for antibodies may reflect false-negative results or low sensitivity of the assays currently used.]

Mutalima *et al.* (2008) conducted a case–control investigation of 148 children aged 0–14 years and diagnosed with Burkitt lymphoma in Malawi. The controls were 104 children admitted to the same hospital with non-malignant conditions or cancers other than haematological malignancies and Kaposi sarcoma. Malaria infection was assessed by questionnaire and ELISA for antibody to the whole schizont. The mean age for cases was 7.1 (SD, 2.6 years) and for controls it was 5.1 (SD, 3.9 years). Among children with data on use of mosquito nets (20% of cases and 24% of controls), the odds ratio for Burkitt lymphoma was 0.2 (95% CI, 0.03–0.9) for those who reported using a mosquito net compared with those who did not. Compared with children who had negative or low titres of antimalaria antibodies (cases, 29%; controls, 53%), the odds ratio for Burkitt lymphoma was 2.4 (95% CI, 1.2–4.4) in those with medium or high antimalaria antibodies (P for trend, < 0.01). [The limitations of the study included incomplete diagnostic verification of cases, and uneven age distribution of cases *versus* controls.]

Asito *et al.* (2010) compared levels of anti-bodies to three *P. falciparum* antigens: the merozoite

Fig. 2.2 Characteristics of cases of Burkitt lymphoma in Africa correlated with mean multiplicity of infection, prevalence, and geometric mean parasite density for *Plasmodium falciparum* malaria



Age group intervals for the cases were plotted according to the age groups used in the malaria papers.
A-B: The percentage of Burkitt lymphoma cases by age (—) and mean multiplicity of *P. falciparum* malaria parasites (---), defined as the average number of distinct genotypes per positive blood sample based on the merozoite surface protein-2 (MSP-2) assessed by polymerase chain reaction in Ghana and the United Republic of Tanzania.
C-D: The percentage of Burkitt lymphoma cases per age (—), the prevalence of *P. falciparum* malaria (…), and the geometric mean parasite density (---) in the general population in Ghana and United Republic of Tanzania. (Age group intervals for the cases are plotted according to the age groups used in the malaria papers.)
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surface protein-1 (MSP-1), liver stage antigen-1 (LSA-1), and apical membrane antigen-1 (APM-1) in 32 cases of Burkitt lymphoma obtained from a large referral hospital and 25 controls enrolled from a region in Western Kenya where malaria is endemic and individually matched on age and sex. No differences between cases and controls were noted in levels of IgG to all *P. falciparum* antigens. This result was specific for antimalaria antibodies because differences were noted between cases and controls for anti-EBV antibodies. [The strengths of this study included examining multiple antibodies to malaria, including those linked to protective immunity to malaria (MSP-1, LSA-1) and histological verification of cases. The limitations included the small sample size, which could have reduced the power of the study to observe statistically significant results, and lack of geographical matching of cases to controls. The cases were more likely to have been treated with antimalarial medication than were the controls and this could explain the inverse association. Also, the focus of the study was EBV, not malaria.]

Guech-Ongey *et al.* (2011) compared antibodies to SE36 antigen in 657 cases of Burkitt lymphoma (92% microscopically confirmed; age 0–14 years) and 498 controls. SE36 is a recombinant protein based on the Pf-SERA5 gene, and a target for protective malaria immunity (Aoki *et al.*, 2002; Okech *et al.*, 2006). The cases were enrolled at the Korle-Bu Teaching Hospital, Accra, Ghana, during 1965–94. The controls were apparently healthy children enrolled from

Table 2.2 Case–control studies examining the association between endemic Burkitt lymphoma and malaria

Reference, Study location, and period	Characteristic of cases of Burkitt lymphoma	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjusted potential confounders Comments
Ziegler *et al.* (1972) Uganda 1967–70	100 histologically confirmed cases (age 0–14 yr); malaria results available only for 17 cases	18 age-, sex-, and tribe-matched hospital-based controls	Malaria parasitemia by microscopy; FAT	FAT ≤ 1:4 FAT 1:8–1:32 FAT ≥ 1:64	3 8 6	1.0 [7.11 (0.82–69.34)][a] [2.29 (0.32–19.10)][a]	FAT performed on samples obtained at admission. Interpretation of FAT levels is uncertain as levels were unrelated to presence of parasitaemia, malaria splenomegaly, disease stage, or response to therapy. The selection of controls was not ideal as matching for age, sex, and residence was not performed. Overlaps partially with Feorino & Mathews (1974) [b]
Feorino & Mathews (1974) Uganda	60 histologically confirmed cases (age 4–15 yr)	60 matched on sex, age, tribe, and residence; age 3–16 yr	IIF and IHA	*IIF* Negative Positive *IHA* Negative Positive	 3 57 21 39	 1.0 [a] [1.35 (0.22–9.66)][a] 1.0 [0.93 (0.41–2.11)][a]	The subjects were a subset of those included in the study by Ziegler *et al.* (1972), thus the results do not represent a totally independent experiment[b]
Nkrumah *et al.* (1979) Ghana	56 histologically confirmed cases (age 4–14 yr)	56 matched on sex, age, tribe and residence	Antimalarial IgG against *P. falciparum* schizont by IIF Antimalarial IgM against *P. falciparum* schizont by IIF	< 1:16 dilution factor 1:16 1:64 1:256 1:1024 1:4096 < 1:4 dilution factor 1:4 1:16 1:64 1:256	0 0 1 8 18 29 35 9 11 0 1	- - - 1.0[a] [0.90 (0.24–3.40)] [1.04 (0.29–3.66)] 1.0[a] [0.32 (0.10–1.00)] [0.28 (0.10–0.80)] - -	This study carefully individually matched the controls to the case on age, sex, tribe and residence and used population-based controls.

Table 2.2 (continued)

Reference, Study location, and period	Characteristic of cases of Burkitt lymphoma	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjusted potential confounders Comments
Carpenter *et al.* (2008) Uganda 1994–99	325 HIV-negative cases confirmed by local histology or cytology (age ≤ 15 yr)	579 HIV-negative controls (age ≤ 15 yr) admitted for orthopaedic conditions (447) or cancers other than lymphoma or leukaemia (132)	Antibodies to *P. falciparum* malaria by IIF; 126 cases and 70 controls	*Antimalaria IIF antibody levels*: Negative Low (1:64 or 1:256) High (≥ 1:1024)	 12 72 42	 1.0 (0.4–2.5) 2.5 (1.6–3.6) 3.4 (1.7–6.7) $P = 0.05$	Age, sex, district, household income, and tribe/slight differences in rural/urban distribution of the cases and age distribution of cases. Questionnaire data on use of mosquito bed net, past history of malaria showed associations compatible with malaria antibody levels.
Mutalima *et al.* (2008) Malawi 2005–06	148 children (age ≤ 15 yr) (including 9 HIV-positive cases), 109 confirmed by local histology or cytology	104 children (age ≤ 15 yr) admitted for non-malignant conditions (11) or cancers other than lymphoma or leukaemia (93)	ELISA for *P. falciparum* schizont extract (*Pf*SE) for 139 cases and 96 controls)	*OD readings used as a surrogate for malaria antibody titres categorized as:* Negative/low (OD < 0.2) Medium/high (OD ≥ 0.2) *P*-value *Bed net use* No Yes	 38 91 12 17	 1.0 2.4 (1.2–4.4) $P < 0.01$ 0.2 (0.03–0.9)	Age, sex, and residence/the controls were younger than cases. Similar to the Uganda study, questionnaire data with mosquito bed net use were consistent with a role for malaria.

Table 2.2 (continued)

Reference, Study location, and period	Characteristic of cases of Burkitt lymphoma	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjusted potential confounders Comments
Asito *et al.* (2010) 2007–08	32 children (age ≤ 15 yr) (1 HIV-positive case), all cases confirmed by local histology or cytology	25 children (age ≤ 15 yr) randomly selected from the community age and sex matched to the case	Malaria measured by microscopy of thick smear, and by luminex bead assay for IgG to AMA-1, MSP-1, and LSA-1 antigens at 1:6400 dilution	Difference in levels of *P. falciparum* IgG titres compared by Mann–Whitney U test.	13% of cases positive for parasites in blood *vs* 68% in controls; Relative titres of IgG to *P. falciparum* antigens similar in cases and controls		The patients were probably treated with antimalarial drugs before admission
Guech-Ongey *et al.* (2011) 1965–94	657 children (age ≤ 15 yr) (none was HIV-positive), 92% confirmed by local histology or cytology	498 children (age ≤ 15 yr) nearest neighbour control from age and sex matched to the case	ELISA for SE36 antibodies; end dilution titres categorized as tertiles:	*Anti-SE36 IgG antibody titres* High Medium Low *P*-trend	 168 217 271	 1.0 1.33 (0.96–1.86) 1.67 (1.21–2.32) $P = 0.002$	Age, sex, enrolment calendar period, and test plate. SE36 is recombinant protein under evaluation as a blood-stage malaria vaccine candidate. High antibody titres have been linked with lower risk of severe malaria and lower mean parasite levels. High titres of anti-SE36 antibodies may be considered as partially protective against severe malaria.

[a] Odds ratios are approximate because matching was ignored.

[b] 60 cases in the Feorino & Mathews study were part of the study by Ziegler *et al.* (1972)

AMA-1, apical merozoite antigen 1; ELISA, enzyme-linked immunosorbent assay; FAT, immunofluorescent antimalarial antibody titre; Ig, immunoglobulin; IHA, indirect haemaglutination assay; IIF, indirect immunofluorescence; LSA-1, liver stage antigen 1; MSP-1, merozoite surface protein; NR, not reported; OD, optical density; SE36, serine repeat antigen 5; *vs*, *versus*

Table 2.3 Case–control studies examining the association between endemic Burkitt lymphoma and haemoglobin electrophoretic patterns

Reference, Study location, and period	Characteristic of cases of Burkitt lymphoma	Characteristics of controls	Haemoglobin genotype	No. of exposed cases	Adjusted potential confounders/comments
Williams (1966) Nigeria 1960–65	100 Yoruban cases histologically confirmed (age 5–15 yr); results presented for 95 cases only with HbAA and HbAS.	331 hospital Yoruban children attending the same hospital over 5 yr with known haemoglobin results, same age and living in a comparable area; results based on 320 controls with only HbAA and HbAS (sickle-cell trait)	HbAA HbAS	78 17	Retrospective study using routinely collected data; hospital controls may have conditions associated with abnormal haemoglobin; study limited to one tribe, although Burkitt lymphoma occurs in other tribes.; results suggest decreased incidence of Burkitt lymphoma in children with HbAS (sickle-cell trait)
Pike *et al.* (1970) Uganda	36 histologically confirmed cases	50 neighbourhood controls matched on age, sex, tribe, and place of residence	HbAA HbAS	30 6	Small sample size; limited power to observe significant associations; results suggest decreased incidence of Burkitt lymphoma in children with HbAS (sickle-cell trait)
Nkrumah & Perkins (1976) Ghana	110 histologically confirmed cases	112 neighbour controls matched by same age, sex, and tribe	HbAA HbAS	90 11	Cases individually matched with one or more unrelated nearest-neighbourhood children of the same age, sex, tribe, and residence; results suggest decreased incidence of Burkitt lymphoma in children with HbAS (sickle-cell trait)
		110 patient-sibling controls	HbAA HbAS	85 12	Siblings of the case, closest in age, were chosen as sibling controls; results suggest decreased incidence of Burkitt lymphoma in children with HbAS (sickle-cell trait)

HbAA; wild-type haemoglobin gene AA; HbAS, variant haemoglobin gene AS (sickle-cell trait)

the neighbourhood of the cases, and matched for age, sex and calendar time. Children with Burkitt lymphoma had statistically significantly decreased mean log end-point dilution titres [0.63 logs lower] than controls (Student *t*-test, $P = 0.019$). Compared with children with high anti-SE36 IgG antibody tertiles, the odd ratios for Burkitt lymphoma were 1.33 (95% CI, 0.96–1.86) for children with medium SE36 tertiles and 1.67 (95% CI, 1.21–2.31) for children with low anti-SE36 tertiles (P for trend = 0.002). The children with Burkitt lymphoma in Ghana may have had lower titres of antibodies to SE36 antigen than matched controls from the same community [suggesting that high anti-SE36 titres may be protective for Burkitt lymphoma, akin to the protective effect suggested for severe malaria] (Aoki *et al.*, 2002; Okech *et al.*, 2006). [The strength of this study was the large size, selection of of age-, sex- and residence-matched controls, and the use of anti-SE36 antimalaria antibodies, which have been associated with reduced risk of severe malaria in epidemiological studies in Africa (Aoki *et al.*, 2002; Okech *et al.*, 2006), to assess exposure to malaria. An inverse association would suggest that antibodies that are protective for severe malaria or malaria parasitaemia may be protective for Burkitt lymphoma. Lack of data on antibody to the whole schizont was a limitation.]

(b) Exposure assessed indirectly by haemoglobin genotype

Three studies indirectly assessed the role of malaria in Burkitt lymphoma by determining haemoglobin type, including HbAS, which is responsible for the sickle-cell trait. Young children with HbAS are substantially protected against severe falciparum malaria (Allison, 1963).

Williams (1966) conducted a retrospective comparison of electrophoretic patterns of haemoglobin protein (which reflect haemoglobin genotypes for sickle-cell disease) in 100 Yoruban children (aged 5–15 years) with microscopically confirmed Burkitt lymphoma during 1960–1965 and 331 Yoruban children of the same age and living in a comparable area treated at the hospital during the same period. The P value for the association between carriage of protective genotypes (HbAS, HbSS, HbAC, HbCC) and Burkitt lymphoma was < 0.10. Restricted to 95 cases and 320 controls with HbAA and HbAS abnormalities (excluding HbSS, HbCC, HbAC, HbCC), children with Burkitt lymphoma had 18% carriage of HbAS compared with 30% in controls (Williams, 1966). [The limitations of the study included use of a retrospective design, use of controls selected from children attending the hospital, which may be related to malaria or abnormal haemoglobin genotypes. Haemoglobin electrophoresis results were based on routine tests, but these may have varied over the 5 years covered by the study. The Working Group noted that authors' decision to exclude children aged 0–4 years was considered reasonable because of survivor bias among the children with the sickle-cell trait. The hospital controls may be biased to children with conditions associated with malaria or HbAS.]

Pike at al. (1970) compared haemoglobin electrophoretic patterns among 36 cases of microscopically confirmed Burkitt lymphoma from the Uganda Cancer Institute with those of 50 control children individually matched on age, sex, tribe and residence. The results suggest a decreased incidence of Burkitt lymphoma among HbAS children.

Nkrumah & Perkins (1976) compared haemoglobin electrophoretic patterns in 110 microscopically confirmed cases with controls individually matched to the nearest neighbouring child of the same age, sex, and tribe ($n = 112$), or to the sibling closest in age to the case ($n = 110$). In subgroup analysis, carriage of haemoglobin C (HbAC) was associated with a slight, but non-significant, protective effect against Burkitt lymphoma (one-sided $P < 0.1$).

2.1.6 Intervention studies

Only one study to control Burkitt lymphoma through malaria intervention has been implemented. Geser *et al.* (1989) reported the results of a study the aim of which was to prevent Burkitt lymphoma through suppression of malaria in children in the Mara region in northern United Republic of Tanzania [the population varied between 177 000 and 200 000 during the study period] where the incidence of Burkitt lymphoma had been monitored through careful registration of cases since 1964 (Eshleman, 1966; Brubaker *et al.*, 1973; Siemiatycki *et al.*, 1980; Geser & Brubaker, 1985). Malaria suppression was implemented in the North Mara region through mass distribution of bi-weekly doses of chloroquine to children aged < 10 years, starting in 1978. The burden of malaria was monitored by survey, and the impact of the programme on the incidence of Burkitt lymphoma was monitored by registering all new cases in North Mara [intervention region] as well as in neighbouring South Mara [non-intervention region].

The prevalence of malaria parasitaemia in the intervention region fell from [24%, prevalence in lowland and highland areas of North Mara] in the baseline survey in May 1976 to 11% in 1977 and 13% in 1978. The prevalence in non-intervention communities was higher and did not fall during the intervention (28% in 1976 to 37% in 1978) (Geser *et al.*, 1989). The fall in the prevalence of malaria parasitaemia was transient and started rising, in part, due to programme failures in the drug distribution system (MacCormack & Lwihula, 1983), and, in part, due to development of chloroquine resistance (Draper *et al.*, 1985), and surpassed the high levels that had prevailed before the programme before 1977. The programme was abandoned in 1981.

Notably, the incidence of Burkitt lymphoma fell rapidly in the intervention villages from about 4 per 100 000 during 1964 and 1977 (range, 2.6–6.9 per 100 000 children) before intervention and fell to about 1 per 100 000 children during 1977 to 1982 (range, 0.5–3 per 100 000). The lowest incidence of Burkitt lymphoma during the entire observation period (0.5 per 100 000) was recorded during the intervention period. However, the incidence of Burkitt lymphoma was falling before the malaria-suppression trial. The fall in incidence was transient, and although it continued falling for about 1–2 years after malaria prevalence started rising, the incidence rose to 7.1 per 100 000 to surpass levels before the malaria-suppression intervention. [Some aspects of this analysis supported the association between malaria and Burkitt lymphoma, but others did not. A causal interpretation would suggest that the lag times would be similar between when incidence of Burkitt lymphoma started to fall after the malaria-suppression intervention was introduced and when it started increasing after malaria suppression was lost. The results were susceptible to errors in case ascertainment due to the short period of intervention and the small numbers of cases of Burkitt lymphoma, despite the large numbers of people participating in the intervention.]

2.1.7 Cofactors

(a) EBV

See Table 2.4

EBV is an established cause of Burkitt lymphoma and is a common childhood infection in sub-Saharan Africa (IARC, 1997, 2012). Carpenter *et al.* (2008) reported an odds ratio of 3.6 (95% CI, 2.3–5.6) and 4.5 (95% CI, 2.3–8.7) for Burkitt lymphoma in children with medium and high titres of anti-EBV antibody, respectively, compared with those with low or negative antibody titres in Uganda. The joint effects of EBV and malaria were examined by estimating the odds ratio for Burkitt lymphoma in children with raised levels of EBV antibody only, antimalaria antibody only, or both EBV and antimalaria antibody. Cases were five times more likely than

controls to have jointly elevated anti-EBV and antimalaria antibodies (OR, 5.0; 95% CI, 2.8–8.9), but not more likely to have elevated antibodies to either EBV or malaria only [but the confidence intervals associated with these odds-ratio estimates were wide].

Mutalima *et al.* (2008) reported odds ratios of 4.1 (95% CI, 1.6–10.1) and 14.8 (95% CI, 5.8–38.5) for medium and high anti-EBV antibody titres compared with low titres. Cases were 13.2 times more likely than controls to have jointly elevated anti-EBV and antimalaria antibodies (95% CI, 3.8 – 46.6, $P < 0.001$) compared with those with low antibodies to both EBV and malaria. The odds ratios were 1.4 (95% CI, 0.3–63) for those with elevated malaria antibodies only and 5.7 for those with elevated EBV antibodies only.

Asito *et al.* (2010) [described in Section 2.1.5] compared levels of IgG to EBV antigens – viral capsid antigen (VCA), EAd, EBNA-1 and Zta – and EBV viral load in plasma in 32 children with histologically confirmed Burkitt lymphoma compared with 25 children matched on age and sex from one village in the study area. Cases of Burkitt lymphoma had significantly increased titres of anti-Zta ($P = 0.0017$) and VCA IgG levels ($P < 0.0001$), and plasma EBV viral loads ($P < 0.0001$) indicative of viral reactivation. Levels of EBNA-1 and EAd-specific IgG were not different between cases and controls. Among the cases, those with tumours in the abdomen had significantly elevated anti-Zta IgG levels ($P < 0.0065$) and plasma EBV viral loads ($P < 0.033$) compared with patients with jaw tumours. [While malaria and EBV are the most widely accepted cofactors for endemic Burkitt lymphoma, the role of a "third" cofactor has been considered to account for several unexplained epidemiological features of endemic Burkitt lymphoma. For example, endemic Burkitt lymphoma predominates in males, but neither malaria nor EBV show significant disparity between the sexes. No additional cofactor has yet been found.]

The findings on the joint effects of EBV and malaria are biologically plausible given that malaria induces polyclonal stimulation of B cells (Chêne *et al.*, 2007) and impairs anti-EBV T-cell responses (Kataaha *et al.*, 1984; Facer & Playfair, 1989; Langhorne *et al.*, 1989). The effect of malaria on EBV control, however, are transient because the EBV load in peripheral blood returns to lower levels when malaria is successfully treated (Lam *et al.*, 1991). Two other issues remain unresolved: (1) are certain EBV strains more lymphomagenic than others? and (2) what is the relevant measurement of malaria exposure? [The Working Group noted that the risk of Burkitt lymphoma is only linked to the more fatal *P. falciparum*.]

(b) HIV

HIV is an established risk factor for AIDS-related Burkitt lymphoma (IARC, 2008). The risk of Burkitt lymphoma in people who are HIV-positive is 50 times higher than in the general population; however, the data relating HIV to endemic Burkitt lymphoma is scant and most cases are HIV-negative (Newton *et al.*, 2001; Mutalima *et al.*, 2008). Furthermore, there are scant published data investigating the joint effect of HIV and malaria on Burkitt lymphoma (IARC, 2012). Despite a high prevalence of HIV in malaria-endemic areas, no epidemic increase in endemic Burkitt lymphoma has been described in Africa. [This might be due to low survival of HIV-infected children in Africa, or underascertainment.] Cases were more likely than controls to be HIV-positive in the study by Mutalima *et al.* (2008) (nine cases and two controls were HIV-positive), but they were not able to examine differences in antibodies to malaria or EBV by HIV status in Malawi.

2.1.8 Other factors

Several studies have investigated other factors or surrogates for malaria in relation to Burkitt lymphoma in malaria-endemic regions, but the interaction with malaria was not specifically examined. These include family/household characteristics (Rainey *et al.*, 2008; Morrow *et al.*, 1974a), rural or urban status (Biggar & Nkrumah, 1979), arthropod-borne viruses (Van den Bosch & Lloyd, 2000), and exposures to plants (Van den Bosch *et al.*, 1993a). In none of these studies was the evidence of malaria infection examined or controlled for.

2.2 Kaposi sarcoma

Kaposi sarcoma is a systemic disease presenting with cutaneous lesions that can also involve the oral cavity, lymph nodes, and viscera. It is caused by Kaposi sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8). It was originally described by Moritz Kaposi, in 1872.

Infection with KSHV alone is not sufficient to cause Kaposi sarcoma. The most important cofactor predisposing a KSHV-infected person to Kaposi sarcoma is HIV co-infection or, to a lesser extent, other immunodeficient states such as iatrogenic immune suppression in organ transplant recipients. Before the HIV epidemic, classic (sporadic) Kaposi sarcoma represented up to 9% of all cancers in parts of sub-Saharan Africa, such as Uganda, in both men and women. The incidence of Kaposi sarcoma in specific geographic areas before the HIV epidemic points to a role of as-yet-unknown cofactors in the etiology of this cancer (IARC, 2012).

Only two cancer registry-based studies have examined the link between classical Kaposi sarcoma and malaria in Italy using case–control methodology (Table 2.5; Geddes *et al.*, 1995; Cottoni *et al.*, 1997). The studies looking at Kaposi sarcoma measured malaria ecologically, using birth in a formerly malaria-endemic area to measure exposure to malaria. [The Working Group noted that the malaria infection relevant in these studies, although unknown, was likely due to infection by *P. vivax*, which was prevalent in Italy. Malaria was declared eradicated in Italy in the 1960s, thus, many individuals may have been exposed from childhood into adulthood. The lack of direct measurement of malaria and conducting the study many years after exposure to malaria complicates interpretation of the results of these studies.]

Geddes *et al.* (1995) investigated the association between classical Kaposi sarcoma and birth in a formerly malaria-endemic area in a case–control study. The cases were 204 patients with histologically confirmed classical Kaposi sarcoma (139 men, 65 women) aged 50 years or older (mean age, 70 years) identified from 11 cancer registries in Italy. The controls were 777 subjects with other cancers, matched for age (± 2 years), sex, and year of diagnosis, identified from the same registry. The odds ratio for Kaposi sarcoma in people born in an area where malaria was endemic was 2.98 (95% CI, 1.97–4.51), and was elevated both in northern (OR, 2.2) and southern (OR, 2.0) Italy, and remained statistically significant in multivariable analyses, including age, sex, registration area, age group, population density in 1936, and altitude. [The weakness of this study was the use of birth region as the best measure for exposure to malaria, which may not reflect actual individual exposure to malaria. The Working Group noted that there was malaria/geographical correlation with Kaposi sarcoma-associated herpesvirus (KSHV), also known as human herpes virus 8 (HHV-8), the established cause of Kaposi sarcoma, which may confound results. However, the strong associations in both northern and southern Italy suggest results may be valid.]

Cottoni *et al.* (1997) investigated the association between a past history of malaria and classical Kaposi sarcoma in a case–control study in northeastern Sardinia. The cases were 40 patients with

Table 2.4 Case–control studies examining the association between Burkitt lymphoma, malaria, and infection with Epstein–Barr virus

Reference, study location, and period	Characteristics of cases of Burkitt lymphoma	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjusted potential confounders Comments
Carpenter *et al.* (2008) Uganda 1994–99	325 HIV-negative cases, confirmed by local histology or cytology (age ≤ 15 yr)	579 HIV-negative controls (age ≤ 15 yr) admitted for orthopaedic conditions (*n* = 447) or cancers other than lymphoma or leukaemia (*n* = 132)	Antibodies to *Plasmodium falciparum* measured by IIF; 126 cases and 70 controls; EBV measured by ELISA	*Antimalarial antibody levels/ EBV antibodies* Low EBV titre: Low antimalarial titre (≤ 1:64) High antimalarial titre (≥ 1:256) High EBV titre: Low antimalarial titre (≤ 1:64) High antimalarial titre (≥ 1:256) *P*-trend	 12 15 22 77 	 1.0 (0.4–2.4) 1.1 (0.5–2.4) 1.0 (0.5–2.2) 5.0 (2.8–8.9) *P* = 0.003	Age, sex, district, household income, and tribe. Malaria assessed in a smaller subset because of loss of samples during transit [related to 09/11/2001 disruptions], but demographical characteristics similar in tested *vs* not tested subjects.
Mutalima *et al.* (2008) Malawi 2005–06	148 children (age ≤ 15 yr) (9 HIV-positive cases), 109 confirmed by local histology or cytology	104 children (age ≤ 15 yr) admitted for non-malignant conditions (*n* = 11) or cancers other than lymphoma or leukaemia (*n* = 93)	ELISA for *P. falciparum* schizont extract for 139 cases and 96 controls); EBV measured by IIF	*Antimalarial antibody levels/EBV antibodies* Low EBV titre: Low antimalarial titre (≤ 1:64) High antimalarial titre (≥ 1:256) High EBV titre: Low antimalarial titre (≤ 1:64) High antimalarial titre (≥ 1:256) *P*-trend	 5 7 32 82 	 1.0 1.4 (0.3–63) 5.7 (1.6–20.7) 13.2 (3.8–46.6) *P* = 0.001	Age, sex, and residence

EBV, Epstein–Barr virus; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence; *vs*, *versus*

classical Kaposi sarcoma and the controls were 120 members of the general population matched on age, sex, and geographical residence. The matched odds ratio associated with a history of malaria was 1.21 (95% CI, 0.51–2.84; 20 exposed cases). The results were similar to those obtained in 1980 from the same group, where a history of malaria was reported by 19 out of 39 patients with classical Kaposi sarcoma, which was similar to that reported by adults of a similar age in the general population (about 50%). [Malaria in this study was likely to be attributable to *P. vivax*. The strength of this study was the ability to directly measure malaria exposure by questionnaire. However, the reliability and specificity of recall for historically remote infections is uncertain, raising concern about exposure misclassification. The study was small (40 cases) and was underpowered.]

2.2.1 Cofactors

KSHV/HHV-8 is a necessary cause of Kaposi sarcoma (IARC, 2012) and HIV is an established risk factor for Kaposi sarcoma. However, no studies have examined the link between malaria, KSHV, HIV, and Kaposi sarcoma.

2.2.2 Other factors

Several studies have investigated other factors or surrogates for malaria exposure in relation to Kaposi sarcoma in malaria-endemic regions, but evidence for interaction with malaria infection has not been examined specifically. The risk factors examined were exposure to volcanic soils (Montesu *et al.*, 1995; Simonart, 2006; Pelser *et al.*, 2009a), plants (Whitby *et al.*, 2007; Pelser *et al.*, 2009b), steroids (Goedert *et al.*, 2002), smoking (Goedert *et al.*, 2002), other parasites (Lin *et al.*, 2008), and genetic polymorphisms (Contu *et al.*, 1984).

2.3 Cancers at other sites

A few sporadic reports have reported associations between malaria and cancer at other sites, including the cervix (Odida *et al.*, 2002), prostate (Thomas, 2005; Elson *et al.*, 2011), nasopharyngeal carcinoma (Yadav & Prasad, 1984; Chen *et al.*, 1990), lymphoma (Ross *et al.*, 1982; Schmauz *et al.*, 1990; Cook-Mozaffari *et al.* 1998; Tavani *et al.*, 2000; Vineis *et al.*, 2000) and liver (Welsh *et al.*, 1976; Lu *et al.*, 1988).

Odida *et al.* (2002) reported a geographical correlation between malarial endemicity and high-grade cervical cancer in Uganda among 457 cases ascertained in 1968–73. Based on 304 cases with complete data, the relative risk of high-grade cervical cancer in areas with severe malaria was 2.04 (95% CI, 1.37–3.04) compared with cases from an area with low malaria endemicity [the completeness of the cancer and population data in this study were uncertain].

Yadav & Prasad (1984) measured titres of antibodies to *P. falciparum* antigens in 22 patients with nasopharyngeal carcinoma and 43 controls. Antimalaria antibodies were present in all except one patient, and 14 patients had titres of> 80 and 4 patients > 640. Compared with controls, the mean titres of antimalaria antibodies for most age groups were higher in the patients. Those patients with high titres of antimalaria antibodies also had high titres of IgA anti-VCA, an antibody that is diagnostic for nasopharyngeal carcinoma [the role of reverse causality and small size of the study were concerns].

Chen *et al.* (1990) investigated the role of malaria in nasopharyngeal carcinoma in Taiwan, China, among 347 cases and in population-based controls enrolled from a household register and matched to cases on age and sex. Self-reported history of malaria was associated with an increased risk of nasopharyngeal carcinoma in males (OR, 2.1; 95% CI, 1.27–3.66) and females (OR, 2.17; 95% CI, 0.82–5.70).

Elson *et al.* (2011) investigated the association between prostate cancer and Duffy antigen/receptor for chemokines (DARC) in a case–control study of 81 patients *versus* age-matched controls enrolled from a phlebotomy laboratory in Jamaica. No association was found between lack of DARC expression and prostate cancer (OR, 1.0; 95% CI, 0.48–2.1). [DARC is required by *P. vivax* for infection, thus lack of expression is protective against infection by this species of *Plasmodium*. The relevance of this study for DARC as a marker of malaria-related exposure was uncertain because direct exposure of patients to malaria was not considered.] [The Working Group noted that the few reports of associations between malaria and cancer for isolated sites were interesting, but insufficient to inform about carcinogenicity attributable to malaria for these sites.]

2.4 Susceptible populations

Populations susceptible to endemic Burkitt lymphoma include children who are born into and live continuously in areas where malaria is holoendemic. Duration and intensity of exposure is important because episodic or seasonal occurrence of malaria, lasting for less than 6 months, does not appear to be closely related to the incidence of Burkitt lymphoma (Burkitt, 1969). Susceptibility to Burkitt lymphoma peaks between ages 5 and 8 years, when peak titres of antimalaria antibodies are attained [given the postulation that latency of Burkitt lymphoma is relatively short, the peak age may be related to a biological window when children are most vulnerable owing to their B-cell physiology, or when the probability of exposure to a particular malaria strain peaks]. In a few small studies in Uganda, adults migrating from areas where malaria was not endemic to hyperendemic or holoendemic areas were at increased risk of the acute consequences of malaria and increased risk of Burkitt lymphoma (Burkitt & Wright, 1966; Morrow, 1985).

3. Cancer in Experimental Animals

There is no animal model for carcinogenesis associated with *P. falciparum*. However, *P. berghei*-infected rodents develop pathologies with symptoms comparable to a certain degree with human malaria. *P. berghei* is highly homologous to *P. falciparum* in most essential aspects of structure, gene content, biochemistry and life cycle. A few studies of carcinogenicity were performed with *P. berghei*, the results of which are summarized in Table 3.1.

3.1 Mouse

A group of 25 Swiss mice [sex not reported] received an intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei* strain K 173. Two weeks after infection, they were fed a diet lacking *para*-aminobenzoic acid (a parasite growth factor) for 2 months and became parasite-free. Immunity was tested and immune mice were killed after 7 months. Seventeen of the 25 animals were reinfected 2 months before euthanasia. A group of 18 unexposed Swiss mice served as controls and were killed at age 8–14 months. An increased incidence of lymphoma (11 out of 25 versus 0 out of 18, [$P < 0.005$]), all involving the thymus, was observed. An immune mouse and a control mouse developed a Rauscher type leukaemia (Jerusalem, 1968). [The Working Group noted the confusing study design, that there was a probable virus contamination in the colony and that spontaneous leukaemias occurred in both groups. This made the study difficult to interpret.]

Four groups of 10–13 male Balb/c mice received (i) an intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei*

Table 2.5 Case–control studies examining the association between Kaposi sarcoma and malaria

Reference, Study location, and period	Characteristic of cases with Kaposi sarcoma	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases	Odds ratio (95% CI)	Adjusted potential confounders. Comments
Geddes *et al.* (1995) Italy 1976–91	204 incident cases with classical Kaposi sarcoma (age ≥ 50 yr), histologically confirmed from 11 cancer registries	777 with other cancers, matched on age (± 2 yr), sex, registry, and calendar year	Birth in an area endemic for malaria (data available on 188 cases and 746 controls)	Birth in an area endemic for malaria No Yes	 83 105	 1.0 2.98 (1.97–4.51)	Age, sex, and registration area Only cases of classical Kaposi sarcoma were evaluated
Cottoni *et al.* (1997) Italy 1991–96	40 cases	120 selected from an electoral register matched on sex, age (± 5 yr), and residence	Malaria history by questionnaire	A history of malaria? No Yes *P*-heterogeneity	 19 20	 1.0 1.207 (0.513–2.842) *P* = 0.67	Matching factors Evaluated classical Kaposi sarcoma

yr, year

yoelii; or (ii) an intraperitoneal injection of an extract of spleen and thymus from mice with lymphoma induced by Moloney leukaemogenic virus (MLV); or (iii) an injection of parasitized erythrocytes followed by an injection of the extract within 5 minutes; or (iv) an injection of parasitized erythrocytes followed by an injection of the extract 10 days later. After 26 weeks, an increased incidence of MLV-induced lymphoma was observed in mice injected with parasitized erythrocytes and injected with the extract within 5 minutes, when compared with mice injected with the extract only (10 out of 12 *versus* 1 out of 11, [$P < 0.001$]). Six of 13 mice injected with parasitized erythrocytes and injected with the extract 10 days later developed MLV-induced lymphoma, but this increase was not statistically significant (Wedderburn, 1970).

Three groups of 10–12 neonatal CFW mice received an intravenous injection of simian virus 40 (SV40), or an intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei yoelli*, or an injection of SV40 followed by an injection of parasitized erythrocytes after 3 months. After up to 12 months, 11 out of 11 mice (100%) injected with both SV40 and parasitized erythrocytes developed sarcomas of the liver and/or spleen compared with 7 out of 10 mice (70%) injected with SV40 only. This increase was not statistically significant. No such tumours were observed in mice injected with parasitized erythrocytes only. In a similar experiment in adult mice, no sarcomas were observed in any group (Hargis & Malkiel, 1979).

3.2 Rat

Four groups of 21–24 male Buffalo rats received: (i) an intraperitoneal injection of mouse erythrocytes parasitized by *P. berghei* and normal diet; or (ii) a diet containing aflatoxin B_1 at a dietary concentration of 2 ppm for 10 weeks; or (iii) an intraperitoneal injection of parasitized erythrocytes 12 days before starting a diet containing aflatoxin B_1 for 10 weeks; or (iv) a normal diet and an intraperitoneal injection of uninfected blood. After 60–82 weeks, the incidence of hepatocellular carcinoma was: 0 out of 22, 13 out of 19 (68%), 5 out of 14 (36%) and 0 out of 19, respectively, in the four groups. The incidence of aflatoxin B_1-induced hepatocellular carcinoma in the group injected concurrently with parasitized erythrocytes was not statistically significant compared with that in the group treated with aflatoxin B_1 only (Angsubhakorn *et al.*, 1988).

4. Mechanistic and Other Relevant Data

4.1 Malaria and the host immune system

4.1.1 Immune response to malaria

Despite extensive efforts, the factors involved in immunity to malaria and pathogenesis of malaria infection are still poorly understood. It is well established that people residing in regions where malaria is endemic acquire immunity to malaria through natural exposure to the parasite. This naturally acquired immunity, which is a non-sterile state of immune protection against the parasite and the clinical disease, results from and is maintained by a continuous exposure to multiple infections. In the human host, the malaria parasite develops in antigenically distinct stages, targeting different cells and tissues, such as the parenchymal cells in the liver and erythrocytes in the blood (see Section 1.1.3 and Fig. 1.1). Several features of malaria and the ensuing immune response suggest that the parasite and the host immune system interact through a myriad of complex mechanisms, which result in the ability of an otherwise functional immune system to eliminate the parasite and protect the

individuals against subsequent infection (Riley *et al.*, 2006; Doolan *et al.*, 2009).

The intensity of malaria transmission determines the development of immunity as a function of age (Fig. 4.1), and the nature of the symptoms therefore depends upon the level of transmission. For example, clinical symptoms related to *P. falciparum* infection become increasingly rare with age in areas where malaria is highly endemic, and where the individual can receive several infected bites each day (up to 1500 infected bites per year). The acquisition of clinical immunity develops step-wise, to severe malaria and subsequently to the clinical symptoms, but immunity to the parasite itself only develops in part and develops slowly (Fig. 4.1). For instance, children aged 5–10 years still frequently carry high levels of parasites in their blood without having symptoms. Adolescents and adults carry the parasite throughout their life, although at very low levels; sterile immunity may not exist. As a consequence, in areas where malaria is holoendemic or hyperendemic, the spleens of exposed children become massively enlarged at an early age, and a correlation between parasite-levels and the size of the spleen has been established (Fig. 4.1).

Natural immune responses to the pre-erythrocytic stages of *P. falciparum* (sporozoite and liver) have been suggested to have limited involvement in malarial immunity (Hoffman *et al.*, 1987; Marsh & Kinyanjui, 2006; Langhorne *et al.*, 2008; Borrmann & Matuschewski, 2011; Schwenk & Richie, 2011). However, other studies have demonstrated that antibodies may contribute to protection against the pre-erythrocytic stages during malaria infection (Hollingdale *et al.*, 1984; Kebaier *et al.*, 2009). Antibodies to sporozoites may act through neutralizing opsonization of sporozoites and/or block the invasion of hepatocytes (Schofield *et al.*, 1987; Nussenzweig & Nussenzweig, 1989; Fidock *et al.*, 1997; Nardin *et al.*, 1999).

The infected hepatocyte is an important target of protective immunity, since the presence of parasites in this MHC class I-expressing cell may render processed parasite antigens more accessible to cell-mediated immune responses (Speake & Duffy, 2009).

During the liver stage, effector functions of $CD4^+$ and $CD8^+$ T-cells have been implicated (Tsuji & Zavala, 2003; Overstreet *et al.*, 2008; Doolan *et al.*, 2009; Wykes & Good, 2009; Tsuji, 2010). There is evidence that the presence of T cells specific for liver-stage antigens and secreting interferon-gamma (IFN-γ) is associated with reduced incidence of malaria (Perlaza *et al.*, 2008; Todryk & Bejon, 2009).

Protective immunity to the asexual blood forms of malaria parasites, the pathogenic stage of the life cycle, involves both cellular and antibody-mediated mechanisms. For *P. falciparum*, a role for antibodies was originally demonstrated by passive transfer of adult immune immunoglobulin G (IgG) to infected children (McGregor, 1964). Antibodies can be effective in protection against blood-stage parasites by various mechanisms. These include opsonization of merozoites before uptake through Fc receptors and/or complement receptors on phagocytes, blocking of invasion of erythrocytes, complement-mediated lysis of infected erythrocytes, inhibition of adherence to the endothelium or neutralization of malaria toxins (Bolad & Berzins, 2000), or by antibody-dependent cell-mediated responses that may kill the parasites (Bouharoun-Tayoun & Druilhe, 1992; Shi *et al.*, 1999). The major IgG subclasses involved in parasite elimination are of the cytophilic type (i.e. IgG1 and IgG3), while the non-cytophilic types (i.e. IgG2 and IgG4) seem to counteract the protective ones (Garraud *et al.*, 2003). However, in some malaria-endemic areas, elevated levels of IgG2 have been related to decreased risk of infection, especially in individuals carrying a specific allele variant of FcgRIIA, which binds IgG2 (Aucan *et al.*, 2000; Garraud *et al.*, 2003; Israelsson *et al.*, 2008; Nasr *et al.*, 2009).

Table 3.1 Studies of carcinogenicity in experimental animals infected with *Plasmodium berghei*

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, Swiss (NR) 9 mo Jerusalem (1968)	- *P. berghei* strain K 173 (1.2×10^6 parasitized mouse erythrocytes), i.p. injection; 2 wk after infection, mice were fed *para*-aminobenzoic acid-free diet for 2 mo and became uninfected. Immunity was tested and immune animals were killed after 7 mo. Some were reinfected 2 mo before killing. *n* = 25 (17 immune reinfected + 8 immune not reinfected) - Unexposed controls *n* = 18	Lymphoma: 11/25[a], 0/18 Rauscher-type leukaemia: 1/25, 1/18	[$P < 0.005$] [NS]	[a] Five additional infected mice had tumours suspected of being lymphoma. All lymphomas involved the thymus. Virus particles (median, 157 nm; not further described) were found in the spleen in 28.5% of exposed mice and 4.5% of controls. Confusing study design.
Mouse, Balb/c (M) up to 6 mo Wedderburn (1970)	- *P. berghei yoelii* (10^6 parasitized mouse erythrocytes in 0.1 mL), i.p. injection *n* = 10 - Extract of enlarged spleens and thymuses of mice with MLV-induced lymphoma in 0.5 mL, i.p. injection *n* = 11 - *P. berghei yoelii* + extract within 5 min, i.p. injection *n* = 13 (1/13 mice died after 24 d) - *P. berghei yoelii* followed 10 days later by extract, i.p. injection *n* = 13	Lymphoma: 0/10, 1/11, 10/12*, 6/13**	*[$P < 0.001$], **[NS]; *vs* extract only-injected group	
Mouse (neonatal), CFW (NR) up to 12 months Hargis & Malkiel (1979)	- SV40 (strain VA45–54, 10^4 $TCID_{50}$/0.1 mL, intravenous injection in the anterior facial vein) *n* = 10 - SV40 followed by 5×10^6 *P. berghei yoelii*-infected mouse erythrocytes (i.p. injection after 3 mo) *n* = 11 - *P. berghei yoelii* only (i.p. injection) *n* = 12	Sarcomas of the liver and/or spleen: - 7/10 (70%; 2 reticulum cell sarcoma, 2 spindle cell sarcoma, 1 pleomorphic sarcoma, 1 poorly differentiated sarcoma, 1 lymphosarcoma) - 11/11 (100%; 2 reticulum cell sarcoma, 2 spindle cell sarcoma, 3 pleomorphic sarcoma, 2 poorly differentiated sarcoma, 2 lymphosarcoma) - 0/12 Tumour latency: 11 months, 9 mo, - Lung metastases: 1, 4, 0	[NS], *P. berghei yoelii* + SV40 group *vs* SV40 group	All tumours expressed SV40 T-antigen

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Buffalo (M) 60–82 wk Angsubhakorn *et al.* (1988)	- 0.5 mL uninfected blood (i.p. injection) + normal diet *n* = 24 - *P. berghei* (10^6 parasited mouse erythrocytes, i.p. injection) + normal diet *n* = 23 - 0.5 mL uninfected blood (i.p. injection) followed by diet containing aflatoxin B_1 at 2 ppm (for 10 wk) after 12 d *n* = 23 - *P. berghei* (i.p. injection) followed by diet containing aflatoxin B_1 (for 10 wk) after 12 d *n* = 21	Hepatocellular carcinoma: 0/19, 0/22, 13/19 (68%), 5/14 (36%)	[NS], *P. berghei* + aflatoxin B_1 group *vs* aflatoxin B_1 group	

d, day; i.p., intraperitoneal; M, male; min, minute; MLV, Moloney leukaemogenic virus; mo, month; NR, not reported; NS, not significant; SV40, simian virus 40; $TCID_{50}$, tissue culture infectious dose 50; *vs*, *versus*; wk, week

Another subclass of great interest in malaria is immunoglobulin E (IgE), for which some reports indicate an association with protection (Bereczky *et al.*, 2004; Farouk *et al.*, 2005; Duarte *et al.*, 2007), and other data suggest an association with pathogenesis (Perlmann *et al.*, 1994, 1997, 2000; Seka-Seka *et al.*, 2004; Leoratti *et al.*, 2008b). Various immune complexes, which consist of either IgE and antigen aggregates, or IgE with IgG-anti-IgE could bind to Fc receptors expressed on monocytes that become activated, giving rise to secretion of tumour necrosis factor (TNF) (Perlmann *et al.*, 1999). The production of TNF is genetically regulated: certain nucleotide polymorphisms in the promoter give rise to overproduction of TNF, which leads to a pathogenic effect, while low production of TNF is associated with protection against malaria (McGuire *et al.*, 1999, Gimenez *et al.*, 2003).

(a) Antigen-presenting cells

Antigen-presenting cells (APCs) are crucial initiators of adaptive immunity. There are three types of professional APCs, namely monocytes, dendritic cells and B cells. APCs interact with pathogens through specialized receptors termed pattern-recognition receptors that have evolved to detect danger signals such as pathogen-associated molecular patterns. A well studied family of pattern-recognition receptors are the toll-like receptors (TLRs), which are expressed differently in different APC populations (Akira *et al.*, 2006; Kawai & Akira, 2010).

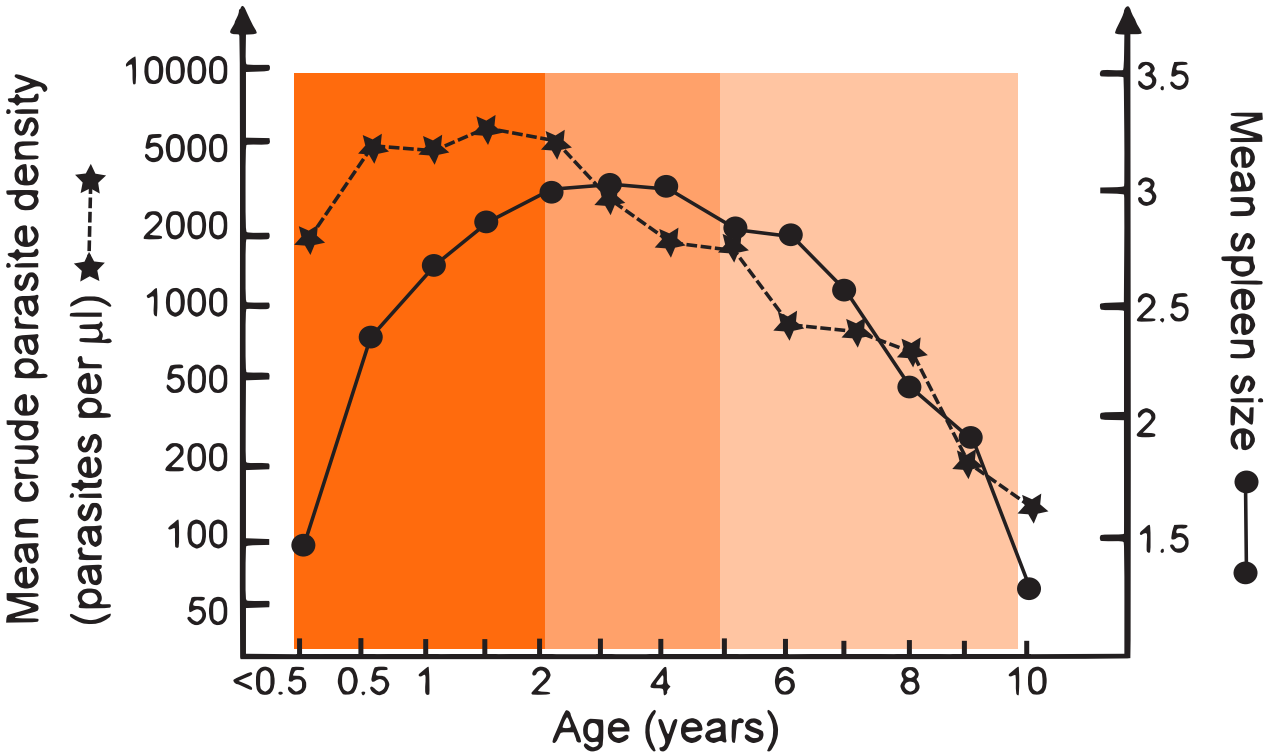
In humans, monocytes and myeloid dendritic cells subsets express mainly TLR1 through TLR6, while TLR7 and TLR9 are expressed in plasmacytoid dendritic cells (Kadowaki *et al.*, 2001, Jarrossay *et al.*, 2001). Recent evidence suggests that TLRs are central mediators of pro-inflammatory responses during malaria (Coban *et al.*, 2007). For example it has been shown that hemozoin, which is a by-product generated after the degradation of haemoglobin by the parasite, impairs both antigen presentation and immunomodulatory functions of the monocytes/macrophages (Schwarzer *et al.*, 1998; Skorokhod *et al.*, 2004) and that *P. falciparum* glycosylphosphatidylinositol activates the TLR9 pathway (Coban *et al.*, 2007). It has been shown that the malaria glycosylphosphatidylinositol anchor activates monocytes to release TNF, IL-6 and IL-12 through the TLR2 pathway (Schofield *et al.*, 2002; Gowda, 2007, Zhu *et al.*, 2009). However, the role of TLRs in the development of immunity to malaria and in the pathology of malaria is not well understood.

Denditric cell-mediated suppression of T-cell functionality has been suggested to be the cause of the poor T-cell mediated immune responses seen in malaria (Ocaña-Morgner *et al.*, 2003; Urban & Roberts, 2003). Observations *in vivo* have shown that children with acute malaria have a lower expression of the human leukocyte antigen (HLA) DR on peripheral blood dendritic cells compared with healthy children (Urban *et al.*, 2001). Moreover, a possible immunomodulatory role has been suggested for the BDCA-3$^+$ subset of myeloid dendritic cells upon malaria infection in Kenyan children (Urban *et al.*, 2006). Observations *in vitro* have shown that exposure of APCs to hemozoin increases the production of both pro- and anti-inflammatory mediators (Coban *et al.*, 2005). Taken together, existing data suggest that the function of various APC subsets is influenced by *P. falciparum* and that immune responses to malaria may be hampered by modulation of APC functions.

(b) Natural killer cells

During human malaria infections, activation of natural killer (NK) cells *in vivo* has been inferred from the observation that NK-cell activity was increased in malaria-infected children and that this activity correlated with parasitaemia and plasma levels of IFN-γ (Ojo-Amaize *et al.*, 1981; Hansen *et al.*, 2007). Another study has shown that NK cells are the first cells to respond by producing IFN-γ after exposure

Fig. 4.1 Occurrence of symptoms of clinical malaria



Average suggestive occurrence of clinical malaria symptoms as a function of age, mean parasite density and mean spleen. Mean parasite density (***) and mean spleen size (ooo) of children in a malaria-afflicted area of holoendemic transmission. The spleen was palpated according to Hackett, with the examiner's hand being pressed gently against the abdominal wall of the child until the spleen is felt. At a size of 3, the spleen was felt inbetween the umbilical level and half-way to the lower border of the thoracic cage, at a size of 2, the spleen was felt inbetween halfway to the lower border of the thoracic cage and the lower border of thoracic cage and at a size of 1 the spleen was felt under the thoracic cage. Reprinted from Chêne et al. (2009), with permission from Elsevier. From (Björkman, 1985).

of human peripheral blood mononuclear cells to *P. falciparum*-infected erythrocytes *in vitro* (Artavanis-Tsakonas & Riley, 2002). In contrast, others have shown that the secretion of IFN-γ by NK cells in response to infected erythrocytes is not detected in all malaria-naive or previously exposed donors. These authors showed that NK cells comprise only a small proportion of IFN-γ-producing cells *in vitro* and that the depletion of NK cells does not affect IFN-γ production after exposure for 24 hours to live *P. falciparum*-infected erythrocytes at the schizont stage. Instead, the predominant source of innate IFN-γ was attributed to gamma/delta T-cells (γδT-cells) that express NK receptors (D'Ombrain *et al.*, 2007). Moreover, a study in Papua New Guinea showed high levels of IFN-γ production *ex vivo* associated with protection from high parasite densities and clinical disease (D'Ombrain *et al.*, 2008). The release of IFN-γ by NK cells seems to require both a direct contact between the infected erythrocytes and the NK cells and be dependent on the presence of accessory cells (monocytes and myeloid dendritic cells), while TGF-β suppresses NK-cell activation (Newman *et al.*, 2006).

There is accumulating evidence that NK T cells play a role in protective immunity against malaria as shown in experimental systems (e.g. malaria in mice). An early study found that the humoral immune response against circumsporozoite protein was strongly diminished in CD1d-deficient mice compared with wild-type mice (Schofield *et al.*, 1999). Since the CD1d is a

lineage-commitment marker of NK T cells, mice lacking this marker have a deficiency in NK T cells. The study further demonstrated that glycosylphosphatidylinositol purified from the asexual blood stages of *P. falciparum* was able to stimulate murine $CD4^+$ NK T cells *in vitro*, suggesting that $CD4^+$ NK T cells act as helper T cells facilitating the production of anti-circumsporozoite protein antibody by B cells *in vivo* (Schofield *et al.*, 1999). The role of NK T cells as helper T cells in the regulation of anti-malarial humoral responses *in vivo* was later confirmed by two independent studies (Molano *et al.*, 2000; Romero *et al.*, 2001). Overall, NK T cells do not seem to have a clear physiological role in inhibiting the pre-erythrocytic stages of the parasite. However, when the NK T cells have been activated by the artificial ligand α-galactosylceramide (α-GalCer), these cells do display an inhibitory effect against the development of liver stages of malaria *in vivo*. This anti-plasmodial activity of α-GalCer-activated NK T cells seems to be mediated by IFN-γ, since the activity is abolished in mice lacking IFN-γ or IFN-γ receptors (Gonzalez-Aseguinolaza *et al.*, 2000).

(c) Gamma/delta T cells

Gamma/delta T cells share characteristics with both NK and αβ NK T cells, such as the capacity to secrete high amount of pro-inflammatory cytokines and cytolytic activity against tumours and pathogens. Increasingly, it is accepted that γδT cells form part of the protective immune response directed against *P. falciparum*. It has been shown that γδT cells inhibit the growth of the asexual blood stages of *P. falciparum* by the granule exocytosis-dependent cytotoxic mechanism (Farouk *et al.*, 2004; Costa *et al.*, 2011). Moreover, studies have shown that *P. falciparum* infection induces expansion of γδT cells to reach up to 30% of circulating T cells in humans (Roussilhon *et al.*, 1990; Ho *et al.*, 1994; Worku *et al.*, 1997).

Subpopulations of γδT cells have been shown to be upregulated to cytolytic functions after interaction with virus-infected cells, like EBV (Häcker *et al.*, 1992, Harly *et al.*, 2011) and cytomegalovirus (CMV) (Knight *et al.*, 2010).

(d) Regulatory T cells

An increase in $CD4^+CD25^+FoxP3^+$ regulatory T cells has been observed in human infected with *P. falciparum* (Walther *et al.*, 2005; Scholzen *et al.*, 2009) and with *P. vivax* (Jangpatarapongsa *et al.*, 2008). The increase in regulatory T cells during malaria infections appears to be associated directly with parasite load (Walther *et al.*, 2005, 2009; Scholzen *et al.*, 2010). Increased levels of $CD4^+CD25^{hi}$ have been correlated with the risk of malaria infection in rural Kenya (Todryk *et al.*, 2008), and *Foxp3* mRNA levels during acute infection with *P. falciparum* in Gambian children inversely correlated with parasite-specific IFN-γ responses after parasite clearance, indicating that induction of regulatory T cells during acute infection could limit the extent of the memory Th1 response (Walther *et al.*, 2009). It is obvious that multiple mechanisms govern the induction, expansion and activation of regulatory T cells during malaria infection, including host-immune mediators such as APCs and immunoregulatory cytokines (Scholzen *et al.*, 2010).

(e) B cells

B cells are important effector cells of the immune system through the production of antibodies against invading microbes and vaccine antigens. In addition to their established effector function as antibody-producing cells, recent studies indicate that B cells also modulate T-cell responses by antibody-independent mechanisms.

Plasma cells reside in specialized niches in lymphoid organs and the bone marrow, where stromal cells provide an environment conducive to survival. Memory B cells preserve and recall previous antigenic experience to prevent or limit reinfection, thus ensuring long-term protection

for each individual and protection of the species through passive transfer from mother to child during pregnancy and after birth.

B cells play a major role in immunity to malaria as demonstrated by early experiments of serum Ig transfer, as mentioned above (Cohen *et al.*, 1961). In mice, memory B cells are generated during primary infection with *P. chabaudi* (Ndungu *et al.*, 2009) and these cells rapidly differentiate to plasma cells upon reinfection with the parasite (Nduati *et al.*, 2010). Acute infection with *P. falciparum* has a profound effect on B-cell function, resulting in hypergammaglobulinaemia, increased plasma concentrations of the B-cell activating factor, and an increase in atypical memory B cells (Asito *et al.*, 2008; Weiss *et al.*, 2009; Kumsiri *et al.*, 2010). In children, antibody responses to malaria antigens are often short-lived and only maintained in the presence of the parasites; the mechanisms underlying changes in B-cell function during acute malaria are incompletely understood. Important receptors for the regulation of antigen-independent activation of B cells are TLRs. In humans, circulating memory B cells express higher levels of TLR6, TLR7, TLR9 and TLR10, whereas naive B cells were shown to express low levels of TLR and to respond poorly to TLR ligands. Transitional B cells in cord blood and in peripheral blood express the highest levels of TLR9 and respond to TLR9 ligation with proliferation, differentiation and antibody production (Capolunghi *et al.*, 2008). Polyclonal B-cell activation during malaria infection is probably due to activation of TLR9 by parasite molecules and ligation of the B-cell receptor by malaria antigens (Bernasconi *et al.*, 2003). Furthermore, high concentrations of B-cell activating factor may inhibit the differentiation of memory B cells to plasma cells in the presence of TLR9 ligands (Darce *et al.*, 2007). [Modulation of the B-cell compartment during and after an acute episode of malaria may contribute to enhanced susceptibility to bacterial pathogens and reduced antibody responses to vaccines included in the Expanded Programme on Immunization (EPI) for children living in malaria-endemic areas.]

B cells secrete cytokines and chemokines, either constitutively or in response to antigens, upon triggering by TLR ligands, T cells, or combinations thereof (Lund & Randall, 2010).

(f) Cytokines

For a successful elimination of parasites, it is important to have an early innate response, including secretion of pro-inflammatory cytokines like IL-12 and TNF-α. For optimal clearance of the parasite, this initial response should be followed by an effective adaptive immune response including high titres of anti-malaria antibodies to clear the parasitaemia. On the other hand, pro-inflammatory cytokines may also play an important role in determining pathology, in particular in the development of severe malaria. In fact, low levels of IL-10 and high levels of TNF-α have been associated with disease severity. In severe cases of malaria among Gabonese children, levels of IL-12 were found to be lower than in the mild cases (Luty *et al.*, 2000). In addition, the development of cerebral malaria has been associated with high plasma levels of IL-1β (Prakash *et al.*, 2006) and higher levels of IL-6 and IL-10 were associated with severe malaria than with uncomplicated malaria (Lyke *et al.*, 2004).

4.1.2 Immunity to malaria in infants

Development of immunity is dependent on exposures to pathogens as well as the age of the host. During childhood, there are several changes in the lymphocyte compartment and these are especially evident from birth until age 2 years. Healthy infants have significantly higher numbers of peripheral CD19+ B cells and CD3+ T cells compared with adults (Denny *et al.*, 1992; Comans-Bitter *et al.*, 1997). Within the lymphocyte compartment, the percentage

of memory B and T cells expands over time and reflects the infants' antigenic history. The relative lack of maturity due to limited exposure to antigens, compounded with an environment that promotes a Th2-like response characterized by minimal inflammatory reactions, leads to a reduced capacity of infants to adequately mount an effective immune response in the first year of life. In addition, infants are unable to respond to thymus-independent antigens until about age 2 years, leaving them especially vulnerable to capsulated bacteria.

Falciparum malaria only elicits protective immunity after several years of continuous exposure, during which time recurring infections and illness occur (Marsh *et al.*, 1989). Thus, children living in malaria-endemic regions of sub-Saharan Africa have the burden of early age of exposure and repeated exposure to falciparum malaria, while their immune system is still developing. That this is problematic is shown by the fact that not only do children aged less than 5 years suffer the highest morbidity and mortality due to infection with *P. falciparum*, they also have the highest all-cause mortality of any age group living in malaria-endemic regions (Snow & Omumbo, 2006). Infants living in a malaria-endemic region of Kenya were found to have elevated levels of transitional B cells and reduced non-class-switched memory B cells compared with infants living in a non-malaria-endemic region (Asito *et al.*, 2011). Expansion of a peripheral memory B-cell subset characterized by expression of the inhibitory ligand FCRL4 and hyporesponsiveness was described in infants as young as age 2 years living in a malaria-epidemic region of Mali (Weiss *et al.*, 2009).

Plasmodium-specific antibody levels rapidly decline in infants after clearance of parasitaemia (Kinyanjui *et al.*, 2007; Akpogheneta *et al.*, 2008) and immunity is acquired only after several years of exposure (Akpogheneta *et al.*, 2008; Crompton *et al.*, 2010). The delay in development of protective immunity to malaria results in repeated infection of infants and young children with *P. falciparum*, which involves continual exposure of their B cells to parasitic antigens.

4.1.3 Malaria and host genetic polymorphisms

Malaria is a complex disease with an etiology that shows a large variation in severity of infection, which is attributable to environmental factors, virulence of the parasite, and host genetics (Mackinnon *et al.*, 2005; Sakuntabhai *et al.*, 2008). Variation in the severity of *P. falciparum* infections includes hypersymptomatic or asymptomatic parasitaemia, severe malarial anaemia, and cerebral malaria, and is suggested to be caused by genetic factors of parasite and host (Garcia *et al.*, 1998; Rihet *et al.*, 1998). Host genetic polymorphisms known to influence susceptibility/resistance to *P. falciparum* include polymorphisms leading to haemoglobinopathies and other erythrocyte disorders. The involvement of polymorphisms in immune-response genes (e.g. HLA, cytokines) is also suspected. This subchapter gives only a brief overview of these polymorphisms, which have been reviewed extensively elsewhere (Verra *et al.*, 2009; Driss *et al.*, 2011).

(a) Haemoglobinopathies/erythrocyte disorders

The genetic basis of some important erythrocyte disorders in the modulation of the clinical course of malaria was first discovered in the middle of the last century. They include the sickle-cell trait (Allison, 1954) and haemoglobinopathies such as thalassaemias and glucose-6-phosphate dehydrogenase deficiency (reviewed by Hill, 1999; Williams, 2006) (see also Table 1.3 and Section 1.2.3).

(b) Immune-response genes

(i) HLA polymorphisms

The immune response and susceptibility to malaria have been the object of extensive investigation using twin studies with the aim of dissecting genetic regulation (Sjöberg *et al.*, 1992; Jepson *et al.*, 1997a, b). These investigations showed that monozygotic twins were always more concordant than dizygotic twins in their responses to malaria, even more so than individuals with identical HLA class II; this implies that genes both inside and outside the HLA class II region are involved in susceptibility to malaria. Further evidence that genetics plays a role in shaping individual immune responses to malaria has come from inter-ethnic comparisons of susceptibility to malaria in West Africa. Epidemiological studies conducted in Burkina Faso and Mali have shown that the Fulani people have lower parasite rates and densities and fewer attacks of malaria than their sympatric ethnic counterparts who live under similar socioeconomic conditions and with comparable intensity of malaria transmission. This is most likely due to their more efficient immune response (Modiano *et al.*, 1996, 1998, 1999, 2001a; Dolo *et al.*, 2005; Verra *et al.*, 2009). The involvement of the classical erythrocyte disorders mentioned above in the stronger protection of the Fulani against malaria seems unlikely, since they are present at lower frequencies in the Fulani (Modiano *et al.*, 2001a). Typing of HLA class I alleles revealed that the Fulani are genetically distant from sympatric groups (Modiano *et al.*, 2001b).

Regarding HLA, associations between the class I haplotype HLA-B53 and the class II haplotype HLA-DRB1*1302-DQB1*0501 and protection against severe malaria have been reported (Hill *et al.*, 1991). These authors identified an epitope of the liver-surface antigen (LSA-1) that binds HLA-B53, providing a possible functional explanation for the association between HLA-B53 and protection from severe malaria (Hill *et al.*, 1992). However, this association could not be reproduced in other countries (Gilbert *et al.*, 1998; Young *et al.*, 2005). The influence of the HLA system on the immune response has been extensively studied in the Gambia. In a comparison of humoral and cellular responses in HLA-identical and HLA-non-identical individuals, non-HLA genes played a more substantial role than the HLA genes (Jepson *et al.*, 1997b). Taken together, the available evidence suggests that it is unlikely that the HLA loci are a major genetic determinant of immune reactivity to malaria (Verra *et al.*, 2009).

(ii) Cytokines and related gene polymorphisms

TNF has been shown to have an important role in the pathogenesis of malaria (Kwiatkowski *et al.*, 1990). In a study conducted in the Gambia, the presence of the 308A allele in the *TNF* promoter region, known to increase the transcription of the gene compared with the 308G allele, was associated with a higher risk of death or severe neurological sequelae due to cerebral malaria. This risk was independent of variation in the nearby HLA class I and II genes (McGuire *et al.*, 1994).

Many other single-nucleotide polymorphisms have been reported, including those in IFN-γ, the IFN-γ receptor, IFN regulatory factor-1 (IRF-1), CD40 ligand, interleukins IL-1 and IL-4, Fcγ receptors, TLRs, mannose-binding protein, complement receptor 1, and intercellular adhesion molecule 1 (ICAM-1). For detailed references to these polymorphisms see Verra *et al.* (2009).

Importantly, results for all single-nucleotide polymorphisms have been difficult to reproduce in different countries, suggesting that different combinations of allelic variants may determine the complex clinical outcomes of severe malaria in different populations.

At present, the multicountry collaborative Malaria Genomic Epidemiology Network (Malaria-GEN) is trying to gain fundamental

new insights into the effects of genetic variations on susceptibility to malaria and molecular mechanisms involved in protection from and pathogenesis of malaria (Malaria Genomic Epidemiology Network, 2008).

4.2 Biological properties of *P. falciparum* antigens relevant to immune dysfunction

The parasite *P. falciparum* resides most of its lifetime in the bloodstream, in the midst of the immune system. When an *Anopheles* mosquito takes a blood meal, the parasite – in its infective stage – is expelled into the circulation and rapidly migrates to the liver where it infects and divides in hepatocytes. On their way to the liver, however, some parasites are trapped in the local lymphoid system, which promotes immune recognition. Subsequently, the infected hepatocyte bursts, and thousands of merozoites are released into the blood stream and swiftly invade erythrocytes. The parasite further develops and divides inside the erythrocyte, which upon rupture releases parasites, initiating 48-hour cycles that may perpetuate for 9 months or longer (see Section 1).

Efforts to develop an effective vaccine against malaria are challenged by the complex life cycle of *P. falciparum*, its capacity to parasitize and hide within the host cells, and its masterful ability to avoid clearance by the innate and adaptive immune responses of the host. The virulence of *P. falciparum* is related to its ability to evade host immunity through clonal antigenic variation of antigens at the surface of the parasitized erythrocyte, and through tissue-specific adhesion of parasitized erythrocytes.

During intra-erythrocytic growth of *P. falciparum*, parasite-derived proteins such as PfEMP1, RIFIN, STEVOR and SURFIN are successively expressed, exported and presented at the surface of the human erythrocyte (Helmby *et al.*, 1993; Baruch *et al.*, 1995; Cheng *et al.*, 1998; Fernandez *et al.*, 1999; Kyes *et al.*, 1999; Winter *et al.*, 2005). PfEMP1, the dominant polypeptide, is an adhesin that enables erythrocytes infected with mature parasite stages to adhere in the microvasculature and sequester, thereby avoiding clearance by the spleen. The parasite thrives in the hypoxic environment of the post-capillary vessels. Consequently, only erythrocytes infected with early-stage parasites are seen in the peripheral blood. Sequestration, depending on endothelial adhesion and binding of parasitized erythrocytes to uninfected erythrocytes and/or parasitized erythrocytes in the context of inflammation, may lead to complete clogging of the microvasculature and to severe malaria. Furthermore, by presenting ligands such as PfEMP1 at the surface of the parasitized erythrocyte, the parasite exposes an Achilles' heel, allowing for host immune recognition of the altered erythrocyte surface (Wahlgren *et al.*, 1999). To evade the mounting immune response, the polypeptides display extensive antigenic variation, simultaneously changing the receptor recognition and the tissue tropism of the infected cell. Thus, the adhesive properties of the exposed variant surface antigen also determine the outcome of the disease.

4.2.1 Antigenic variation

PfEMP1 is a large protein (200–360 kDa) encoded by a family of approximately 60 *var* genes (Scherf *et al.*, 2008). Each individual parasite only expresses a single variant gene at a time, maintaining all other members of the family in a transcriptionally silent state according to a very precise, but yet to be determined, counting mechanism (Chen *et al.*, 1998a, Scherf *et al.*, 1998). The same *var* gene is expressed during many parasite generations. Switching of expression to another variant PfEMP1 species occurs at a rate of about 1%, and allows the parasite to avoid immune clearance and to prolong the period of infection.

The *var* genes are composed of two exons, a conserved intron and a 5′ upstream flanking sequence (ups). Almost all PfEMP1s share some conservation in their structure: an N-terminal segment, variable numbers of Duffy binding-like domains, one or two cysteine-rich interdomain regions (CIDR), a transmembrane domain, sometimes a C2 domain; and a conserved intracellular acidic terminal segment (Baruch *et al.*, 1995; Chen *et al.*, 2000; Kraemer & Smith, 2006). Although the PfEMP1 proteins differ extensively in their amino-acid composition and binding properties, they have related protein architectures. As an example of PfEMP1 organization and binding phenotype, the PfEMP1 expressed by the parasite clone FCR3S1.2 is shown in Fig. 4.2 (see also Chen *et al.*, 2000).

4.2.2 B-cell dysfunction

Chronic infection with *P. falciparum* leads to a severely dysregulated B-cell compartment. The existence of malarial B-cell mitogens was suggested decades ago by the clinical presence of hypergammaglobulinaemia (Abele *et al.*, 1965; Greenwood & Vick, 1975), elevated titres of auto-antibodies (Adu *et al.*, 1982), and the frequent occurrence of Burkitt lymphoma (Greenwood *et al.*, 1970). Moreover, extracts derived from cultured *P. falciparum* induce B-cell proliferation and antibody production *ex vivo* (Greenwood *et al.*, 1979; Gabrielsen & Jensen, 1982; Kataaha *et al.*,1984).

During intra-erythrocytic proliferation of *P. falciparum* (for details see above, and Fig. 4.2), PfEMP1 is successively expressed, exported and presented at the surface of the human erythrocyte membrane. Parasitized erythrocytes have the ability to bind to several receptors present on B cells, such as non-immune human immunoglobulins (IgG and IgM) (Scholander *et al.*, 1996, 1998; Flick *et al.*, 2001; Rasti *et al.*, 2006; Barfod *et al.*, 2011), heparan sulfate (Chen *et al.*, 1998b; Barragan *et al.*, 2000), CD31/PECAM1 and CD36 (Treutiger *et al.*, 1997; Chen *et al.*, 1998b). The parasitized erythrocytes and the cysteine-rich interdomain region 1 α (CIDR1α) domain of PfEMP1 from the parasite clone FCR3S1.2 harbour a multiadhesive phenotype that is able to bind to CD31/PECAM-1, CD36 and IgM (Fig. 4.2; Chen *et al.*, 2000). Heparan sulfate is present on all B cells, but CD31/PECAM-1 expression is mainly associated with early developmental stages of B cells in the secondary lymphoid organs (Jackson *et al.*, 2000), while CD36 seems to be differentially expressed on B-cell subsets during development and in response to antigens (Won *et al.*, 2008).

PfEMP1 is included in the family of Ig-binding proteins in which the binding activity resides in different parts of the molecule, depending on the particular PfEMP1-species (Donati *et al.*, 2004). For example, the Duffy binding-like domain 2β (DBL2β) is the adhesive ligand of the parasite clone TM284, while CIDR1α is the ligand for the FCR3S1.2 clone (Chen *et al.*, 2000; Flick *et al.*, 2001; Donati *et al.*, 2004). PfEMP1-CIDR1α binds to both non-immune IgM and IgG, and to IgG from human and other animal species, and is also able to bind to the Fc and Fab parts of both IgM and IgG (Donati *et al.*, 2004). Characterization of the crystal structure of the PfEMP1-CIDR1α subdomain from the "Malayan-camp parasite" strain indicates that it is composed of a bundle of three α-helices that are connected by a loop to three additional helices (Klein *et al.*, 2008). Although there is very wide diversity in PfEMP1 sequences, there is still structural similarity between the CIDR and Duffy binding-like domains (Singh *et al.*, 2006; Singh *et al.*, 2008), suggesting that PfEMP1 is a polymer of three-helix bundles. Interestingly, the binding pattern and crystal structure of CIDR are similar to those of another microbial Ig-binding protein, protein A of *Staphylococcus aureus* (Graille *et al.*, 2000).

The structural and functional similarities between protein A and PfEMP1 are important for the effect of malaria infection in the B-cell

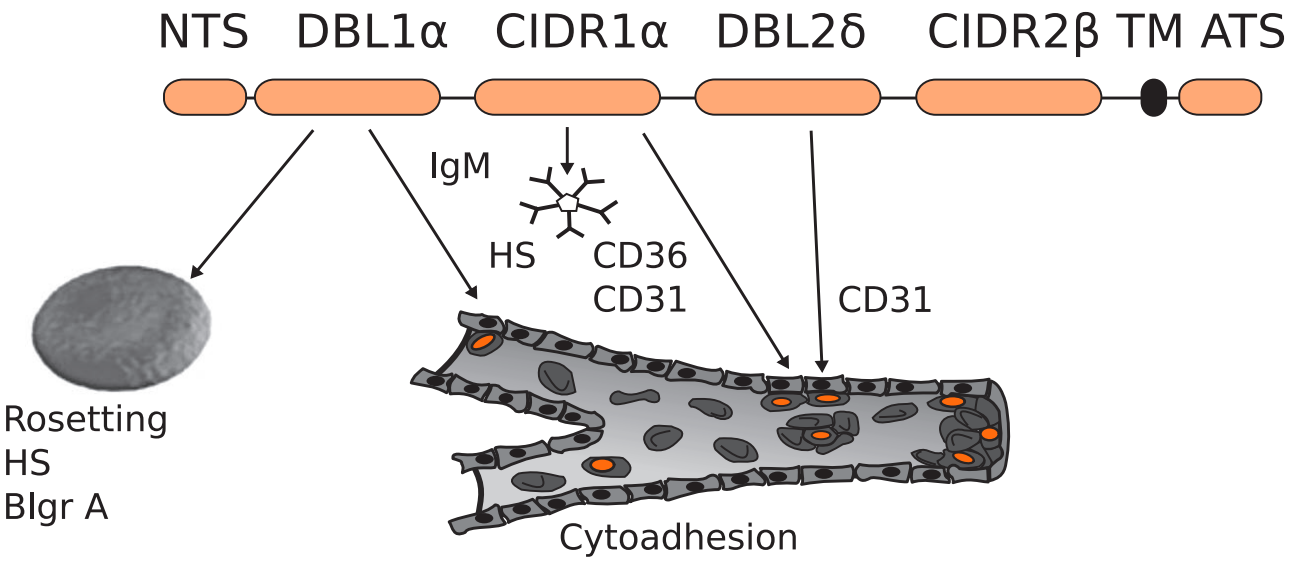
compartment. Microbial Ig-binding proteins are produced by protozoa, viruses, and bacteria, and play important physiological roles (Chen *et al.*, 1998b; Graille *et al.*, 2000). During the infectious process, Ig-binding proteins may act in an evasion mechanism to divert specific antibody responses (Daniel-Ribeiro *et al.*, 1989; Léonetti *et al.*, 1999). CIDR1α is a polyclonal B-cell activator that binds to purified B lymphocytes from non-immune donors, inducing activation, proliferation, and antibody secretion (Donati *et al.*, 2004, 2006a). These effects are predominantly observed in memory B cells. The CIDR–B-cell interaction is partially mediated through the binding to surface Ig (Donati *et al.*, 2006a). PfEMP1 affects the dynamics of germinal-centre B cells by increasing the proportion of cycling cells, inducing proliferation, and protecting B cells from apoptosis (Donati *et al.*, 2006a). The mechanisms include upregulation of genes involved in immunological signalling pathways, cell growth/apoptosis, and transcription. The known anti-apoptotic genes that were upregulated included *CALB1*, *DNAJB1* (previously called *Hsp40*), *GSTM2*, and *BNIP1*, which encodes the BCL2/adenovirus E1B 19 kDa interacting protein. CIDR1α also downregulated the expression of *BCL2L1* (previously known as *BCL-xl*) and *BCL2*, and increased the expression of *XIAP* (Donati *et al.*, 2006a).

During the erythrocytic growth phase, the *P. falciparum* parasite contains TLR9 ligands (Parroche *et al.*, 2007) known to induce proliferation of memory B cells (Bernasconi *et al.*, 2003; Kumsiri *et al.*, 2010). CIDR increases the expression of TLR7 and TLR10, and sensitizes B cells to TLR9 signalling (Simone *et al.*, 2011). Despite its ability to bind surface Ig, CIDR-induced B-cell activation does not activate LYN and/or phosphotyrosine pathways associated with B-cell receptor signalling. It rather induces phosphorylation of downstream kinases such as ERK1/2, p38 and the IKK complex (Simone *et al.*, 2011). B-cell activation can also be potentiated by the presence of elevated levels of IL-10, IL-6 and TNF (Lyke *et al.*, 2004) during severe malaria; it can also be produced *in vitro* by lymphocytes exposed to parasitized erythrocytes (Wahlgren *et al.*, 1995).

In vitro, exposure of B cells to *P. falciparum* schizont extracts induces mRNA expression of activation-induced deaminase (AID), and Ig class switching (Potup *et al.*, 2009). Infants living in malaria-endemic regions have alterations in their B-cell subset distribution, B-cell homeostasis, longevity, quantity and quality of humoral responses based on age and on the dynamics of malaria transmission (Asito *et al.*, 2008; Weiss *et al.*, 2009). Impaired humoral immune protection associated with prenatal or chronic exposure to *P. falciparum* is common in children living in malaria-endemic regions (Chelimo *et al.*, 2005; Scott *et al.*, 2005). A recent study demonstrated that children from a malaria-endemic area had higher overall numbers of B cells, reduced populations of circulating memory B cells, and an expanded population of immature transitional B cells, compared with infants living an area with unstable malaria transmission (Asito *et al.*, 2011). Expansion of transitional B-cell populations has been demonstrated to correlate to impaired humoral immunity (Cuss *et al.*, 2006).

The marked effect of malaria infection on B cells not only pertains to the nature of the malarial antigens, but also to the biology of the infection. A large proportion (83%) of parasitized erythrocytes in fresh isolates bind non-immune Igs (Scholander *et al.*, 1998) and could therefore interact in the peripheral blood with B cells, *via* their surface Igs. Erythrocytes parasitized by *P. falciparum* have the potential to directly interact with B cells at different anatomical sites, and to induce activation and differentiation into antibody-secreting cells. Mature stages of infected erythrocytes sequester in the vasculature to avoid clearance by the spleen. Under stringent blood-flow conditions, large numbers of infected erythrocytes fail to adhere to the vascular endothelium and end-up trapped in the spleen

Fig. 4.2 Molecular architecture of PfEMP1



The molecular architecture of PfEMP1 encoded by the var1 gene of the parasite clone FCR3S1.2 depicting its receptor specificities including those present on B cells such as heparan sulfate (HS), CD31/PECAM1 and CD36.
ATS, acidic terminal segment; Blgr A, blood group A antigen; CIDR, one or two cysteine-rich interdomain regions; DBL, Duffy binding-like domain; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; HS, heparan sulfate; IgM, immunogloblin M; NTS, N-terminal segment
Reprinted from Chêne *et al.* (2009) with permission from Elsevier. From Pettersson (2005).

where B lymphocytes can represent up to 50% of the splenocytes. Parasitized erythrocytes and their constituent Ags could thus interact in the spleen with B cells displaying a variety of surface phenotypes, Ag-binding repertoires and signalling profiles (Engwerda *et al.*, 2005). Noteworthy, during malaria infection the splenic architecture is profoundly changed, and the splenomegaly observed after malaria infection partially results from a prominent migration of macrophages into the white pulp regions of the spleen. These changes may alter the homeostasis of the B-cell compartment (Urban *et al.*, 2005).

In summary, *P. falciparum* affects the B-cell compartment through mechanisms that include: (a) polyclonal activation *via* B-cell receptor- and TLR9-engagement leading to increased AID activation, Ig production and switching, proliferation and survival, resulting in expansion of the B-cell compartment; (b) diversion of specific antibody responses, both as result of polyclonal activation and persistent activation; (c) dysregulation of the development of B-cell subsets, which leads to higher numbers of total B cells, a reduced population of memory B cells and higher numbers of transitional B cells; and (d) these mechanisms contribute to a persistent activation of B cells, which may result in impairment of B-cell functions.

4.2.3 Immune evasion

The parasitized erythrocytes of children and those in the placenta of pregnant women frequently carry non-immune IgM and IgG at their surfaces, where they have been found to participate in the sequestration of the parasitized erythrocytes (Scholander *et al.*, 1996, 1998; Flick *et al.*, 2001; Rasti *et al.*, 2006). The PfEMP1 protein VAR2CSA was found to be involved in immunoglobulin binding, placental adhesion and pathogenesis (Rasti *et al.*, 2006). Antibodies generated to this adhesion inhibit binding *in vitro* (reviewed in Scherf *et al.*, 2008). Further, recent evidence indicates that the capacity of the

parasitized erythrocytes to bind non-immune IgM also allows them to evade immune recognition through the masking of PfEMP1-specific IgG epitopes by nonspecific IgM. Binding of non-immune IgM to erythrocytes infected by parasites expressing the PfEMP1 protein VAR2CSA blocked subsequent specific binding of human monoclonal IgG to the Duffy binding-like domains of this PfEMP1 variant. Taken together, the results indicate that the affinity of VAR2CSA for non-specific IgM has evolved not only to allow placental sequestration of the parasitized erythrocyte, but also to evade acquired protective immunity without compromising VAR2CSA function or increasing the susceptibility of the parasitized erythrocytes to complement-mediated lysis (Barfod *et al.*, 2011).

4.3 Other infectious agents associated with falciparum malaria and human cancer

4.3.1 EBV and Burkitt lymphoma

EBV is a ubiquitous oncogenic gamma-herpes virus that infects and persists in more than 90% of the adult population worldwide. African children are infected early in life and nearly all have sero-converted by age 3 years, while in affluent countries primary infection is often delayed until adolescence (Biggar *et al.*, 1978a, b; Piriou *et al.*, 2012). EBV was originally identified in the tumour cells of a Burkitt lymphoma and was among the first viruses to be associated with the pathogenesis of a human cancer; its etiological role in the causal association with Burkitt lymphoma is established (IARC, 2012). After primary infection, EBV enters into a lifelong persistent infection, characterized by low-level periodic lytic reactivation and viral shedding into saliva for transmission. This persistence is usually benign and only causes disease when the host–virus balance is upset; it relies on a balance between viral latency, viral replication, and host immune responses. The EBV genome contains approximately 100 open reading frames (ORFs) of which only few have been characterized with regard to expression and function. EBV establishes various forms of latency in which different sets of EBV-encoded antigens are expressed, depending on the differentiation stage, location and origin of the infected cell. Latently infected B lymphocytes express nine proteins, i.e. six nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP) and three membrane proteins (LMP-1, LMP-2A, LMP-2B). In addition, two types of non-coding RNA are expressed, the EBV-encoded RNAs and the BamH1A rightward-transcript micro-RNAs (miR-BARTs) (see review, Rickinson & Kieff, 2007; Forte & Luftig, 2011). In contrast, the majority of Burkitt lymphomas express only one virally encoded protein, EBNA-1. These two patterns of gene expression are often referred to as latency III and latency I, respectively, and they are achieved by the use of different promoters to generate alternative primary transcripts from which the different EBNA mRNAs are spliced (reviewed in Rowe *et al.*, 2009). *In vivo*, EBV latency is established in resting memory B cells, infectious virus is produced when memory B cells switch from the latent to the lytic phase, which seems to occur after the differentiation from memory to plasma cells (Laichalk & Thorley-Lawson, 2005). These memory B cells seem to express none of the viral latent proteins and are invisible to the immune system.

Endemic Burkitt lymphoma, the most frequent paediatric cancer in sub-Saharan Africa, is a high-grade B-cell lymphoma characterized by the consistent presence of EBV (Epstein *et al.*, 1964, 1965; zur Hausen *et al.*, 1970), and a chromosomal translocation involving the human proto-oncogene c-*MYC* on chromosome 8 and the *Ig* loci (located either on chromosome 2, 14 or 22) that result in deregulated expression of the c-MYC protein (reviewed by Klein, 2009). Endemic Burkitt lymphoma seems to originate

from a germinal centre-derived B cell or a memory B cell. The hypermutation undergone by tumour cells is a feature of germinal-centre reaction during B-cell activation and differentiation, and the Ig gene break-point to which *MYC* is transferred in endemic Burkitt lymphoma occurs at the V(D)J region, suggesting that the translocation occurs during the process of somatic hypermutation and class-switch recombination (Thorley-Lawson & Allday, 2008). The somatic hypermutation and Ig recombination involve the use of a mutation-generating enzyme, AID (reviewed by Pavri & Nussenzweig, 2011).

The role of EBV in lymphomagenesis seems to relate to different mechanisms and properties of EBV-related products. EBV latent proteins induce expression of AID (He *et al.*, 2003). Another suggested role for EBV has been to confer resistance to apoptosis, with evidence of anti-apoptotic potential being reported for several genes, including EBV-encoded RNAs (Komano *et al.*, 1998; Nanbo *et al.*, 2002), EBNA-1 (Kennedy *et al.*, 2003), and BHRF1 (a BCL2 homologue protein usually associated with the lytic virus cycle; Kelly GL *et al.*, 2009), and with evidence of virally mediated epigenetic repression of BIM (also known as BCL2L11), a proapoptotic human tumour suppressor that is a member of the BCL2 family (Paschos *et al.*, 2009).

(a) Malaria and Burkitt lymphoma

The endemicity of *P. falciparum* malaria varies greatly, as does the incidence of endemic Burkitt lymphoma. Indeed, endemic Burkitt lymphoma only occurs where malaria transmission is high, within the so-called "lymphoma belt" of sub-Saharan Africa, and the high-transmission areas of Papua New Guinea. Furthermore, in the areas and countries where endemic Burkitt lymphoma occurs, it only arises among people living in areas with the highest transmission, the so-called holoendemic or hyperendemic areas. Conversely, the incidence of endemic Burkitt lymphoma is lower in areas where malaria is less endemic (mesoendemic, hypoendemic). For example, there is little malaria or endemic Burkitt lymphoma in the northern and southern parts of Africa. This suggests a dose–response relationship between the presence of *P. falciparum* malaria and endemic Burkitt lymphoma. In order to develop Burkitt lymphoma, a child has to be exposed to continuous infection, and chronic malaria since birth, as occurs in holoendemic or hyperendemic areas of Africa or New Guinea. Thus, having few or even multiple infections with *P. falciparum* does not predispose a child to endemic Burkitt lymphoma, it is the chronic nature of infection that is likely to increase the risk of developing this disease. There is no evidence that the other malaria parasites (*P. vivax, P. ovale, P. malariae, P. knowlesi*) are involved in the genesis of Burkitt lymphoma (see Section 2).

Antimalarial treatment in holoendemic areas has been associated with a decrease in the incidence of endemic Burkitt lymphoma (Burkitt, 1969; Morrow *et al.*, 1976). A malaria intervention trial was conducted in United Republic of Tanzania from 1977 to 1982 to monitor the prevalence of endemic Burkitt lymphoma and malaria, but the results were inconclusive due to methodological issues (Geser *et al.*, 1989). Still, a high average annual rate of incidence (4.7 per 100 000 population) of Burkitt lymphoma prevailed in the holoendemic area. All cases were detected in the lowlands, with none occurring on the high plateau situated more than 1500 m above sea level, although 17 cases would have been expected if the rate found in the lowlands had also prevailed in the highlands. Correspondingly, it was also found that malaria endemicity was considerably higher in the lowlands (Geser *et al.*, 1989). Other attempts to determine whether relative peaks in malaria incidence act as a trigger for endemic Burkitt lymphoma – by analysing space–time clustering or seasonality of cases – have been inconclusive (Ogwang *et al.*, 2008). However, analytical models have found high-risk clusters

of incidence of endemic Burkitt lymphoma within a malaria holoendemic region, indicating the heterogeneity of malaria exposure within the same area (Rainey *et al.*, 2007b). These data support the role of intense malaria transmission in the etiology of endemic Burkitt lymphoma (Rainey *et al.*, 2007b).

The heterogeneity in the relationship between malaria endemicity and incidence of endemic Burkitt lymphoma suggests that the timing of co-infection and the duration of infection with *P. falciparum* at the individual level may determine the outcome of EBV dysregulation leading to endemic Burkitt lymphoma (Moormann *et al.*, 2011). This assumption is supported by the fact that the average age of patients with endemic Burkitt lymphoma in hyperendemic areas is between 6 and 8 years *versus* 16.2 years in low-transmission areas (Burkitt & Wright, 1966). The chronology of EBV and *P. falciparum* co-infections in relation to incidence of endemic Burkitt lymphoma in malaria holoendemic areas is depicted in Fig. 4.3. Immigrants from areas where malaria is hypoendemic, who migrated to holoendemic areas, developed endemic Burkitt lymphoma in late adolescence and adulthood (Burkitt & Wright, 1966). Moreover, development of endemic Burkitt lymphoma before age 2 years is extremely rare, although the reason for this is unclear.

P. falciparum has been clearly shown to be a powerful B-cell mitogen. As a consequence of polyclonal B-cell activation, hyper-gammaglobulinaemia is induced by the infection and is likely to be responsible for the high levels of IgM and IgG in children at age 5–10 years (Greenwood & Vick, 1975; Whittle *et al.*, 1990); these levels are three to four times higher in children in highly endemic areas than in children in non-endemic areas. This age range is also the age at which the incidence of endemic Burkitt lymphoma peaks (Mwanda *et al.*, 2004).

No other parasite, prion, bacterium, or helminth has been associated with the development of endemic Burkitt lymphoma, although some of these agents are very common in the underprivileged populations that are affected by this form of cancer.

(b) Malaria and EBV

Although co-infection with *P. falciparum* and EBV has long been implicated in the genesis of endemic Burkitt lymphoma, the relative contribution of these various mechanisms is not yet fully understood. A prospective study on more than 40 000 Ugandan children found very high antibody titres of antibodies to the EBV viral capsid antigen (VCA) in children who subsequently developed endemic Burkitt lymphoma. This led to the hypothesis that infection with EBV early in life results in an infection that is poorly controlled and increases the risk of developing the tumour (De-Thé *et al.*, 1978). Recent studies compared EBV load and time of EBV primary infection in two Kenyan districts with different patterns of malaria transmission. The mean age of infection in the holoendemic area was 7.28 months, with 35.3% of the children infected before age 6 months *versus* 8.39 months, with 12.2% being infected before age 6 months, in a district with limited transmission of malaria. Modelling analysis showed that residence in holoendemic areas and younger age at first EBV infection were significant predictors for having a higher EBV load throughout the observation period (Piriou *et al.*, 2012).

The contribution of falciparum malaria to the pathogenesis of endemic Burkitt lymphoma depends on the nature of the malaria antigens and their direct effect on the B-cell compartment and persistence of EBV, and on the impairment of EBV-specific T-cell immunity during infection. Infection with *P. falciparum* has immunosuppressive effects, as reflected by the impairment of macrophage function and antigen presentation (dendritic-cell inhibition), diminished specific T-cell response, induction of regulatory T cells and high plasma levels of pro-inflammatory

cytokines (IL-6, TNF) and regulatory cytokines (IL-10, TGFβ) (reviewed by Cunnington & Riley, 2010). IL-10 and TGFβ decrease EBV-specific T-cell responses (Altiok *et al.*, 1990; Bejarano & Masucci, 1998).

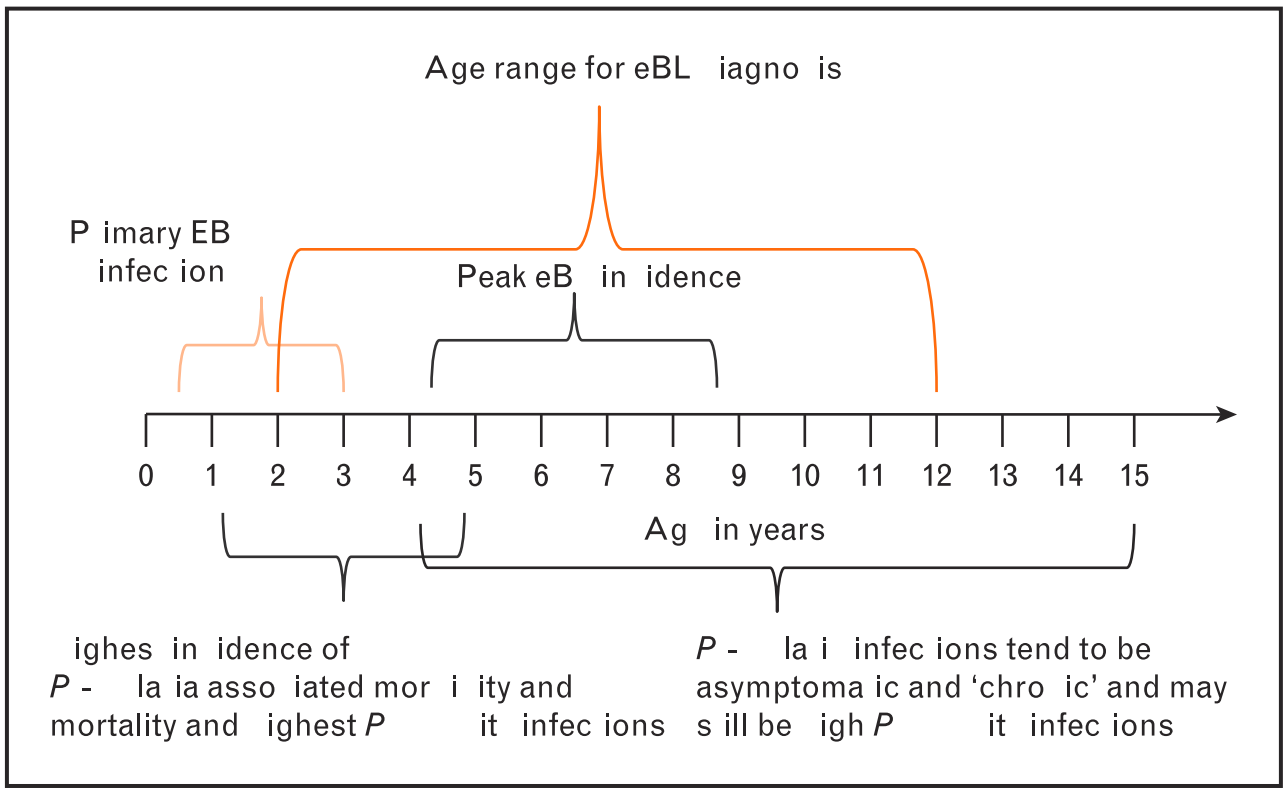
In immunocompetent individuals, EBV-specific T cells restrict viral replication, prevent the switch to lytic production, and limit the number of latently infected B cells (reviewed in Rickinson & Kieff, 2007). EBV is activated when the immune system is compromised (reviewed by Hopwood & Crawford, 2000). During malaria infection, the virus/host balance is changed in favour of the virus. Impairment of immune control of EBV in patients with acute malaria infection leads to increased numbers of circulating EBV-carrying B cells (Whittle *et al.*, 1984; Lam *et al.*, 1991), viral reactivation, and increased viral burden, as demonstrated by quantitative measurement of EBV DNA (Moormann *et al.*, 2005; Rasti *et al.*, 2005; Donati *et al.*, 2006b; Njie *et al.*, 2009). Comparison of levels of EBV DNA in blood from children living in two areas with different malaria-transmission intensities showed a correlation between viral load and the endemicity of malaria (Moormann *et al.*, 2005). Detection rates and levels of EBV DNA were also shown to be higher in children with acute malaria than in control children without malaria (Donati *et al.*, 2006b).

Studies in Kenya demonstrated an age-related loss of T-cell IFN-γ responses to lytic and latent EBV antigens, but predominantly to lytic antigens, in children living in areas of holoendemic malaria (Moormann *et al.*, 2007). T-cell responses against lytic, but not latent, EBV antigens seem to decrease gradually among malaria-exposed children aged 5–9 years, compared with other age groups and non-malaria-exposed children (Moormann *et al.*, 2011).

The impact of malaria on the control of EBV persistence seems to be evident only before immunity to malaria is fully acquired. Children, but not adults, living in malaria-endemic areas have high serum levels of EBV DNA, an indicator of active EBV replication (Rasti *et al.*, 2005). Endemic Burkitt lymphoma is a childhood tumour with a peak incidence at between age 5 and 8 years, an age range at which malaria infection may impair the virus/host balance. Moreover, the onset of endemic Burkitt lymphoma is marked by high antibody titres, indicative of viral replication (Henle *et al.*, 1969). EBV reactivation can be monitored by the appearance of antibodies against VCA and BZLF1, which are markers of EBV reactivation (Yamauchi *et al.*, 1998; Dardari *et al.*, 2000). A study that examined the effect of chronic infection with *P. falciparum* on EBV antibody titres reported higher and more persistent IgG titres to EBNA-1, VCA and BZLF1 in children from a malaria holoendemic area than in children from a hypoendemic area (Piriou *et al.*, 2009).

In vivo, latent EBV in infected B cells can be reactivated to enter the lytic cycle by various cellular signalling pathways, including ligation to B-cell receptors and plasma-cell differentiation (Laichalk & Thorley-Lawson, 2005). Viral reactivation can also be triggered *in vitro* in EBV-carrying cell lines by anti-human Ig and by several chemical agents and drugs (IARC, 2012). Lytic replication begins by the expression of the intermediate–early EBV lytic activator genes *BZLF1* and *BRLF1*, followed by translation of late genes encoding structural components such as VCA. The first demonstration that EBV can be reactivated directly by another pathogen was published by Chêne *et al.* (2007), who showed that PfEMP1 is able to directly drive a latently infected B cell into EBV replication. Infected erythrocytes and the PfEMP1 domain CIDR1α bound to the EBV-carrying B-cell line Akata led to a lytic-cycle reactivation and an increase in viral load, independent of cell proliferation (Chêne *et al.*, 2007). Viral reactivation was also induced in latently infected B cells derived from the blood of healthy EBV carriers and patients with endemic Burkitt lymphoma.

Fig. 4.3 Chronology of *Plasmodium falciparum* malaria and Epstein–Barr virus infections in relation to incidence of endemic Burkitt lymphoma



eBL, endemic Burkitt lymphoma; EBV, Epstein–Barr virus; Pf, *Plasmodium falciparum*
From Moormann *et al.* (2011). Reprinted with permission from Wolters Kluwer Health.

The molecular mechanisms underlying the EBV switch from latency to the lytic cycle may result in part from the interaction of PfEMP1 with surface Igs, and possibly other molecules, mimicking the effect achieved by B-cell receptor cross-linking with anti-human Igs (Chêne *et al.*, 2007) and triggering viral reactivation in a similar way as anti-human Ig induces viral production in EBV-carrying cell lines (Takada, 1984). The main interactions between B lymphocytes and infected erythrocytes/malarial antigens most likely occur in the secondary lymphoid tissues *in vivo*, such as the spleen where memory B cells, potentially latently infected by EBV, could return to undergo additional rounds of maturation. The disruption of the secondary lymphoid organs that characterize malaria infections may favour the triggering of viral reactivation in EBV-carrying lymphocytes by malarial antigens.

The preferential activation of the memory B-cell compartment by malarial antigens is of importance given that EBV resides in a latent state in memory B cells, and that differentiation from memory to plasma cells results in switching the latent to the lytic phase (Babcock *et al.*, 1998; Laichalk & Thorley-Lawson, 2005). Therefore, the increased Ig production and differentiation induced by stimulation with malarial antigens may also contribute to the increased viral production that characterizes malaria infection.

4.3.2 KSHV

KSHV, also known as HHV-8, is the etiological agent of Kaposi sarcoma (IARC, 2012). The risk of Kaposi sarcoma is significantly increased by immune suppression, such as that caused by HIV, leading to increased viral load and shedding (Engels *et al.*, 2003; IARC, 2012). Geographical and temporal variation in the incidence of Kaposi sarcoma and the prevalence of KSHV among immunocompetent individuals, indicate that other (co)factors may be important in facilitating both transmission and disease, e.g. environmental cofactors (Ziegler, 1993; Dedicoat & Newton, 2003; Whitby *et al.*, 2007; Dollard *et al.*, 2010; IARC, 2012) and presence of mosquitoes and other blood-sucking arthropods (Coluzzi *et al.*, 2003; Ascoli *et al.*, 2006).

The association between malaria and KSHV was suggested by epidemiological analysis of the distribution of Kaposi sarcoma in Africa before the AIDS epidemic (Cook-Mozaffari *et al.*, 1998). However, cross-sectional analysis of the incidence of Kaposi sarcoma in Latina (a central province of Italy where malaria was endemic until the 1930s), and in Malta (where malaria was not endemic), showed similar results, suggesting that malaria endemicity is not likely to increase the risk of disease (Serraino *et al.*, 2003). Studies in Ugandan women found that seropositivity to KSHV was significantly associated with malaria parasitaemia, hookworm and *Mansonella pestans* (Wakeham *et al.*, 2011). It seems therefore that other infections that are also prevalent in malaria-endemic areas may be contributing factors.

A recent hypothesis suggests that the immunosuppressive effect of antimalarial drugs e.g. chloroquinine and quinine derivatives, may play a role in the high incidence of Kaposi sarcoma in malaria-endemic areas (Ruocco *et al.*, 2011). The iatrogenic immunosuppression caused by these drugs is long-lasting; traces of chloroquinine can be found several years after the last ingestion (Meyer *et al.*, 2010).

The evidence for host immune dysregulation in the pathogenesis of Kaposi sarcoma is based on evaluation of high-risk populations (e.g. HIV-infected and post-transplant patients) (Grülich *et al.*, 2007), as well as an increasing understanding of specific immune defects. Risk of Kaposi sarcoma is strongly associated with defects in cellular immunity. Epidemiological and clinical studies suggest that KSHV viraemia is a risk factor for development of Kaposi sarcoma (reviewed by Uldrick & Whitby, 2011). KSHV-infected patients who develop Kaposi sarcoma showed a reduced KSHV-specific CD4 response as measured by an IFN-γ enzyme-linked immunosorbent spot assay, specific for KSHV-derived peptides (Guihot *et al.*, 2006).

There are insufficient data available to evaluate the precise role of malaria in KSHV infection. However, it is possible that in in the same way that malaria impairs the immune control of EBV persistence, malaria could also lead to KSHV reactivation and increased prevalence of KSHV, with increased incidence of Kaposi sarcoma and other lymphoproliferative disorders.

4.3.3 Other human herpes viruses

Human herpes viruses are widely distributed pathogens. In immunocompetent individuals the clinical outcomes of infection are generally benign, but in immunocompromised hosts, primary infection, reinfection, or extensive viral reactivation can lead to critical diseases.

It has been suggested that acute infection with *P. falciparum* induces HSV-1 and VZV reactivation (Cook 1985; Regunath *et al.*, 2008; Sowunmi *et al.*, 2008). Recently, the effect of acute *P. falciparum* malaria infection on reactivation and shedding of all known HHVs including HSV-1, HSV-2, EBV, VZV, CMV, HHV-6, HHV-7 and KSHV (HHV-8) was analysed in a retrospective study among Ugandan children. Quantification of viral loads in blood and saliva demonstrated that malaria infection increased the load of EBV

in the blood and of HSV-1 in the oral cavity. HHV-6, HHV-7 and CMV were frequently detected in the malaria-infected children, but acute malaria was not found to be associated with reactivation of the β herpes viruses (Chêne *et al.*, 2011). [The sample size in this study was too small to draw any conclusion on the impact of malaria on reactivation of VZV and HHV-8.]

4.4 *Plasmodium* infection in experimental animals

Murine models for malaria infection do exist, employing parasites such as *P. yoelii, P. berghei*, and *P. chabaudii* (Serguiev & Demina, 1957; Landau & Killick-Kendrick, 1966), but there is a lack of studies on the induction of B-cell tumours or B-cell hyperplasia. A major limitation of these models is that repeated infection with *P. falciparum* cannot be reproduced because the murine *Plasmodium* induces sterilizing immunity. On the other hand, *P. falciparum*, the parasite associated with endemic Burkitt lymphoma, is specific for humans. Injection of human erythrocytes into immunodeficient mice results in greater than 90% of circulating erythrocytes that are human and can be infected by *P. falciparum* (Badell *et al.*, 2000; Angulo-Barturen *et al.*, 2008; Jiménez-Díaz *et al.*, 2009). This model is useful for the study of antimalarial drugs.

Transplantation of human cord-blood $CD34^+$ progenitor cells to newborn $Rag2^{-/-}\gamma_c^{-/-}$ mice leads to *de novo* development of major functional components of the human adaptive immune system (Chicha *et al.*, 2005). The model has already been developed for EBV infection and the results support the model of EBV persistence *in vivo*. Intriguingly, in cases that were characterized by nodular and diffuse proliferation similar to infectious mononucleosis, EBV also induced clonal expansion and ongoing somatic mutations without germinal-centre reactions (Cocco *et al.*, 2008). More recently, the same model was used to investigate the effects of HTLV-1 infection *in vivo*, where alterations in human T-cell development were observed (Villaudy *et al.*, 2011). No animal model has been developed for efficient development of the human erythrocyte, and there is no suitable model available that incorporates all the key facets of both *P. falciparum* and EBV infections. The mouse transplanted with the human adaptive immune system could be further developed to support the injection of *P. falciparum*-infected erythrocytes selected *in vivo* to grow in immunodeficient mice and escape phagocytosis. Another possibility could be the use of murine malaria in $CD34^+Rag2^{-/-}\gamma_c^{-/-}$ mice, or the use of purified malarial antigens such as CIDR. Other aspects could also be tested, like the functional consequences of the interaction of *P. falciparum* with TLR9 on EBV-infected B cells.

4.5 Susceptible populations

In human malaria, certain processes are critical to disease development, and genetic variants that disrupt these processes can protect against the disease. The invasion of erythrocytes by malaria parasites plays a central role here. However, the few studies that evaluated potential effects of haemoglobin polymorphisms on the risk of developing Burkitt lymphoma have not been conclusive (see Section 2.1.5(b)).

4.6 Mechanisms of lymphomagenesis

It is in the context of an uninterrupted presence of malaria parasites, a continuous activation of the immune system, splenomegaly and concurrent infections by viruses, bacteria and parasites that endemic Burkitt lymphoma emerges at high frequency. The determining factors that bring about endemic Burkitt lymphoma are, as currently understood, the malaria parasite and EBV.

Different mechanisms may explain the incremental contribution of chronic and acute infection with *P. falciparum* in the lymphomagenesis of endemic Burkitt lymphoma.

On the one hand, malaria infection induces B-cell proliferation and activation, and protects against apoptosis, thereby increasing the latently infected B-cell pool. On the other hand, malaria infection increases virus production, and impairs EBV-specific responses. Increased viral reactivation may result from modulation of T cells, NK cells and humoral responses that limit the progression to lytic replication (Precopio *et al.*, 2003; Moormann *et al.*, 2007), and from direct activation of the lytic cycle resulting from specific interaction between malarial antigens and latently infected B cells involving CIDR, and TLR9 engagement. After expansion, memory B cells can differentiate to antibody-secreting plasma cells. *P. falciparum* schizonts and infected erythrocytes have the potential to interact directly with B cells, thereby inducing proliferation and differentiation into antibody-secreting cells, concomitant with viral reactivation. *P. falciparum* has a TLR9 ligand (Parroche *et al.*, 2007) and TLR9 signalling synergistically enhances proliferation of EBV-infected B cells (Zauner & Nadal, 2012).

The association of EBV with endemic Burkitt lymphoma is probably determined by the age at which EBV infection occurs. The timing of co-infection and duration of *P. falciparum* infections at the individual level seems to determine the outcome of EBV dysregulation leading to endemic Burkitt lymphoma. This assumption is supported by the fact that the average age of patients in hyperendemic areas was found to be 8.1 years, whereas in low-risk areas it was 16.2 years, and immigrants from malaria hypoendemic areas who migrated to holoendemic areas developed endemic Burkitt lymphoma in late adolescence and adulthood (Burkitt & Wright, 1966). Moreover, development of endemic Burkitt lymphoma before age 2 years is an extremely rare event. These effects are more pronounced in children living in areas with perennial transmission where they are repeatedly infected with *P. falciparum* and harbour chronic, asymptomatic, and often untreated infections.

Increased viral load may increase the risk by multiple non-exclusive mechanisms at different levels:

- infecting bystander B cells and augmenting the pool of latently infected B cells;
- increasing activity of AID, which enhances the possibility that translocations will occur;
- providing an anti-apoptotic environment that tolerates the *MYC* translocation (Kelly & Rickinson, 2007);
- exhaustion of T-cell immune responses. In mouse models it has been shown that the frequency of infections has a greater effect on immune function since the greater the number of antigenically heterologous infections, the greater the decrease in overall T-cell activity. Acute and persistent infection can induce loss of memory T-cell function to previously encountered non-cross-reactive viruses (Doherty, 1993; Kim & Welsh, 2004).

Endemic Burkitt lymphoma is one of the most rapidly dividing tumours, thus the triggering event must occur shortly before clinical presentation. The frequency of the molecular event leading to malignancy must be low, as the annual incidence of endemic Burkitt lymphoma in holoendemic areas is on average only 5–10 (ranging up to 22) per 100 000 children, implying that the oncogenic event is rare.

Thus, EBV and malaria together exert effects on the human host that increase the likelihood of *MYC* translocation, and that synergize with the de-regulated MYC protein to enhance the proliferative capacity and survival of the tumour. The contribution of malaria-induced activation and enhanced survival of B cells, plus the EBV-driven proliferation and facilitation of mutation in

memory germinal-centre B cells may assist the *MYC–Ig* translocation to persist, giving rise to a Burkitt lymphoma progenitor cell.

5. Summary of Data Reported

5.1 Exposure data

Malaria in humans is caused by four well-characterized strict human *Plasmodium* pathogens: *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. A fifth pathogen, *P. knowlesi*, infects both humans and monkeys. The greatest burden of morbidity and mortality is associated with *P. falciparum*. While all people living in equatorial regions of the world are at risk of malaria, most cases (> 90%) of falciparum malaria and death occur in children aged < 5 years in sub-Saharan Africa. Across sub-Saharan Africa and in Papua New Guinea, malaria transmission is highly endemic, although there is great local variation. Transmission of endemic malaria is categorized according to several parameters, including entomological inoculation rate, parasite prevalence and splenomegaly in children aged 2–10 years. Holoendemic malaria transmission can be defined as parasite prevalence of > 70% and splenomegaly in > 80% of children, and there is no sterilizing immunity from a primary infection. Host genetic variation influences disease susceptibility. Where malaria transmission is endemic, *Plasmodium* infection in children recurs frequently. Antibodies are clearly important in protection against *P. falciparum*; however, correlations of humoral protection have not been well defined.

P. falciparum has a complex life cycle that involves both human and *Anopheles* mosquito hosts. After the bite of a *Plasmodium*-infected mosquito, the parasite travels to the liver; this stage of development is asymptomatic. It is only when the parasite is released from infected liver cells and invades erythrocytes that clinical illness occurs. Falciparum malaria can be uncomplicated, with fever and chills, or associated with complications such as severe acute anaemia, cerebral malaria, adult respiratory distress syndrome and acidosis. Interventions such as use of bednets and intermittent prevention and treatment for malaria have been widely used to reduce the burden of malaria. Drug resistance remains a problem, and no vaccines are currently available.

5.2 Human carcinogenicity data

5.2.1 Endemic Burkitt lymphoma

All the studies reviewed for endemic Burkitt lymphoma were conducted in sub-Saharan Africa, where infection with Epstein–Barr virus (EBV), an established cause of the disease, is largely ubiquitous and occurs at an early age.

Evidence consistent with an etiological role for holoendemic malaria caused by infection with *P. falciparum* in relation to endemic Burkitt lymphoma comes from a variety of sources. In 1962, Dennis Parsons Burkitt highlighted a very strongly specific geographical association between Burkitt lymphoma and holoendemic malaria on the African continent. Since then, a substantial body of evidence has accumulated from multiple correlation studies strongly linking the incidence of Burkitt lymphoma to areas where *P. falciparum* transmission is more intense. These ecological studies carry substantial weight in this context because of global and local contrasts in exposure (e.g. there are areas where either everybody or nobody is exposed).

Early case–control studies were inconclusive due to small sample size, inadequate exposure assessment at the individual level, or poor methodology. Uncertainties in some of the serological measurements employed continue to be a limitation, even in recent case–control studies. Nevertheless, two studies that measured antibodies to the whole schizont (considered as a

measure of exposure) found an increasing risk of endemic Burkitt lymphoma with increasing titres of antimalaria antibody; they also demonstrated the combined effect of malaria and EBV infection.

5.2.2 Other cancers

There was no clear evidence of an association between infection with *P. falciparum* and cancer at any other site.

There were no data available on cancer associated with species of *Plasmodia* other than *P. falciparum*.

In summary, evidence on the association between holoendemic malaria caused by infection with *P. falciparum* and cancer is strongly supportive, but not conclusive.

5.3 Animal carcinogenicity data

In a study in male Balb/c mice, intraperitoneal injection of *Plasmodium berghei yoelli* promoted induction of lymphoma by Moloney leukaemogenic virus. In a limited study in Swiss mice, intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei* strain K173 increased the incidence of lymphoma. In a third study in neonatal CFW mice, intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei yoelli* resulted in a non-statistically significant promotion of SV40-induced sarcomas of the liver and/or spleen. In one study in male Buffalo rats, intraperitoneal injection of mouse erythrocytes parasitized with *P. berghei* did not promote aflatoxin B_1-induced hepatocellular carcinoma.

5.4 Mechanistic and other relevant data

Different mechanisms may explain the incremental contributions of EBV and of chronic infection with *P. falciparum* in the lymphomagenesis of endemic Burkitt lymphoma:

- The timing of co-infection and the duration of malarial infections at the individual level seem to determine the outcome of EBV dysregulation, leading to endemic Burkitt lymphoma. The endemicity of malaria infection correlates with the viral load, while it inversely correlates with the average age at which primary infection with EBV occurs.
- EBV is recognized as a necessary agent in the pathogenesis of endemic Burkitt lymphoma.
- The malaria parasite *P. falciparum* reactivates EBV both *in vitro* and *in vivo*.
- *In vitro*, malarial antigens such as PfEMP1 (*P. falciparum* erythrocyte membrane protein), cause polyclonal B-cell activation, enhance B-cell proliferation and differentiation, and alter different cellular pathways that inhibit apoptosis. These mechanisms lead to increased survival of the B cells that are the target for the specific chromosomal *MYC* translocation that characterizes endemic Burkitt lymphoma.
- *In vivo*, infection with *P. falciparum* modifies the B-cell subset composition, with expansion of immature transitional B cells and an increase in the activity of the enzyme activation-induced deaminase (AID), which generates mutations involved in immunoglobulin affinity and immunoglobulin class-switching. The increased survival and maturation further augment the EBV load.
- Several latent and lytic EBV-associated proteins increase the activity of AID and prevent apoptosis, and thus these viral proteins can additionally augment the likelihood of developing endemic Burkitt lymphoma by preventing apoptosis of B cells that carry the *MYC* translocation.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *limited evidence* for an association between malaria caused by infection with *P. falciparum* in holoendemic areas and cancer. A positive association has been observed with endemic Burkitt lymphoma.

6.2 Cancer in experimental animals

There is *inadequate evidence* in experimental animals for the carcinogenicity of *P. berghei*.

6.3 Mechanistic and other relevant data

There is strong evidence that the malaria parasite *P. falciparum* reactivates EBV, which is the known cause of endemic Burkitt lymphoma. In addition, infection with *P. falciparum* augments the pool of B cells in which this tumour arises.

6.4 Overall evaluation

Malaria caused by infection with *P. falciparum* in holoendemic areas is *probably carcinogenic to humans (Group 2A).*

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